Adverse Events with Biomedicines

Giuseppe Tridente

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Prevention Through Understanding

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Giuseppe Tridente
University of Verona
Verona
Italy

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Ex bono malum
Ex malo bonum

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Contents

Abbreviations

5FU	5-Fluorouracil
A&U	Angioedema and urticaria
AA	Aplastic anemia/allergic asthma
AAO	American Academy of Ophthalmology
AAV	ANCA-associated vasculopathy
ACCP	American College of Chest Physicians
ACS	Acute coronary syndrome
ACV	Acyclovir
ADA	Anti-drug antibody
ADC	Antibody-drug conjugate
ADCC	Antibody dependent cell cytotoxicity
ADR/ADE	Adverse drug reaction/event
ADRS/ARDS	Acute distress respiratory syndrome
AE/AR	Adverse event/reaction
AFND	Acute febrile neutrophilic dermatosis
AID/sAID	Autoimmune disorders/secondary
AIHA/AHA	Autoimmune hemolytic anemia
ALCL	Anaplastic large cell lymphoma
ALL	Acute lymphoblastic leukemia
ALP	Alkalyne phosphatase
ALS/G	Anti-lymphocyte serum/globulin
ALT	Alanine transaminase
AMD	Age-related macular degeneration
AMF	Autocrine motility factor
AML	Acute myeloid leukemia
ANA	Anti-nuclear antibody
ANC	Absolute neutrophil blood count
ANCA	Anti neutrophil cytoplasmic antibody
APC	Antigen presenting cell
APRIL	Another proliferation inducing ligand
ARDS/ADRS	Acute respiratory distress syndrome
AREG	Amphiregulin
ARF	Acute renal failure
AS	Ankylosing spondylitis

ASCT	Allogenic stem cell transplant
AST	Aspartate transaminase
ATE	Arterial thromboembolic events
ATG	Anti-thymocyte globulin
AZA	Azathyoprine
B cells	Bone-marrow dependent lymphocytes
B-PLL	B-cell prolymphocytic leukemia
B&J	Bone and joint
BAE	Biological adverse event
BBB	Blood brain barrier
BBW	Black box warning
BC/mBC	Breast cancer/metastatic
BCC	Basal cell carcinoma
BCL	B-cell leukemia/lymphoma
BCMA	B cell maturation antigen
BCRP	Breast cancer resistant protein
BFU-E	Burst forming unit erythroid
BKV	Polyoma virus BK
BMd	Bone marrow depression/hypoplasia
BRB	Blood retinal barrier
BRM	Biological response modifiers
BR3	BlyS receptor 3
BRVO	Lateral branch retinal vein obstruction
BUN	Blood urea nitrogen
c-Fms	Transmembrane receptor tyrosine kinase for M-CSF
C&P	Chemical injury and poisoning
CAD/CAO	Coronary artery disease/occlusion
CAPS	Cryopyrin associated periodic syndromes
CC	Colon cancer
cCTL	Cutaneous T cell lymphoma
CD	Crohn's disease
CDC	Complement dependent cytotoxicity
CDR	Complementary-determining region
CEP	Chronic eosinophilic pneumonia
CERA	Continuous erythropoietin receptor activators
CF	Cystic fibrosis
CFC/CFU	Colony forming cell/unit
CGD	Chronic granulomatous disease
CHD	Congenital heart disease
CHF	Congestive heart failure
CHOP	Cyclophosphamide, vincristine, prednisolone, adriamycin (doxorubicin)
CHP	Cytophagic histiocytic panniculitis
CHVP	Cyclophosphamide, doxorubicin, teniposide/etoposide, prednisone

CID	Chronic immune-mediated disease
CINCA	Chronic infantile neurological cutaneous articular syndrome
CIT	Chemotherapy-induced thrombocytopenia
CKD	Chronic kidney disease
CLL	Chronic lymphocytic leukemia
CLS	Capillary leak syndrome
CMV	Cytomegalovirus
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
COPD	Chronic obstructive pulmonary disease
CPK	Creatine phospho kinase
CRC/mCRC	Colo-rectal cancer/metastatic
CRF	Chronic renal failure
CRP	C-reactive protein
CRS/CRRS	Cytokine release (related) syndrome
CRVO	Central retinal vein obstruction
CS	Corticosteroids
CSF/-1R/M-/G-/GM-	Colony stimulating factor/type 1 receptor/macrophage/granulocyte
CSS	Churg-Strauss syndrome
CTCAE	Common terminology criteria for adverse events classification (grade 1–5)
cTCL	Cutaneous T cell lymphoma
CTLA-4	Cytotoxic T lymphocyte antigen-4
CVA/CVD	Cerebro-vascular accident/disorder
CVE	Cardio-vascular events
CVP	Cyclosporine, vincristine, prednisolone
CYA/CsA	Cyclosporin A
D&G	Dental and gingival
DC	Dendritic cell
DIC	Disseminated intravascular coagulation
DIH	Drug-induced hepatitis
DIHS	Drug-induced hypersensitivity syndrome
DIPG	Diffuse intrinsic pontine glioma
DIRA	Deficiency of IL-1Ra
DLBCL	Diffuse large B cell lymphoma
DLT	Dose-limiting toxicity
DMARD	Disease-modifying antirheumatic drug
DME	Diabetic macular edema
DRAE	Drug-related adverse event
DRESS	Drug reaction with eosinophilia and systemic syndrome
dsDNA	Double-stranded DNA
DT	Diphtheria toxin
DVT	Deep vein thrombosis
E/T	Embolia/thrombosis

EBV	Epstein-Barr virus
ECL	Electrochemiluminescence
EFD	Ejection fraction decrease (cardiac)
EGF/EGFR	Epithelial growth factor/receptor
ELAM	Endothelial-leukocyte adhesion molecule (E-selectin)
EM	Erythema multiforme
EMA/EMEA	European Medicines Agency
EOC	Epithelial ovarian cancer
EpCAM	Epithelial cell adhesion molecule
EPO/EpoR	Erythropoietin/receptor
ERA	Enthesitis-related arthritis
Erb	Erythroblastic leukemia viral oncogene (EGFR, HER)
EREG	Epiregulin
ESA	Erythropoiesis stimulating agents
ESI	Events of special interest/emergency severity index
EUV	Eudravigilance, Europe
FA	Folinic acid
FAERS/AERS	FDA adverse events reporting system
FBS	Foreign body sensation (eye)
FC	Fludarabine, cyclophosphamide
FCAS	Familial cold autoimmune syndrome
FCUS	Familial cold urticaria syndrome
FDA	Food and drug administration, USA
FGF/FGFR	Fibroblast growth factor/receptor
FCL	Follicle center lymphoma
FL	Follicular lymphoma
FLS	Flu-like syndrome
FLT3	Fms-like tyrosine kinase-3
FMF	Familial mediterranean fever
FOLFIRI/FOLFOX	5-FU, leucovorin, irinotecan/oxaliplatin
FP	Fusion protein
FTC	Fallopian tube cancer
GA	Gout arthritis
G-CSF	Granylocyte colony stimulatory factor
G-CSF/GM-CSF	Granulocyte/monocyte colony stimulatory factor
GBM	Glioblastoma multiforme
GBS	Guilliain-Barrè syndrome
GC/mGC	Gastric cancer/metastatic
GCV	Ganciclovir
GFR	Glomerular filtration rate
GI	Gastro-intestinal
GIP	Gastro-intestinal perforation
GIST	Gastro-intestinal stromal tumor
GN	Glomerulonephritis
GU	Genito-urinary

GvH/GvHR/GHVD	Graft versus host/reaction/disease
HA	Hemolytic anemia
HAART	Highly active antiretroviral therapy
HACA	Human anti-chimeric antibodies
HAHA	Human anti-human antibodies
HALT	Hormone ablation therapy
HAMA	Human anti-mouse antibodies
HB-EGF	Heparin-binding epithelial growth factor
HBV/HCV	Hepatitis viruses
HCC	Hepatocellular carcinoma
HCL	Hairy cell leukemia
HE	Hemorrhagic events
HER1,2..	Human epidermal growth factors
HES	Hypereosinophilic syndrome
HFS	Hand-foot syndrome (palmar-plantar erythrodysesthesia syndrome)
HGF	Hepatocyte growth factor
HIF	Hypoxia-inducible factor
HL	Hodgkin's lymphoma
HLH/HPS	Hemophagocytic lymphohistiocytosis/syndrome
H&N	Head and neck
HNC	Head and neck carcinoma
HS	Hidradenitis suppurativa
HSCT	Hematopoietic stem cell transplantation
HSTCL	Hepatosplenic T cell lymphoma
HSV	Herpes simplex virus
HT	Heart transplant
HUS/aHUS	Hemolytic uremic syndrome/atypical
HZV	Herpes zoster virus
IBD	Inflammatory bowel disease
ICAM	Intracellular adhesion molecule
ICH	Intracranial hemorrhage
IDS	Immunodeficiency syndrome
IFL	Irinotecan/fluorouracil/leucovorin
IFN	Interferon
IGF-1	Insuline-like growth factor 1
IL-1, 2, 3	Interleukins
IL-1Ra	IL-1 receptor antagonist
IL-1RAP	IL-1 receptor accessory protein
ILD	Interstitial lung disease
IMID	Immune-mediated inflammatory disease
INN	International nonproprietary name
INR	International normalized ratio (prothrombin time)
IOP	Increased intraocular pressure
IP	Intraperitoneal administration

IR/IRR/IRS	Infusion (related) reaction/syndrome
IrADR/IrADE	Immune-related adverse drug reaction/event
IRIS	Immune reconstitution inflammatory syndrome
ISR	Injection site reaction
ITAM	Immunoreceptor activation motif
ITCP/ITP	Immune thrombocytopenia
ITIM	Immunoreceptor tyrosine-based inhibitory motif
Itk	IL-2 inducible leukocyte tyrosine kinase
IV	Intra-venous administration
IVI	Intra-vitreal injection
JAK	Janus associated kinase
JCA	Juvenile chronic arthritis
JIA	Juvenile idiopathic arthritis
JRA	Juvenile rheumatoid arthritis
JC/JCV	John Cunningham polyomavirus
KGF	Keratinocyte growth factor
Kit/c-kit	Tyrosine kinase receptor, or CD117, or stem cell factor receptor
KLH	Keyhole limpet hemocyanin
KRAS	Kirsten rat sarcoma viral oncogene (V-Ki-ras2)
KSHV	Kaposi sarcoma-associated herpesvirus
LAK	Lymphokine-activated killer cells
LBL	Lymphoblastic leukemia/lymphoma
LCF	Lymphocyte chemoattractant factor
Lck	Lymphocyte specific protein tyrosine kinase
LCV	Leukocytoclastic vasculitis
LFA-1	Lymphocyte function-associated antigen-1
LFT	Liver functional tests (ALT/AST,etc)
LIF	Leukemia inhibitory factor
LLS	Lupus-like syndrome
LRTI/D	Lower respiratory tract infections/disorders
LT-α/-β	Lymphotoxins
LT	Liver transplant
LVD/LVCD/LVEF	Left ventricular cardiac dysfunction/ejection fraction
mAb	Monoclonal antibody
MAC	Membrane attack complement complex
MACE	Major adverse cardiovascular event
MAF	Macrophage activating factor
MAH	Market authorization holder
MALT	Mucosal associated lymphoid tissue
MAPK	Mitogen-activated protein kinase
MAS	Macrophages activation syndrome
MCL	Mantle cell lymphoma
MCP-1	Monocyte chemotactic protein-1
MDS	Myelo-dysplastic syndrome

MF	Mycosis fungoides
MF/MFN	Myelofibrosis/myeloproliferative neoplasm
MI	Myocardial infarction
MIP-1,2	Macrophage inflammatory proteins
MLC/MLR	Mixed lymphocyte culture/reaction
MM	Malignant melanoma
MMF	Mycophenolate mofetil
MMP3	Matrix-metallopeptidase-3
MO	Malignant osteoporosis
MOF	Multi-organ failure
MOMP	Mitochondrial outer membrane permeabilization
MPA	Microscopic polyangiitis
MR	Mucocutaneous reactions
MRA	Myeloma receptor antibody
MS	Multiple sclerosis
MTD	Maximum tolerated dose
MTX	Methotrexate
MWS	Muckle-Wells syndrome
NCCN	National Comprehensive Cancer Network
NESP	Novel erythropoiesis-stimulating protein
NF	Nephrotic syndrome
NHA	Non-emolytic anemia
NHL	Non-Hodgkin's lymphoma
NK	Natural killer cells
NKCL	NK cell lymphoma
NMO	Neuromyelitis optica
NMSC	Non-melanoma skin cancer
NODAT	New onset diabetes after transplantation
NOMID	Neonatal onset multisystem inflammatory disorder
NPC	Nasopharyngeal carcinoma
NPSLE	Neuropsychiatric SLE
NSAID	Non-steroidal anti-inflammatory drug
NSCLC	Non-small cell lung carcinoma
oJIA	Oligoarticular JIA
ON	Optic neuritis
ONJ	Osteonecrosis of the jaw
OP	Osteoporosis
OPG	Osteoprotegerin
P&R	Peritoneal and retroperitoneal
PAMP	Pathogen associated molecular patterns
PAPA	Pyogenic arthritis, pyoderma gangrenosum, acne syndrome
PAR	Perennial allergic rhinitis
PBPC	Peripheral blood precursor cells
PBSCT	Peripheral blood stem cell transplant
PC	Prostate cancer

PDGF/PDGFR	Platelet-derived growth factor/receptor
PE	Pulmonary embolism
PEDF	Pigment epithelium-derived growth factor (ocular)
PEG	Polyethylene glycol
PG	Pyoderma gangrenosum
PGF/PlGF	Placental growth factor
PJIA	Polyarticular juvenile idiopathic arthritis
PK/PKD	Pharmacokinetics/dynamics
PM/DM	Polymyositis/dermatomyositis
PML	Progressive multifocal leukoencephalopathy
PMN; PNP/m	Peripheral motor neuropathy
PMO	Postmenopausal osteoporosis
PNP	Peripheral neuropathy
PNS	Paraneoplastic syndrome
PNH	Paroxysmal nocturnal hemoglobinuria
PPC	Primary peritoneal cancer
PPES	Palmar-plantar erythrodysesthesia syndrome
PPMS	Primary progressive multiple sclerosis
PRCA	Pure red cell aplasia
Ps	Psoriasis
PsA	Psoriatic arthritis
PSN; PNP/s	Peripheral sensory neuropathy
Psoralens	Drugs containing chemicals reacting to ultraviolet light
PTCA	Percutaneous transluminal coronary angioplasty
PTEN	Phosphatase and tensin homolog
PTLD	Post-transplant lymphoproliferative disorder
PUVA	Psoralen and ultraviolet A therapy
RA	Rheumatoid arthritis
RANK/RANKL	Receptor activator of nuclear factor kappa-B receptor/ligand
RBS/nRBC	Red blood cells/nucleated RBC
RCA	Red cell aplasia
RCC/mRCC	Renal cell carcinoma/metastatic
ReA	Reactive arthritis
REMS	Risk evaluation and mitigation strategy—program
RPLS/PRES	Reversible posterior (leuco)-encephalopathy syndrome
RRMS	Relapsing remitting multiple sclerosis
RTK	Receptor tyrosine kinases
RVO	Retinal vein obstruction
SAA	Serum amyloid A
SADE/SADR	Suspected adverse event/reaction
SAE	Serious adverse event
sALCL	systemic anaplastic large cell lymphoma
SAR	Seasonal allergic rhinitis
SC	Subcutaneous administration

SCC	Squamous cell carcinoma
SCCHN	Squamous cell carcinoma, head and neck
SCLC	Small cell lung carcinoma
SCF	Stem cell factor
ser	Serious
sev	Severe
SIE	Serious infection event
SIR	Standardized incidence ratio
SIRS	Systemic inflammatory response syndrome
SJIA	Systemic juvenile idiopathic arthritis
SJS	Stevens-Johnson syndrome
SLE	Systemic lupus erythematosus
SM	Systemic mastocytosis
SOC	Standard of care (therapy)
SRA	Soluble receptor analogue
SRE	Skeletal-related event
SS	Sjögren's syndrome
SSLS	Serum sickness-like syndrome
STATs	Signal transducers and activators of transcription
SUSAR	Suspected unexpected serious adverse reaction
SzS	Sèzary syndrome
T cells	Thymus-dependent lymphocytes
TAC	Tacrolimus
TACE	TNF-α converting enzyme
TACI	Transmembrane activator and calcium modulator and cyclophilin ligand interactor
TB	Tuberculosis
TCC	Thrombocytosis
TCL	T cell lymphoma
TCP/TP	Thrombocytopenia
TCR	T cell receptor
TEAE	Treatment emergent adverse event
TEN	Toxic epidermal necrolysis
TGA	Therapeutic Goods Administration, Australia, New Zealand
TGF	Transforming growth factor
TIA	Transient ischemic attack
TKI	Tyrosine kinase inhibitor
TLS	Tumor lysis syndrome
TNF	Tumor necrosis factor
TPO	Thrombopoietin
TRAF	TNF receptor-associated factor(s)
TRAP	TNF receptor-associated periodic syndrome
TSH	Thyroid stimulating hormone
TVA	Thrombosis of vascular access

TYK	Tyrosine kinase
UC	Ulcerative colitis
UF	Unknown frequency
ULN/LLN	Upper/lower limit of normal
URI/URTI/URTD	Upper respiratory (tract) infection/disorder
URTD	Upper respiratory tract disorders
uSpA	Undifferentiated spondyloarthritis
UT/UTI	Urinary tract/infection
VEGF/VEGFR	Vascular endothelial growth factor/receptor
VIT	Venom immunotherapy
VOD	Hepatic veno-occlusive disease
VP1	Viral protein1 (JCV)
VTE	Venous thrombotic event/thromboembolism
WBC	White blood cells
W&E	Water and electrolytes
WG	Wegener granulomatosis

Part I
General Aspects

Introduction

1

The amount of biological molecules already in use in human therapy and the consistency of the promised new entries demand attention to clinicians, oncologists, rheumatologists, immunologists, allergologists, to health care professionals, and to public health surveillance. It is reasonable to assume that, together with the new entries and their massive therapeutic expansion, an increasing number of known adverse events and new reactions are to be expected, and will need to be observed, possibly prevented, and controlled. In fact, most of these new "Biomedicines" interfere with cell receptors, cytokines, chemokines, cell recognition molecules, and intracellular signaling that influence a number of crucial functions, including immune and inflammatory reactions, not only as positive consequence of expected beneficial effects, but also as negative outcomes due to homeostatic unbalance produced at various levels in a complex systemic network of cells and soluble factors.

By studying the mechanisms of action of this new class of drugs, and by considering their structure and the physiological role of the respective targets, it is possible to attempt a pathogenetic definition and even venture predictions on the typologies of adverse human reactions generated during their intervention. For example, by inhibiting or enhancing specific functions restricted to a highly sophisticated cell subset, such as interfering with the binding of a single cytokine to its natural receptor, some first-line ineludible consequences can be presumed, both beneficial and harmful. Furthermore, the glycoproteic structure of many biomedicines is easily recognized by the recipient's immune system. Their "foreignness", although mitigated by "humanization" procedures and structuring of fully human therapeutic molecules, still raise a variety of immune reactions capable to elicit destructive consequences for the patient and for the biomedicine itself. However, the systemic nature of most biological basic functions and the complexity of interactions triggered by "receptor-oriented" drugs leave a large margin of unpredictability, which is in the everyday's medical experience with old and new drugs. Therefore, the accumulated experience on direct observation of adverse events during therapy with available biomedicines is as much fundamental

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for a comprehensive and prospective vision of this important section of iatrogenic pathology.

The purpose of this monograph is the collection of information on any observed type of adverse event produced by each member of this new drug class, and to provide a framework of their safety profiles with respect to typology, structure, mechanism of action, and immunogenicity.

In particular, the proposed analysis refers to:

Biomedicines: not all of them, but only the most recent officially entered in human therapy and their capacity of inducing whatsoever **Adverse Event**.

The work purpose: understanding for better prevention, monitoring and control of adverse reactions; possibly contributing to the development of better drugs providing benefits with the lowest possible risk.

Some relevant notes concerning the adopted terminology, typology, and classification of Adverse Events (AEs) together with the criteria followed for the selection of data sources and documentation are preliminary provided.

Basic information on each group of the examined biomedicines, namely **Monoclonal antibodies, Fusion proteins, and Cytokines** is then summarized, followed by a comprehensive report on each examined product. Finally, a drug class analysis on the collected material is attempted.

An electronic data sheet in excel format for most relevant examined products are also part of the work. These files can be downloaded from (http://extras.springer.com) and include more detailed safety data, together with additional basic information on product characteristics, pre- and postmarketing AEs classified according to frequency and system/organ targeting. Data on excipients and selected information on drug interactions and associations have been added, in order to better evaluate their possible concurrence in the AEs insurgence. In fact, the frequent use of biomedicines in association with other agents is expanding, mainly in oncology and rheumatology, thus posing problems of synergism not only in terms of efficiency, but also of safety.

Altogether, data on 35 Monoclonal Antibodies, 7 Fusion proteins, 23 Cytokines including 8 Interferon products, 3 Interleukins, 9 Hemopoietic Stimulatory Factors, 2 Epidermal Growth Factors, and 1 recombinant Cytokine Receptor Analog, all officially approved for human therapy or for diagnostic purposes by the US Food and Drug Administration (FDA), and/or by the European Medicines Agency (EMEA, or EMA after December 2009) are reported. For simplicity, the acronym EMEA will be the only used in this volume.

1.1 Definitions

Before entering the analysis, it is important to convene on some definitions currently used in the field, since in the last decades there has been an accumulation of terms and acronyms, that are often differently used to define the same class of drugs and related events, or to gather different phenomena under the same acronym, and even to assign different definitions to the same event.

1.1.1 Drugs as Biological Derivative: Biomedicines

Terminology on this field is rather overwhelming and may pose some interpretation problems.

Biologics, in the FDA definition include a wide range of substances and tools, such as vaccines, blood and blood components, allergenics, gene and cellular therapies, somatic cells, tissues and recombinant therapeutic proteins.

Other sources define ***Biologicals*** as biological-derived proteins present in the human body, which can be extracted or synthesized in the laboratory to be used in therapy. ***Biosimilars*** has been alternatively used to define drugs with structures similar to those of molecules naturally present in the (human) body, or drugs of biological origin/similarity that have gone out of the patent cover time. The latter definition seems now to prevail, indicating any therapeutic biomolecule produced after patent expiry, provided that companies follow strict quality and safety regulations imposed by national and international authorities. An increasing demand for their recognition as alternate therapeutics, and obvious worries on their safety have brought authorities to re-consider regulations and restrictions in more clear-cut formulations, although the debate is still open.

Biological Response Modifiers **(BRM)** are exogenous (mainly of bacterial origin) or endogenous naturally occurring substances produced in small quantities as response to infection and other diseases. These substances are now synthesized and used for modulating responses in various diseases, primarily as non-specific enhancers of immune attack in therapy against cancer. Endogenous BRM also include, or are meant as, cytokines and cell growth factors. However, the BRM definition seems to be gradually disused, or mainly restricted to indicate biological immune modulators.

Cytokines are a large and heterogeneous group of extracellular peptides binding to specific receptors at targeted cell surface, thus starting an intracellular signaling cascade exerting pleiotropic effects. They are secreted by immune and non-immune cells and regulate both innate and adaptive immune response, cell movement, and communication inside and beyond the immune system. In the present terminology, cytokines include: *Interferons* (IFNs), exerting anti-viral and immune regulatory effects; *Interleukins* (ILs), acting as intercellular cross-talk molecules; *Tumor Necrosis Factors* (TNFs), implicated in tumor regression and inflammation; *Hemopoietic Stimulatory Factors* (HSF), promoting proliferation and differentiation of hematogenous stem cells, *Growth Factors* (GF) acting on epithelial, endothelial and mesenchymal cells, and *Chemokines*, characterized by their ability to induce chemotaxis or chemokinesis in leukocytes. The latter group is more often not included among cytokines, and is considered a separate group of structurally different regulators of cell movement.

Monoclonal antibodies **(mAbs)** are a group of genetically engineered molecules closely related to natural glycoproteins produced by the immune system (immunoglobulins). They are mostly used as anti-neoplastic drugs or as inhibitors

of immune reactions thought to be crucial in the development of some immunological and autoimmune diseases.

Soluble receptors analogs **(SRA)** define synthetic analogs of cell receptors with various functions. They are mainly used as competitors of natural ligands or inhibitors of the original receptors' function, when injected in their soluble form (decoy receptors).

Fusion proteins **(FPs)** are chimeric products of a fusion gene, i.e., a hybrid gene derived from the joining of two different genes originally coding for separate proteins. Translation of a fusion gene results in a single polypeptide, which may carry functional properties of both original proteins.

All these definitions are partially overlapping. In fact, some mAbs and SRA act as BRM molecules. FPs may act as decoy receptors for cytokines, or as growth factors, all being included in the "Biologics" category because of their original presence/production by living cells. All mentioned categories have important representative products already included among the human therapeutic agents.

In this work, the term ***Biomedicine*** embraces any product of biological origin or structurally pertinent to the living matter introduced in human therapy. However, this monograph will not refer to all available substances of this class. In fact, some of them, like human proteins, immunoglobulins, vaccines, hormones, and blood/serum components of different origin, either extracted or synthesized, have been experienced in human therapy for a long time and their properties as well as their capacity as AEs inducers are well known. Therefore, the analysis will focus on the most recent derivatives, namely ***Monoclonal Antibodies***, ***Fusion Proteins***, and ***Cytokines***, because of their raising importance in human therapy and the parallel concern for drug-related adverse events induced at different levels of gravity.

1.2 Adverse Events

In addition to the expected therapeutic effects, side effects, adverse reactions of different origin, changes in the homeostasis of the treated organism, modifications of the underlying disease, either spontaneous or drug-induced, frequently occur as an ensemble of signs, which are often difficult to relate to a precise drug-induced mechanism. The picture is even more complicated, since in modern therapies, including those employing biomedicines, multi-drug associations are frequent, making the assessment of each drug's responsibility even more difficult. Nonetheless, when new drugs are involved, every effort to individuate and define any single undesired event becomes crucial not only for patient's safety, but also for a better evaluation of risk/benefits of each therapeutic agent involved. For example, in multi-drug different protocols experienced in oncology or in autoimmune pathologies, sometimes the best efficacy clashes with the highest capacity of induction of most violent and life threatening syndromes. In other occasions, in the presence of an equal efficacy of a single drug in two different diseases, the burden of adverse events makes the application worth or unfeasible. Therefore, the

capacity to discriminate among single unwanted events, recognizing the possible pharmacologic inducer, becomes fundamental for the best therapy adjustment and for attempting new formulations of old and new drugs.

In conclusion, while in a first phase of observation, mainly in the premarketing phase, adverse events are better evaluated as a global package of processes contrasting the therapeutic effect of a new drug, at later times, when additional information has accumulated in the long run, the necessity of investigating each relevant adverse effect becomes more important to adjust each therapeutic present or future opportunity.

1.2.1 Typology of Adverse Events

In principle, ***Adverse Events*** **(AEs)** identifies any untoward medical event associated with the use of a drug, whether or not considered drug-related, while ***Adverse Reactions*** **(ARs)** identify undesirable effects, reasonably associated with the use of a drug, that may occur as part of the pharmacological action of the drug or may be unpredictable in its occurrence. In practice, these two terms are often used as synonyms. ***Adverse Drug Events*** **(ADEs)**, ***Adverse Drug Reactions*** **(ADRs)**, or ***Drug-related Adverse Events*** **(DRAEs)** may all be referred to every noxious and unintended response to a medical product at any dose, implying that a causal relationship between the medical administration and the event is at least possible, and in any case cannot be ruled out.

However, in particular occasions these definitions are considered slightly different, according to the mentioned proper meanings of AEs vs ARs, being the latter more strictly related to a specific investigator's assessment.

These definitions are in turn slightly different from the terms agreed by the WHO consensus conference: as suggested by FDA and similarly yet not identically by EMEA, they would fit better for events collected from clinical preapproval drug studies [1]. In fact, these data are the basic parameters on which the AEs profiles of individual drugs are depicted at approval, and are fundamental for the analysis provided in this volume as well. They are subsequently enriched by postapproval studies and postmarketing observations, mostly collected in different clinical and observational situations.

An additional yet less common definition, ***Side Drug Effects*** **(SDE),** is reported as an expected/known effect of a drug unrelated or even paradoxical with respect to the therapeutic expectance. Finally, the ***Treatment-emergent Adverse Events*** **(TEAEs)** refers to AEs emerging during the treatment observational period, whether or not related to the drug in study.

In principle, ***Side Effects*** (Events, Reactions) relates to any event caused by a drug other than the intended therapeutic effect, whether beneficial or not, while ***Adverse Effects*** imply harmful consequences related to the event, whether or not related to the administered agent. Unfortunately, they are sometimes used as synonyms.

It must be underlined that AEs definition also includes any reaction occurring due to overdosage, either intentional or accidental, to drug abuse or withdrawal, and even to a significant failure of expected pharmacological action. Therefore, it is often difficult to discriminate among all these effects and other unwanted, unexpected, undesirable adverse events, those that are strictly related to standard and professional administration of the drug, mostly when considering data included in postmarketing databases, case reports, or uncontrolled studies. In fact, even in controlled trials the adopted protocols for AEs evaluation are not homogeneous, or are not detailed when data are published, which makes even more difficult to properly pool and compare data, a highly demanded opportunity, especially when rare diseases are under investigation. Noteworthy, parallel comparisons and non-inferiority trials among biomedicines are rare, and long-term observations are still limited.

On this basis, for the purpose of the present analysis, the rule of reporting the whole typologies of adverse events for each drug has been adopted, keeping the premarketing studies separated from the postmarketing observations, as well as from the spontaneous case reports and clinical care experiences, as much as possible. Moreover, in analogy to the unifying definition of Biomedicine adopted to identify all bioproducts employed in human therapy, ***Adverse Events*** **(AEs)** is used as embracing all possible reactions observed after any drug administration. However, despite a different terminology is often used by investigators when describing drug-related events, their definition frequently is not provided. In such cases, the investigator's specific terminology is adopted, given that such events can either be drug-related, or such relation cannot be excluded.

Some subgroup definitions have been occasionally used to identify particular types of drug events:

Immune-related Adverse Drug Reactions **(IrADRs)** are defined as events determined by the interaction of a drug with the immune system, mostly related to *hypersensitivity mechanisms* of all type (I-IV). Long-lasting treatments may also induce *autoimmune reactions*, either at laboratory level (asymptomatic) or as clinical expression/exacerbation of *autoimmune diseases*. Immune-related events appear related to the immunogenicity of a drug rather than to its mechanism of action, e.g., to the structural capacity of being recognized as a "foreign" antigen by the immune system. In this case, genetically determined favorable backgrounds (atopy, unbalanced immuno-surveillance, HLA associations, etc.) are important concauses of the development of immune/autoimmune drug-induced effects.

Immune-related Adverse Events **(IrAEs)** has been referred to an overboosting of the immune system reactivity due to a direct drug stimulation of specific immune receptors, or to the blocking of endogenous regulatory controls, such as CTLA-4 for T cells (see ipilimumab, Chap. 25). Alternatively, IrAEs are observed as a rebound of immune reactivity after discontinuation of an immunosuppressive biomedicine (see natalizumab, and IRIS, Chap. 3, 27). Differently from IrADRs, these events are more specifically related to the drug mechanism of action.

1.2.2 Classification of Adverse Events

There are different proposed/used classifications of AEs, which alternatively take into account:

- **Frequency** (very common/common/uncommon/rare/very rare);
- **Probability** (definitive/probable/possible/doubtful);
- **Severity**, graded from 1 to 5 (mild/moderate/severe, life threatening, or disabling/death) according to Common Terminology Criteria for Adverse Events (CTCAE) classification;
- the **System Organ Classification** (SOC), based on specific systems and/or organs involved in the event [2, 3].

In 1994, the Council for International Organizations of Medical Sciences (CIOMS) decided that the Medical Dictionary for Drug Regulatory Affairs (MedDRA) would be the basis for drug regulatory purposes. Entries from Adverse Reaction Terminology (WHO-ART) and Coding Symbols for Thesaurus of Adverse Reaction Terms (COSTART) were also included to develop an international unified medical terminology.

All of them are equally useful in relation to the search needs and the field of application. While the probabilistic classification finds particular advantages in the field of Adverse Events Following Immunization (AEFI)—since the cause/effect association is more difficult to prove when reactions may appear at rather long distance such as after a single or few shots of vaccine—the other three are widely but not uniformly employed for drug AEs evaluation, with different interpretation of severe/serious terminology assigned to related events. The terms are obviously not synonymous, although sometimes they are used as such.

***Severe AEs* (-)** according to the Common Toxicity Criteria (CTCAE v 4.03, June 2010) correspond to grade 3, while serious/life-threatening reactions are grade 4, and deaths related to AEs are grade 5. Often AEs data are referred as serious over grade 3 [3, 4]. Severity should then be related to intensity of the reaction, not necessarily to its gravity (seriousness). For example an eyelid edema can be severe, while a laryngeal edema is usually serious or life threatening.

***Serious AEs* (SAEs)** are medical occurrences that are life threatening, require hospitalization or its prolongation, and results in persistent/significant disability/incapacity, congenital abnormality/birth defect, or death. They all are usually classified as grade 4, and only recently deaths have been separately considered as grade 5 (since CTCAE v.3, August 2006). In other situations AEs are referred as $\geq$grade 3 or $\geq$grade 4 to include severe/serious events, or serious events and deaths, respectively.

1.2.3 Adverse Drug Events

Adverse drug experiences have been defined in different ways by national and international organizations (WHO, EMEA, FDA, OHRP) over time, in relation to the intended use of the definition itself (adverse drug reporting, data collection in clinical trials, product labeling, etc.). There are substantial differences among Agencies' regulations, not only in the definition of events but also on those to be reported, timing of reporting duty, and appointed Authority to which reports shall be addressed.

The modalities of data collection and assessment in trials and in other less controlled observational clinical situations are dissimilar as well. Therefore, the analysis of collected data under different circumstances is complex and often data are hard to compare. The efforts to reach a general consensus for unifying terms and definitions have not been totally successful [4–7].

The definition of ***Adverse Drug Event*** **(ADE)** as a noxious and unintended response to a medicinal product related to any dose used in humans (ICH E 2A) is the one adopted by this work, and includes the more limited definition proposed by WHO (Technical Report 498, 1972), which considers adverse reactions occurring only at doses normally used in man for prophylaxis, diagnosis, or therapy of disease or for modification of physiological function.

Similarly, the definition of ***Serious Adverse Drug Event*** **(SADE)** refers to any adverse drug experience occurring at any dose resulting in one of the following outcomes: death, life threatening event; hospitalization or prolonged existing hospitalization; persistent, significant disability/incapacity; congenital anomaly/birth defect; any event jeopardizing the treated subject that may require medical/surgical intervention to prevent one of listed outcomes.

The adoption of SADE was preferred to avoid confusion with ***Suspected Adverse Drug Reaction*** **(SADR)** definition, launched in 2003 to be used for trials mostly in prelicensing phases. The proponent Agency agreed to cancel such terminology in 2010 (FDA, *MMM November 2010*) for the evident negative impact on classification and reporting systems, particularly when investigating a new drug (IND). The discussion on this matter brought to new safety rules and new definitions for ADEs, which better keep separated safety reports from clinical trials and from postmarketing safety reporting.

Similarly, the additional terminology of ***Suspected Unexpected Serious Adverse Reaction*** **(SUSAR)** has been proposed by EMEA for safety monitoring and clinical trials reporting, with questionable utility.

Taken together, the need for urgent simplification and unification of terminology on adverse events is still demanded, despite repeated calls and attempts [1–6]. Most of all, the adoption of a unique terminology would reduce heterogeneity in observational protocols, and would facilitate data analysis and comparative evaluations, which at present are still hard to perform.

1.2.4 Off-Label Observations

In addition to the experiences performed along with the official therapeutic indications for each biomedicine, it is important to evaluate, in general terms, some of the most relevant experiences in the off-label investigations, particularly focusing on safety data. This information is often collected in controlled trials, but more frequently is based on uncontrolled small groups, individual case reports, and on postmarketing spontaneous reporting. Provided that such information is not reciprocally comparable, nor with clinical trials and studies concerning official therapeutic settings, their contribution to depict a wider safety framework and possibly individuate potential new signals is not irrelevant, since often reflects situations closely related to everyday practice in clinical care.

Therefore, this work includes selected off-label observations in the overall safety panorama for each examined biomedicine, as separate information to be considered in addition to in-label information and postmarketing emerging settings, especially when new, serious, or unexpected AEs were encountered.

1.2.5 Postmarketing Surveillance

Postmarketing observations are usually reported to surveillance authorities, such as FAERS/FDA, EUV/EMA, Health Canada, AIMS (Australia), JCAHO (Japan), etc., mainly on spontaneous basis during clinical care in the postmarketing activity, while they are often mandatory during pre- and postmarketing trials individuated or requested from licensing authorities.

For the purpose of this volume, the consulted databases have been the FAERS/FDA at *www.fda.gov* and of EMEA/EudraVigilance (EUV) at *www.adrreports.eu*. FAERS data are also available at the DrugCite website (*www.drugcite.com*), which provides a search engine where reported AEs can be found through the international non-proprietary name (INN) or brand names of each FDA approved drug.

EUV has become publicly available quite recently (June 2012); this database was in fact previously collecting only SAEs reports, but from such date it has been opened to any AE report. Data classified according the SOC system from EU and extra-EU reports are now publicly accessible.

Occasionally, other sources of information has been collected from public registries on specific diseases, from FDA risk evaluation and mitigation strategies (REMS) investigations, and other postmarketing focused investigations, mostly when organized in the form of controlled trials.

Additional available websites, also consulted for safety warnings, alerts, and safety updates were: *www.tga.gov.au/safety/daen.htm*; *www.pmda.go.jp*; *www.mhra.gov.uk www.adversevents.com*; *www.medsafe.govt.nz*.

Although much effort has been made at collecting AEs report from different sources, difficulties encountered in the attempt of harmonizing and comparing data remain a crucial problem to be solved. Among others, it must be considered that all

databases are partially overlapping, and therefore some reports can be differently coded by Agencies, and by manufacturers who in turn send their reports to them.

Therefore, in the present work, whenever specific sources are used, related information is recognizable and quoted. Pooled and comparative analyses have been limited and generally employed for a better understanding of the overall safety profile of single biomedicines, as well as for direct comparison of safety profiles of different products, while drug-class comparisons have been performed when considered particularly instructive for identification of differences in the respective safety profiles.

1.2.6 Adverse Events Analysis

The analysis of the safety profile has focused on three drug classes, ***Monoclonal Antibodies***, ***Fusion proteins***, and ***Cytokines***, representing the core of the new therapeutic approach with biomolecules, mainly in the following clinical areas: oncology, autoimmune and autoinflammatory diseases, and hemopoietic stimulation. In these areas the impact of biomedicines is impressive. Despite the analysis of most AEs safety profiles showed a wide tolerability and manageability, especially when compared to alternative standard chemotherapy and other immunosuppressive treatments, it became evident that biomolecules are not less harmful just because of their biological nature, and even fully human engineered molecules were not freed from the capacity of inducing unwanted reactions, not even the most serious of adverse events. Indeed, these products can be highly harmful, although in a restricted number of cases, and therefore the understanding of such capacity is crucial to prevent and mitigate their effects, and to possibly identify the origin of the AEs in order to address new drug developments toward more efficient and safe tools.

In performing the analysis, this work has particularly focused on two basic aspects: the **mechanisms of action** of the examined biomedicines, trying to pinpoint the possible pathogenetic roots of the generated AEs, and **immunogenicity,** the basis of immuno-mediated adverse reactions.

New agents in preapproval stages, although of great interest from the investigational point of view, are not sufficiently consolidated for establishing a solid safety profile. Published data from ongoing advanced studies have been excluded as well, since in most cases they relate to short-term observations and official validation of data is lacking. In particular, the analysis has been restricted to biomedicines officially approved by FDA and/or EMEA, although additional data of some products approved elsewhere have been reported, when considered relevant for safety peculiarities and comparisons. By contrast, certain discontinued products have been included, when considered instructive for the understanding of AEs insurgence and impact, or for the evaluation of biomedicines developments to improve safety.

As for data analysis, the adopted strategy focuses primarily, yet not exclusively, on pre- and postapproval most significant controlled trials. Nevertheless, relevant open studies, case reports, and observation from clinical care have been also considered as additional information. In fact, as already mentioned, while controlled trials are the basis of both efficiency and safety evaluations, they mostly refer to homogeneous preselected cohorts of subjects with particular attention in excluding comorbidities and other additional risk factors, while the other sources investigate more heterogeneous populations, yet closer to clinical practice. Therefore, even when data are not comparable, they are considered usefully complementary.

Finally, particular attention has been given to severe and serious events, for obvious reasons.

Collected AEs pertaining to each biomedicine are usually referred to the ***SOC Classification*** and/or to ***Frequency***, since these two methodologies are widely used in clinical trials and in drug approval request procedures. However, in some instances data collected according to one system for one biomedicine are not easily comparable with another biological of the same class collected according to the other criterion. In some cases, reports include most frequent/common AEs according to SOC classification, while less frequent events are listed according to their frequency or vice versa, thus making the comparison even more difficult.

In this volume, AEs to each biomedicine, indicated with its INN, are reported under the respective chapters, as well as in the electronic data sheet (excel), which can be downloaded for more detailed information on most relevant drugs. In both sections, data are arranged according to SOC and Frequency Classes, thus allowing to spot at a glance the most targeted systems and the organs involved, in terms of AEs quality and multiplicity, and to individuate the clusters of most frequent and associated signs involving different targets at the same time.

At present, the major areas of intervention with biomedicines include oncology and autoimmune diseases (mainly rheumatic disorders). Among 35 commercialized monoclonal antibodies, 16 are employed in oncology, 9 are indicated for autoimmune disorders, 3 in immunosuppression and graft rejection/prophylaxis, and 7 in other conditions (asthma, autoinflammatory diseases, AMD, osteoporosis, PNH, cardiac disorders, RSV infection). Among the 7 available fusion proteins, 4 are employed in autoimmune diseases, 1 in autoinflammatory diseases, 1 in AMD, and 1 for renal graft rejection/prophylaxis. Among 23 cytokines in therapy, 8 interferon formulations are employed in oncology, HCV/HBV hepatitis, MS, CGD, and malignant osteoporosis; hemopoietic stimulatory factors are mainly used in severe anemic and myelosuppressive states mainly as consequence of chemotherapy or for myeloablative procedures; 1 pluripotent stem cell factor is used as an ex vivo (but also in vivo in some countries) stem cell expander; 2 different epidermal growth factors are used for wound healing (diabetic ulcers) or for severe postchemotherapy oral mucositis, respectively.

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Adverse Drug Events to Biomedicines

2

Adverse Drug Events (ADEs) are commonly differentiated in two classes: **Type A**, as predictable reactions related to the pharmacological properties of the drug in study; **Type B**, as unpredictable events in predisposed individuals. The former group includes side effects, toxicity of overdose, secondary effects, and drug interactions. Overall, they contribute for about 80–90 % of ADEs. The latter group includes hypersensitivity (immunological) reactions, and non-specific reactions, such as pseudo-allergic (anaphylactoid) reactions and idiosyncrasy, all contributing for the remaining 10–20 % of events [1, 2].

Type A reactions are typically related to the *pharmacological action* of the agent, expressed by its known or presumed mechanism(s) of action, while Type B events are usually unwanted and sometimes unexpected accessory phenomena derived from the interaction of the agent with the recipient's environment.

Hypersensitivity may derive from all the known four types of immune-specific reactions, namely the three antibody-mediated, Type I (IgE-mediated, or allergy), Type II (IgG and/or IgM-mediated cell cytotoxicity), Type III (immune complex deposition), and the cell-mediated Type IV reaction induced by T cells (delayed hypersensitivity).

Non-specific reactions may derive from a variety of mechanisms, including defective enzyme functioning, aspecific mast cell degranulation and, in the present view, as a consequence of a cytokine network imbalance causing mild to serious, local or systemic syndromes with potential life threatening outcomes (see Chap. 3).

Two additional types of reaction less frequently used consist in Type C events associated with long-term therapy, and Type D events referred to carcinogenic and mutagenic drug with long-term effects.

While Type A reactions are preferably dose-related, Type B events usually are not; in fact, the latter type may be triggered by extremely low doses of the causative agent.

Alternatively, they all may induce *Tachyphylaxis*, defined as an acute decrease of drug response and reactivity related to both dose and rate of administration, leading to desensitization, and to rebound phenomena following treatment.

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Idiosyncratic reactions are usually referred to non-immune, unexpected, abnormal events not related to the peculiar pharmacologic action of the responsible agent.

Drug metabolism dysfunction may cause ADEs, mostly due to accumulation of detoxified metabolites expressing direct cell and tissue damage, or indirect induction of immune responses by binding to macromolecular (proteic) endogenous components.

Therefore, a number of unexpected, unpredictable AEs are difficult to classify, since most of the responsible mechanisms are not known, or more parameters are involved in a single reaction, thus leading to their grouping according to the mentioned SOC or Frequency classification criteria.

Recently, a different approach considered not only the intrinsic properties of the agent as the leading criterion for classification of AEs, but also other concomitant properties, such as time, dose, severity, and individual peculiarities (genetic, biopathological conditions) conferring different susceptibility. In particular, the addition of individual susceptibility to the more familiar dose-timing conditions widely experienced in the evaluation of adverse events to vaccines, takes in proper consideration important variants such as the genetic background, age, sex, physiological (pregnancy) and underlying pathological conditions, and exogenous factors (**Dose-Time-Susceptibility -DoTS- classification**). Although such tridimensional approach is more realistic and appropriate for biomedicines as well, it has not gained much diffusion in the field [3]. On this basis, ADEs can be identified by a number of clinical syndromes which appear more frequent and relevant than others. Among them, there are *CNS toxicities* (acute toxic confusion), *respiratory disorders* (asthma, pulmonary fibrosis), *cardiovascular events* (tachycardia—torsade de points—), *hemopoietic toxicity* (aplasia, agranulocytosis, aplastic anemia), and *neuropsychiatric disorders* [4].

However, the spectrum of adverse *cutaneous drug eruptions*, not considered in the previous report, is also fundamental for the ADEs evaluation of old and new drugs, including biomedicines [5]. Elevated sensitivity, prompt detectability, and potential gravity of some skin lesions, together with the possibility of discriminating distinct ADEs on the basis of easily performable histopathological analyses, make the dermatological observation crucial for the understanding, prevention, and control of drug-induced reactions. Unfortunately, most of the cutaneous expressions of drug harm are not followed by dermatologists during this kind of studies.

An additional and relevant problem relates to the assessment of *ADEs in pediatric age*. In fact, experience on this age setting with biomedicines is even more limited and is associated with additional risks. Over-dosing, the absence of pharmacokinetic studies performed in premarketing stages, the influence on growth and development, and of long-term exposure in chronic diseases are among them. There is no ADE classification that takes into consideration such aspects. In fact, most of the premarketing trials either exclude pediatric subjects, or enroll quite small groups, even when the disease in study pertains to this age (i.e., genetic disorders such as autoinflammatory diseases); in most cases the efficacy profile analysis prevails on the safety profile [6]. In consideration of the expanding use of

Table 2.1 Criteria for adverse drug events classification

Criterion		Typology		Manifestation
Class	Type A	Predictable	Dose Toxicity	Hepatic failure
		Common	Side Effects	Collateral pharmacoactivity
		Drug-related	Drug interactions	Synergistic toxicity
			Secondary Effects	Diarrhea (xenobiotic imbalance)
	Type B	Unpredictable	Intolerance	Low threshold pharmacoactivity
		Uncommon	Hypersensitivity	Anaphylaxis, Arthralgia
		Drug-unrelated	Pseudoallergic	Radiocontrast reaction
			Idiosyncratic	Anemia (enzymatic deficiency)
Dose	Dose–response	Dependent	Independent	
		Related	Time change	Increasing toxicity
		Unrelated		Decreasing/paradoxical toxicity
Time	Time-response	Dependent	Independent	
		Injection rapidity	Dose change	Acute toxicity
		First Dose	Accumulation	Toxicity
		Peaking Dose	Drug interaction	Synergistic, unexpected
Frequency		Type	Range	
		Very common	> 10 %	Injection site reaction
		Common	1–10 %	Pyrexia, diarrhea
		Uncommon	0.1–10 %	Vasculitis
		Rare	0.01–0.1 %	Anaphylaxis
		Very rare	< 0.01 %	Leucoencephalitis
SOC		System Organ Groups	Subgroups	Blood/Anemia/Grade (1-5)
Combined		SOC	Frequency	Bidimensional AEs comparison
		Group	Range	

(continued)

Table 2.1 (continued)

Criterion		Typology		Manifestation
DoTS		Mechanism of action	Clinical evidence	Tridimensional AEs comparison
	Dose	Yes/No	Yes/No	
	Time	Yes/No	Yes/No	
	Susceptibility	Yes/No	Yes/No	

new therapies in *off-label conditions*, the relevance of the problem is even more concerning for these young patients. In an interesting, yet not very recent study on 1419 children, 45 % of them was exposed to off-label treatments, associated to a significant increased risk to develop ADEs [7].

Tables 2.1 and 2.2 summarize the criteria for ADEs' classification more frequently adopted.

Is the mentioned conventional approach of AEs evaluation feasible also for biomedicines? Probably not. These agents are a relatively new class of therapeutics carrying specific risks. They are derived from living sources; production and purification result in more complex procedures; and minor modifications can result in major differences, both in efficacy and safety. A few changes in the glycosylation of a monoclonal antibody or a fusion protein, may modify its binding capacity and immunogenicity. With this respect they are more comparable to vaccines and allergenic products. Moreover, they have an extraordinary capacity of influencing the complex network of cytokines, directly interfering with their receptors and ligands. In fact, application requirements for biomedicines' approval were soon adapted as ad hoc Biological License Applications (BLA) or similar, but the body of pre- and postmarketing safety regulatory actions remained practically the same. Moreover, due to their promising expectancies in lethal diseases lacking efficient therapy, accelerated approvals were released and important safety problems could be individuated only in the postmarketing experience. Between 1995 and 2007, a total of 174 biological products were approved (136 in US; 105 in EU; 67 in both). Up to 2008, 81 regulatory actions/alerts were issued on 41 biomedicines (24 %), along with 46 letter to health care professionals, 17 direct healthcare communications, and 19 BBWs [8]. First in drug class approvals were exposed to more safety regulatory actions, while last issued biomedicines, such as mAbs, fusion proteins, and cytokines, that are primarily considered in this volume, had a higher risk of ADEs induction compared to early issued hormones, such as recombinant insulin, somatotropin, and immunoglobulins.

Even the simple Type A and Type B distinction of ADEs appears questionable for biomedicines. For example, typical hypersensitivity reactions could be triggered by direct stimulation of cytokine receptors or by non-covalent interactions with

Table 2.2 System organ classification categories (°)

Blood and lymphatic system disorders
Cardiac disorders
Congenital, familial, and genetic disorders
Ear and labyrinth disorders
Endocrine disorders
Eye disorders
Gastrointestinal disorders
General disorders and administration site disorders
Hepatobiliary disorders
Immune system disorders
Infections and infestations
Injury, poisoning, and procedural complications
Investigations
Metabolism and nutrition disorders
Musculoskeletal and connective tissue disorders
Neoplasms benign, malignant and unspecified (incl. cysts and polyps)
Nervous system disorders
Pregnancy, puerperium, and perinatal conditions
Psychiatric disorders
Renal and urinary disorders
Reproductive system and breast disorders
Respiratory, thoracic, and mediastinal disorders
Skin and subcutaneous tissue disorders
Social circumstances
Surgical and medical procedures
Vascular disorders

(°) evs.nci.gov/ftp1/CTCAE v 4.03

immune receptors, without triggering a conventional immune response. This peculiarity was observed also for smaller therapeutic molecules acting as haptens when bound to endogenous protein carriers [9]. In fact, the homeostatic alteration produced by biomedicines bypassing immune-mediated mechanisms, may explain the higher frequency of hypersensitivity-like reactions observed during treatment, in the absence of a significant induction of sensitization signs and of antibody production [10].

Consequently, **new classificative criteria** are needed and are being proposed, which selectively consider AEs encountered during administration of mAbs, fusion proteins, and cytokines.

In particular, one classification identifies five groups of ADRs to biomedicines: (α) *Immunostimulation* (infusion reactions, direct substance-dependent effects, cytokine release); (*β*) *Immunogenicity* (Type I-IV hypersensitivity reactions; anti-drug antibody formation); (*γ*) *Immunodeviation* (immunosuppression, autoimmunity); (*δ*) *Cross-reactivity*; (ε) *Non-immune adverse reactions* [11].

The major novelty in such classification relates to the attention dedicated to immunogenic substance-specific reactions, as referred to the three fundamental classes of new biomedicines, namely monoclonal antibodies, fusion proteins, and cytokines. Therefore, in analogy to the known CTCAE grading, a severity I-V scale has been put aside for Type *α* and Type *β* reactions.

On this basis, Table 2.3 reports a modified version of the proposed ADRs classification, with the aim of underlining the necessity of pursuing a better approach to the peculiarities of biomedicines. The reported approach is rather preliminary and too complex to be routinely applied to prospective investigations, and even more to retrospective analyses.

Recently, a mechanistic approach to "**Biological Adverse Events**" (BAE) has been proposed [12]. Two pathogenetic mechanisms are identified, *Pharmacological* and *Non-Pharmacological,* including two subgroups, respectively. The former identifies reactions derived from the intended interaction of the biomedicine with its target. They are distinct in "expected biology" and "new biology" events. Non-pharmacological events basically identifies "immune-mediated" responses and "non-immune mediated" reactions. Examples of pharmacologically mediated toxicity include infections subsequent to immunosuppression, inhibition of vascularization, cardiotoxicity, and massive cytokine release. Non-pharmacological immune events relate to all hypersensitivity reactions and to autoimmune diseases, while non-immune reactions are referred to Fc-mediated acute phase reactions. Table 2.4 reports a modified version of BAE classification.

Although this approach also appears preliminary, it clearly indicates that a better characterization of BAE with respect to ADEs may become feasible, and ensure a more precise identification of the pathogenetic routes of biomedicines.

In conclusion, while waiting for more stringent classifications and possibly differentiated procedures of pharmacovigilance for biomedicines, the evaluation of their ADEs still remains in the general framework of the **System organ classification** (SOC) of the **Frequency classification**. In fact, they allow collection of any encountered AEs under a unified terminology identified in the MedDRA hierarchy, where anatomical, physiological, etiological, and investigational interventions are grouped. Within each category AEs are graded in terms of severity on 1–4 or 1–5 scale, according to the typology of the observed event. In fact, not all grades are appropriate for all AEs, and therefore some are grouped in fewer grades. For example, stroke is graded from 1–5, while generalized muscle, ataxia, and some investigational events are graded from 1–3, syncope is only grade 3, and cerebral edema is only grade 4.

Table 2.3 Classification of adverse drug reactions to biomedicines (a)

Class	Mechanism of action	Type	Effectors	Manifestation
Alpha	Immunostimulation	Infusion reactions	Cytokine release and Complement activation	CRS, FLS
		Injection reactions		Erythema, Dyspnea, Hypotension,
				Arthralgia, Systemic signs
Beta	Immunogenicity	I	IgE	Anaphylaxis, Rash, Urticaria
		II, III	IgG, IgM	Serum Sickness, Arthralgia
		IV	T cells	Cytotoxicity, Exanthema
				Autoimmunity
Gamma	Immune deviation	Immunosuppression	Receptor/Ligand blockage	Infections Tumors
				Virus-associated tumors
		Immune imbalance	Th1,Th2, Treg	Autoimmunity
				Hypersensitivity induction
				Disease exacerbation
Delta	Cross antigenicity	Bystander aggression	Cross-reactive Abs, T cells	Exanthema, Skin toxicity
				Autoimmunity
Epsilon	Non-immune	CYP 450 inhibition	Cytokine release	Cardiovascular, ATE, VTE
		Anaphylactoid reactions		Xeroderma

(a) Modified from Sherer K et al. (2010) JDDG 8:411–426
Type I–IV hypersensitivity (Gell and Coombs classification); CRS: cytokine release syndrome; FLS: flu-like syndrome; ATE,VTE: arterial, venous thromboembolic events

The frequency parameter is a practical and simple evaluation, and includes the following categories: Very common events (>10 %); Common events (1–10 %); Uncommon events (0.1–1 %); Rare events (0.01–0.1 %), and Very rare events (<0.01 %).

The combination of the two criteria is still the basis for all types of AEs classification in all sorts of clinical situations, and has been adopted for data

Table 2.4 Biologic adverse events (BAE)[a]

Pharmacological			
Expected biology		**New biology**	
Type	**Target**	**Type**	**Target**
Inhibition of wound healing	VEGF	CRS	Massive T cell activation
Hypoglycemia	Insulin	Cardiotoxicity	HER2
Infections	Immunosuppression	Thrombosis	VEGF
Non-pharmacological			
Immune response mediated		**Non-immune response mediated**	
Type	**Target**	**Type**	**Target**
Hypersensitivity	Non-self epitopes	TLS	CD20
Autoimmunity	Self epitopes	Inflammation	Acute phase proteins

[a] Modified from Clarke JH, Adverse Drug Reactions. Handbook Exp Pharmacol,Uetrecht J(ed) Springer 2010, pp 453–474
CRS: cytokine release syndrome; TLS: tumor lysis syndrome. See Chap. 3

collection in the electronic data sheets annexed to this volume. On this basis, the analysis of the safety profile has focused on three drug classes, **Monoclonal Antibodies**, **Fusion proteins**, and **Cytokines**, representing the core of the new biological therapeutics in oncology, autoimmune and autoinflammatory diseases, and hemopoietic stimulation.

In this monograph, such data have been arranged in distinct *Sections*, and in *Downloadable Associated Files* provided for the most relevant examined biomedicines. In the first section, data of each product include a short *History* on basic safety studies leading to approval. Such data setting is fundamental to understand the amount of experience accumulated in the premarketing phase, and the subsequent evolution and evaluation of safety profiles during the postmarketing expansion.

A short, albeit detailed, section on the *Mechanism(s) of Action* follows, since many pathogenetic roots of ADEs are linked to their expression. A brief analysis of the *Immunogenicity* of the drug in study follows. These two aspects are instructive not only for the understanding of therapeutic risks, but also as useful guidelines for the development of better medicines. Interestingly, by following the progressive modulation of immunogenicity during the development of new monoclonal antibodies, the overall progress in the safety of biomedicines becomes tangible and educational. However, lessons have also been learned from unexpected reactions due to minimal underestimated molecular variations, such as in the glycosylation of antibodies or of fusion proteins. The *Adverse Events* main section is dedicated to the *safety general profile* of each agent, depicted in relation to its officially approved indications. Whenever applied in different pathological situations, attention has been given to substantial differences and *specificities with respect to the standard profile*, usually assessed on the major disease representative of the group (e.g.

Rheumatoid Arthritis for rheumatic diseases, etc.). Subsequently, *additional experiences*, mainly concerning long-term studies, subpopulation differential reactivities, and studies of particular relevance in confirming initial safety trends, or the appearance of new signals, are reported. When available, particular attention has been given to experiences from the clinical practice, in unselected groups of patients, where the background pathology and the presence of comorbidities make a great difference with the cohorts of patients selected for clinical trials, thus offering to evaluate safety aspects closer to the reality of clinical care.

The following section is dedicated to *Off-label experience*, including controlled studies conducted in clinical trials, as well as in clinical care and in case reports, whenever indicative of relevant safety concern. These individual/small group studies on off-label conditions are often of more concern than utility. However, in some circumstances they may show potential alert signals or suggest new therapeutic indications, requesting immediate attention and proper confirmation in controlled studies.

With a similar aim, data from the *Postmarketing surveillance* databases, in particular the FAERS and the EUV database, have been consulted and reported. When considered of particular interest for safety understanding, information on developing drugs strictly related to the biomedicine in study are reported (e.g., the unsuccessful evolution of palivizumab into motavizumab). In fact, attempts to improve efficacy, such as by increasing the affinity of the product for the respective target, are not necessarily independent from a relevant modification of the safety profile, making the acquired clinical improvement unacceptable. Finally, some *Remarks* on most relevant safety peculiarities of the agent, including peculiarities within the same drug class, are provided.

The downloadable *Electronic Sheet Dataset* includes additional safety information, organized according to SOC and frequency classifications.

In the upper part of the downloadable sheet, *general data* on brand name(s), typology, target(s), class (Anatomical Therapeutic Chemical—ATC) and function, therapeutic indications, and major approval dates are reported, followed by essential product information and the major involved *Mechanism(s) of Action*. *Excipients* present in each commercial product have also been reported due to their known potential role in AE reactivity, and with the aim of providing all the useful information to help in understanding and evaluating the documented AEs reported in the same file.

In the second part, *safety information* has been reported according to SOC classification, and has been slightly modified to better fit the purpose of this volume. For example, the "Immune system disorders" SOC group has been divided into "allergy"/"immunology" "immunogenicity", and "autoimmune" subgroups. *Pre- and postmarketing data acquisition* from studies and reports has been considered separately, and major sources of information have been reported. In particular, data have been collected from scientific papers and reviews, official reports, case studies, clinical studies, trials—mainly of Phase II and III—AE alerts and documents from official producers, when available. The reported AEs have also been classified according to their frequency (common, uncommon, rare, etc.)

in order to allow a quick representation of the whole spectrum of detected reactions. *Spontaneous Reports* from the two major *postmarketing surveillance* databases (FAERS, EUV) have been separately reported, and organized according to the same frequency grading.

Finally, *BBWs and additional relevant warnings* along with most common and most serious events have been inserted in the last section, followed by essential information on *Drug interactions* and *Associations,* and some *Comments* completing the whole safety profile of each biomedicine.

The information provided in the electronic data sheets may help in quick searching for specific AEs and in comparing drug classes. Being such data sheets provided in the excel format, users may integrate the downloaded file with their personal experience.

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Systemic Syndromes with Biomedicines

3

Since the introduction of biomedicines in human therapy, a number of systemic reactions have been more frequently reported as important adverse events following treatment. Some monoclonal antibodies, interleukins, receptor inhibitors, and growth factors may preferably induce acute, violent, early events with an overall low frequency, but with serious and life-threatening capacity. In fact, these reactions represent one of the major limitations to therapeutic efficacy of this new set of drugs. Different mechanisms of action, only partially known, are implicated in the various syndromes, although most of them may be related to highly specific receptor targeting causing immediate massive release of specific factors, such as in the cytokine release syndrome, or of intracellular active components of neoplastic cells, such as in the tumor lysis syndrome.

However, these syndromes are not exclusive of bio-therapeutic interventions, and may occur during the development of various pathologies and as complications unrelated to drug administration. Moreover, the therapeutic intervention may enhance the expression of underlying, low/asymptomatic conditions rather than inducing per se the evoked systemic manifestations. Therefore, among the adverse events of biomedicines, the syndromes reported in Table 3.1 should be particularly taken into account, and be carefully considered in terms of etiopathogenesis as well.

3.1 Capillary/Vascular Leak Syndrome (CLS/VLS)

The capillary leak syndrome (CLS) was first described in 1960 as sudden episodes of collapse due to a massive, albeit reversible, transfer of plasma into extravascular compartments causing shock, and edema [1]. Later on, it has been related to cytokines' action on vascular permeability and identified also as vascular leak syndrome (VLS) [2]. In its acute phase (leak phase), up to 70 % of plasma is extravasated. *Prodromic signs* such as malaise, weight gain, fatigue, weakness and myalgia may occur; pyrexia, abdominal pain, diarrhea and vomiting may follow.

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Table 3.1 Systemic syndromes

Denomination	Acronym	Mechanisms of action/ expression	Manifestations	Bio-inducers (*)
Capillary (vascular) leak syndrome	CLS	Endothelial damage/ apoptosis Endothelial and leukocyte activation Cytokine release	*Leak phase*: hypotension, peripheral edema, hemoconcentration, hypoalbuminemia, oliguria Complications: ischemia, stroke, DVT, renal failure, rhabdomyolysis *Post-leak phase*: visceral edema Complications: pulmonary and cardiopulmonary edema	Interleukins (IL-1, IL-2, IL-3, IL-4) Interferons (IFN-α, IFN-β1b) Monoclonal antibodies (alemtuzumab, basiliximab, bevacizumab, catumaxomab, daclizumab) Growth Factors (oprelvekin) Immunotoxins (denileukin-diftitox)
Reversible posterior leukoencephalopathy syndrome	RPLS	Local (brain) CLS	Cerebral edema, cephalea, visual loss, seizures, hypertension	Monoclonal antibodies (bevacizumab, certolizumab, Rituximab, ustekinumab)

(continued)

Table 3.1 (continued)

Denomination	Acronym	Mechanisms of action/ expression	Manifestations	Bio-inducers (*)
Cytokine release syndrome	CRS	Cytokine storm; T cells (mainly CD28+) B-cells and monocytes massive activation	*Early phase*: cephalea, nausea, vomiting, diarrhea, chills, pyrexia, hypotension *Secondary/late phase*:cardiorespiratory and renal disorders, DIC cytopenia, ARDS, cardiovascular shock, pulmonary edema, renal/hepatic disorders, neuro-psychiatric events *Flu-like syndrome*: cephalea, pyrexia, chills, muscular pain/weakness	Interleukins (IL-1, IL-2, IL-3, IL-6, TNF-α) Monoclonal antibodies (muromomab, tositumomab, rituximab, alemtuzumab, catumaxomab)
Infusion reaction syndrome	IRS	CRS, hypersensitivity, anaphylaxis (IgE), intolerance, direct toxicity, anaphylactoid reactions	Hypotension, pyrexia, chills, bronchospasm, dyspnea, tachycardia, nausea/vomiting, rash/ urticaria, angioedema, other cardiovascular disorders, ARDS	Most infused biomedicines, IgE-mediated (muromomab, cetuximab, panitumumab)
Tumor lysis syndrome	TLS	Massive tumoral cell lysis and consequent, release of K, P, nucleic acids	Oligouric renal failure, arrhythmias, hypotension, cardiac failure, neuro-muscular disorders, hyperkalemia, hyperphosphatemia, hyperuricemia elevated LDH, pyrexia, secondary hypocalcemia	Monoclonal antibodies (alemtuzumab, brentuximab gemtuzumab, ipilimumab, ofatumumab, rituximab)
				"Double TLS storm" (muromomab, ibritumomab, ofatumumab, rituximab, tositumomab)
				CLS-CRS-TLS "Shock waves" (alemtuzumab)

(continued)

Table 3.1 (continued)

Denomination	Acronym	Mechanisms of action/ expression	Manifestations	Bio-inducers (*)
Systemic inflammatory response syndrome	SIRS	Activation/release of proinflammatory cytokines	Pyrexia/hypothermia, leukocytosis/ leukopenia, tachycardia/tachypnea	Pro-inflammatory cytokines (IL-1, IL-6, IL-8, TNF-α, IL-8) Integrins (ICAM) Monoclonal antibodies (catumaxomab)
Macrophage activating syndrome	MAS	Unknown. genetic defects of T and NK cells High levels of macrophage stimulating factors (M-CSF, MCP-1, IFNγ, interleukins (IL-6, IL-12) IL-18, TNF-α, IL-2), receptors (IL-2R)	Hepatosplenomegaly, encephalopathy, pancytopenia, coagulative, disorders, increased ferritin, elevated non remitting pyrexia, hematophagocytosis, LDH elevation, hyponatremia, hypertriglyceridemia, hypoalbuminemia	Monoclonal antibodies (alemtuzumab) Cytokine receptor analogues (anakinra)

(continued)

Table 3.1 (continued)

Denomination	Acronym	Mechanisms of action/ expression	Manifestations	Bio-inducers (*)
Immune reconstitution inflammatory syndrome	IRIS	Therapy discontinuation. immune response Dysregulation/rebound after drug-induced, immunosuppression. T memory/naive imbalance Hyperproduction of inteleukins (IFNγ, IL-2, IL-6, IL-12)	*Infectious:* worsening/unmasking, neutrophil rebound *Non infectious:* inflammatory and autoimmune exacerbation, cutaneous reactions (papular urticaria, SLE), GBS, acute porphyria	Monoclonal antibodies (natalizumab, infliximab, adalimumab)
Progressive multifocal leucoencephalopathy	PML	JC virus reactivation Local (brain) IRIS-PML T cells, B cells and plasma cells increase	Leukoencephalitis signs: vision loss, paralysis, cognitive disorders Alien hand syndrome	Monoclonal antibodies (efalizumab, infliximab, rituximab, natalizumab) Fusion proteins (belatacept)

* Examples of direct and indirect events related to biomedicines administration; DIC: disseminated intravascular coagulation; ARDS: acute respiratory distress syndrome. See also list of acronyms.

The *Leak phase* is characterized by prolonged hypotension, edema (face, trunk, extremities) hemoconcentration, hypoalbuminemia, oliguria, thirst, and cool skin. Complications such as ischemia, renal failure, stroke, deep vein thrombosis, and rhabdomyolysis may also occur at this stage.

During the *postleak phase*, symptoms revert rapidly; fluids are recruited into circulation and diuresis increases; the massive fluid rebound induces diffuse visceral edema, usually not present in the leak phase. Therefore, pulmonary edema and cardiopulmonary failure are the consequent complications during the postleak phase. Their severity and frequency may be influenced by the usual high volumes of fluids administered during the leak phase to compensate extravasation.

CLS is a rare, acute, unpredictable cyclic event with intervals from days to decades, albeit stereotyped in each patient. The diagnosis is based on the simultaneous occurrence, regardless of severity, of at least two of the following signs: edema, hypoalbuminemia, and/or hypotension occurring at the beginning of a cycle of treatment, associated to signs of hemoconcentration in the absence of apparent cardiac dysfunction. However, this diagnostic approach may tend to overestimate the incidence of CLS, since these signs are common to underlying diseases and to other associated complications, such as hypersensitivity reactions.

Reversible Posterior Leukoencephalopathy Syndrome (RPLS) is at present considered as a localized rare brain-capillary leak syndrome associated with hypertension, fluid retention, and cytotoxic damage on the vascular endothelium.

A very rare *chronic CLS* has also been postulated, characterized by noncyclic peripheral edema and hypoalbuminemia, in the absence of hypotensive acute crisis.

The pathogenesis of these syndromes is substantially unknown. Endothelial injury and apoptosis or cell retraction have been suggested on the basis of morphological and functional studies, although not conclusive. The vascular damage may involve activation of endothelial cells and leukocytes, intercellular adhesion, and most importantly the massive release of cytokines and inflammatory mediators. Their effect greatly increases vascular permeability allowing fluids, proteins, and electrolytes to flow into interstitial spaces, producing edema, hypoxia, and multiple organ failures (mainly pulmonary, cardiac, and renal). Therefore, multifactorial mechanisms have been postulated as: (1) initial toxic effects on vascular endothelium integrity; (2) activation of endothelial cells and leukocytes; (3) additional secretion of cytokines and inflammatory mediators consequent to cell activation; (4) increased damage by activated leukocytes and secondary reaction of newly formed mediators [3–5].

CLS has been observed in various human pathologies, such as sepsis, trauma, lymphoma, monoclonal gammopathy, burns, pancreatitis, and as a consequence of bone marrow or stem cell transplantation, as well as subsequent to nonbiological anti-neoplastic drugs (cyclosporine, cyclophosphamide, mitomycin C, cytosine arabinoside, gemcitabine, and docetaxel) and dermatological (acitretin) treatments.

Since the introduction of biomedicines in human therapy, CLS has been more frequently reported as an important AE following treatment. Some monoclonal antibodies, interleukins, receptor inhibitors, and growth factors may induce CLS with low frequency but at serious/severe levels.

As for *CLS induced by biomedicines*, three aspects appear more relevant in understanding the pathogenesis: (a) the endothelial cell retraction with released cells interconnections; (b) the observed association of IgG monoclonal gammopathy with CLS, not related to therapy; and (c) the direct effect of biomolecules (cytokines, antibodies, and inflammatory mediators) exerted in vitro and in vivo on vascular endothelial cells [2, 5]. These mechanistic factors may be differently represented in specific clinical conditions, but indicate the common basic pathogenetic conditions, namely the capillary physical leakage induced by apoptosis and oxidation injury, the structural characteristics of the inducer agent, and the effectors of CLS [6].

Among cytokines, interleukins (IL-2, IL-3, IL-4) and interferons (IFN-α, IFN-β1b) were first identified as potential CLS inducers. Among monoclonals, after a mouse antiGD3 ganglioside IgG3 antibody (B24) preliminary tested against melanoma, the murine muromonab stimulated a strong cytokine production in vitro and induced relevant CLS reactions in vivo [7–9]. Among the most recent biomedicines, alemtuzumab, basiliximab, bevacizumab, catumaxomab, and daclizumab can induce CLS with different degrees of gravity. Similarly, cases of CLS have been observed with early IL-1, IL-2, and Il-4 experiences in human therapy, with stimulatory and growth factors, such as oprelvekin, filgrastim, pegfilgrastim, sargramostim, even at low doses and with immunotoxins, such as denileukin diftitox [10; see also this volume, at respective drug descriptions].

Overall, CLS represents one of the major limitations to therapeutic efficacy of cytokines and of monoclonal antibodies, together with the related cytokine release syndrome [9]. Notably, these reactions were also found to be strictly related to the respective drug-specific therapeutic actions and therefore have been indicated as possible predictor markers for efficacy (catumaxomab) [11].

3.2 Cytokine Release Syndrome (CRS)

In principle, any action determining a massive T lymphocyte/Monocyte activation may produce a “Cytokine Storm”, better defined as cytokine release syndrome (CRS) consisting of an immediate immission into circulation of proinflammatory and cytotoxic cytokines which rapidly elicit systemic and dramatic signs. Although initially observed after mouse-derived mAbs infusions, more frequently as mild to moderate reactions, subsequent and prolonged experimentation encountered dramatic consequences, even with fully humanized mAbs [9, 12, 13].

Candidate targets able to induce CRS are those widely diffused on T cells (CD3, CD52), CD20, activated T cells (CD25), B cells (CD20), and monocytes (CD52). However, not all biomedicines are associated with CRS. For example, basiliximab

is considered as a low inducer of CRS because it targets the IL-2Rα chain lacking the capacity of intracellular signaling and therefore being not able to burst violent cytokine releases, such as those causing CRS. Alternatively, some biomedicines may induce less typical reactions reported in anecdotal episodes and in the post-marketing settings yet not identified as CRS.

After early experience with muromonab (mouse-anti human CD3), licensed in 1986/87, CRS was observed with another murine mAb (anti human CD20, tositumomab), but also with chimeric (anti-CD20, rituximab), humanized (anti-CD52, alemtuzumab), and with hybrid rat/mouse tri-functional bispecific (anti-EpCAM and anti-CD3, catumaxomab) mAbs. Finally, an anti-CD28 fully humanized mAb (TGN1412), rapidly and almost simultaneously injected in six volunteers in a Phase I trial, caused an even more dramatic cascade of immediate, long lasting, and life-threatening events. The initial response was characterized by cephalea, nausea, vomiting, diarrhea, chills, pyrexia, and hypotension accompanied by high levels of cytokines into circulation. A second phase showed increasing cardio-respiratory and renal dysfunctions, associated with disseminated intravascular coagulation. A profound lympho/mono-cytopenia followed. Finally, a prolonged cardiovascular shock and severe clinical signs of acute respiratory distress syndrome (ARDS) completed the complex dramatic feature of the syndrome.

On this basis, the etiopathogenesis and the clinical expressions of drug-induced CRS became more evident, and great concern was raised against biomedicines and their preclinical testing procedures. The CD28 positive T lymphocytes were the major targets and releasers of the pathogenetic cytokines. Moreover, the timing of infusion was a critical factor, and it became clear that even fully humanized mAbs could not avoid CRS at the most severe grades. Later on, the reason of failure to predict the cytokine storm in these subjects was ascribed to the lack of CD28 antigen on the surface of CD4+ effector memory T cells in animal species employed in preclinical investigations [14].

In the clinical experience, CRS signs usually appear after the first infusions as mild/moderate malaise, with a cohort of milder symptoms now recognized also as *Flu-like syndrome* (FLS), characterized by pyrexia (non-infective, sometimes hyperthermia), cephalea, tremor/chills, nausea/vomiting, diarrhea, abdominal pain, muscle/joint aches, and generalized weakness. Less frequently, FLS may evolve into more serious (occasionally fatal) with additional signs including cardio-respiratory events (dyspnea, bronchospasm/wheezing, tachypnea, respiratory arrest/failure/distress, cardiovascular collapse, cardiac arrest, angina/myocardial infarction, chest pain/tightness, tachycardia, hypertension, hemodynamic instability, hypotension, shock, heart failure, pulmonary edema, ARDS, hypoxia, apnea and arrhythmias, and hypertension), transient renal and renal allograft dysfunction (oliguria, creatinemia), transient hepatic abnormalities (transaminases increase), and neuropsychiatric events (dizziness, confusion, depression, seizures, paresis/plegia, deliria, somnolence/lethargy/coma, deliria, hallucinations, and hypotonia).

Not all signs are present in every patient, even when expressing highest degrees of severity, neither they appear with all involved biomedicines. FLS is also observed in patients treated with IFNs, IL-1, IL-2, IL-3, and TNF-α.

Nonetheless, all forms of CRS are usually reversible and can be mitigated/controlled by slow drug infusion and appropriate therapies, according to the grading of severity.

In a number of studies, CRS has been clearly associated to specific mechanisms of action of some mAbs [9, 15]. In particular, the anti-CD3 activity initially leads to massive activation of the T cell compartment, with consequent abundant release of proinflammatory and cytotoxic cytokines initiated by the binding on immune and tumor cells, before expressing toxic and apoptotic effects on the same cells. In the case of alemtuzumab, in vitro testing showed that CRS is IgG isotype dependent and that IgG1—the most used isotype in mAbs production—induces the highest levels of cytokine release. Pyrexia and hyperthermia are mostly related to IL-1, IL-6, and TNF production. In particular, hyperthermia seems to be more related to IL-6 release, but independent from PGE2 production, e.g., from the usual inducer pathway of pyrexia [16]. However, CRS expression, even at moderate levels, seems also to correlate with efficacy of treatment, giving to this syndrome a potential predictive value, which can be assessed in vitro only on human cells [15, 17].

The potential stimulatory effect of single biomedicines can be now selectively tested in some assays, and their capacity to induce CRS seems to correlate with the response in vitro. However, in the case of the trifunctional antibody catumaxomab, this activity was only observed in significant amounts when the antibody was incubated in vitro with blood cells in the presence of the target (EpCAM positive colon tumor cells) [18]. Therefore, intercellular binding and/or additional releases of other CRS–inducing factors from tumor cells might play additional roles in CRS manifestation, particularly when a high burden of specific tumor targets are involved. Major effects were seen in releasing TNF-α and IL-6 in the presence of EpCAM-positive tumor cells, with a smaller activity on IL-2 and a nonsignificant action on IL-12 and IL-1. In this case, no histamine release or complement activation was observed during experiments, thus indicating the exclusive role of cytokines in the development of typical CRS. Altogether, the in vitro cytokine release stimulation on effector cells and the protective effect exerted by some corticosteroids in the same in vitro assay are a further proof-of-concept of mAb-mediated CRS pathogenesis and of the efficacy of steroid (pre) medication in mitigating its effects in vivo.

Therefore, the possibility of preventive checking by in vitro methods should be taken into consideration when CRS is expected to occur due to the underlying pathology or to the specific administered drug, becoming crucial in preventing from the dramatic “Cytokine Storms” [15, 17, 18].

3.3 Infusion Reaction Syndrome (IRS)

IRS is mostly related to CRS, but also involves other reactions such as hypersensitivity, direct toxicity, drug intolerance, and anaphylactoid reactivity. The reaction occurs with most systemic cancer treatments and usually appears rapidly. Hypotension, pyrexia, chills/rigors, bronchospasm, dyspnea, tachycardia, nausea, vomiting, urticaria, and/or rash are the most common signs. Serious events such as cardiac dysfunctions/insufficiency, myocardial infarction, cardiac and respiratory arrest, syncope, pulmonary infiltrates, ARDS, angioedema, and anaphylactoid shock may be associated at lower frequencies. Obviously, not all signs appear simultaneously and are of the same severity. Usually, they appear shortly after the first intravenous drug infusions, and have a mild to moderate intensity, with tendency to decrease with subsequent doses. Less frequently IRS are serious and fatal.

In the experience with biomedicines, these reactions are frequent and ultimately they appear as cytokine-mediated reactions of different intensity. However, they can be prevented by appropriate prophylactic and symptomatic therapy, and by dose grading of the drug. In a minority of cases, Type-I hypersensitivity reactions (IgE-mediated) were observed, such as after muromonab (29 %), cetuximab (3–13 %), and less frequently after panitumumab and basiliximab administrations. Interestingly, antidrug IgE antibodies were preferably directed to oligosaccharides and in some cases were present as preformed antibodies directed to the same antigens.

The subcutaneous administration significantly reduces signs and severity of IRS, but not their overall frequency. In particular, pyrexia elevation and incidence are not appreciably reduced.

Local reactions at site of injection are common, albeit mild/moderate, and tend to disappear in days or weeks. They are generated by a series of mechanisms, including local cytokine release, immune-mediated reactions, immediate or delayed, and by irritative reactions to various drug components [19].

3.4 Tumor Lysis Syndrome (TLS)

The syndrome was described in 1929 in chronic leukemia, as an acute oliguric renal failure associated with hyperkalemia, followed by hyperuricemia, hyperphosphatemia, secondary hypocalcemia, elevated LDH and pyrexia, in adult and pediatric patients with high load tumors at elevated cell turnover. Usually these types of tumors, either hematologic or solid, undergo rapid spontaneous and massive cell lysis, which liberates ions and toxic metabolites affecting at first the renal function, followed by a life-threatening multisystem organ failure. Clinical consequences, due to electrolytes' abnormalities and acute toxic overload, progressively affect cardiac, muscular, hepatic, and neurological conditions. The syndrome can be fatal although in most cases is reversible and preventable [20–22].

Spontaneous TLS may occur mainly in high-grade lymphomas (Burkitt's, NHL), AML/ALL, and CLL. Interestingly, spontaneous TLS can be triggered by local events, such as infiltration of leukemic T-ALL cells in the renal parenchyma producing acute kidney failure, even in an aleukemic phase of the disease [23].

Cytotoxic chemotherapy, radiation therapy, occasionally glucocorticoid therapy, and biomedicines have further enlarged the category of solid tumors undergoing *secondary TLS* (hepatoblastoma, neuroblastoma grade IV, renal cell cancer, gastro-intestinal stromal tumors, pancreatic neuroendocrine tumors, and melanoma) and in particular to those combining a high rate of turnover with high sensitivity to specific treatments. In fact, cancer cells have an abnormally high amount of potassium, phosphorus, and nucleic acids. The breakdown of the latter in the liver produces hyperuricemia, mainly affecting the renal function, while secondary hypocalcemia occurs because of serum calcium binding to the elevated amounts of phosphates in the bloodstream. The subsequent calcium/phosphate unbalance produces arrhythmias, hypotension, and cardiac failure; hyperkalemia increases renal injury and impairs cardiac and neuromuscular functions.

The main *difference between spontaneous and posttreatment TLS* is that the former are also associated with particular high levels of hyperphosphatemia and related consequences. A possible explanation has been related to re-usage of released phosphates during spontaneous TLS by newly growing tumor cells, which is avoided in secondary TLS because of the prolonged action of administered cytotoxic drugs. Therefore, an acute renal failure with hyperkalemia and hyperphosphatemia and oligo/anuria in patients with a large tumor burden during therapy is highly indicative of secondary TLS.

In 1960, a "*prodromic TLS*" was identified, with 25 % increase of electrolytes and uric acid associated with signs of renal injury. This phase may occur from 3 days before to 7 days after cytotherapy initiation, showing creatinine increase, cardiac (arrhythmia, sudden death) and neurological signs (seizures), which can be controlled by appropriate therapy (anti-uric, hemodialysis). Importantly, adequate surveillance and therapy can prevent the evolution of the prodromic phase into clinical TLS.

The experience with biomedicines and other recent drug classes, such as protein kinase inhibitors and a proteasome inhibitor, confirmed that the potential susceptibility to TLS is particularly linked to tumor/patient bio-specificities, more than to drug class characteristics or specific mechanisms of action. Among mAbs, alemtuzumab (CD52), brentuximab (CD30), gemtuzumab (CD33), ipilimumab (CTLA-4), ofatumumab, and rituximab (CD20) have induced TLC or have alerted for possible potentiality of induction, due to their high efficiency in massive tumor cell destruction. Clearly, being their targets quite different, a specific pathway of destruction has been excluded. However, some of these agents, such as IL-2 and anti-CD3 (muromonab) or CD20 (ibritumomab, ofatumumab, rituximab, and tositumomab), have the capacity of activating their target cells soon after therapy initiation and before destroying them. Therefore, in the presence of relevant tumor burdens with high cell turnover, such as T cell lymphomas and acute leukemia, they may cause, although infrequently, a "*double storm*" in sequence, first through

massive *cytokine release (CRS)* and then by vast *tumor cell lysis (TLS*). Very rarely, as documented for example during antiCD52 (alemtuzumab) therapy, *CLS, CRS, and TLS* can follow like *incoming shock waves*.

Since these episodes are rare, they can remain a mere potential risk at clinical level, provided that timing prevention occurs.

Understanding TLS at theoretical level remains important for identifying biomedicines' combined mechanisms of actions that induce violent AEs, such as T cell activation causing high endothelial toxicity (widely cytokine-dependent), and massive destructive capacity of tumor burden.

Taken together, these aspects are currently a major limitation to the therapeutic utility of most active biomedicines, yet they also represent a master lesson and a crucial point for future development.

3.5 Systemic Inflammatory Response Syndrome (SIRS)

SIRS is an acute progressive reaction resulting from the activation of proinflammatory cytokines caused by infectious and other noninfectious stimuli such as ischemia, major trauma, surgical trauma, and therapy. According to a largely accepted official definition (ACCP) pyrexia (>38 °C) or hypothermia (<36 °C), leukocytosis ($>12\times10^9$/l) or leukopenia ($<4\times12\times10^9$/l), increased heart rate (>90) and respiratory rate (>20 or PaCO2 <32) are the cardinal signs, and at least two of them must support the diagnosis. *Infectious SIRS* may proceed to sepsis, severe sepsis, septic shock, and multiple organ dysfunction syndrome (MODS), as defined by ACCP since 1992.

Nonseptic SIRS evolves into a multiorgan dysfunction and eventually failure. Among them, renal failure, gastrointestinal bleeding, anemia, deep vein thrombosis, disseminated intravascular coagulation (DIC), electrolyte abnormalities, and hyperglycemia are the most relevant signs. Overall, SIRS occurs frequently (35 %) in acute hospitalized medical patients, is moderately related to initial infection, and highly related to mortality [24, 25]

Initiators of SIRS include a number of factors, such as bacterial endotoxins, immune (anaphylaxis) and complement-mediated systemic disorders (DIC), hypoxia, endothelial vascular injury, and cytokine releasing agents including biomedicines with a peculiar capacity of induction of CLS/CRS manifestations.

In principle, all cytokine releasers may induce the syndrome; the most relevant involved in the syndromic progression are TNFα, IL-1, IL-6, IL-8, and possibly IL-8, and IL-17. However, other molecules may be involved, such as integrins. Interestingly, an anti-integrin monoclonal antibody blocking the CD11d receptor for ICAM3 (CD50) and ICAM1 (CD106) reduces multiorgan signs of SIRS [26, 27].

SIRS has been observed within 24 h after catumaxomab infusion, showing severe tachycardia, pyrexia, leukocytosis, and dyspnea, that resolved with symptomatic therapy. Overall, SIRS is rarely diagnosed during treatments with biomedicines, including those actively inducing cytokine storms, presumably because

of the difficulty to distinguish SIRS from CLS/CRS events, bacterial-induced sepsis, and septic shock frequently encountered among AEs, all sharing various mechanistic routes [18].

3.6 Macrophage Activating Syndrome (MAS)

MAS acronym was proposed in 1993 and identifies a serious, life-threatening complication of rheumatic diseases, more frequently observed in the *Systemic juvenile idiopathic arhritic forms (SIJA)*, also known as acquired hemophagocytic lymphohistiocytosis (HLH), included in the group of hemophagocytic syndromes (HPS). Rare primary *inherited forms* of the disease are also known (Chédiak-Higashi Syndrome). MAS is also a complication of lymphoma (mainly NHL), SLE, Kawasaki disease, and of autoinflammatory inherited periodic fever disorders. The syndrome also occurs after EBV and CMV *acute infections* and is characterized by an impaired or absent function of NK cells, and of cytotoxic T lymphocytes. This unbalanced situation seems to induce a persistent antigen-driven activation, leading to a consistent production of cytokines that stimulate macrophage proliferation and activity.

The clinical features of MAS include an intense macrophage hemophagic activity, mostly evident in the bone marrow, developing into pancytopenia, coagulative disorders, hepatosplenomegaly, encephalopathy, rapidly increasing ferritin levels, and elevated nonremitting pyrexia. Additional laboratory abnormalities include high levels of LDH and triglycerides, hyponatremia, and hypoalbuminemia. Frequently, the clinical evolution is acute and dramatic, with a high rate of mortality.

At present, the syndrome is not considered rare as it used to be, since subclinical forms have been more recently detected with a frequency of up to 40 % of cases in SJIA. The etiology of noninfectious forms is unknown. Genetic defects in T and NK cells cytotoxicity, the latter related to perforin deficiency encountered especially in SJIA, have been identified. High levels of macrophage stimulating (M-CSF, IFNγ, and MCP-1), or macrophage-derived (IL-6, IL-12, IL-18, and TNFα) cytokines, and of T-derived products (IL-2 and IL-2R) are usually present, while IL-1 is not always elevated, although its role in developing the syndrome is revealed by beneficial effects of IL-1 antagonist, such as anakinra. However, a number of iatrogenic inducers/boosters have been suspected, such as acetylsalicylic acid and other NSAIDs, gold salts, sulfasalazine, and biomedicines with high capacity of cytokine release, including TNF-α releasers. Interestingly, the latter seems to play an additional role in dyscoagulative disorders [28–31]. Cases of MAS or exacerbation of underlying states of HPS, including cytopathic histiocytic panniculitis (CHP) have been reported after treatment with etanercept, rilonacept, tocilizumab, anakinra, and alemtuzumab [32, 33]. However, a direct relation between treatment and MAS induction is not always evident. For example, in cases where MAS was associated to alemtuzumab a reactivation of EBV and CMV

viruses occurred, thus indicating a possible immunosuppressive effect of the biomedicine as an indirect cause of MAS activation. In other clinical situations, such as after anakinra treatment, the agents seemed to ameliorate MAS, but in other occasions they acted as inducers of macrophage activation. In one case of CHP evolving into a severe HLH with elevated circulating IFNγ, IL-12, IL-4, and IL-18, but also of the antiinflammatory IL-10, etanercept was partially effective, yet produced aphasia and hemiparalysis that resolved after treatment discontinuation [34]. The subsequent treatment with anakinra was very effective in controlling the syndrome. Such biomedicine was equally effective in Still's disease and SJIA, but in other cases of JIA failed or led to induction of MAS [35–37]. It must be stated that anecdotal reported cases of similar conditions are difficult to compare per se. Observations during controlled studies are rare and often refer to different clinical situations.

Overall, the cytokine cascade defined as CRS appears as the common denominator of all syndromes associated with treatment, and in particular with biomedicines administration.

According to the induced agent typology and the individual clinical situation, CRS may be differently modulated from a mild FLS to the impressive cytokine storms (CRS, SIRS), or associated with toxic endothelial-directed events (CLS), with hypersensitivity reactions (IRS), with systemic toxicity generated by massive neoplastic destruction (TLS), or with prevalent macrophage activation (MAS).

3.7 Immune Reconstitution Inflammatory Syndrome (IRIS)

While the previously mentioned syndromes are a direct consequence of therapy with some biomedicines, IRIS is the consequence of therapy discontinuation. This syndrome is defined as a dysregulated inflammatory response to noninfectious and infectious agents occurring during immune recovery, after an induced state of immunodeficiency. The syndrome is characterized by a new-onset of worsening symptoms in a phase of immune function restoration, showing aggravated or new infection, and inflammation signs.

Unmasking IRIS defines an occult undiagnosed infection(s), which appears after immunologic recovery. This feature is usually observed in patients recovering from drug-induced neutropenia.

Paradoxical IRIS defines a worsening of a known infection (opportunistic), already receiving treatment, which deteriorates during immune system recovery, in spite of appropriate concurrent therapy.

Infectious IRIS—initially observed in HIV patients undergoing multiple highly active antiretroviral therapy (HAART)—can also develop during other infectious (mycobacterial, herpetic infections, HBV, HCV, CMV, JCV and parvovirus infections, opportunistic infections) or noninfectious conditions (rheumatic diseases, SLE, GBS, AIDS-related lymphoma, autoimmune thyroiditis, sarcoidosis, and other granulomatous reactions).

The pathogenesis of IRIS consists in rapid and exuberant immune-inflammatory response of the host directed to resident microbial agents, or in an aspecific noninfective homeostatic rebound, causing a consistent increase of CD8 + T cells, macrophage infiltration, and necrosis.

Among the clinical manifestations there are signs of infective reacutization, such as mycobacterial lymphadenitis, recurrence of opportunistic pulmonary infection, and viral hepatitis reactivation, as well as noninfectious inflammatory and autoimmune exacerbations of underlying diseases [31, 38].

Noninfectious IRIS may cause cutaneous manifestations (papular urticaria, eosinophilic folliculitis, Sweet syndrome, Reiter's syndrome, and SLE), and noncutaneous disorders (GBS, radiculopathy, acute porphyria, Castleman disease, and NHL) [39].

The pathogenetic framework of IRIS consists in a rapid recovery of multiple immune functions, but the specific mechanisms involved are less clear. The syndrome may occur during antiretroviral treatment when the CD4 + cell burden rapidly increases, or after immunosuppressive treatment discontinuation. During reconstitution of the immune system, not only the number of these cells is increased, but their subtypes recover with different kinetics and peripheral redistribution; memory CD4+ cell appear to anticipate the recovery of naive T lymphocytes (of months), regulatory T cells (Treg) appear compromised by previous therapies, and an exuberant production of interleukins (IFNγ, IL-2, IL-6, IL-12 primarily) follows, with the known activation inflammatory signs caused by the cytokine storming. However, other factors may be related to IRIS insurgence, such as VEGF signals. In one case report of TB-IRIS granulomatous infection causing retinal detachment, bevacizumab (an anti-VEGF mAb) successfully controlled the complication [40].

Overall, IRIS pathogenesis implies a complex interaction between an underlying antigen precipitant (infectious or endogenous)—the entity of immune reconstitution rebuilding a strong reactivity against the antigen—and possible host genetic yet unknown factors [41].

The immune response is predominantly of the granulomatous type, but the cell components may vary, being predominantly of the CD4+ type, with a variable association of CD8+ cells, such as in HIV-associated sarcoidosis. An additional characteristic during the immune reconstitution phase results in localized inflammation (where disseminated is typical) or in an exaggerated intensity of the inflammatory response, when the recovery of circulating T cell level has not reached normal values yet [39].

As for the rare association of IRIS to biomedicines administration, a peculiar localized IRIS form has been observed in the CNS, showing features of *Progressive Multifocal Leukoencephalopathy* (PML), soon after therapy cessation with natalizumab in MS patients.

Brain histology showed an extensive infiltration of T cells, particularly CD8+ T cells, and plasma cells. This feature, together with a low number of JCV-positive cells within the same areas, is considered as specific of "*IRIS-PML*". In fact, the number of T cells in this form was 8–9 times higher than in PML cases

observed in MS patients, as well as for plasma cells and B cells, which were practically absent in PML not related to natalizumab administration. Notably, these parameters were inversely correlated with JCV-infected cells [42].

A number of TB-IRIS were observed after TNF-α antagonists (infliximab, adalimumab) discontinuation. In one of these patients the reaction was associated to the recovery of cell-mediated reactivity to tuberculin, and to the capacity of organizing granulomatous lesions at pulmonary level. All patients recovered, and in one case with life-threatening manifestation; the monoclonal therapy was reintroduced with beneficial effects. The amelioration was related to inhibition of granulomatous organization allowing a better antibiotic diffusion in pulmonary lesions [43, 44].

Another and intriguing systemic syndrome has recently been observed after ipilimumab administration, related to the induction of **immune-related (mediated) adverse events (IrAES, or IMAE)** as a consequence of therapy exerting an enhanced activity on immune effector mechanisms. In this case the inhibition of a natural inhibiting signal mediated by CTLA-4, triggers a number of multiorgan serious and fatal inflammatory processes (hepatitis, enterocolitis, dermatitis etc.), driven by the massive activation o T cells (see Chap. 25). IrAEs are highly concerning, yet to be fully investigated and understood before attemping to locate them in a precise AEs framework.

The accumulated knowledge of these syndromes has offered great opportunities to put in action solid steps for their prevention. In fact, they are all infrequent adverse events related to the administration of biomedicines, but most of them can be serious and life threatening. However, their occurrence has become a mere rarity due to effective prevention and the experience of oncotherapists.

It is not easy to differentiate some of these events from hypersensitivity reactions and anaphylactoid reactions, as well as from underlying disease-related disorders. Although showing a variety of differential expressions, *the common denominator of these systemic syndromes* appears so far based on *cytokine release, cytokine dysregulation, and cytokine rebound*. They also represent a master lesson for the development of future biomedicines, having faced some failures and dramatic experiences [14]. Most of all, they have confirmed that experimentation on animal models is not sufficient to predict even frequent and life-threatening events, while in vitro efforts in finding the minimal anticipated biological level (MABEL) seems now more relevant in order to determine the initial dose for first attempts of in vivo administration [45].

More attention should be given to avoid preactivation of cytotoxic targets before their destruction, especially when rich of biologically active molecules or toxic metabolites. Finally, highest affinity bindings and highest concentrations of biomedicine/cell may not be the real goal, when targets overexpress antigens shared by normal cells at lower concentration. Lower concentration may still kill the neoplastic cells and spare a higher number of normal cells [46].

Table 3.2 reports biomedicines showing inducer capacity of one or more of the mentioned systemic syndromes.

Table 3.2 Biomedicines as inducers of systemic syndromes and related local syndromes

Syndrome	**Systemic**							**Local**			**Comparators**	
Type	CLS	CRS°	TLS	IRS	MAS	SIRS ^		IRIS	PML	RPLS	Anaphylaxis	PM reports[a]
Subtype						NI	I					
Monoclonals												
Abciximab	–	–	–	4	–	4	22	–	–	–	<1 %	4400
Adalimumab	5	–	2	62	1	57	1195	X, 13	X, 50	X, 9	<0.01 %	130505
Alemtuzumab	X, 11	X, 11	X, 38	≥10 %	X	8	109	–	X, 30	–	<1 %, 11	3590
Basiliximab	X, 7	X, 10	–	–	–	20	94	–	1	3	32	2106
Belimumab	–	–	–	≥10	–	–	1	–	–	–	<1 %, 5	659
Bevacizumab	16	37	26	X, 101	–	2	398	–	14	105	116	21880
Brentuximab	–	–	X, 3	≥10 %, 2	–	–	8	–	X, 11	–	–	122
Canakinumab	–	1	–	–	–	–	7	–	–	–	–	403
Catumaxomab	–	X	–	–	–	X	–	–	–	–	–	–
Certolizumab	–	–	–	2	–	–	60	–	1	–	44	12087
Cetuximab	–	–	8	≥10 %, 509	–	3	141	–	2, 19	9	X, 219	7983
Daclizumab	X	–	–	–	–	1	30	–	–	–	X, 12	1141
Denosumab	–	–	–	1	–	–	7	–	–	–	3	1150
Eculizumab	–	–	–	X, 30	–	1		–	7	–	7	7407
Efalizumab	1	–	2	–	–	–	20	–	X, 26	–	10	3318
Gemtuzumab	3	X, 1	X.67	≥10 %, 191	–	–	276	–	–	–	X, 30	3106

(continued)

Table 3.2 (continued)

Syndrome				**Systemic**					**Local**		**Comparators**	
Type	CLS	CRS°	TLS	IRS	MAS	SIRS ^		IRIS	PML	RPLS	Anaphylaxis	PM reports[a]
Subtype						NI	I					
Monoclonals												
Golimumab	–	–	–	1	–	–	27	–	3	–	<1 %, 3	2259
Ibritumomab	–	2	5	<1 %, 5	–	–	74	–	X, 23	1	X, 4	1504
Infliximab	10	2	–	≥10 %, 5373	–	34	1065	X,7	X, 84	33	≤1, 685	79722
Ipilimumab	–	X	X^^	10	–	–	5	–	–	–	–	938
Natalizumab	–	–	–	≥10 %, 610	–	10	527	X,834	X, 258	X	< 1 %, 395	90168
Ofatumumab	–	X	X, 1	≥10 %, 11	–	–	8	–	X, 3	–	< 1 %	261
Omalizumab	–	1	–	2	–	–	30	–	–	–	< 0.1 %	10189
Palivizumab	2	–	–	–	–	–	66	–	–	–	< 0.01 %, 21	7221
Panitumumab	–	–	–	≥10 %, 47	–	–	12	–	–	–	X, 7	1279
Pertuzumab	–	–	–	–	–	–	–	–	–	–	–	–
Ranibizumab	–	–	–	1	–	1	33	–	–	–	7	–
Rituximab	5	X, 25	X, 122	≥10 %, 226	–	11	222	–	279	15	99	9758
Tocilizumab	–	–	–	≥10 %, 35	X	3	84	–	1	–	< 1 %	3437
Tositumomab	–	–	–	≥10 %, 8	–	–	2	–	–	–	X	182
Trastuzumab	31	3	12	≥10 %, 99	–	4	119	–	–	10	X	9576
Ustekinumab	–	–	–	3	–	–	31	–	–	5	< 0.1 %	3130

(continued)

Table 3.2 (continued)

Syndrome	**Systemic**								**Local**		**Comparators**	
Type	CLS	CRS°	TLS	IRS	MAS	SIRS ^		IRIS	PML	RPLS	Anaphylaxis	PM reports[a]
Subtype						NI	I					
	–	–	–	–	–	–	–	–	–	–	–	–
Fusion proteins												
Abatacept	–	–	1	< 10 %, 43	–	2	55	–	X, 6	X	< 0.1 %, 57	5327
Aflibercept	–	–	–	–	–	–	–	–	–	X	–	104
Alefacept	3	–	–	–	–	–	22	–	1	–	X?	3561
Belatacept	–	–	–	–	–	–	–	–	X	–	–	39
Etanercept	5	2	7	35	X, 8	–	–	–	X, 40	–	169	166330
Rilonacept	–	–	–	–	X	10	679	–	–	–	–	15
Romiplostim (TPO)	2	–	–	4	–	8	224	–	–	–	12	10346
Cytokines												
Aldesleukin (IL-2)	30	1	X	10	–	–	–	1	–	5	10	1562
Denileukin-DT (IL-2)	≥10 %, 48	3	9	7	–	1	8	–	–	–	2	404
Anakinra (IL-2R)	–	–	–	X, 2	X	2	33	–	–	–	18	1968
IFN-α	15	–	X	1	–	–	24	2	5	–	15	8129
IFN-β	6	–	–	3	–	2	91	–	5	6	35	14298

(continued)

Table 3.2 (continued)

Syndrome	**Systemic**							**Local**		**Comparators**	
Type	CLS	CRS°	TLS	IRS	MAS	SIRS ^	IRIS	PML	RPLS	Anaphylaxis	PM reports[a]
Subtype						NI I					
IFN-γ	–	–	–	–	–	– 7	–	1	–	–	779
Growth factors											
Darbepoetin(epoetin-α)	6	1	–	27	–	11 340	–	3	19	61	12714
Filgrastim (rG-CSF)	X, 45	37	51	17	1	– –	8	21	19	57	12759
Sargramostim (rGM-CSF)	–	1	–	4	–	– –	–	1	–	15	927
Oprelvekin (IL-11)	15	–	–	1	–	– 10	–	–	–	3	781

°: as severe reactions, excluding mild FLS. ^NI: noninfectious; I: infectious. ^^: in ulcerative colitis. [a] : number of postmarketing consulted reports
%: refer to data in trials, and absolute numbers refer to postmarketing reported cases. X: not quantified, reported in controlled studies

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Part II
Monoclonal Antibodies

Monoclonal Antibodies

4

Since their initial production, Monoclonal Antibodies (monospecific clone-derived antibodies, shortly mAbs) have first expanded as outstanding laboratory tools for research and then as highly valuable therapeutic agents.

Muromonab-CD3 (Orthoclone, OKT3) was the first to be clinically applied to prevent acute transplant rejection in 1986. Thereafter, an important improvement for their applicability at human level was achieved by the production of "chimeric" and "humanized" mAbs to reduce the immune reactions against the rodent-derived components. In fact it was soon evident that the injection of fully murine mAbs, despite showing extraordinary efficacy, caused rapid and complex reactions in patients, including serious allergic and systemic inflammatory effects, and the production of human anti-mouse antibodies with consequent rapid inactivation and removal of the injected mAb from circulation.

Although able to reduce such unwanted effects, mouse-human chimeric mAb at different degree of "humanization" could not completely avoid them, and even fully humanized mAbs could not achieve total compatibility, since human-anti-human antibodies were raised under different therapeutic regimens.

Because of these difficulties, the production of new mAbs for human treatment initially developed at a rather slow level. However, in the last decade, and in particular in the last few years, progressive major developments have led to a rapid increase in mAbs production and approval for human use. In fact, these new products have developed fewer allergic/inflammatory reactions and non-specific immune responses, mainly by steadily reducing the amount of non-human (mostly mouse gene-derived) portions within the mAb structure, but also by cutting off the terminal sequences of the human portion, such as the Fc fragment, or changing its glycosylation, or even by eliminating part of the Fab fragment.

Between 2008 and 2010, 34 mAbs were available for a number of human diseases, 12 were humanized mAbs and 8 were fully humanized. Among these, 5 of the "old" murine mAbs were radiolabeled or conjugated with cytotoxic "bullets" to be used as diagnostics or for short-term therapeutic interventions.

By the end of 2010, 10 new mAbs were approved while a few of the previous ones started to be withdrawn, on the basis of industrial motivations or safety

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concerns, although not necessarily from all markets. Discontinuation of their production was not always followed by the licensure withdrawal.

By the end of 2012, the majority of 35 mAbs proposed for human therapy was approved by FDA and/or EMEA, or designated as orphan drugs, 5 were discontinued, 1 was approved elsewhere (nimotuzumab) and study in a restricted program in U.S. was allowed.

Most of these licensed products (15) are dedicated to anti-cancer (solid tumors and leukemias) therapy, while 10 were licensed for inflammatory and autoimmune diseases (mostly for rheumatoid arthritis). Finally, 4 other mAbs found application in other pathologies such as retinal maculopathy (1), osteoporosis (1), RSV infection (1), and paroxysmal nocturnal hemoglobinuria (1).

This monograph deals with safety evaluations on all licensed mAbs and some withdrawn/discontinued products, because of their educational interest in the expression of Adverse Events (AEs). In contrast, investigational products, including a large number of mAbs actively studied in the various pre-approval stages, are not considered in the present volume because of the limited safety experience so far accumulated, and the absence of validated data from official authorities.

In the next few years, provisional industrial reports indicate that a consistent number of new mAb will be commercialized. At present, about 150 new mAbs are being tested for clinical use at different stages of experimentation. In particular, about 70 mAbs are in Phase II, and 11 in Phase III, mostly for the treatment of solid tumors, lymphomas, leukemias and myeloma. However, the number of mAbs directed against inflammatory and autoimmune diseases is growing more rapidly than in the past. Among them, about 40 % are directed to rheumatoid arthritis and psoriasis, while other are targeting ulcerative colitis, Chron's diseases, and asthma. Over 60 mAbs in study are directed to other targets, such as metabolic disorders (diabetes), and CNS diseases (beta-amyloid). Among these, 25 mAbs are studied as treatments of infectious diseases and a few are targeting cardiovascular diseases. In 2013, 10 new mAbs with cancer indication and 20 with non-cancer indications, over about 350 products in the clinical pipeline, are good candidates for the market [1].

These areas, whenever successfully acquiring at least some of the mAbs under study, clearly indicate a widening of usage and application in numerous cohorts of patients of all ages. They will demand particular attention as the main growing class of biomedicines.

4.1 Structure and Typology

Monospecific clone-derived antibodies are usually obtained by in vitro fusion of a myeloma (murine, human) cell line with normal spleen cells from a pre-immunized animal (mouse, rabbit) to a specific antigen (pure), against which the mAb is intended. The cell fusion is induced by physical (electroporing) or chemical (polyethylene glycol) agents. The successfully fused hybrid cells (hybridomas)

gain from the myeloma the capability of perpetual proliferation and antibody production, while obtaining from the animal sensitized B lymphocytes the mono specificity against which the raised mAb is directed. Hybridoma cells must be subsequently selected and grown into specific media (HAT), which allows their preferential expansion, while both the original partners are progressively unable to survive. The HAT selectivity against myeloma cells is based on their defect in HGPRT, an enzyme necessary for nucleic acid salvage. This medium, in fact, inhibits the ex novo synthesis of nucleic acids, thus blocking the duplication and growth of myeloma cells. On the other hand, normal murine cells are not able of perpetual duplication in vitro (only cancer cells are immortal) and therefore, hybridoma cells are the most survivors at the end of selection with both saved properties (immortality and mono-specific antibody production). However, these cell mixtures need to be further selected in proper growing media by limiting dilution procedures in order to individuate at single cell level the best mAb producers. Passed in suitable media, in the presence of cell feeder layers (such as fibroblasts) when occurring, single cell hybridomas can be grown in unlimited and large quantities for commercial use. They can also be grown in vivo by injecting in the peritoneal cavity of suitable animals (mice) from where a highly enriched ascitic fluid can be obtained for further purification, although this procedure is basically limited to experimental laboratory use.

The following mAb purification procedures are crucial to obtain a monospecific, highly purified mAb for laboratory or clinical use. These steps are also critical to eliminate contaminants and remnants of the previous induction, selection, and initial growth of productive hybridomas. Among those coming from in vitro culturing there are cell debris, proteins, nucleic acids, lipids, anions, media components such as growth factors, hormones, transferrin, but also other components derived or produced by the same mAb secreting clones, such as cytokines, and microbial contaminants. All these components must be also eliminated for their potential role as additional producers of side effects and allergic reactions. Centrifugation, ultrafiltration, dialysis, exchange chromatography, size exclusion chromatography, protein A/G affinity chromatography, and affinity purification and elution—using the same antigen to provide exquisite specificity of the final mAb—are different or alternative steps according to type and final quality of the product needed. Usually, a final protein precipitation of the purified mAb is performed with low concentration of sodium or ammonium sulphate, followed by salt elution by dialysis. The final purity is checked by chromatography, electrophoresis and capillary electrophoresis, before adjusting to proper concentrations required by single and addition of adequate excipients.

As already known for vaccines and other drugs, the presence of final production remnants, contaminants and excipients must be taken unto high consideration for the evaluation of AEs following in vivo diagnostics and therapy with mAbs, together with those that may be induced by the structure or by the action of the employed mAbs.

As previously mentioned, the original murine-derived mAbs were highly immunogenic, and therefore the industrial focus moved to chimeric, humanized, and fully humanized mAbs. However, not all monoclonal antibodies designed for human administration need to be humanized, particularly those designed for short-term inoculation (diagnostics, short therapy). In fact some of non-humanized mAbs are still in use for human interventions.

4.2 Chimeric and Humanized mAb

The humanization process of mAbs may alternatively consist in two procedures: the assembly of chimeric molecules or the production of non-human antibodies, whose protein sequences are progressively made more similar to the antibodies produced naturally in humans.

Typical chimeric antibodies have a murine Fab fragment spliced into a human Fc. However, they are still easily recognized as "foreign" structures from the human host. In order to further humanize the product, recombinant DNA technologies were employed to raise hybrid antibody molecules having the majority of the stem structure (Fc fragment and the proximal part of Fab segment sequences) of the human type, and the antigen-recognizing domain (distal Fab segment sequence) of murine type.

4.3 Fully Humanized mAb

Recent technological developments have made the production in vitro of fully humanized mAbs possible, in the attempt of further reducing side effects and major adverse reactions observed with either chimeric or partially humanized products. These mAbs are currently produced by phage-display technology to provide almost humanized (or human-like) antibodies in transgenic mice. The former need a screening process to individuate specific mAbs against normal or "pathological" antigens to be targeted. Alternatively, a free radical enzymatic labeling is used to individuate the "best fit" mAb.

Recombinant humanization procedures may render these molecules progressively human-like even up to the distal sequences of the Fab segment, leaving to the murine counterpart only some of the Complementarity Determining Regions (CDR) responsible for the highly specific recognition of the Antigen Determinant.

An alternative and additional approach to reduce AEs among mAbs and fusion proteins was the truncation of the Fc fragment, when the therapeutic effect was not critically linked to the expression of CDC (cell cytotoxicity)and/or ADCC (antibody-mediated cell cytotoxicity). In this case the shortage of the half-life of the remaining Fab portion is compensated by pegylation of the remaining Fab fragment, leading to products with reasonable durability and a

lower AEs potentiality. For example, certolizumab is a pegylated recombinant humanized Fab fragment (91 kD) composed of a single light and heavy chain derived from a murine IgG2a antibody, directed against soluble and transmembrane TNF-α. The absence of the Fc fragment avoided CDC and ADCC-dependent reactions. Truncation can be even more aggressive, involving the hinge region and part of the Fab fragment. Abciximab is a smaller fragment (47.6 Daltons) consisting in a disulfide-linked dimer of an Fd heavy chain fragment and an intact light chain.

Nonetheless, the protein sequences of these antibodies still remain distinct from homologous antibodies naturally occurring in humans, and are therefore potentially immunogenic when administered to patients. It must be stressed that even in the case of acquisition of full homology, these molecules will still be recognized as non-self structures by any recipient's immune system (except for monozygotic twins), due to individual genetically determined differences in their sequence. Side effects derived by the functional action of the mAb on the target cannot be avoided as well [2–4].

In conclusion, from the rodent (mouse, rat) initial mAbs to the roughly half chimeric mouse-human mAb, to the most humanized products, AEs have been remarkably reduced but cannot be eliminated even with the latest sophisticated products. Perhaps, best results were obtained in the more prolonged half-life of the fully humanized mAbs after injection, rather than avoiding most serious and frequent adverse reactions [3–5].

Table 4.1 International Nonproprietary Naming (INN 2009)

Monoclonal Antibodies					
Prefix Variable	**Substem A** Target		**Substem B** Source		**Suffix** Constant
random -	b(a) -	bacterial	a	rat	-mab
	c(i) -	cardiovascular	axo	rat/mouse	
	f(u) -	fungal	e	hamster	
	k(i) -	interleukin	i	primate	
	l(i) -	immunomodulating	o	mouse	
	n(e)-	neural	u	human	
	s(o) -	bone	xi	chimeric	
	tox(a) -	toxin	xizu	chimeric/humanized	
	t(u) -	tumour	zu	humanized	
	v(i) -	viral	v(i)	viral	
Prefix: euphonious, distinctive name. Suffix: invariable for all mAbs Substem A: the target may be molecule, cell, organ. Substem B: source species of mAb					

4.4 Nomenclature

The International Nonproprietary Names (INN) Programme [6, 7] (doc.09.251, 2009) has recently updated the nomenclature of monoclonal antibodies (Table 4.1). The procedure adopted by WHO helps in the recognition of a few characteristics of each mAb. In general, suffixes are used to identify a class of medicines; all monoclonal antibody pharmaceuticals end with the suffix -mab. However, different preceding affixes are used depending on the structure and function of the medicine. These are officially called "substems" and sometimes erroneously "infixes", but are actually suffixes. Moreover, the nomenclature has evolved, by introducing new variants and shortages in the use of suffixes (now identified as old and new). An example is reported below.

Example			
Efa	li	zu	mab
distinctive	immunomodulating	humanized	monoclonal

This nomenclature is also used for fragments of monoclonal antibodies, such as antigen binding fragments and single-chain variable fragments.

Before INN document 09.251, other substems A (Targets) were used, which are reported here in order to help in recognizing older denominations of mAb. In particular, some were longer or slightly different synonyms of the present official stems, such as ba(c) = b(a); ci(r) = c(i); fu(ng) = f(u); ki(n) = k(i); li(m) = l(i); ne(u)(r) = n(e); toxa = tox(a); o(s) = s(o); vi® = v(i). Other substems referred to specific targeted tumors, such as co(l) = colon; go(t) = testicular; go(v) = ovarian; ma(r) = mammary; me(l) = melanoma; pr(o) = prostate; tu(m) = miscellaneous. All these substems will not be used in future.

4.5 Basic Structure, Targets and Mechanism of Action

The majority of the examined medicinal monoclonals pertain to the IgG1 isotype (26/35). Among them there are 4 murine, 5 chimeric, 12 humanized, and 5 fully human products. Seven monoclonals are IgG2 (4 murine, 1 humanized, and 2 fully human) and 2 are humanized IgG4. As expected, the most recent ones are fully human mAbs, while the older are murine or chimeric.

The major difference between IgG isotypes for the purpose of mAb structuring relates to their capacity of complement activation (high in IgG1, low in IgG2, virtually absent in IgG4) via the C1q binding on the Fc portion. These efforts were aimed at the reduction of complement dependent immune reactions, when not necessary for the action of the structured mAb. For example, gemtuzumab was

structured on the IgG4 isotype to transfer the toxic ozogamicin into myeloid leukemic cells, in the absence of potentially disturbing Fc-mediated immune reactions. Similarly, when these aggressive mechanisms are not demanded, or are considered potentially dangerous, the choice of IgG2 isotype also offers a relatively inactive structure for Fc-mediated effector functions.

The choice of IgG1 is basically determined by two known properties of this isotype: the activation of the classical pathway of complement exerting effective CDC after binding the first factor of the complement cascade (C1q); the capacity of induction of ADCC after binding at cell surface specific epytopes (by the variable regions), and to effector cells (by the Fc fragment to its receptors). Stability in serum, glycosylation differences, and induced changes are considered additional basic conditions to develop more efficient and less immunogenic products.

The subsequent manipulation of these isotypes also showed some peculiarities. For example, humanization of the variable regions appeared to be more effective in reducing overall immunogenicity, although the overlapping induction of human anti-chimeric (HACA) and human anti-human (HAHA) anti-mAbs indicated that this complex procedure seems to be less effective than expected and recognition of the Fc antigenicity could still make the difference. Moreover, in some circumstances the humanization process seemed to 10-fold reduce the affinity for the target, compared to the chimeric structure, and to increase the half-life of about 5 times, such as for daclizumab and basiliximab [8–10].

Table 4.2 reports essential information on mAb targets, their typology and expression at cellular level, together with the INN names of all approved products, including some of the discontinued ones, because of their relevance to the scope of this volume.

The development of monoclonal antibody therapy is based on their high binding specificity for epitopes expressed on selected cell targets, either for determining their destruction, as for modulating their function by interfering with specific receptors. As for the neoplastic targets, various strategies are employed: the recruitment of natural effectors such as complement, natural killer (NK) cells, and macrophages to destroy the target; the administration of antibodies inducing apoptosis; the use of mAbs to deliver exogenous toxic agents (immunotoxins, drug-antibody conjugates, enzyme-antibody conjugates capable of activating drugs, bi-specific antibodies recruiting exogenous agents, and radioisotope-antibody conjugates).

In addition, the modulation of humoral and cellular immune mechanisms can be induced by monoclonal antibodies in oncology and in autoimmune and inflammatory diseases with different and opposite scopes, namely by inducing immunostimulation or immunosuppression. These effects can be achieved by blocking the binding of a natural ligand (antagonist) or by delivering a surrogate signal to the cell (agonist). The consequences of this signaling depend on the cell type involved.

Table 4.2 Targets typology and expression of monoclonal biomedicines

Target	Typology	Main Expression	Monoclonal
CD20	Bp35	pre-B, B	Ibritumomab
			Ofatumumab
			Rituximab
			Tositumomab
TNF-α	s- and tm-cytokine	Soluble and on T, M/Mθ, NK	Adalimumab
			Certolizumab
			Infliximab
			Golimumab
EGFR	tm class I RTK (Her family)	Epithelia	Cetuximab
			Nimotuzumab
			Panitumumab
CD25	tm IL-2R-α subunit	aT, aB, THY, MYpr, ODC	Basiliximab
			Daclizumab
EpCAM	tm-adhesion glycoprotein	Epithelia[b]	Catumaxomab
			Edrecolomab
HER-2 (CD340)	tm class I RTK (Her family)	Epithelia	Pertuzumab
			Trastuzumab
VEGF	growth factor (RTK) gp	Ep, E, R, F, Mθ, NEU	Bevacizumab
			Ranibizumab
α-4β1, α-4β7	integrins (mainly VLA-4)	T, B, M, Mθ, Bas, E	Natalizumab
BLyS (BAFF)	cytokine (TNF family)	Soluble	Belimumab
CD3	T3 antigen TCR co-receptor	T	Muromonab
CD11a (LFA-1)	LFA-1R integrin	T, B, Mθ, N	Efalizumab
CD30[a]	gp (TNFR family)	Th2	Brentuximab
CD33	tm-adhesion gp	MY, M, ERpr,	Gemtuzumab
CD41	GpIIb,IIIa, integrin-α2b	Thrombocytes	Abciximab
CD52	non-modulating gp	T,B, M/Mθ, NK(50 %)	Alemtuzumab
CTLA-4 (CD152)	Ig superfamily tm-receptor	aT	Ipilimumab
IL-1β	interleukin IL-1β	Soluble	Canakinumab
IL6R (CD126/130)	s- and tm-cytokine receptor	Soluble and on T, B, G, F, Mg	Tocilizumab
IL-12/IL-23	p40 subunit	Soluble	Ustekinumab
RANKL	RANKL (TNF family)	OB, OC, BMSC, other[c]	Denosumab

(continued)

Table 4.2 (continued)

Target	Typology	Main Expression	Monoclonal
C5	Complement factor 5	Soluble	Eculizumab
IgE	free IgE (Cε3 domain)	Soluble	Omalizumab
RSV	A antigen of F viral gp	Respiratory Syncytial Virus	Palivizumab

A: astrocytes; aT,aB: activated stages of respective cell lineage; Bas: basophils/mast cells; BMSC: bone marrow stromal cells; Bp35: B lymphocyte-restricted differentiation antigen; CD25: IL-2Rα; CTLA-4: cytotoxic T cell antigen-4; DC: dendritic cells; E/pE: endothelia cells/precursors; ERpr: erythroid precursors; F: fibroblasts; G: granulocytes; gp: glycoprotein; K: keratinocytoes; LC: Langerhans cells; LFA-1:lymphocyte function-associated antigen-1; M: monocytes; Mθ: macrophages; Mg: microglia; MY/MYpr: myeloid cells/precursors; N: neutrophils; NEU: neurons; NK: natural killer cells; OB/OC osteoblasts/osteoclasts; ODC: oligodendrocytes; R:renal cells; RANKL: receptor activator of nuclear factor k ligand; RTK: receptor tyrosine kinase; SC: synovial cells; TCR T cell receptor; THY: thymocytes; Th2: T helper 2 subset lymphocytes; tm: transmembrane; VLA-4: very late a-4 integrin

[a] Ligand (CD30L) on aT, B, G, epithelial thymus; [b] also in esthesioneuroblastoma; [c] prostate, mammary epithelia, T, DC, LC, B cells

See also list of acrornyms

However, the same actions can determine the insurgence of unwanted reactions, which can affect the therapeutic action causing discontinuation and produce serious consequences for the patient. Some of these expressions are most frequently mild and localized, but may become systemic and life threatening, as described in the next sections of this volume.

Frequently, AEs induced by these biomedicines show a wide variability and unpredictability. Most events show etiopathogenetic expressions related to the mechanism of action of each mAb, or to a specific drug class of biomedicines. The inter-subject variability is also rather consistent, possibly reflecting specific conditions, such as the tumor burden rapidly destroyed by highly efficient monoclonals, or the non-linear pharmacokinetics often expressed by these agents. In fact, being proteins, their clearance is mostly ruled by phagocytic/histiocytic brake down, more than by hepatic enzymatic pathways or renal filtration, thus showing high variability in relation to the state of activation and potential drug-related impairment of the phagocytic compartment. Similarly, dosage variability and accumulation depend from the plasma concentration of soluble targets of monoclonals, that can be rapidly modified by massive cell killing, and/or by rapid release of bioactive molecules (e.g. cytokines) causing relevant adverse reactions, and substantial waving in individual response to therapy. Actually, these aspects may be considered a peculiarity of the monoclonal drug class.

Finally, an additional peculiarity in AEs expression with these agents is related to the rapid and frequent recovery after discontinuation of therapy, and even to the induction of rebound states caused by the removal of agonistic/antagonistic receptor blocking, soon after therapy suspension.

4.6 Therapeutic Indications

Monoclonal antibodies for human therapy are mainly employed in oncology, and in autoimmune and inflammatory diseases. Cancer and arthritis account approximately for 75 % of the global production. However, the areas of application are rapidly expanding, mainly towards chronic non-neoplastic conditions.

In the present analysis, 17 monoclonal antibodies with oncological indications (Table 4.3), 9 indicated for autoimmune and inflammatory diseases (Table 4.4), and 10 with other indications (Table 4.5), including one used also to treat bone metastases, have been evaluated. Most of them are approved in US and/or in EU. However, a few products subsequently withdrawn, or discontinued, or not officially approved in these Regions have been also included, when considered of particular interest for the understanding of safety evolving in the whole drug class.

The major indications in oncology include chronic leukemia (alemtuzumab, ofatumumab, rituximab), lymphoma (brentuximab, ibritumomab, ofatumumab, rituximab, tositumomab), gastro-intestinal epithelial tumors (bevacizumab, cetuximab, nimotuzumab, panitumumab), squamous head and neck carcinoma (cetuximab, nimotuzumab), renal cell carcinoma (bevacizumab, cetuximab, panitumumab), non-squamous lung carcinoma (bevacizumab, cetuximab), breast carcinoma (bevacizumab, trastuzumab), CNS blastomas (bevacizumab, nimotuzumab), melanoma (ipilimumab), and bone metastases from solid tumors (denosumab).

Rheumatic diseases are the predominant targets of the second large class of monoclonals, and in particular rheumatoid arthritis (adalimumab, canakinumab, golimumab, infliximab, tocilizumab) and psoriasis/psoriasic arthritis (efalizumab, golimumab, infliximab, ustekinumab).

Three monoclonals are indicated for the control of graft rejection (basiliximab, daclizumab, muromonab), although two of them have been recently discontinued.

Finally, individual products are indicated for cardiovascular disorders (abciximab), osteoporosis (denosumab), paroxysmal nocturnal hemoglobinuria (eculizumab), RSV infection (palivizumab and its modified, albeit discontinued formulation motavizumab), asthma (omalizumab), and adult macular degeneration (ranibizumab).

Therefore, while in some areas the accumulated safety experience is based on a number of products targeting the same disease, in other pathologies a single monoclonal antibody is currently available.

As expected, off-label treatments include a much wider spectrum of tumors, autoimmune diseases, inflammatory disorders, cutaneous pathologies of unknown etiology, etc.

The present volume in the next sections reports a more detailed analysis of the mentioned monoclonal antibodies experienced in human therapy, including some discontinued or withdrawn products, because of their relevance with respect to the expression of adverse drug reactions. Primary attention will be dedicated to adverse events expressed during officially recognized therapy indications.

Table 4.3 Monoclonals and monoclonal-associated biomedicines in oncology

INN	Trade name Company	Target/ Isotype	Indications FDA and/or EMEA	Approval[a] FDA/EMEA
Alemtuzumab	(Mab) Campath Genzyme; Bayer	CD52/IgG1 k	B-CLL	
Bevacizumab	Avastin Roche	VEGF/IgG1 k	CRC, NSCLC, BC, RCC, GBM, EOC, FTC, PPC	2004
Brentuximab-vedotin	Adcetris Seattle Genetics	CD30/IgG1 k	HL, sALCL, cTCL	2011/2012
Catumaxomab (bispecific trifunctional hybrid)	Removab Fresenius	EpCAM,CD3/ IgG2a, 2b	Malignant ascites, EOC, GC	OD 2009/OD 2004 2009 EMEA
Cetuximab	Erbitux BMS Ely-Lilly; Merck	EGFR/IgG1k	CRC, SCCHN, NSCLC	2004
Denosumab	Prolia, Xgeva Amgen; GSK	RANKL/IgG2	Bone metastasis (from solid tumors)	2010/2011
Edrecolomab	Panorex GSK	EpCAM/ IgG2a	CRC	NA (1995, Germany)
Gemtuzumab-ozogamicin[b]	Mylotarg Pfizer Wyeth	CD33/IgG4 k	AML	2000/NA
Ibritumomab-tiuxetan-Y^{90}	Zevalin Biogen	CD20/IgG1 k	NHL	2002
Ipilimumab	Yervoy BMS	CTLA-4/ IgG1 k	Melanoma	2011
Nimotuzumab	Theracim,Theraloc CIM, Innogene-Kalb, YM Biosc., Bicon	EGFR (3A)/ IgG1 k	SCCHN, GBM, HNC, NSCLC, BC Pancreatic and Esophageal cancer	OD/OD 2004 2008-2010 (in other countries)
Ofatumumab	Arzerra GSK	CD20/IgG1 k	B-CLL	2009
Panitumumab	Vectibix Amgen	EGFR/IgG2 k	CRC	2006
Pertuzumab	Perjeta Genentech	HER2/IgG1 k	BC	2012
Rituximab	Rituxan Genentech Biogen Idec; Roche; Zenoaq	CD20/IgG1 k	NHL, B-CLL, RA, DLBCL, WG, MPA, AAV	1997
Tositumomab-I^{131}	Bexxar Corixa, GSK	CD20/IgG2a λ	NHL	2003
Trastuzumab	Herceptin Roche	HER2/IgG1 k	BC, GC, GE	1998

[a] Initial approval date. Some of the reported indications approved at a later time. [b] Withdrawn from US, 2010. *OD* orphan drug, *NA* not approved
For targets and therapeutic indications see text and list of acronyms

Table 4.4 Monoclonal antibodies in autoimmune and inflammatory diseases

INN	Trade name Company	Target/Isotype	Indications FDA and/or EMEA	Approval[a] FDA/ EMEA
Adalimumab	Humira Abbott	TNF/IgG1 k	RA, PsA, AS, CD, Ps, JIA, VC	2002/2003
Belimumab	Benlysta HGS, GSK	BLyS/IgG1λ	SLE	2011
Canakinumab	Ilaris Novartis	IL-1β/IgG1 k	CAPS	2009
Certolizumab-pegol	Cimzia UCB	TNF/Fab (IgG2a)	RA, CD[a]	2008/2009
Efalizumab[b]	Raptiva Genentech	CD11/IgG1 k	Ps	2003/2004
Golimumab^	Simponi Merck	TNF/IgG1 k	RA, PsA, AS	2009
Infliximab	Remicade J & J, Merck	TNF/IgG1 k	CD, UC, AS, RA, Ps, PsA	1998/1999
Tocilizumab	(Ro)Actemra Roche	IL-6R/IgG1 k	RA,SIJA, PJIA	2010/2009
Ustekinumab	Stelara Janssen-Cilag	IL-12, IL-23/ IgG1λ	Ps	2009

[a] Initial approval date. Some of the reported indications approved at a later time
[b] Withdrawn in 2009 from US and EU markets; ^: status to be determined in US; not approved in EU
For targets and therapeutic indications acronyms see text and list of acronyms.

Table 4.5 Monoclonal antibodies with other indications

INN	Trade name Company	Target/Isotype	Indications FDA and/or EMEA	Approval[a] FDA/ EMEA
Abciximab	Reopro Centocor, Lilly	CD41/Fab (IgG1 k)	Cardio-vascular	1993/1994
Basiliximab	Simulect Novartis	IL-2R/IgG1 k	Rejection	1998
Daclizumab[b]	Zenapax Roche	IL-2R/IgG1 k	Rejection	1997/1999
Denosumab	Prolia, Xgeva Amgen	RANK-L/ IgG2 k	PMO, SRE	2010
Eculizumab	Soliris Alexia	C5/IgG2,4 k	PNH	2007
Muromonab-CD3[c]	Orthoclone-OKT3 Janssen-Cilag, J & J	CD3/IgG2a	Rejection	1986
Natalizumab	Tisabri Biogen	α4-integrin/IgG4	MS	2004
Omalizumab	Xolair Novartis	IgE/IgG1 k	Allergic asthima	2003/2005
Palivizumab	Synagis Medimmune, Abbott	RSV/IgG1 k	RSV infections	1998/1999
Ranibizumab	Lucentis Genentech, Novartis	VEGF/Fab (IgG1 k)	AMD (wet)	2006/2007

[a] Initial approval date. Some of the reported indications approved at a later time
[b] Production discontinued in 2009; [c] discontinued in 2010; NA: not approved
For targets and therapeutic indications acronyms see text and list of acronyms.

However, additional data will be also evaluated in some off-label experiences when relevant for the scope of the study.

Overall, these data may contribute in evaluating the entire spectrum of the acknowledged AEs of this drug class, and offer a reference basis for new products in advanced development, while waiting for more consolidated data on their AEs potentialities in a wider range of possible indications [1].

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Abciximab

5

Abciximab (ReoPro®, Centocor, Eli Lilly) is an IgG1k Fab anti-GPIIb/IIIa chimeric monoclonal antibody (mAb) approved by FDA in 1993, by Health Canada in 1996, and by TGA (NZ) in 2005. In Europe the product was approved in some countries (UK, RoI), and under specific concertation procedures, but did not receive the final clearance from EMEA.

Abciximab was first approved on the basis of eight Phase I–II trials and one pivotal Phase III trial (EPIC), as an adjunct to prevent cardiac ischemic complications in patients undergoing percutaneous transluminal coronary angioplasty (PTCA). In 1997, on the basis of additional pivotal trials (EPILOG, CAPTURE) it was extended to patients with unstable angina resistant to conventional therapy in urgent need of PTCA. Other selective trials (EPISTENT, TARGET, GUSTO IV/V, etc.), along with other subgroup studies on repeated administrations and their effect on immunogenicity, allowed to better refine the dosage of associated heparin and indicated better strategies to reduce major AEs in a number of coronary artery disease (CAD) related interventions. Overall, up to 2010, 13 trials examined the effect of abciximab versus placebo and 10 trials evaluated abciximab versus other inhibitors of platelet aggregation or in association with CAD-related interventions [1–5].

5.1 Mechanism of Action

GPIIb/IIIa is a heterodimeric member of the integrin family of adhesion molecules consisting of two transmembrane Type I glycoproteins, integrin-α-2b (CD41) and integrin-β-3 (CD61). CD41 undergoes a post translational cleavage allowing the disulfide linkage of one light and one heavy chain, both joining CD61 to form the

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Ca-dependent complex GPIIb/IIIa (CD41/CD61) receptor for fibrinogen, von Willebrand factor, and other adhesins participating to the cross-link platelet aggregation process. CD41 is also expressed in early stages of hematopoiesis and on mature megakaryocytes.

Abciximab is a disulfide-linked dimer of the c7E3 IgG Fd heavy chain fragment and the intact Lk light chain of the chimeric human-murine 7E3, specifically binding to human GPIIb/IIIa. The murine Fc fragment was removed to minimize immunogenicity. Abciximab binding prevents the natural ligands from reaching the GPIIb/IIIa receptor, possibly through its steric modification, thus inhibiting the platelet aggregation process. The binding site appears to be located in the specificity-determining loop of the GPIIb/IIIa I-like domain.

Abciximab also binds to a few other cellular surface structures with similar affinity: vibronectin ($\alpha v\beta 3$) present on platelets and on leukocytes, vascular endothelia, and smooth muscle, thus inhibiting its pro-coagulant activity, cell activation, and proliferation; activated Mac-1 receptors on monocytes and neutrophils, thus inhibiting monocyte adhesion, Mac-1 expression, and the formation of circulating leukocyte-platelet complexes; $\alpha M\beta 2$ integrin expressed on leukocytes, thus reducing their infiltration and inflammation induction in areas of myocardial ischemia. However, the high-affinity binding to these receptors is reversible. Therefore, abciximab can be redistributed among platelets, endothelial cells, and leukocytes. [2, 6–8]. Furthermore, abciximab seems also to promote dissolution of newly formed platelet aggregates in vitro, implying that could help in dispersing coronary mural thrombi in vivo [9].

5.2 Adverse Events

Abciximab-related AEs are mainly concerned with *hemorrhagic complications* (spontaneous and induced by surgical interventions) associated with *hypotension* and *acute thrombocytopenia* (TCP), possibly as a consequence of immunogenicity of the chimeric mAb.

Bleeding is the most common and expected adverse event, given the specific biological effects of abciximab, the concomitant administration of heparin and aspirin, and the undergoing invasive coronary procedures. In fact, >70 % of bleeding occurs at femoral artery access site, while spontaneous bleeding develops more frequently in gastrointestinal and in genitourinary tracts, and less frequently in the retroperitoneal spaces. Major bleeding (according to TIMI Study Group criteria), mostly serious and sometimes fatal, ranged 11–14 % in initial trials with standard doses of heparin, which subsequently was reduced and adjusted to body weight with a significant reduction of severe hemorrhagic complications. Usually, mAb-related events occur within 36 h from treatment. Overall, the maximal incidence of major bleeding was 2–3-fold higher than with placebo in high-dose treatments (abciximab bolus plus infusion and standard dose of heparin). Adoption of low-dose heparin regimens drastically reduced both frequency and severity of

bleeding. A peculiar major complication regarded the intracranial hemorrhage (ICH), initially as high as 4 %, which was also lowered to 0.2–0.3 % after the heparin dosage adjustment, reaching levels of the placebo groups. Pulmonary alveolar hemorrhage was rarely observed [10]. Overall, the reduction of global bleeding was mainly related to heparin dosage reduction, but also to a better general surgical and clinical care.

Hypotension is the second serious and life threatening event, mostly coinciding with major bleeding (14–20 %). This event may be associated to bradycardia (4.5 %) and other cardiac dysfunctions usually at frequencies lower than 1 %.

Acute thrombocytopenia is the third relevant AE ranging 1–3 % that was detected in trials and in subsequent clinical experience. In a re-administration registry study, any degree of TCP reached 5 % (serious 2 %). A drastic decrease of platelet counts occurs in minutes or hours from initial treatment, causing bleeding in various sites, frequently moderate but also as severe (0.5–1 %) and life threatening. Usually, a gradual recovery starts in few hours, reaching platelet baseline values within 3–4 weeks. The sharp increase of TCP incidence after a second injection was 2–4-fold high compared to first injection, thus suggesting a possible implication of immune-mediated mechanisms. In particular, immediate TCP occurrence has been attributed to preexisting antibodies against the murine portion of abciximab, while episodes appearing after 5–10 days are thought to be due to newly formed anti-mAb antibodies.

Finally, in some studies, pseudo-TCP due to in vitro clumping of platelets in EDTA formulations was not associated with bleeding phenomena [1, 2].

Other frequent events are bradycardia, lumbalgia, cephalea, nausea, vomiting, pyrexia, and puncture site pain. Other serious and rare events are cardiac tamponade, pulmonary (mostly alveolar) hemorrhage, and ARDS.

5.3 Immunogenicity and Immune Thrombocytopenia

An interesting study was performed in a small number of CAD patients to assess the immunogenicity of abciximab (bolus + infusion) after one or more administrations [1].

Pharmacokinetics and pharmacodynamic effects were compared to immune response to mAb, bleeding, and induction of immune thrombocytopenia (ITCP). The presence of HACA/HAMA was ascertained after the first injection, and only negative patients who had been followed up for 12 weeks were reinjected at week 14. Therefore, only 29/41 patients received a second injection. Among concomitant medications, aspirin (but not heparin) was given in vicinity of both treatments. Table 5.1 summarizes the results. HACA and HAMA incidence and titers resulted increased after the second injection performed only in negative subjects (+1, HACA borderline). Most HACA peaks appeared earlier than HAMA in the same subjects. However, a few were positive only for HAMA and only some of them developed HACA titers afterwards. Positivity was present in some patients up to 15–18 months. In some HACA and/or HAMA positive patients, the immune

Table 5.1 HAMA/HACA positivity after reinjection of abciximab

Treatment	Patients		Antibody positivity			AEs
	N	%	Peak (w)	Titer	Duration (m)	Type-incidence
I Injection°	41	100	–	–	–	Bleeding[a] 20 % (2.25/P)
HACA	7	17	4–8	1:50–1:800	3–15	1 ITCP[b] 1 pseudo-ITCP
HAMA	10	24	12	2, Borderline	8–18	–
HACA/HAMA	5	12	–	–	2–24	1 Facial dermatitis
HACA → HAMA	5	100	12	–	–	–
HAMA → HACA	2	20	Early	–	9–18	–
II Injection	29	100	–	–	–	Bleeding[a] 31 % (1.22/P)
HACA	7	24	1–2	1:50–1:6400	3–15	1 ITCP, hematuria [c]
HAMA	9	31	–	1:20–1:10240	12–15	–
HACA/HAMA	9	–	–	–	–	Facial dermatitis relapse
HACA → HAMA	7	78	–	–	–	–
HAMA	2	–	–	Borderline	–	–
HAMA → HACA	1	–	–	–	–	–

°: 8 subjects (20 %) were HACA+ before treatment; 5 had a sharp titer decrease after Ist injection suggesting immune complex formation and elimination, without clinical signs. No allergic/anaphylactic reactions occurred

a: mostly mucosal lasting 5',moderate; no therapy

b: HACA at baseline before treatment. Possible immuno consumption-related ITCP

c: immune-mediated event. Increase (21 %) of neutralizing antibody titer anti-murine V region

reactivity was associated with the insurgence of AEs, being bleeding the most frequent event, which increased from 20 to 31 % with reinjection. Overall, HACA positivity developed in 5 subjects within 12 weeks (1 after 2 weeks), and 2 more subjects became positive after 4 and 6 months, respectively.

After the fourth injection, positive subjects reached 44 % (16/36). The HACA positive status was associated with an increased risk of TCP, but not to serious allergic reactions including anaphylaxis, nor to changes in clearance or pharmacodynamic alterations after repeated injections. Three cases of ITCP, one after each injection, and one case of pseudo-TCP (platelet swelling/drop in EDTA, but not in citrate preparations) were also observed, all possibly related to the presence of anti-mAb antibodies. In fact, the pseudo-TCP and one case of ITCP after the second injection were cleared as drug-related, while the third, showing soon after the first injection, was considered related to immune consumption (immunocomplex formation). Finally, the case of facial dermatitis, which was reactivated by the second injection, developed antibody titers only after reinjection.

A subsequent analysis of 9 ITCP patients after a second dose of abciximab showed that each of them had a strong IgG recognizing platelets coated with the same mAb [11]. Five of them had also IgM antibodies. However, similar but not identical IgG were present in 77/104 healthy subjects (74 %). In fact, only the

patient's anti-mAb antibodies were specific for the murine sequences of c7E3 molecule and were responsible for severe ITCP upon injection of abciximab, while those of healthy subjects seemed to recognize any human IgG Fab. The Authors could also exclude pseudo-TCP acting in their cohort, as well as their responsibility into in vivo bleeding phenomena.

In a larger analysis on 500 patients receiving a second therapeutic administration of abciximab, no cases of anaphylaxis, nor major bleeding or deaths were observed. TCP occurred in 4.6 % of cases and HACA occurred in 22/454 tested subjects (4.8 %) after the first injection, and in 82/432 (19 %) subjects after the second administration. These antibodies were not neutralizing [12].

All together, these data strongly support the responsibility of anti-mAb antibodies in determining ITCP and subsequent bleeding adverse events during treatment with abciximab, and the existence of an increased risk after reinjection. Therefore, these events are apparently not primarily related to the abciximab mechanism of action, which remains a facilitator of bleeding, but to the immune reactivity against the murine part of this mAb. However, when compared to the initial immunogenicity of full murine 7E3 Fab, where HAMA were produced from 15 up to 52 %, the chimeric product showed a much reduced immunogenicity, although still able to justify immune-mediated AEs such as ITCP.

5.4 Off-Label Experience

As expected, off-label use of abciximab is restricted to cardiovascular and vascular cerebral disorders. In particular, studies on cardiac and arterial thrombosis, LVEF deficiency, acute MI, and cerebral vasculature thromboembolism did not show new or unexpected AEs. However, off-label intracoronary administration in high-risk patients with drug-eluting stents showed an increase in late stent thrombosis, MI, and death. This is why FDA in 2006 raised an alert for late adverse outcomes [13]. In some instances intra-arterial abciximab administration has been performed to treat thromboembolism (www.off-label.com).

5.5 Postmarketing Surveillance

No relevant additional signals have been reported in the literature and in the postmarketing settings. In FAERS, among 2,564 reports most common events regarded cardiovascular and hematological disorders, and infections. The average AEs/patient ratio was 3.4.

Among 2,293 EUV reports, the most common events were cardiovascular disorders and death, with an average AEs/patient ratio of 1.8.

In both systems, immune-related AEs, such as allergy, hypersensitivity, anaphylaxis, and anaphylactic shock were present in 0.1–0.3 % of reports.

5.6 Remarks

Overall, the major AEs occurring during abciximab treatment are bleeding, hypotension, TCP, ITCP, and their complications (mainly ICH, stroke). These features are not exclusive of abciximab, but common to other GPIIb/IIIa antagonists [14].

Although there are still some concerns about the consistent immunogenicity of this chimeric mAb, no serious allergic events were encountered during studies, while a few spontaneous reports have signaled anaphylactic reactions.

In spite of the high number of HACA/HAMA (mostly IgG) positive subjects reported in the readministration studies, hypersensitivity reactions seemed to occur at a lower frequency.

No specific IgE response to abciximab has been so far reported.

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Adalimumab

6

Adalimumab (Humira®, Abbott) is a recombinant fully human IgG1/2 k anti-TNFα monoclonal antibody approved in 2002 by FDA and in 2003 by EMEA (simultaneous submission). During the following years, this monoclonal spread over 62 countries to be used in a wide series of immune-mediated inflammatory diseases (IMID). Initially indicated for the treatment of rheumatoid arthritis (RA), between 2005 and 2008 it was progressively extended to psoriasic arthritis (PsA, 2005), ankylosing spondylitis (AS, 2005), Crohn's disease (CD, 2007), plaque psoriasis (Ps, 2008), and juvenile (3–17 years old) idiopathic arthritis (JIA, 2008). During 2012, FDA extended the indication to ulcerative colitis (UC), and EMEA to UC, to moderate forms of CD and other inflammatory bowel diseases (IBD), and to adult patients with axial spondyloarthritis (SpA) with or without signs of AS. In 2013 EMEA approved the extension to pediatric CD, and to JIA patients 2–17 years old. Up to 2012, over 70 trials were performed for the official therapeutic indications, including pivotal trials submitted for drug approvals. Overall, most data come from RA studies (over 14,000 subjects), followed by the more recent ones on CD and Psoriasis (over 3,000 subjects each), and to pediatric forms of CD and JIA (about 200 each).

In particular, pivotal studies include: four major Phase III trials for RA on 2,070 patients (studies DE009 known as ARMADA, DE011, DE019, DE031); two controlled trials (M03-403/433 CLASSIC I; M04-691 GAIN) and one maintenance study (M02-404 CHARM) on 1,478 CD patients; two controlled trials (M06-826; M06-827) on 1,092 UC patients, and their extension open-label study (M10-223); two Phase III studies (M02-518, M02-570) on 315 PsA patients and one open-label extension (M02-537); one Phase II trial (M02-528) on 147 Ps patients and its extension (M02-529), one Phase III extension study (M03-658), one additional Phase III trial (M03-656 REVEAL) on 1,212 Ps patients, and one Phase III long-

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term study (M04-716 CHAMPION) on 271 patients; two controlled studies (M03-606, M03-607) on 397 AS patients; one controlled study (M10-791) on 192 axial SpA; one study (DE038) on 171 cases of polyarticular JIA. The extension to pediatric CD was based on Phase III study M06-806 on 192 patients 6 to 17 years of age, and on its extension open-label study M06-807. The pediatric JIA extension was based on one open-label study (M10-444) on 32 subjects 2 to 4 years of age.

This was the basis for depicting the safety profiles in each disease. Most studies adopted the SC route for the administration of adalimumab. Since approvals to the different IMID were released in the following years, some data may be also considered as off-labels, according to the stage of approval extensions. Nonetheless, off-label use of adalimumab is still consistent, but mainly remains in the area of chronic IMIDs. It is estimated that off-label use in this area covers about 10 % of treatments [1–8].

6.1 Mechanism of Action

The tumor necrosis factor (TNF) family is a group of 19 cytokines mainly involved in apoptosis, including TNFα and lymphotoxins (LTα, previously TNF β, and LT-β). Their structures are homotrimeric (the former) or heterotrimetric (the latter), and are recognized by specific receptors (TNF-R1; TNF-R2). TNFα (also identified as TNF, being the pivotal molecule of the group) is expressed at the cell surface, mainly on activated macrophages and T lymphocytes, and can be cleaved by a TNFα converting enzyme (TACE) in a soluble form, which is considered the mature expression of this cytokine. However, the transmembrane precursor (tmTNF, 26 kDa) acts also as a bipolar molecule that transmits signals both as a ligand and as a receptor in a cell-to-cell contact fashion, while the soluble form (sTNF, 17 kDa) acts also at distance by interacting with the same receptors. However, sTNF binds to TNFR1 with a 30-fold higher dissociation rate compared to TNFR2. Therefore, much of the sTNF linked to the TNRF2 is promptly released and possibly captured by TNFR1. After shedding, mediated by TACE, both receptors are capable of neutralizing TNF in solution, thus acting as potential natural TNF antagonists. This effect is controlled by TACE inhibitors via metallopeptidase-3 (MMP3). TNFR1 is ubiquitous (except for RBC) and constitutionally expressed, whereas TNFR2 is generally inducible and preferentially expressed on endothelial and hematopoietic cells. Macrophages, T and B cells, NK cells, neutrophils, endothelial cells, smooth muscle cells, osteoclasts, and fibroblasts produce TNF as a result of innate and adaptive immune responses induced by exogenous molecules from bacteria, viruses, but also by immune complexes, hypoxia, and trauma. However, the primary source of TNF in immuno-inflammatory processes is the monocyte/macrophage lineage. TNF release, in turn, stimulates the secretion of cytokines (IFNγ, IL-1, 6, 8, 17, G-CSF), chemokines (MCP-1), adhesion molecules (ICAM-1, E-selectin), and inflammatory proteins (MIP-1 and 2), acting also on leukocyte activation/mobility and on endothelial permeability. The production of TNF is regulated by feedback loops initiated by

TNF-induced factors. IL-1, IFNγ and IL-2 induce TNF production, while IL-10, prostaglandins and corticosteroids downregulate their production by inhibiting transcription of TNF mRNA. Therefore, TNF is a key pro-inflammatory cytokine with a central role in inflammatory processes. TNF plays a crucial role also in granuloma formation and maintenance.

In healthy humans, circulating TNF is hardly detectable. However, in patients with acute infections, septic shock, or chronic inflammatory and autoimmune diseases, TNF levels are rapidly and consistently increased, being detectable also in serum, stools and synovial fluid. TNFRs or TNF antagonists, can bind to tmTNF at cell surface. This binding induces reverse signaling, which in turn triggers cell activation, cytokine suppression, or apoptosis of the tmTNF-bearing cells. This peculiarity may be also responsible of some AEs induction.

Adalimumab is a recombinant fully human IgG1/2 k anti-TNFα monoclonal antibody. The high affinity binding to soluble and transmembrane forms of TNFα, not occurring when they are already bound by the receptors, inhibits their interaction with TNF-RI (p55) and TNF-RII (p75) specific receptors at cell surface, thus blocking TNFα-induced inflammatory and immune responses.

The assumption of a potential beneficial effect in IMIDs mostly came from the elevated level of TNFα detected in synovial fluids of RA, JIA, PsA, AS patients and in Ps plaques during active disease. In fact, two inflammatory cytokines, TNF and IL-1, are critical in the progression of inflammatory synovitis and articular matrix degradation. In vitro, adalimumab has lytic effects on cells expressing TNFα, in the presence of complement (CDC). It also modulates the expression of some adhesion molecules (ELAM-1, ICAM-1) responsible for leukocyte migration. Therefore, it exerts anti-inflammatory and anti-proliferative activities. Interestingly, some differences in clinical efficacy among TNFα antagonists have suggested that mechanisms of action of these biologicals may be more complex and dissimilar, possibly playing different roles in different IMIDs [9–11]. In addition to soluble TNFα blockade and CDC, adalimumab shows also CDC, ADCC, apoptotic and cytokine suppression activity mediated by transmembrane TNFα blockade. The capacity of forming immune-complexes (drug-TNFα, anti-drug Abs) varies among different anti-TNF biomedicines, while the involvement of other targets (such as LTα for etanercept) may explain the efficacy in case of patients non-responding to another antagonist [12]. The variety of mechanisms of action pertaining to different anti-TNF antagonists (etanercept, infliximab and adalimumab) seem also relevant in raising peculiar AEs, such as granulomatous infections (TB, opportunistic) appearing with different frequencies after anti-TNF therapy [13].

Finally, the unexpected increase of cutaneous Langerhans cells in the healing psoriasic lesions during treatment evidenced a possible anti-inflammatory role of these cells, and an additional mechanism of action for adalimumab. Interestingly, the cell restoration density was already evident within 7 days of adalimumab treatment, when clinical response was not yet evident [14].

Therefore, as for AEs induction, it can be expected that the complexity of drug action on crucial inflammatory pathways and the pathogenetic variability within the IMID group will produce a wide panel of adverse events, different both in typology and severity.

6.2 Immunogenicity

Although structured as a fully human antibody, adalimumab exerts an immunogenic reaction, as expressed by the induction of HAHA. A considerable variability was observed even among healthy volunteers (0–33 %). In RA studies, about 5 % of the 1,062 tested patients undergoing various therapeutic regimens showed neutralizing antibodies, albeit at low titer. However, when associated to MTX their presence lowered to about 1 %, while in monotherapy raised to 12 %. Similar levels were found in AS patients, while increased levels (16 %) were found in JIA treated subjects, with similar lower levels (6 %) in the presence of MTX and higher levels (26 %) in monotherapy. PsA patients had about 12 % antibodies when in monotherapy, but their suppression in the presence of MTX was less pronounced (7 %) than in RA patients (1 %). CD and UC patients showed anti-adalimumab antibodies in about 5 % of cases.

The detection of such antibodies is difficult during therapy, especially when high levels of adalimumab are still in circulation. For example, UC patients, showing and average positivity of 5 %, and Ps patients showing 8 % positivity for the same anti-drug antibodies, had 21 % positivity in a portion of treated subjects showing low levels of the biomedicine in circulation at the time of testing. In the more recent studies on pediatric CD, HAHA were present in 3.3 % of patients (182 screened), while in pediatric JIA they were detected in one patient of the 15 tested subjects (6.7 %). These last data were similar to those found in the previous DE038 study (11 % HAHA on 171 tested subjects). As expected, rates of positivity were lower in patients receiving concomitant MTX (4.7 % vs. 17.4 % without MTX).

Although no apparent correlation to the general AE profile was found, their role in specific events, mainly in the long-term administrations, is still to be elucidated [1–8, 15].

6.3 Adverse Events

Due to the wide spectrum of diseases in which adalimumab is employed with different therapeutic regimens it is difficult to depict a general safety profile for the whole group. Since the global trial's experience spans over 12 years, AEs frequencies are usually reported as percent of encountered events and as rates of AEs/patient/year (AEs/PY) or standardized incidence rates (SIR). Because of the complexity and data variability within the IMID group, most data refer to serious adverse events/patient/year (SAEs/PY). Due to the wide diffusion of anti-TNFα

therapy in IMIDs, AEs data have been also collected in a series of Registers in UK (BSRBR), Germany (RABBIT), Spain (BIOBADASER), and Sweden (ARTIS). These Registers give the possibility of comparing different TNFα antagonists with the adverse events, mostly when serious. Therefore, a great variability of data is encountered in this area and the general profile should be considered with caution.

A warning about cases of *tuberculosis* (TB) was issued from the beginning. However, by the end of 2008 FDA requested a Black Box Warning (BBW) on *serious infections*, and *malignancies* were added since 2009. Additional warnings include *allergy, including anaphylaxis, HBV reactivation, demyelinating diseases (exacerbation or new), cytopenias, heart failure, and lupus-like syndrome (LLS).*

The most common typologies are URTI, injection site reactions, cephalea and rash. FLS was observed in 7 % of cases [1–7].

Serious infections were the most frequent events (SIEs), with greatest rates described in RA and CD. The overall rates in adult RA, PsA, AS, CD, UC, and Ps were 4.6 per 100 P/Y (on 7,304 exposed patients) versus 3.6 per 100 P/Y of controls (4,232 patients). Both conventional and opportunistic infections were observed from bacterial, viral, fungal, and parasitic agents. Frequently, they were rather disseminated than localized, and sometimes fatal. Histoplasmosis resulted fatal in about 20 % of cases on 240 reports. Initial SIEs global rates were estimated around 1 %. In a recent review of over 23,000 IMID patients from 71 trials with up to 12 years exposure to adalimumab, overall rates ranged from 0.2 to 0.7 SAEs/100 PY, with consistent differences among IMIDs [14]. As for their typology, the most common events included *pneumonia, appendicitis, UTI, gastroenteritis, cellulitis, and herpes zoster infections*. Gastrointestinal tract abscesses were observed at high rate only in CD. Global therapy discontinuation due to infections ranged from 18 to 32 %, and was mostly caused by pneumonia (7 %), bacterial arthritis (3 %), and cellulitis (3 %).

TB infections, either as reactivation or new episodes, were of special concern after the very early clinical observations. However, after the introduction of mandatory tuberculin test as pre-enrolling screening, it showed a progressive decrease of incidence from initial 1.5/100 PY to 0.2–0.3/100 PY.

Opportunistic infections, (<01/100PYs) excluding TB and oral candidiasis, were rather rare and mostly encountered in RA patients, but not in AS, PsA, and PS trials.

Overall, data coming from observational national registries, including the outcomes of different anti-TNFα therapies, report SIEs rates from 3.8 to 6.4/100PYs, which are higher than data on adalimumab and other drugs coming from trials and clinical review studies. This is not surprising, since there are differences in data collection methodologies, and also due to the fact that registries include data from unselected patients treated with heterogeneous regimens, that cannot be compared to data from selected cohorts of patients enrolled in clinical trials.

When assessing *malignancies* rating, comparisons are usually performed with general population or specific diseased populations, rather than with inside controls. This mainly happens when considering rare events in long-term observation, where placebo controls are no longer available.

Furthermore, population background levels are provided only in a few sites on earth, and usually most of them are US-based or related to partially matching populations. However, the fact that many trials are already multicenter and globally widespread introduces further bias in the analysis. Among other influential parameters, race, sex, and age are usually relevant. Therefore, these comparisons, when intended to evaluate malignancies as drug-induced SAEs, tend to be confounded by the high background levels of neoplasms, especially when they refer to specific pathologies with a significant rate of oncogenic potential. For these reasons, evaluation of malignancies induced or aggravated by biomedicines is difficult.

This is the case of some IMIDs (e.g., RA), especially in young patients affected by specific tumors such as lymphomas [1–6, 14]. Nonetheless, starting from initial trials, lymphomas were 2–3 fold higher than in general population. However, patients in this group, mainly those with highly active RA, are known to have a higher risk of lymphoma and acute/chronic leukemia, the latter being two fold higher than background levels of general population.

Usually, global rates of malignancies tend to exclude the lymphoma/leukemia group, melanomas, and non-melanoma skin cancers (NMSC), which are all considered in separate categories. Overall, rates of *malignancies across all indications* detected in trials with adalimumab were 0.7/100 PY, except for *lymphoma* (0.1/100 PY) and *NMSC* (0.2/100 PY). The risk of *melanoma* (<0.1/100 PY) appeared higher among Ps patients (0.2/100 PY), while *NMSC* risk seemed more frequent (0.3/100 PY) in AS treated patients.

Noteworthy, malignancies were not observed in JIA, and no *hepato-splenic T-cell lymphoma* (HSTCL) cases were reported in trials with adalimumab, although some were reported in the FAERS postmarketing setting.

On this basis, the risk of malignancies after long-term treatment with adalimumab can be considered low but real, and higher than backgrounds at least for lymphoma in RA, for NMSC in RA, Ps, and CD, and for melanoma in Ps patients. It is difficult to obtain clear-cut data on this matter, for the mentioned reasons, and due to interferences with other therapies and accidental exposures. Therefore, according to the current body of knowledge, the risk for the insurgence of lymphomas, leukemia, and other malignancies cannot be excluded in patients treated with a TNF-antagonist, mostly in long-lasting active forms of IMID demanding prolonged exposures to therapy, especially in children and adolescents [16].

As for other relevant SAEs, *demyelinating disorders, lupus-like syndrome (LLS), cardiac heart failure (CHF),* and *systemic skin reactions* are to be considered. Overall, rates in trials have been retrospectively estimated as 0.1–0.2 SAEs/100 PY. Demyelinating disorders, such as optic neuritis, GBS, and PML have been occasionally encountered in trials and in postmarketing reports. In particular, no cases of confirmed PML were signaled after adalimumab treatments in trials, while there were spontaneous reports from various sources (47/126,829–0.04 %).

Occasionally observed *LLS* and *CHF* were both more frequent in AS (0.1/100 PY), while only CHF was higher in RA patients (0,2/100 PY). More recently, serious CHF was reported in Ps. Moreover, seven cases of skin reactions, including four cases of erythema multiforme (EM), two cases of Stevens-Johnson's

syndrome (SJS), and one case reporting both EM and SJS were observed. An additional case of EM was described in the literature. Most patients recovered after drug discontinuation. In two patients adalimumab was the only medication administered (FDA Drug Safety Newsletter Winter 2008).

Taken together, the safety data indicate RA as the pathology at higher risk of developing SAEs among IMIDs. It must be stressed, however, that most safety data (over 65 %) have been provided by RA studies and patients were observed for a longer period. Nonetheless, serious and opportunistic infections are prevalent in RA, as well as infections related to discontinuation of therapy. CHF rate is two fold higher than in other IMIDs. Similarly, the number of lymphomas was higher in RA, where all NHL occurred. Possibly the risk of malignancy in RA appears also at earlier times compared to other IMIDs, and has been related to prolonged active forms of the disease. However, the risk of lymphoma is higher than general population background, yet it is not high compared to RA population not treated with adalimumab. In the more recent studies on pediatric CD (M06-806, 192 subjects) about 53 % of patients during the 4 weeks induction period reported any AEs, which appeared to be dose dependent. Infections were observed in 14 % of cases and were more frequent as URTI. Two serious infections occurred, but no opportunistic/TB cases were observed. Injection site reactions were 11.5 %. Severe AEs were about 5 % including GI disorders, injection site reactions, and three cases of severe CD exacerbation. Overall drug-related events (mostly viral URTI, injection site reactions, and fatigue) were estimated around 20 %. During the 52-week maintenance regimen, drug related events increased and were mostly represented by CD worsening, which resulted to be more frequent in the low-dose treated group (72 events per 100 P/Y vs. 59 events with higher dose of adalimumab). However, infections were more frequent in the high-dose group (60 % vs. 49.5 %) and tended to be more severe. while allergic reactions were less frequent with low-dose treatment. No cases of TB, CHF, demyelinating disorders, LLS (1 case in M06-807 study), and malignancies emerged during maintenance treatment. Three cases of psoriatic worsening were also observed in the whole study.

Overall, no new signals emerged from these pediatric studies, and the overall profile was considered similar to the approved indications, including adult CD.

The experience in the active polyarticular JIA 2 to 4 years of age from the ongoing study M10-444 on 32 patients treated for 24 weeks, and interim data from subjects continuing up to week 60, were put in context with previous data from DE038 study on 171 JIA patients aged 4-17 years. Approximately 85 % of treated patients showed at least one TEAE, but were considered related to adalimumab in only 25 % of cases (inj. site reactions, ear infection, laryngitis, pneumonia, viral pharyngitis, URT congestion, pyrexia, cystitis, and rash). Four cases of severe AEs were considered not related to the study drug. URTI, nasopharyngitis, cough and pyrexia were more frequently encountered ($\geq$ 5 %) during the 24 weeks observation period, with a slight increase up to week 60. One patient showed JIA exacerbation, and one reported signs of RA at week 60. The overall safety profile appeared to be acceptable and similar to children of higher age (study DE038),

with a slight increase of serious infections (9 % vs. 6 % respectively). No new and/or unexpected signs were observed.

To ensure further information on both new pediatric indications, long-term observations, including a Registry for pediatric CD (Study P11-282) to be enrolling 500 patients, and safety surveillance on ongoing programs for CD and JIA have been activated on AEs of special interest.

6.4 Off-Label Experience

The off-label administration of adalimumab is wide and expanding, although essentially occurring in the area of IMID and particularly in dermatologic diseases. In a wide analysis on data collected from the Spanish Registry BIOBADASER, which collects on- and off-label treatments with biomedicines in rheumatic diseases, off-label administrations of TNFα antagonists were used in chronic arthritides (CA), such as spondyloarthritis, enteropathic arthritis, seronegative chronic polyarthritis, seronegative chronic oligoarthritis, Still's disease, juvenile uSpA, ReA, SAPHO syndrome, juvenile AS, and in chronic immune-mediated diseases (CIDs) including Behçet's disease, uveitis without rheumatic disease, vasculitis, SLE, PM/DM, sarcoidosis, relapsing polychondritis, systemic sclerosis and related connective tissue disorders. Overall, 11 % of filed patients received an off-label treatment. AEs occurrence and related discontinuations were higher mainly in the group of CIDs (0.6 AEs/patient), where AEs were almost two fold more frequent than in RA [17].

Adalimumab was sparely used in this series (13 % of off-label treatments), mostly within the CA group, where infliximab was the most employed in the whole off-label group. However, when separate analysis was performed excluding infliximab, the profile of AEs did not change. Overall, there were no new signals coming from off-label treatments and adalimumab was not the most aggressive biomedicine.

As for off-label use in dermatologic diseases, adalimumab has been used in anecdotal cases of pyoderma gangrenosum, hidradenitis suppurativa, sarcoidosis, vasculitis, multiple familial trichoepithelioma, reticulohistiocytosis subcorneal pustular dermatosis and the already mentioned Behçet's disease. These reports have not raised particular nor new concern on AEs [18].

Finally, a peculiar aspect of TNF inhibitors relates to the possibility of reactivation of latent infections. A recent review analysis identified 35 cases of HBsAg positivity prior to initiation of therapy, among which 6 cases were treated with adalimumab (17 %), 17 received infliximab and seven etanercept [19]. Interestingly, the rate of virus reactivation and the appearance of clinical signs were consistently different between treatments. In particular, major and serious signs of reactivation came from infliximab. No cases of clinically symptomatic HBV reactivation or hospitalization were reported when adalimumab or etanercept were used. The former had increased level of ALT in one case, and signs of HBV replication in another

case. Once again, these differences were related to different mechanisms of action of the three TNFα antagonists, among other possible diversities.

6.5 Postmarketing Surveillance

In the postmarketing, spontaneous reports infections are predominant. In the FAERS database (over 77,000 reports) infections rate is about 7 %. In particular, HBV reactivation has been repeatedly reported, and therefore subsequently included in official label warnings. Leukemia and HSTCL are reported as rare events.

Among over 21,000 (20,500 serious) reports in EUV database, about 46 % relates to infections and 17 % to malignancies. Melanoma, lymphoma, NMSC and leukemia were signaled with more frequency. Eleven cases of HSTCL were also reported (0.05 % of total reports). Among infections, the most common were pneumonia (924), TB (499), sepsis (451), cellulitis (240), HZV infections (199), disseminated TB (155), and septic shock (127).

6.6 Remarks

Most recent data analyses are in line with previous safety observations on adalimumab and parallel those of other TNF antagonists, with minor albeit interesting variations both in efficacy and AEs expression. Such differences could suggest more flexible therapeutic strategies for obtaining a better risk/benefit balance with these biomedicines.

Infections remain the most common and important adalimumab-related AEs.

The risk of malignancies after long-term treatment can be considered low, although higher than backgrounds at least for lymphoma in RA, for NMSC in RA, Ps, and CD, and for melanoma in Ps patients. It is difficult to obtain clear-cut data on this matter, as already mentioned, due to confounding factors both in the treated and background populations.

However, the existence of a real risk of increasing malignancies cannot be excluded in these patients, although their exposure to more than one potential oncogenic factors, especially in long-lasting active forms of IMID, and particularly in children and adolescents who are at higher risk of lymphoma and leukemia, makes challenging the specific individuation of responsibility on the treatment in study.

Therefore, on the basis of the current knowledge, a possible risk for the development of lymphomas, leukemia, and other malignancies in patients treated with adalimumab, as with other TNF-antagonists, must be taken into serious consideration.

These cautions become even more relevant when considering the wide diffusion of this biomedicine and the large spectrum of treated diseases at all ages, especially in clinical care where unselected and less controlled populations are encountered.

Immunogenicity of this fully human monoclonal antibody remains a concern. In fact, HAHA detection is not infrequent when testing and clinical conditions allow better evaluations. Due to the beneficial clinical effect of MTX combination and its capacity of reducing the anti-mAb response production as well, the associated therapy has been recommended, leaving adalimumab monotherapy to MTX intolerant patients [8, 15, 16].

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Alemtuzumab

7

Alemtuzumab (Campath®, MabCampath®, Genzyme) is an IgG1k anti-CD52 humanized monoclonal antibody (mAb) that was first licensed in March 2001 by FDA. EMEA granted its approval in July 2001 and Health Canada in November 2005. The initial indication was limited to B-CLL previously treated and resistant to alkylating agents. Starting from 2007, alemtuzumab was approved also as first-line therapy of B-CLL. So far, it has been experienced in over 60 countries.

Initial therapeutic attempts, performed up to 1995 on 527 subjects and conducted by Burroughs Wellcome, regarded both leukemia/lymphoma and non-neoplastic conditions (rheumatoid arthritis, renal transplant rejection). They were aimed at taking advantage of the profound depletion of T cells caused by this mAb. In fact, CD52 is also highly expressed on T-CLL (100 %), HCL, ALL (79 %), and NHL (94 %) other than on B normal and neoplastic cells. Among 21 trials conducted by the end of 2004, seven related to CLL, six to lymphomas and other types of leukemia, four investigated rheumatoid arthritis, three were compassionate studies, and one was conducted on kidney transplant recipients. However, the encountered severe hematotoxicities led to discontinuation of many of these attempts and the approved indication remained restricted to B-CLL.

Pivotal studies for initial approval consisted in three single arm trials enrolling 149 patients, and in particular one Phase II study (CAM211) enrolling 93 CLL patients (86 with B-CLL), Study CAM009 on 24 CLL patients (22 B-CLL), and Study CAM005 on 32 B-CLL patients. All subjects had been previously treated with alkylating agents and were refractory to fludarabine. The subsequent approval for B-CLL first-line therapy was based on one Phase III trial (CAM307) enrolling 297 (149 exposed) patients [1–6]. In most cases alemtuzumab is administered intravenously (IV), while in a number of studies and in clinical care is also administered subcutaneously (SC).

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In August 2012, EMEA decided to withdraw the marketing authorization for alemtuzumab, allowing patients in need of treatment for B-CLL to receive it through specific access programs. Nonetheless, off-label applications are still frequent and relate to different neoplastic and non-neoplastic conditions. More recently, two main ongoing studies (CARE-MS I, CARE-MS II) have evaluated the efficacy on multiple sclerosis (MS), and a new application of alemtuzumab, under the name of Lemtrada, has been submitted during 2012 to FDA (accepted for review in January 2013) and to EMEA.

7.1 Mechanism of Action

CD52 is a non-modulating glycoprotein of 21–28 kDa expressed on virtually all normal and malignant T and B lymphocytes, NK cells (>50 %), most monocytes, macrophages, a portion of dendritic cells, and granulocytes (<5 %). In the bone marrow, lymphoid progenitors stain strongly with alemtuzumab, while uncommitted and myeloid-committed progenitors are weakly positive. Erythrocytes and platelets are negative. In other non-hemopoietic tissues, relevant binding is present on cutaneous dendritic cells and T lymphocytes. Additional positivity was also encountered in lymphoid primary and secondary organs, and on some male sexual organs (epididymis, seminal vesicles) and mature sperm cells. In lymph nodes the germinal centers stain weakly. These bindings were considered Fab-specific. However, non-specific Fc binding was also detected in a wide range of organs and tissues. An average expression of 5×10^5 CD52 molecules/cell has been reported for lymphocytes.

Alemtuzumab is an IgG1k anti-CD52 humanized monoclonal antibody binding to CD52 cell surface nonmodulating glycoprotein. Upon binding, there is a profound depletion of CD52+ cells. A transient loss of CD52 cell expression was also observed during treatment. In a study sub-group included in the CAM307 trial, 2/139 patients reported a complete loss of CD52, which recovered in both cases prior to disease relapse. Therefore, unstable negative clones seem to be produced by this treatment.

The proposed mechanisms of action, based on in vitro studies, involve antibody-mediated cell cytotoxicity (ADCC), complement dependent cytolysis (CDC), and apoptosis. The first is considered the most relevant effector function in vivo. Other actions such as opsonization, T cell activation, cytokine release, non-specific complement activation, and induction of T cell anti-tumor activity are considered less relevant for the specific therapeutic action, but may be important for AEs induction. With this respect, some pharmacokinetic aspects of alemtuzumab accumulation and clearance are important as well. The latter was shown to be nonlinear and dependent on the amount of CD52+ cells (i.e. tumor burden). Hence, the half-life increases with dosing, due to saturation of clearance pathways and progressive reduction of tumor mass capturing the mAb. Therefore, the specific mechanism of action and the pharmacodynamics of alemtuzumab are crucial for

AEs typology and expression not only during treatment, but also for a wide post-treatment phase, due to the mAb long lasting activity [7, 8].

After alemtuzumab discontinuation, B cell recovery tends to precede T cell recovery, the number of B cells may exceed baseline values, and CD8+ T cell subset tends to reappear before CD4+ lymphocytes. However, the physiological balancing may take a considerable length of time (years) to reach normal values. Therefore, emerging autoreactive B cell clones, either T-independent or as a consequence of the relative absence of regulatory T cells, may expand and raise autoimmune phenomena after monoclonal lymphocyte depleting regimens.

7.2 Immunogenicity

The initial IgG2a rat anti-CD52 monoclonal antibody was genetically engineered by inserting six complementary-determined regions (CDR) into a human IgG1k molecule to reduce its immunogenicity. Therefore, the immunoreactivity against alemtuzumab is expected to be relatively low. In fact, the raise of anti-alemtuzumab antibodies has been estimated either in first (8 %) or in second-line (2 %) B-CLL therapy, and appears to be not relevant for AEs induction. The production of neutralizing antibodies is usually a minor fraction and seems not to be involved in the generation of AEs, as well. However, a higher response (30–50 %) was observed in RA patients, mainly when treated with SC injections. Noteworthy, such route of administration is known to produce more effective sensitizations to antigens, possibly due to a better concentration and presentation by Langerhans cells to T lymphocytes in the local environment. Moreover, in other experiences, SC administration gave a lower rate of response, which was attributed to a more effective induction of anti-mAb response [9, 10].

7.3 Adverse Events

Adverse reactions to alemtuzumab in B-CLL, as well as in various off-label and new special trials, remain the most important therapeutic limitation.

The general safety profile is primarily based on data obtained from the mentioned three primary studies, including 149 B-CLL patients previously treated with alkylating agents, and from the additional study on 147 untreated patients, which was presented in 2007 for approval extension to naive B-CLL patients [4]. Additional information comes from previous three Phase I-II studies including 175 patients, compassionate programs conducted on 177 patients, seven open oncology studies, three studies on RA (140 patients), and from postmarketing reporting.

Alemtuzumab-related adverse events in B-CLL patients are mainly expressed as *acute infusion reactions*, *infections,* and prolonged *cytopenias*. *Tumor lysis syndrome* (TLS) and *progressive multifocal leukoencephalopathy* (PML), although rarely encountered in the postmarketing experience, are additional serious complications. TLS was first included in the 2004 label update, and a warning about

monitoring and treatment suspension in the presence of signs suggesting PML has been recently added [6].

Infusion reactions (pyrexia, rigors/chills, nausea, hypotension, urticaria, dyspnea, rash, emesis, bronchospasm) occurred at the highest rate during the first week of treatment. In clinical trials, severe reactions (≥grade 3) have been estimated to be 35 % in patients previously treated with alkylating agents, and 10 % in untreated subjects. Serious and fatal events observed in the postmarketing settings included ARDS, pulmonary infiltrates, cardiac functional and ischemic disorders, angioedema and anaphylactoid shock.

The overall absolute frequency of infusion reactions in controlled studies is difficult to assess, due to systematic albeit variable premedications routinely performed and to a non-systematic reporting of mild-moderate events. While all patients receive antipyretics and antihistamines, about half of them also receive glucocorticoids, which usually are not advised in oncologic patients under different immunosuppressive treatments. Therefore, serious events (SAEs) are better estimated in trial records, since they receive much attention in the study, and reporting is mandatory.

In some clinical trials and clinical care, alemtuzumab has been administered by subcutaneous (SC) injections, usually for a more prolonged period. Local reactions (erythema, edema, pruritus, pain), often associated with pyrexia, and systemic reactions may appear as well. They are usually observed during the first 2 weeks, the latter with lower frequency and milder expression in case of IV administration. Hypotension, cutaneous reactions, and a number of constitutional signs are virtually absent after SC administration. However, pyrexia remains frequent (70 vs. 85 %), although considerably reduced in severity (2 vs. 14 %) [11, 12].

It is known that CLL is accompanied by immunosuppression, inherent to the disease and worsened by cytostatic treatments. Bacterial and viral *infections* are therefore common, and are the major cause of death.

Serious and sometimes fatal bacterial, fungal, viral, and protozoal infections have been reported as related to alemtuzumab, either in trials or in postmarketing reporting. The overall incidence ranges 23–80 % in different studies, and SAEs reach 50 %, with no significant differences among previously treated or naive B-CLL. Opportunistic infections are also frequent (17–43 %) and include pneumocystis pneumonia (PCP), aspergillus, HZV, CMV, candidiasis, mucomycosis, and JC virus reactivation (PML) [5, 13]. Since the immunosuppressive effect is not strictly dose-dependent, infection may appear at any stage of treatment and post-treatment, with repeated episodes of different etiology. CMV reactivation and subsequent infections have been followed with particular interest in these patients. CMV viremia was found to be as high as 66 %, and consequent infections appeared to be surprisingly higher (16 %) in naive patients than in previously treated ones (6–8 %). However, in protocols applied to untreated subjects, CMV detection and infectivity reporting were mandatory, while in other pivotal studies they were mostly recorded only when classified as serious. In fact, when only serious events were compared, the incidence in the two groups was similar [3, 5, 6]. It must be stressed that, as it happens for symptomatic premedication of

infusion reactions, the potential sensitivity to infections is likely to be in part masked by routine anti-microbial prophylaxis. Remarkably, more than 70 % of all infections remained of unknown etiology in most studies. Overall, their average rate was estimated to be over 1.8 infections/patient. In SC treatments, rates of CMV and non-CMV infections were similar [12].

Due to the massive destruction of circulating WBC, *cytopenia* is the central phenomenon related both to therapeutic effect and to AEs genesis. In particular, it derives from the profound and prolonged lymphopenia induced by alemtuzumab. A massive destruction of T cells is present in almost 100 % of cases, producing a rapid and abundant release of cytokines that are mainly responsible of the acute infusion reaction, *cytokine release syndrome* (CRS) and of similar systemic syndromes (see Chap. 3). The profound lymphopenia impairs the immune resistance to infections, including the opportunistic ones, while the rapid destruction of the neoplastic cell burden (mainly represented by malignant B lymphocytes) causes the nephrotoxic TLS. Therefore, these AEs are strictly related to the specific action of adalimumab, and theoretically are difficult to be avoided. However, they can be mitigated through different strategies, such as premedication and anti-microbial prophylaxis (for infusion reactions and infections, respectively), or administration rules (subcutaneous injection; dose-graduation; tumor burden pre-reduction) to globally reduce their overall negative impact. Finally, a peculiar risk of severe and profound lymphopenia is related to potential *transfusion-associated graft versus host disease* (TA-GVHD), usually avoided by the previous radiation of transfused material [14].

Other cytopenias, mainly neutropenia present in 75–85 % of cases (febrile neutropenia 5–10 %), further increase the risk of infections (bacterial in 40 % of cases). During SC treatments, neutropenia occurred at lower levels (56 vs. 70 % IV). Thrombocytopenia (over 70 %) can be serious in 57 % of cases, causing purpura and infrequent hemorrhagic fatalities. General hematotoxicity, expressed by pancytopenia, bone marrow hypoplasia, and aplastic anemia, is rare although serious.

As for AEs/SOC typology, they mainly involve the immune system, and the respiratory and dermatological compartments; less frequently, although with occasional severity, the cardiovascular and gastrointestinal systems are involved.

As for AEs timing, immediate events (hours to days) mostly relate to infusion and hypersensitivity reactions, while delayed reactions (weeks to months) pertain to cardiac function (insufficiency, failure), neuro-psychiatric disturbances (GBS, depression), and secondary malignancies. Overall, the majority of alemtuzumab-related reactions appear as early (days to weeks) events.

Fatalities are mostly related to infections, being higher in pretreated patients (16 %) than in patients receiving alemtuzumab as first-line drug (2 %). Fatal infections include viral meningitis, listeria meningitis, legionella pneumonia, CMV, PCP, EBV, and associated lymphoproliferative disorder, appearing at any stage of treatment and long after therapy.

Overall, the safety profile in previously treated and naive B-CLL patients is similar, although a lower incidence to induce severe reactions—mainly as drug-related severe infections—among the latter suggests a milder occurrence [15].

It must be stressed that since data are heterogeneous, attempting to compare profiles of different studies is difficult and inconvenient.

Since SC alemtuzumab injection showed comparable efficacy with a lower toxicity in CLL, this method of administration has become the preferred one. However, Health Canada did not approve such route [12, 14, 16, 17].

7.4 Off-Label Experience

Alemtuzumab therapeutic interventions, although officially limited to B-CLL, continue to expand. A number of therapeutic uses have been experienced, both in neoplastic and non-neoplastic diseases, either in studies or in current clinical care. Alemtuzumab has been mainly employed in non-B CLL, T-PLL, ALL, TCL, and pTCL. Non-neoplastic experiences are reported in in hemopoietic stem cell transplantation (HSCT), immunosuppression, renal transplant, bone marrow conditioning, and various autoimmune disorders, including MS. The latter indication is currently under evaluation from FDA and EMEA, on the basis of efficacy and safety data collected in two trials (CARE-MS I; CARE-MS II).

7.4.1 Neoplastic Off-Label Experience

Although results on *T cell prolymphocytic leukemia* (T-PLL) remain poor, alemtuzumab effects against this aggressive form of leukemia have been repeatedly investigated, since CD52 antigen is highly expressed on its cells. At present, five ongoing trials have enrolled 185 patients, while previous experiences have been recently reviewed [18].

In a previous report on eight single arm studies [19], poor results were associated with a high risk of AEs. Most common encountered AEs were serious thrombocytopenia 32 %, serious neutropenia 17 %, infections 25 %, and mild infusion reactions. However, more recent reports experiencing alemtuzumab as single agent have showed more encouraging results and reduced AEs rates [20]. About 50 % of these patients were naive T-PLL; nine of them had been previously treated in a study of the same group investigating IV vs. SC routes. Although the latter induced less AEs, the former method was chosen on the basis of clinical response. Nonetheless, it was found that infections were lower (10 %) in these patients than in CLL (40 %) cured by the same institution. Antimicrobial prophylaxis and symptomatic premedications respectively reduced opportunistic infections and infusion reactions. They also showed that debulking strategies to reduce tumor burden were not advisable, because of the increase in hematotoxicity.

Therefore, it seems that a proper use of alemtuzumab as monotherapy, instead of second-line therapy, remarkably reduces frequency and severity of AEs.

In principle, T cell lymphomas, including *peripheral T cell lymphoma* (pTCL), should be particularly suitable for therapy with alemtuzumab due to high CD52

density expressed on their neoplastic cells. However, the rate of serious AEs resulted particularly relevant. AEs encountered in a pilot study on 14 patients with relapsed or chemotherapy-refractory pTCL were mild/moderate infusion reactions (64 %), including urticaria and bronchospasm (7 %), during the first infusion. However, hematotoxicity resulted much relevant, since four patients suffered pancytopenia (one resolved) and two of these cases developed hemophagocytosis. Interestingly, one of them was reverted by mAb therapy discontinuation. Infections were serious and included opportunistic complications (three cases fatal), and CMV reactivations (37 %), with pneumonitis (29 %). In particular, two pulmonary aspergillosis (one fatal), one HZV infection (fatal), and one infection associated with TB (fatal) were observed. Due to a total of five fatal AEs in a short period, considered to be drug-related (36 %), the study was halted [21].

In a second investigation, including 20 patients with pTCL treated with consistent doses of alemtuzumab, AEs during therapy consisted of a relevant number of infections (70 %), mostly serious (86 %), neutropenic fever (40 %), and CMV reactivation (35 %). One CMV disease (retinitis) developed 1 year after therapy discontinuation. Three patients developed secondary EBV-related lymphoma (two of them after two months and the other after one year), and one developed CMV retinitis after one year from the end of treatment [22]. Similar experiences were reported even with lower doses of alemtuzumab. Therefore, due to the high incidence of CMV reactivation (approximately 30 % of patients in the two reported studies) and pneumonitis, compared to various T-lymphomas not treated with this mAb, a specific role of alemtuzumab was suspected.

Interestingly, susceptibility to AT-GVHD after alemtuzumab administration seems higher than after rituximab. In fact, irradiation of transfused blood materials is only recommended with the former, thus indicating its stronger and prolonged lymphopenic effect [14].

As for other relevant AEs signs, pancytopenia was observed at an unexpected high rate (29 %) and severity (100 %) in pTCL, compared to B-CLL (5–6 %; serious 3 %) and other neoplastic forms treated with alemtuzumab [11].

Since hemopoietic stem cells (CD34+) do not express CD52, additional toxicity and/or peculiarities of this lymphoma may be postulated. Furthermore, hemophagocytosis is known to be associated with T-lymphoma, although at lower expected rates, and has been related to EBV reactivation. However, other EBV-related lymphomas (HL, BKL) are not associated with hemophagocytosis. EBV reactivation and induction of lymphoproliferative diseases has been also encountered in these patients after months from the end of therapy, as it happens also with other immunosuppressive therapies, in kidney transplant recipients treated with alemtuzumab. Therefore, despite this anti-CD52 mAb is expected to destroy also EBV+ B cells, the subsequent imbalance in reconstitution of various lymphocyte cell classes may result in a new outbreak of EBV+ cells, more prone to proliferate in the absence of fully operative immunosurveillance [22]. In conclusion, particularly severe *pancytopenia associated with hemophagocytosis,* and *viral reactivations* may represent additional specific signals of alemtuzumab treatment in T

cell lymphoma patients, apparently developing hematotoxic secondary effects, due to additional mechanisms of action.

7.4.2 Non-Neoplastic Off-Label Experience

Major information on AEs expression derive from the experience in non-neoplastic conditions, consisting in the treatment of some autoimmune diseases and in lymphoablative procedures mainly followed by autologous HSCT. The most relevant signal coming from these experiences is the insurgence of *secondary autoimmune disorders* (sAID), reported in the range of 5–30 %, and including autoimmune cytopenias, thyroiditis, rheumatoid arthritis, lupus-like syndrome, Factor VII and Factor VIII hemophilia, and myasthenia gravis.

In *myeloablative/lymphoablative treatments* with alemtuzumab prior to HSCT, sAID occurrence has been reported (2–5 %) after autologous and allogeneic HSCT for nonmalignant and malignant conditions, and cytopenia was the most frequently reported event.

One retrospective (1996–2006) study on 155 patients undergoing auto-HSCT for various autoimmune primary disorders identified six patients having SLE (3), MS (2), or SSc (1) as primary disease, developing sAID *distinct* from their underlying autoimmune diseases after a median time of 8.5 months; four of them (67 %) had been previously treated with alemtuzumab and developed autoimmune cytopenias. In particular, they developed autoimmune thrombocytopenia (2), hemolytic anemia (1), and neutropenia (1). The remaining 2 cases were treated with anti-thymocyte globulin (ATG) and developed Factor VIII hemophilia. Overall, sAID complications were 16 % with alemtuzumab (4/25), 2 % with ATG (2/102) and 0 % without lymphoablative treatments [23].

Interestingly, the underlying mechanisms seem paradoxical and complex. A genetic propensity to develop autoimmunity, the unbalanced lymphocyte reconstitution after HSCT, and the combination of such status with the mAb-dependent lymphocyte depletion seem to act as powerful inducers of sAID, even when the primary autoimmune disease seems to be controlled by therapy. In fact, all but one of these patients developed sAID despite achieving remission of the primary autoimmune disease. With this respect, a prolonged lymphocyte depression induced by alemtuzumab and a delayed T cell reconstitution compared to B cells, both induced by HSCT and mAb treatments, may facilitate the appearance of uncontrolled new auto-reactive clones. Since alemtuzumab also affects dendritic cells, monocytes, and in part NK cells (all sharing the CD52 targeted antigen), such profound immune dysregulation may greatly enforce the possibility of developing new and even rare autoimmune disorders. Noteworthy, when employed in the absence of HSCT, such as in solid organ transplantation (see below), the frequency of sAID related to alemtuzumab was not particularly evident, thus indicating a peculiar synergistic effect of HSCT and alemtuzumab treatment in inducing autoimmune disturbances, especially in genetically "autoimmune-prone" patients.

Although not approved by FDA and EMEA, off-label use of alemtuzumab as inducer agent in *solid organ transplants* represents approximately the 10 % of its overall use. Among these, the *kidney transplant* is the major representative class, since there is little evidence on the beneficial role of alemtuzumab in liver and pancreas/islet transplantation.

Some interest has grown in small-bowel and multi-visceral transplantation, given the morbidity of acute rejection in this field. However, data on AEs are anecdotal and have not evidenced substantial peculiarities.

Beyond rejections in the first two years after transplant, infections are the major challenge. However, it is still unclear whether alemtuzumab effectively increases the risk of infection in solid organ transplant recipients or not. Hematologic toxicity and infusion reactions in transplant recipients appear with low frequency and limited severity compared to hematologic patients. One reason is that the former only receive single-dose therapy, while heavy multi-dose regimens are used in hematologic malignancies [24].

In 1986, alemtuzumab was the first biomedicine employed in renal transplant as immunosuppressor. The first report appeared in 1990, and the first long-term one was published in 2005, after a five year follow-up in 33 renal transplants.

According to the second report, infections (33 vs. 18 %), and in particular HZV infections (15 %) were more frequent after treatment, but differences were not statistically significant.

Skin cancer (9 %) and two autoimmune disorders (hemolytic anemia, hypothyroidism) were also observed in this group of patients. Moreover, one subject developed a fatal plasmacytoid lymphoma (a type of PTLD) three years after transplantation. Therefore, the emerged safety data were considered to be consistent with the alemtuzumab standard profile observed in AID treatment, yet with a reduced frequency of serious infections and the unexpected insurgence of PTLD [25].

In a subsequent study, safety and induction efficacy of alemtuzumab were compared with basiliximab and with ATG. Serious infections resulted to be higher (35 %) in the former than in the comparator mAb (22 %). In patients at high risk of rejection, ATG showed a rate of infections higher (81 %) than alemtuzumab (60 %). However, the overall infections rates—as well as those of CMV, BKV, and EBV—were similar compared to conventional therapy. Interestingly, the degree of lymphocyte depletion was not correlated with the rate of encountered AEs, nor with type and site of infection [26].

A large retrospective study on infections after solid organ transplants (82 % kidney, 12 % kidney pancreas, 3 % liver, 2 % pancreas, 1 % liver-kidney) in 726 patients treated with alemtuzumab reported an overall rate of 33 %, equal to that reported with ATG treated subjects, while basiliximab-treated patients developed infections in 40 % of cases. Ten percent of the overall infections were fungal, being this rate reported also for the basiliximab-treated group. However, disseminated fungal infections were 68 % in alemtuzumab and 30 % in basiliximab. Therefore, while basiliximab induced a slightly higher number of infections than

the other drugs, alemtuzumab caused more systemic fungal infections (mostly candida) and a higher rate of CMV viremia [27].

In another large and recent meta-analysis, infections and PTLD appeared comparable to other immunosuppressive treatments. However, the time of observation for PTLDs insurgence was considered too short, since they usually appear after 10 years from transplant [28].

Similarly, a retrospective analysis on 357 *pancreas transplant* (alone or combined with kidney graft) recipients treated with alemtuzumab associated with daclizumab and MMF, or with ATG and muromonab, to eliminate CD52 negative T cells, detected severe infections (70 %) and cytologic abnormalities in the bone marrow, together with hematologic disorders (AIHA, ITCP, RCA) in 20 recipients (6 %) within two years from therapy initiation. Nine cases of AIHA and 11 cases of RCA were diagnosed. In the latter group, seven RCA patients had also an associated hemolytic component. Most patients had autoantibodies and complement bound to erythrocytes. Severe infections analyses were restricted to the 20 patients having hematological complications, and indicated as main causes CMV (50 %) and BKV (20 %). No Parvovirus B19 was detected [29].

Interestingly, potential synergistic effects between alemtuzumab, daclizumab, and MMF were implied in the impairment of T cell subsets balance leading to autoimmune hematological disorders and virus reactivation.

As for *liver transplants*, it has been shown that alemtuzumab-treated patients had significantly elevated levels of HCV replication, causing an increase in related mortality as a consequence of lymphocyte depletion [30].

Although the overall risk of infection does not seem to be increased with the use of alemtuzumab, as compared to other immunosuppressive treatments, the occurring infections appear to be more severe and more likely to be disseminated. When used at higher regimens, such as for the treatment of rejection, the risk of opportunistic and unusual infections was three fold higher than the background of solid transplant recipients.

However, compared to hematologic patients treated with the same mAb, infections were low and usually mild, possibly due to a lower intensity of treatment. Noteworthy, fungal infections were associated with an excess mortality that in alemtuzumab patients was high compared to ATG, and low compared to basiliximab.

Alemtuzumab, as other biomedicines, has been experienced in various forms of *uveitis, including Behçet's syndrome*, with alternate fortune. The main concerns derive from the induction of sAID, such as Grave's disease and ITCP.

In a study on 18 patients, moderate infusion reactions (28 %) and hypothyroidism (33 %) were observed.

More recently, a retrospective study on 20 patients treated with alemtuzumab since 1998, reported that 25 % of them developed infusion reactions, with only one drug-related discontinuation, while six patients developed new thyroid dysfunctions. Interestingly, no drug-related infections were registered. Altogether, the incidence of the thyroid disorder ranged around 30 % in the two studies [31–33].

Similarly, in *peripheral neuropathy* and *chronic inflammatory demyelinating polyneuropathy* (PNP, CIDP) alemtuzumab mainly promoted sAID. Among seven CIDP treated patients, one developed a severe rash and three developed sAID. In particular, two of them developed anti-thyroperoxidase (TPO) antibodies, associated in one case with anti-TSH R antibodies, followed by Grave's disease after three years. One patient developed a fatal AIHA 18 months after treatment, although the cause of death remained obscure. Neither ITCP cases nor typical infusion reactions were detected in these patients. However, the typical unbalance in CD4/CD8 lymphocyte reconstitution was observed [34].

Since 1990s, alemtuzumab has been used in refractory *rheumatoid arthritis* (RA) with some evidence of temporary efficacy. Four early trials and a number of studies have not definitively assessed a positive risk/benefit, but off-label occasional applications are still on course. AEs were similar to those experienced in other autoimmune diseases, with opportunistic leading infections. These studies constantly signaled a profound and long lasting lymphocyte depletion with unbalanced reconstitution of T and B cell compartments and of CD4/CD8 T cell subsets, as observed in the previously mentioned disorders. Therefore, the major concern relates to the long-term potential consequences of such dysregulation in the immune system. Interestingly, sAID observed after the administration of alemtuzumab are predominantly antibody-mediated, and they respond to B cell depletion therapies. Similar features have been also observed after HSTC. Possibly, the RA model may fit better then other pathologies to evaluate long lasting consequences of a drug-induced unbalanced immune system.

A recent report examined 20 RA patients, treated with alemtuzumab between 1991 and 1994, at 12 years after treatment [35]. These patients still had a significantly low total lymphocyte count mainly dependent from the CD+ T cell and NK cell subsets. Within the former set, naive and central memory subsets were reduced, while effector memory CD4+ cells seemed unaffected. A similar subsets pattern was observed for CD8+ cells. Total B cell levels were comparable to controls, except for the CD5+ subset, which was significantly reduced. Immunoglobulin levels and response to vaccines were within the range of controls. The role of CD5+ B cells is still under investigation and may be related to autoimmune disorders. In the case of RA, a low rate of autoimmune disorders seems to correlate with the reduction of this subset. However, a good response to vaccines and a low rate of infections in these patients suggest a reasonable reconstitution of immune reactivity even in the presence of persistent imbalance of some T and B cell subsets.

Much interest is devoted to the possible therapeutic effects of alemtuzumab on *multiple sclerosis* MS. Quite recently, two trials (CAM-MS I; CAM-MS II) completed their observation on relapsing-remitting multiple sclerosis (RRMS) with encouraging results, and applications to FDA and EMA have been submitted for the alemtuzumab/Lemtrada® approval. In these trials alemtuzumab was administered as one annual dose for two consecutive years. Most common reported AEs were infusion reactions (90 %; 3 % as SAEs) and infections (67–77 %), usually mild to moderate, with no life threatening or fatal events. However, about 20 % of cases developed autoimmune thyroid abnormalities. A consistent increase

in immune thrombocytopenia initially had raised concerns, causing temporary discontinuation of the investigation in 2003, but at the end of study resulted less concerning (1–2 %).

Previous reports repeatedly showed a consistent frequency of sAID in these patients exposed to alemtuzumab. A survey on 248 MS treated patients revealed that 22 % of them developed sAID, being thyroid the most frequent target (16 %). Hematologic, renal, and dermatologic autoimmune disorders, including anti-GBM renal disease, were recorded with a peak appearance at 12–18 months after treatment. No cases were observed over 60 months after treatment. However, in some cases signs of sAID persisted up to 5 years. No relation was found with the total dose or interval of administered mAb, or with sex and age. Asymptomatic autoantibodies were also detected [35]. ITCP raised particular interest for its frequency (6/1000 P/Y vs. 00.2-0.04/1000 P/Y of the general adult population) and delayed appearance (more than 10 months after therapy), although with a self-limited course in about 80 % of cases and a good response to conventional therapy [36]. Interestingly, the imbalance of T lymphocytes during reconstitution was different in MS and RA treated patients. In particular, MS patients showed a biphasic profile, with an anticipated raise of CD4+ memory/regulatory T cells—possibly driven by IL-7 levels—followed by a normalization phase, where naive T cells progressively raised [36–39].

Nonetheless, the peripheral lymphocyte reconstitution remained impaired for long time compared to other ablative treatments (after one year, CD4+ T cells were still 50 % of baseline levels).

7.5 Postmarketing Surveillance

In the FAERS database providing about 4,000 reports acknowledged by the end of 2012, viral infection was the first reported class of AEs (40 %), followed by any infections, WBC abnormalities, immune disorders, and fungal infections. CMV infections were included in 230 reports, followed by 45 ITCP, 34 TLS, and 27 PML.

Similarly, in the EUV database 18 % of the over 430 reports referred to viral infections, and 26 % of them related to CMV infections. Five TLS, three CLS, three ITCP, two cases of JCV infection, and three cases of anaphylaxis were also reported. Seven cases of GBS, and three cases of PNP were registered among the nervous system disorders.

7.6 Remarks

Alemtuzumab is a potent lymphocyte depletory agent, and has been widely used for B-CLL treatment as well as in a series of off-label diseases. The analysis of induced AEs has revealed a number of complex disturbances, mostly related to its

mechanisms of action and affecting various systems and organs. Rapid, profound, and long lasting cytopenia, mainly as lymphopenia, is the pivotal cause of infusion reactions and infections, to be considered the main expression of drug-related adverse events.

The lowering of hematotoxicity and of some constitutional signs in naive patients undergoing alemtuzumab monotherapy indicates that the previous chemotherapy and/or the different clinical condition of pretreated patients are involved in increasing the incidence of AEs, yet not of SAEs.

Off-label experience is particularly wide and prolonged, thus allowing the observation of additional drug-related AEs among which sAID is the most important and new issue. Although particularly violent and aggressive in a minority of cases, most AEs have shown to be manageable and in part preventable through accurate symptomatic pre-medication and anti-microbial prophylaxis. T cell destruction and imbalanced post-treatment reconstitution are respectively considered responsible for early acute cytokine-mediated reactions such as infusion reactions and CRS, and for secondary delayed interventions such as sAID related to improper reconstitution of B and T cell compartments. As for sAID insurgence, two pathogenetic mechanisms have been evoked. The first relates to a genetically determined abnormal production of IL-21, a potent inducer of T cell apoptosis and cell cycling, in some subjects detectable even before treatment [37]. The second observation relates to the kinetics of the lymphocytes compartment's reconstitution, which can be delayed and incomplete up until 12 years after treatment, with anticipated reappearance of some B cells (except for CD5+ subset) followed by CD8+ T cells, and a persistent scarcity of CD4+ lymphocytes. Altogether, they seem to establish a situation particularly favorable to let autoreactive B cell clones appear and expand [33].

As for future consideration, these data suggest that more selective and less persistent depletive effects may be more promising for further reducing the incidence and severity of these drug-related events. In fact, depletion of T cells in B-CLL treated patients does not favor the control of disease and enhances adverse and long lasting conditions.

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Basiliximab

8

Basiliximab (Simulect®, Novartis) is a chimeric murine/human IgG1k directed to CD25, which is selectively expressed on activated T lymphocytes (aT). This monoclonal antibody is specifically indicated for the prophylaxis of acute renal graft rejection. It was licensed by FDA in 1998 for adult and pediatric (2–15 years old) use, and within the same year also by EMEA, for adult treatment; the pediatric indication (1–17 years old) followed in 2000.

Pivotal investigations include three open label Phase I-II dose-finding studies (B101, B105, B106) conducted on 94 kidney transplant recipients, and two Phase III trials (B201, B352) on 363 kidney transplant recipients for assessing the reduction of rejection rate. Additional studies were dedicated to triple immunosuppressive regimens (INT-10, INT-11, US-01) on 61 kidney transplant pediatric recipients (B152, C102), while two supportive studies enrolled 482 adult liver transplant recipients (C304, INT-13). More recently, an additional Phase IV study on 202 pediatric patients (DE01) was conducted to demonstrate the superiority of standard triple immunosuppressive regimen (CYA, Prednisone, MMF) when associated with basiliximab [1–6]. At present, this biomedicine is approved in over 52 countries.

8.1 Mechanism of Action

CD25 is a high affinity IL-2 receptor (IL-2R) α chain component (p55, Tac antigen), which is part of the IL-2 binding site. The chain is specific for IL-2R and has a short intracytoplasmic tail, which is unable to induce intracellular signaling. It associates with CD122 (IL-2R-β chain) forming functional heterodimeric high affinity receptors for IL-2, expressed on activated T and B cells, on some thymocytes and myeloid precursors, and on olygodendrocytes.

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IL-2R is composed of three subunits (α, β, γ). Only after T-cell activation induced by IL-2, the subunits assemble to form either the intermediate-affinity IL-2R (β, γ_c dimer) or the high-affinity IL-2R (α, β, γ trimer) receptors. Therefore, CD25 is expressed on T cells only upon activation, and enters only in the high affinity receptor assembly. IL-2/IL-2R binding causes T cell proliferation and differentiation, cytokine production, cytotoxicity, and B cell help.

Blocking of ligand-receptor interaction specifically interferes with the T cell-mediated immune responses. Their pathogenetic counterpart is involved in acute allograft rejection, in graft-versus-host disease (GVHD), and in some autoimmune diseases.

Basiliximab (Simulect®, Novartis) is a chimeric murine/human IgG1k monoclonal antibody directed to CD25 (primate IL-2Rα). Due to the high affinity binding, it acts as an antagonist and competitor of IL-2, thus inhibiting IL-2 signaling and consequently inhibits T-cell proliferation and functioning. Concurrent ADCC by its Fc portion increases the immunosuppressive effect by clearing bound aT cells. While in circulation at saturating doses (serum levels exceeding 0.2 μg/ml), occurring within 35–60 days at standard dosage, the blocking is consistent and also impairs immune responses to specific antigens. However, the effect is reversible as soon as the optimal dose is lowered, and CD25 expression returns to pre-therapy levels in 1–2 weeks after treatment discontinuation. The interaction between this chimeric antibody and IL-2R is complex and particularly strong. The epitope targeted by basiliximab is located on the extracellular domain of CD25, and overlaps with daclizumab binding site. A recent crystallographic and plasmon resonance analysis of the Fab/IL-2Ra complex showed that the binding affinity of basiliximab to this receptor is over 70-fold higher (0.14 nM) than the natural binding of IL-2 (10 nM) [7, 8].

8.2 Immunogenicity

A specific anti-murine mAb response (HAMA) was observed in 3.5 % of cases in a cohort of 339 patients, while anti-idiotype (HACA) response was 1.2 %. These data were confirmed by subsequent clinical experiences. However, a more consistent response came from subjects who had later been treated with muromonab-CD3 (18 %). Therefore, the latter fully murine monoclonal antibody, although highly immunosuppressive, seemed to enhance the sensitization against murine structures of the chimeric basiliximab [1–3]. Noteworthy, HAMA can be found in naive individuals of the general population. Moreover, an interesting case report indicated that sensitization after retreatment with basiliximab might involve a specific IgE response, which could produce an anaphylactic shock [9]. Therefore, basiliximab immunogenicity can be considered of low level, yet able to induce serious events.

The response to exogenous antigens, such as vaccines, is usually reduced in immunosuppressive treatments. A recent study on the impact of anti-T cell therapy on the response to influenza vaccines in 60 kidney transplant recipients (38 treated with basiliximab) evidenced a trend toward lower reactivity to the vaccine

components, although no statistical difference either in seroconversion rate (73 %) or in antibody titer was reached. Similar data (68 %) were obtained after ATG administration [10].

8.3 Adverse Events

The safety profile of basiliximab is essentially based on initial observations conducted on adult and pediatric kidney transplant recipients. These trials enrolled 590 adult patients treated with basiliximab in association with other immunosuppressive drugs. The cohort of pediatric patients was smaller (41 patients).

Overall, differences between the reported AEs in basiliximab (7–40 %) and placebo (8–39 %) groups were not significant.

Similarly, the incidence of most serious events such as *infections* (76 %) and *malignancies* (1 %) were comparable. Lymphoma and post-transplant lymphoproliferative disorders (PTLD) ranged 0.1–0.3 %, with no apparent increase over the background levels. In one study, PTLD were reported as 18 % in the study group, and as 5.4 % in the placebo group [6].

Follow-up observations, which lasted up to 5–7 years, confirmed equal incidences in both groups, although no beneficial effects were obtained on renal transplant long-term outcomes.

Most common (>20 %) AEs in *adult patients* included *gastrointestinal disorders* (>70 %), *infections* (76 %, serious 26 %), mainly as UTI and URTI, *constitutional signs*, *hypertension,* and *anemia*. CMV infections were frequent and appeared with comparable incidence (15 % vs. 17 % in controls) as well. Similarly, overall serious events were generally not higher than in placebo groups. However, infections were increased in all patients receiving multi-drug immunosuppression. Moreover, patients who early discontinuated concomitant immunosuppression therapies or had to be re-administered with basiliximab, showed a higher risk of developing hypersensitivity reactions [1–4].

In a 6-year long-term retrospective and comparative analysis between basiliximab and ATG (anti-thymocyte globulin), both in combination with tacrolimus and steroids, conducted on 120 renal transplant adult recipients (60 in each group), the overall AEs profile resulted milder in the basiliximab-treated group. In particular, infections (33 % vs. 58 %), CMV infections (5 % vs. 22 %), and hematological complications, such as thrombocytopenia (2 % vs. 37 %), anemia (20 % vs. 57 %), and leukopenia (23 % vs. 60 %) were significantly lower in the basiliximab-treated cohort than in the ATG-treated arm. However, the rate of fatal bacterial infections (two cases each) was comparable in the two groups [11].

The most common events (>20 %) in *pediatric patients* were *UTI*, *hypertrichosis*, *rhinitis, pyrexia, hypertension, URTI, viral infections, sepsis and constipation, diarrhea, and gum hyperplasia*, and were similarly distributed among treated and control groups. However, a slightly higher frequency of any infection was observed in the study group (95 % vs. 90 % of controls), including CMV infections (13 % vs. 9 %), URTI (35 % vs. 27 %) and UTI (35 % vs. 23 %).

Serious infections showed a similar profile (53 % vs. 48 %). Four cases of *anaphylactic reaction* occurred in the treated cohort, and two were observed in the placebo group. These differences did not reach statistical significance, except for toxic nephropathy (14 % vs. 4 %) and abdominal pain (11 % vs. 2 %), but indicate the induction of a deeper immunosuppression after basiliximab exposure. Finally, four discontinuations only occurred in the study group (ATE/rejection; two hypersensitivity reactions; malignant hyperthermia/rhabdomyolysis) [5, 6].

Overall, the introduction of basiliximab in combined therapy did not increase AEs resulting from underlying disease or from concurrent administration of other immunosuppressive agents, nor induced new safety signals. Interestingly, basiliximab was not associated with injection site reactions, or with major systemic cytokine-related disorders (CRS), although similar events were reported in the postmarketing settings. Long-term experience (at least 7 years) did not show different safety profiles or new signals.

8.4 Off-Label Experience

Basiliximab has been mostly used as induction therapy in recipients of other transplanted organs, such as liver and lung, for the management of GVHD, and as a general immunosuppressant in a number of immune-related, autoimmune and inflammatory diseases. A few trials and anecdotal experiences concerning dermatologic diseases and myasthenia gravis (MG) have been reported.

As for *liver transplantation*, in a long-term study on pediatric recipients conducted on 54 patients up to 52 months after transplant, no adverse events related to basiliximab therapy were reported. In fact, some patients suffered hypertension and renal toxicity, which were higher in controls, while hepatic toxicity and CNS complications were absent in the study group and present in controls [12].

In a large review on adult and pediatric liver transplants ranging 1950–2009, basiliximab was mainly used as induction therapy, to reduce/avoid the use of steroids or global anti-T mAbs known to produce serious long-term adverse effects. The overall safety profile was improved by the basiliximab administration, which allowed to shorten/avoid treatment with steroids or the use of global anti-T mAbs (ATG/OKT-3) conventionally used in steroid-resistant patients. In particular, in all the 18 examined trials no cases of CRS, usually occurring with anti-CD3 administration, were observed. AEs incidence, and in particular infections and other serious adverse events, did not change in basiliximab-treated groups. Therefore, basiliximab appears to be a safer substitute of steroids and of global anti-T agents, exposing to a lower risk of infection, HCV recurrence, malignancy, or other adverse effects [13].

Basiliximab has been successfully used in *heart transplantation* for two purposes: induction of immunosuppression, or as “rescue” therapy in case of severe rejection. The overall trend is toward a consistent low incidence of adverse events with basiliximab compared to OKT3, yet at similar rates of rejection, when used for induction therapy and associated with conventional immunosuppressive therapy.

The former biomedicine confirmed an incidence of drug-related AEs comparable to that of placebo. In fact, none of the AEs was considered related to basiliximab and only five minor events (two cases of cephalea, diarrhea, low grade pyrexia, and one confusional syndrome) among the AEs prespecified in the study were registered [14].

As for *lung transplants*, a large retrospective review on mono and bilateral transplants treated with basiliximab prior to transplant showed beneficial effects, and no statistical differences in the incidence of common serious events, such as bronchiolitis obliterans, infections including CMV, or survival were reported [15]. Therefore, these patients stand at high risk of pulmonary opportunistic infections due to the immunosuppression, and over time develop pulmonary hypertension and progressive bronchiolitis obliterans, which remains the leading cause of death [16].

Overall, the safety profile of basiliximab in off-label organ transplants remains within the range of the on-label experience in the prophylaxis of kidney transplant rejection.

The experience of basiliximab in *non-transplant clinical conditions* mainly focused on ulcerative colitis (UC), cutaneous disorders, and MG.

In a small non-controlled trial, the enrolled patients (10) had steroid-resistant *ulcerative colitis* (UC) and were followed for 24 weeks after a single dose of basiliximab. UC improvements appeared in 9 subjects, but most of them relapsed shortly. All patients showed minor AEs (pyrexia, paresthesia, transient lethargy and photosensitivity, lumbalgia, and URTI). No steroid-related side effects (facial edema, weight increase) were increased by the pretreatment with basiliximab, although in vitro tested steroid sensitivity of lymphocytes of all patients was increased. These studies were subsequently enlarged to 20 more patients, and confirmed in frequency and typology of expressed AEs [17]. In a larger and more recent study on 149 patients, drug-related AEs were still mild-moderate, but slightly higher than in placebo (28 % vs. 25 %), as for serious events' incidence (6 % vs. 4 %). Gastro-intestinal disorders were the most frequently reported AEs, (13 % vs. 8 %). Infections remained low (8 %) and equal to controls. Discontinuation rate was higher in the study group (9 % vs. 2 %), yet in vitro steroid sensitivity was not increased [18].

Overall, the spectrum of AEs in UC was moderate as expected, but efficacy was more limited than previous expectancy.

Anecdotal cases of epydermolysis bollosa, erosive lichen planus, and psoriasis (pustular, palmo-plantar) treated with basiliximab and daclizumab have been reported [19]. These off-label attempts were mainly directed to cases resistant to conventional therapies. Partial success was obtained, yet these patients had to discontinue the therapy due to relevant AEs, cost of treatment, or limited clinical efficacy [20].

Finally, an interesting case report in a quite different pathological context relates to off-label use of basiliximab in *myasthenia gravis*, administered on the assumption that anti-aT lymphocyte activity could interfere with the specific pathogenesis of this autoimmune disease. However, the safety profile showed

increased infections as the primary drug-induced AE, leading to therapy discontinuation.

Infections were reported as several bacterial sinusitis and tonsillitis, along with two episodes of bacterial pneumonia. Cephalea also appeared after infusions lasting 2–3 days [21].

8.5 Postmarketing Surveillance

Postmarketing observations evidenced hypersensitivity reactions, including anaphylaxis, anaphylactoid reactions, and CRS, as rare events. These data had already been reported in the official labeling [1, 2].

In the FAERS database, over 1,500 reports registered by the end of 2012 on basiliximab administrations included about 9,300 AEs (6.4 AEs/report), being infections (6 %), viral infections (5 %), renal disorders (5 %), and immune disorders (4 %) the most frequently registered events. In particular, 167 reports included CMV infections as the most frequent disorders of this category.

In the EUV database, 1,215 (1,209 serious events) reports were registered by the end of 2012, and included 5,512 AEs (4.5 AEs/report). Infections were the most frequent event (18 % of reported AEs; 0.8 infection/report), followed by immune disorders (8 %). Among the former, there were 144 CMV infections, 32 cases of pneumonia, 26 EBV, and 23 HZV infections. Moreover, 122 malignancies were also signaled, including 30 PTLD as the most frequent proliferative event. Interestingly, 17 cases of anaphylaxis/anaphylactic shock and 3 cases of CRS were also reported.

8.6 Remarks

Basiliximab targets only activated lymphocytes (aT) sparing the resting pool of T cells, and inducing low rates of mild-moderate AEs. One possible explanation of such moderate safety profile is the lack of intracellular signaling ability of CD25, the specific target of basiliximab, which is not able to burst per se acute toxic effects or CRS, although some cases were reported in the postmarketing setting. These qualities render the molecule theoretically applicable to a wide range of immune and autoimmune disorders, where the insurgence of additional immune-related AEs is highly undesirable. However, the best basiliximab response has been proved only in the context of transplantation, and in particular on the prophylaxis of organ graft rejection, where aT cells are clearly involved in allo-recognition and initiation of strong immune reactions. In addition to the low capacity of AEs induction, basiliximab gives the opportunity of reducing alternative treatments at higher AEs potential, such as steroids and global anti-T mAbs without lowering the efficacy of associated therapies, and thus leading to a significant reduction of overall drug-induced AEs.

Interestingly, basiliximab peculiarities indicate that most of the rejection events generated in the aT-cell compartment, while AEs related to other wide-range anti-T agents (OKT3, and similar) rather derive from the destruction/inhibition of the large resting pool of such cells, than from their action on the crucial pool of aT-cells, which eventually represent the real optimal target for most immune mediated disease control.

Moreover, basiliximab seems to show, together with a milder safety profile, a more generalized control of the aT cell pool with respect to agents interfering with single specific pro-activating T cells signals (abatacept, alefacept, belatacept). In fact, the inhibition of individual pro-activating signals do not exclude that other pathways may still induce a partial activation of the T-cell compartment, with due consequences on the underlying disease and on the overall safety profile. On the other hand, a residual activation of T cells may as well mitigate immunosuppressive effects on such vital compartment, thus reducing the insurgence of infections or malignancies escape. A fine-tuning of these alternatives may be still possible by combining proper therapies and may be instructive for future drug development.

Basiliximab does not induce acute violent cytokine releases possibly because of inactive direct CD25 cell signaling, and most probably because of the selective action on a limited amount of T cells.

These aspects, mainly related to the high T-cell selectivity and high receptor affinity, make this molecule worth of further developments [7]. In fact, basiliximab (and the similar daclizumab) surfaces are rather flat for an adequate confronting with IL-2R and could be further ameliorated by conformational modifications to enhance complementarity and possibly efficiency.

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Belimumab

9

Belimumab (Benlysta®, GSK), previously known as LymphoStat-B and produced by Human Genome Science, is a fully human IgG1λ monoclonal antibody approved by FDA in March 2011, by EMEA in June and by Health Canada in July 2011, for the IV treatment of adult patients with active, autoantibody-positive, systemic lupus erythematosus (SLE), who are also receiving standard therapy.

It is the first biomedicine exclusively dedicated to active SLE, and is specifically directed to the B lymphocyte stimulating/activating factor (BLyS, or BAFF), thus inhibiting growth and differentiation of B cells into immunoglobulin-producing plasma cells.

In the nine studies submitted for initial approval, 3,535 SLE patients were enrolled and 80 % of them were treated with belimumab. In particular, pivotal studies included two Phase II trials (LBSL02, Study 1070) and two pivotal Phase III studies (C1056/BLISS-76; C1057/BLISS-52) investigating efficacy and safety in 2,133 SLE patients (1,458 treated by IV route) enrolled in 21 countries. While most patients received IV administrations, the SC route was investigated in a Phase I bioavailability study (C1058) on 19 healthy subjects and in Study 1070 on 28 SLE patients. However, this route of administration was not put under evaluation of the Authority. Supportive data were included in three open-label extension studies (LBSL99, C1066, C1074), enrolling only IV treated SLE patients.

At present, among 34 studies, 11 of them are completed, including those concerning RA and other immune and B cell proliferative disorders, and 12 are still recruiting [1–6].

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9.1 Mechanism of Action

BLyS is a cytokine of the TNF family, constitutively expressed by cells of myelocyte/monocyte origin, by activated neutrophils, T and DC cells, and specifically binding to B lymphocytes. BLyS is organized in trimers, cleaved by furin and then released into circulation as a soluble B cells stimulator factor. Its expression can be potentiated by inflammatory cytokines (IL-2, TNFα, and IFNγ). BLyS can bind to three different receptors, BR3, TACI, and BCMA, but is the sole ligand for the former one, on which it binds with higher affinity. The latter two receptors receive signals also from another proliferation-inducing ligand (e.g., APRIL). BLyS-BR3 interaction is necessary for the newly formed naive B cells, bursting signals of viability that antagonize apoptosis during the transitional negative selection of potential autoreactive B cell clones, and thus allowing alloreactive clones survival, expansion, differentiation and migration to peripheral lymphoid organs/tissues. However, excess BLyS can rescue autoreactive clones that may progress in maturation.

Belimumab is a fully human IgG1λ monoclonal antibody binding with high affinity to soluble BLyS. The initial prototype molecule was obtained in the NS0 cell line, after screening for V regions in a phage library of single chain antibodies binding to human BLys of 43 neutrophil healthy donor's pool. The selected clone was then converted to full-length human immunoglobulin (LymphoStat-B), subsequently named belimumab.

When belimumab blocks BR3 on naive B cells, APRIL can still induce proliferation on mature, antigen-activated B cells, on memory cells, and on long-lived plasma cells through transmembrane TACI and BCMA signal transduction pathways. Therefore, BLyS affects predominantly the early stages of B cell selection and maturation.

Elevated serum levels of BLyS are present in SLE patients. Transgenic mice overexpressing this soluble factor develop disorders similar to human SLE and to Sjögren syndrome. The production of autoantibodies, mainly anti-DNA antibodies, has been correlated with BLyS-sensitive/T-independent, B lymphocytes. SLE and RA subjects develop T-independent autoantibodies. On this basis, BLyS may prominently figure in the development of autoimmune disease, particularly of SLE. Total immunoglobulin levels (mainly IgM), autoantibodies (anti-dsDNA) and B cell subsets (CD19+, CD20+), including a peculiar subset (such as CD19/CD27b/CD38b-positive, transitional B lymphocytes), are reduced during belimumab therapy, while memory B cells, T cells and complement factors (C3, C4) raise. However, the transient rise of memory B cell normalizes within 2 weeks after treatment, while duration of T cells and complement increase is present up to 52 weeks [1–4, 7–10].

9.2 Immunogenicity

Being belimumab specifically directed against the Ig-secreting compartment, it is expected that this capacity may interfere with the potential immune humoral reactivity against this mAb. In fact, during treatment, there is a dose-related reduction of circulating immunoglobulin (Ig) levels. IgM levels decrease by 18 % starting from week 8, and drop to 66 % during therapy, up to 52 weeks. IgG are lowered by 6 % and drop to 14 % after the same period. IgA are less involved (3 % decrease) and tend to remain stable with time. Similarly, there is a progressive decrease of total B cells, identified as CD19+ cells (19–32 %), reaching 58 % reduction after 76 weeks.

A recent analysis of belimumab effects on immunologic biomarkers of SLE showed a tendency to the normalization of pre-existing Ig levels and of complement factors (C3, C4), and to a gradual conversion to seronegative status for a number of specific autoantibodies [8].

Initial experience on anti-drug antibody induction came from Phase I-II studies and from the following two Phase III trials. HAHA were detected in 1–13 % of cases [1]. Subsequently, HAHA were reported in 0.7–5 % of cases, mostly as neutralizing, with titer trends inversely related to belimumab dose [4]. In fact, higher doses tended to lower the HAHA positivity (0.7–0.9 %), possibly as a consequence of the interference of circulating mAb in the titration test, and/or of higher immunosuppressive mAb-induced effect. However, in persistent-positive subjects (7–25 %, dose-related) infusion reactions on administration day were mild to moderate, and a few of them were reported as serious/severe AEs. Experience with vaccine's immunogenicity (tetanus, pneumococcal, streptococcal, and influenza) appeared not modified, yet collected data were very limited [2, 9].

9.3 Adverse Events

Overall, 17 studies included safety data on SLE, the pivotal being LBSL02, C1056, and C1057 trials. Basic investigations started in 2003 and were completed in part by 2010, while some trials have been expanded and are still ongoing. From this experience, a warning was raised for *infusion reactions*, *serious infections*, *hypersensitivity reactions* including anaphylaxis, *psychiatric disorders*, *malignancies,* and increased *mortality*. At least one AE/patient was observed in 93 % of the treated subjects, with at least one serious event in 18 % of cases.

Infusion reactions overall rates were slightly higher in belimumab-treated patients than in placebo recipients (17 vs. 15 %, respectively). However, serious AEs rates were comparable (0.5 vs. 0.4 %). The most common AEs expressions related to constitutional (nausea, cephalea) and cutaneous reactions.

Hypersensitivity was reported in 13 % of cases. *Anaphylaxis* occurred in 0.6 % of treated patients (0.4 % in controls). Most episodes appeared during infusion or shortly after, and could hardly be distinguished from other infusion-related events

(See IRS, Chap. 3). Moreover, the concomitant use of corticosteroids and the premedication prophylaxis may mask part of these events.

Overall, infections in clinical trials revealed a higher incidence (71 %) in groups treated with belimumab than in controls (67 %). The most frequent episodes in study groups were UTI (14 %), URTI (5–7 %), and influenza (7 %). Serious events (6 %), sometimes fatal, included pneumonia, UTI, cellulitis, and bronchitis, not dissimilar from controls in frequency (5.2 %) and typology.

A higher frequency of *psychiatric disorders* was detected after belimumab treatment (16 vs. 12 %), being depression the main encountered expression (6 vs. 5 %). Serious events, including suicide, ranged around 1 % and raised some concern. By the end of 2010, additional information for suicidal events on the entire exposed population identified three suicides and four attempts. It must be noted that psychiatric disturbances are common in SLE patients (cognitive 55–80 %, mood 14–57 %, and psychotic 8 %), but patients with a positive history had been excluded from trials. Unfortunately, long-term studies providing a proper comparison between these data and the background rates of the SLE population are lacking.

Malignancy ratings ranged 0.2–0.4 % in all groups. Exposure-adjusted rate for malignancy was calculated as 0.9/100 PY (0.7 excluding NMSC).

The main cause of drug-related *mortality* was infection related to immunosuppressive activity and to underlying disease, followed by cardiovascular accidents and suicide. During the controlled period of three clinical trials, eight fatalities were observed in the study groups, and six cases in placebo. Death exposure-adjusted rates were calculated as 0.79/100 P/Y. These data appear similar to the SLE patient's cohort. Long-term data are expected from the ongoing trials [1–6].

Overall, the initial experience with belimumab has shown a rather safe profile. Infections were the most common and important AEs, followed by general constitutional signs, including pyrexia. Psychical imbalance has raised particular concern. Drug-related causes of therapy discontinuation (6–7 %) resulted slightly higher than controls, being the most frequent related to infusion reactions. Due to the lack of sufficient long-term data, the consequent evaluation on protracted impairment of antibody production is still pending.

9.4 Off-Label Experience

Belimumab has soon been employed in a number of off-label conditions related to the increased B cell proliferation and to other autoimmune disorders. However, initial studies on RA (LBRA01, LBRA99, and 1089), Sjögren syndrome (2), prerenal transplant desensitization (2), and Waldenstrom's macroglobulinemia (1) were initiated before approval and were included in the application to FDA as additional information [1, 6].

At present, a number of ongoing trials are investigating Waldenstrom's macroglobulinemia, RA, idiopathic membrane glomerulonephritis, Sjögren syndrome, cutaneous systemic sclerosis, chronic immune thrombocytopenia, desensitization of renal transplant recipients, prevention of kidney rejection, vasculitis, and myasthenia gravis. Part of these trials is also dedicated to special off-label forms of SLE (lupus nephritis, pediatric lupus), and to potential racial differences in efficacy (afro-american, north-east asian). Finally, two extended trials have further evaluated the SC route of administration in SLE, and one of them is still recruiting patients.

Two preliminary studies (C1058, C1070) initially tested safety and efficacy of the *SC route of administration in* healthy volunteers (19) and in *SLE* patients (56). Data were included in the application, but this route was not evaluated.

In one of the recently completed study (C1070), the 56 patients were treated with two different and repeated SC doses of belimumab. Total pooled serious AEs reached 11 %. Myocardial infarction, bradycardia, and retroperitoneal hemorrhage ranged 2 % each. Infections (54 %, serious 9 %) included cellulitis, gastroenteritis, pneumococcal sepsis, pyelonephritis, and subcutaneous abscess (2 % each). Although apparently less prone to AEs induction, the safety of this route remains to be established.

Data from Phase II studies (LBRA01, LBRA99, C1089) on *RA patients* were also included in the SLE application, but were considered only for safety evaluations and mortality rates. Overall, they were reported as not significantly different to placebo for adverse events, serious adverse events or laboratory abnormalities. Infusion reactions were rare. Belimumab significantly reduced levels of circulating B cells and of rheumatoid factor.

Study LBRA01 was extended to 283 patients (214 treated) up to 5 years, and some results were released in February 2012. Any AEs were 54 %, with predominant mild infections (30 %), mainly as UTI and URTI, musculoskeletal and GI disorders, minor nervous (dizziness, cephalea) and respiratory disorders (about 5 %). Serious AEs were reported as 11 % and included muscular/skeletal disorders (3 %), infections (<1 %), and malignancies (1.4 %). Interestingly, no cases of depression were observed.

A recent literature search up to October 2012 showed that the overall safety profile of belimumab in RA was assuring, yet the efficacy outcomes provided from the mentioned trial were not satisfactory [12].

As for *Sjögren syndrome*, it is known from preliminary studies that levels of BLyS in 49 patients were significantly higher than in other autoimmune rheumatic diseases, including SLE. Moreover, a peculiar subset of B cells (transitional B lymphocytes) seems particularly represented in both diseases [13]. This allowed the starting in 2012 of two trials, in France and Italy, which at present have completed patients' recruitment (NCT01160666 and NCT01008982).

9.5 Postmarketing Surveillance

The initial postmarketing reports enlisted fatal anaphylaxis after belimumab administration. A few spontaneous reports (586) from FAERS by the end of 2012 indicated constitutional signs, gastro-intestinal, respiratory, dermatologic and neurological disorders, and infections as the most signaled events.

In the EUV database, 271 reports included the same categories as the most frequently involved. Gastro-intestinal signs mainly related to nausea/vomiting (32/22), and diarrhea (28). Noteworthy, eight cases of anaphylaxis, one anaphylactoid reaction, and eighteen hypersensitivity reactions were included. Infections (140) were represented by pneumonia (23), and UTI (12). At cutaneous level, pruritus/rash, (17) and urticaria (15) were predominant.

9.6 Remarks

The recent experience with belimumab focused safety concerns on infections, increase of psychiatric disturbances, and a higher mortality. Mortality rate was nearly doubled in treated groups, although confidence intervals were not dissimilar between the two classes. However, mortality seems to be lower than in the average SLE population. It must be noted that, as usually planned in controlled trials, the enrolled groups are selected and cannot be compared to the general SLE population encountered in clinical care.

Rates of serious infections were not dramatically increased, possibly because of the sectoral immunosuppressive effect of belimumab. In fact, this drug should mainly act on the T-independent immunoglobulin effector arm of the immune system, leaving natural and acquired humoral immunity, as well as cellular immunity, intact [10]. Infections resulting in death occurred in 0.3 % of treated patients versus 0.1 % of placebo. A consistent reduction of Ig, mainly of the IgM class was observed, but it was not strictly related to infections' increase or to serious infections rates.

Unexpected results came from the increase of psychiatric disorders (16 % vs. 12 % of placebo treated patients), including depression and suicide. Serious depression was reported in 0.4 % in the treated groups, versus 0.1 % of placebo. It is known that the disease also affects CNS, but these patients were excluded from trials.

Infusion reactions and most concomitant hypersensitivity reactions, including anaphylaxis, were higher in belimumab treated patients (13–17 %) compared to controls (11–15 %). Apparently, malignancies were not increased, but the observation interval was short, since the most relevant long-term studies are still ongoing. However, a recent report examining safety after 4 years of belimumab exposure for the treatment of SLE, with 1,165 cumulative P/Y, has showed that AEs (severe/serious AEs, infusion reactions, infections, malignancies, grades 3/4 laboratory abnormalities) were stable or declined during the 4 years of belimumab

exposure. The most common AEs included nausea, diarrhea, pyrexia, URTI, insomnia, pain, and depression. Serious infusion reactions were rare: only one occurred during the 4-year follow-up. Serious infection rates decreased from 5.9 to 3.4/100 PY, and no specific type of infection predominated [11].

Taken together, the acceptable profile of belimumab may have some explanation in its mechanism of action. In fact, BLys/BR3 is not the only pathway acting on B cell survival, since both APRIL/TACI and APRIL/BCMA signals are not disturbed by belimumab. Moreover, the partial blockade may also explain the moderate efficacy encountered in some clinical conditions. The importance of inhibiting the APRIL-dependent pathways was also suggested by their role in lupus nephritis, where a correlation was found between the serum levels of APRIL and the renal disease activity. Therefore, new attempts have been recently directed to obtain a total inhibition of such transmembrane signals, by a new experimental fusion TACI-Fc protein, atacicept, blocking both BLys and APRIL. Indeed, the double blocking produced a more pronounced reduction of serum Ig and a remarkable increase of serious infections in lupus nephritis treated patients, leading to an anticipated end of the Phase II-III trial [14]. In particular, 3 out of the 4/6 patients treated with atacicept developed serious IgG lowering below the protocol-defined discontinuation level, associated with pneumonia (*Haemophilus influenzae*) and complicated by empyema, septicemia and pneumothorax in one patient. Two more patients developed Legionella pneumonia and Bacillus bacteriemia, respectively. Lymphopenia (CD4+) and C3 imbalance were also observed in some of them. The effect could be related to the drug in study, and not to the associated immunosuppressive therapy, since it was evident in two different therapeutic associations, namely with MMF and MTX [15].

These aspects shed new light not only on the role of BLys unilateral blocking, but also warned against widened blockades leading to dangerous rather than to beneficial consequences.

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Bevacizumab

10

Bevacizumab (Avastin®, Genentec) is a recombinant humanized monoclonal IgG1k antibody specifically binding to soluble vascular endothelial growth factor (VEGF-A), and thus inhibiting its interaction with the respective receptors Flt-1 (VEGFR-1), KDR (VEGFR-2), and Ftl-4 (VEGF-3) located at the surface of endothelial cells.

Clinical studies began in 1997 by testing the potent anti-angiogenic effect of this mAb in about 30 types of human tumors. However, the first approval by FDA was granted in 2004 only for metastatic colorectal cancer (mCRC), on the basis of a pivotal Phase III study enrolling 900 mCRC patients, and of additional safety data from 1,400 patients treated in other trials. EMEA approval followed in 2005, for the same indication. In the following years, the approval was extended to other forms of tumors and in particular to non-squamous, non-small cell lung cancer (NSCLC) in 2006, to metastatic breast cancer (mBC) in 2008, to metastatic renal-cell carcinoma (mRCC), and to glioblastoma multiforme (GBM) in 2009. However, the indication for mBC granted by EMEA in 2007 and by FDA in 2008 under accelerated approval program, was subsequently withdrawn in 2010 by the latter Agency due to safety and efficacy concerns. The indication for GBM was not endorsed by EMEA (2009). Finally, treatments of epithelial ovarian cancer (EOC), fallopian tube cancer (FTC), and primary peritoneal cancer (PPC) were approved by EMEA in 2012.

Bevacizumab has being investigated in more than 450 clinical trials and 30 different tumor types; over 370,000 patients have been treated worldwide. Pivotal trials include three active-controlled studies on mCRC (AVF2107g, AVF0780g, and AVF2192g) on 605 exposed patients (ep), and two additional studies (NO16966 and E3200 on 992 ep). Selected trials were also conducted on patients affected by mRCC (BO17705, AVF2938, AVF0890 on 419 ep), NSCLC (E4599

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and BO17704 on 914 ep), GBM (AVF3708 on 163 ep), and mBC (E2100 and AVF3694g on 731 ep). Two subsequent confirmatory trials (AVADO, RIBBON1) were requested to confirm accelerated approval for mBC, but results were considered not sufficient to endorse such indication.

Most of the information on AEs were derived from these studies. However, a number of additional investigations were also conducted on other tumor typologies, such as AML (2 Phase II trials on 57 ep), LDBCL (MAIN Study on 720 ep; S0515 on 73 ep), CRC as adjuvant therapy (NSABP C-08 and AVANT trials on 3246 ep), pancreatic cancer (Phase II trial on 306 ep), epithelial ovarian, fallopian, and primary peritoneal cancer (GOGO2180 on 1,248 ep; BO17707 on 764 ep), which were also considered for the overall safety analysis. However, scarce observations are available on GBM treatment in the pediatric population (PBTC-022 on 30 ep). Part of these trials was cumbersome and not satisfactory for efficacy profiles, as evidenced by selective and alternative granted approvals. Nonetheless, this complex and heterogeneous mass of results allowed to obtain a detailed safety profile of bevacizumab, estimated from over 3,500 patients with various malignancies, mostly treated in clinical trials and in association with chemotherapy [1–9].

10.1 Mechanism of Action

The vascular endothelial growth factor (VEGF) family is a soluble 45-kDa group of cytokines (six homodimeric glycoprotein isoforms) that includes five ligands (VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PGF) derived from a gene splicing. The family member recognized by bevacizumab is VEGF-A, the most active variant, which mediates its effects by binding to two tyrosine kinase receptor (TYKR) isoforms, VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1), while the third receptor (VEGF-3) responds to VEGF-C and D ligands. VEGF-A is expressed in four major isoforms (VEGF121, 165, 189, 206) and in five minor isoforms (VEGF145, 148, 162, 183, and 165b). Moreover, VEGF165b acts as an inhibitory factor binding to VEGFR-2, and VEGF110 is a smaller biologically active ligand derived from the proteolytic cleavage of VEGF121 and VEGF165.

Fibroblasts, neutrophils, endothelial cells, and T cells produce VEGF molecules. Their synthesis is stimulated by hypoxia, nitric oxide, and protein kinase C. VEGFR-1 and VEGFR-2 are expressed on progenitor and mature endothelia, but also on monocytes, macrophages, neurons, renal glomerular, preglomerular, and peritubular cells. VEGFR-2 is considered the most important angiogenic factor in the family, while VEGFR-1 seems to act as its modulator/competitor, and VEGFR-3 is predominantly expressed on lymphatic endothelium and shows lymphoangiogenic properties. These receptors are transmembrane Ig-like structures with a predominant extracellular portion (7 domains), and the intracellular tail containing one TYK domain. VEGFR-2 can be also cleaved as a soluble form (sFlt-1) and acts as a physiological competitor of the membrane-bound VEGFR-1 and VEGF-2.

The natural ligand's synthesis can be stimulated by local hypoxia, which produces the hypoxia inducible transcriptional factor (HIF) capable of enhancing nearby angiogenesis. Overall, the system generates signals for homeostatic regulation, survival, and activation directed to endothelial cells, regulates angiogenesis and vascular permeability, but also exerts neurotrophic and survival-promoting effects on neural and glial cells, and on the renal epithelial/vascular district.

The VEGF network also plays an important role in embryonic and postnatal vasculogenesis, skeletal muscle regeneration, cardiac remodeling, endochondrial bone formation, in the female reproductive cycle, and in kidney function. These additional features may help in understanding the overall safety of VEGF-blocking biomedicines, including bevacizumab.

VEGF ligands are also produced by various epithelial tumors, thus ensuring their proper vascularization and growth. The oxygen and nutrient requirements of rapidly proliferating tumors release HIF, leading to production of VEGF and stimulation of adjacent capillaries. Their endothelial cells become migratory, invasive and proliferative, thus digesting the extracellular matrix and migrating to extravascular spaces. Tip cells lead migration until they make contact with another growing sprout. The contact stops migration and converts them into tubule-like structures, giving rise to a network of capillary vessels, which ultimately ensure tumor survival and growth. Most of the involved VEGF signaling is mediated by VEGF-A/VEGFR-2 interaction.

Bevacizumab is a recombinant humanized monoclonal IgG1k antibody specifically binding to soluble VEGF-A, thus inhibiting its interaction with the respective receptors on endothelial cells. The antibody contains the complementarity determining regions (CDRs) of a murine anti-VEGF antibody in a human framework, and contains approximately 10 % of murine protein.

The binding sequesters VEGF-A and prevents from VEGF-2 activation, resulting in an anti-vascular and anti-angiogenic action. Since tumors rely on blood vessels to get the nutrients they need to survive, the drug is thought to work by preventing the formation of new blood vessels that feed the tumor. They are also able to secrete endogenous VEGF and therefore activate the "angiogenic switch", thus generating new vessels, changing vascular permeability and hemodynamic responses. Moreover, angiogenesis is a fundamental step in the transition of tumors from a dormant to a more aggressive stage. Increased levels of VEGF expression have been found in many human malignancies. Sustained blockade of VEGF signaling is therefore directly acting on tumor neo-vasculature, likely restraining its growth. This activity results complementary and adjuvant to cytotoxic therapies, directly targeting the tumor cells. Interestingly, bevacizumab seems to sensitize the tumor vasculature to chemotherapy-induced damage, thereby enhancing the activity of both agents. It also helps in keeping these tumors in a dormant state, by preventing the recruitment of new vessels and by "normalization" of the preexisting tumor vasculature; this provides more stable and less permeable structures, limiting the hematogenous spread of metastases as well.

Finally, VEGF/VEGFR binding at ocular level may induce endothelial cell proliferation and vascular hyperpermeability, which contribute to the development and progression of the neovascular (wet) form of adult macular degeneration (AMD) and similar ocular disorders. Such pathogenetic property led to the development of ranibizumab, the Fab fragment of bevacizumab, subsequently modified to bind with a higher affinity to the same VEGF-A epitope. Meanwhile, a wide off-label use of bevacizumab in ocular diseases related to neoangiogenesis occurred (see ranibizumab, Chap 34).

In order to better understand the mechanism of intervention of anti-VEGF agents and the framework of their safety profiles, it must be stressed that the VEGF/VEGFR system is not the only angiogenic/neoangiogenic regulator, and hypoxia is not the only VEGF inducer. In fact, other toxic/degenerative mechanisms frequently related to tumor growth, and a number of inflammatory cytokines (IL-1a, IL-6, EGF, TGF, PDGF, and IGF-1) may act as additional promoters of angiogenesis [10–12].

10.2 Immunogenicity

Although this monoclonal contains approximately 10 % of murine protein, it did not induce the expected acute early reactions, even during short infusion regimens. A potential risk for immunogenicity was expected, but no significant data had been previously documented in pivotal trials, except for a general assertion that high titers of anti-mAb antibodies were not detected on 500 tested patients. Immunogenicity and its relation to AEs induction was not determined in major trials and scarcely appeared in the postmarketing reporting [1, 8]. However more recently, hypersensitivity reactions, including anaphylactic shock, rash, and urticaria were reported in the postmarketing settings, including 2 cases of IgE-mediated Type I hypersensitivity (see below).

10.3 Adverse Events

Bevacizumab has been employed in a wide variety of tumors and can induce a number of relevant AEs. Initial studies have attempted preliminary treatments in about 30 different histotypes of malignant tumors. At present, bevacizumab is officially indicated in US for the treatment of four malignant solid tumors (mCRC, NSCLC, GBM, and mRCC) and in EU for the treatment of seven (mCRC, mBC, NSCLC, mRCC, EOC, FTC, and PPC). The multiplicity of their histotypes, the wide spread of associated therapies, the different adopted protocols in pivotal trials and supportive studies, the strategies adopted for safety data collection and the clinical differences of underlying pathologies, render difficult to embrace the entire physiognomy of the AEs developed during bevacizumab treatments. Along with acquired experience, the prescribing information of this biomedicine has been repeatedly expanded and revised to update safety information. For example, ATE events and

infusion reactions (2004), GI perforation (2006), RPLS and nasal septum perforation (2006), non-GI fistula formation (2007), proteinuria, hemorrhage (2009), ovarian failure, ONJ and VTE, and bleeding during anticoagulant therapy (2011) were progressively introduced among the warnings in official labels. Therefore, most of these signs came from postmarketing observations and spontaneous reporting.

Nonetheless, a general safety profile could be identified as a framework of events common to all groups of pathologies, while some events were considered more peculiar for specific tumors, either for typology or frequency [1–9].

A BBW on *gastrointestinal perforations*, *surgery and wound healing* complications, and *hemorrhage* was issued since approval. Additional most relevant events related to bevacizumab treatment are: *non-GI perforations, thromboembolic events* (*ATE/VTE*)*, hypertension and hypertensive crisis, infections, leuco/neutropenias, cardiac functional events* (*CHF, LVEF*)*, proteinuria/nephrotic syndrome, general constitutional disturbances, infusion reactions, and reversible posterior leukoencephalopathy syndrome* (*RPLS*). Their frequency and relevance are different among the tested tumor categories, and are more easily evaluable when reported as severe/serious, since their reporting was included in all protocols, although with some variability in their grading classification and in some prespecified events to be recorded in single protocols.

The *most common events* (any grade) include asthenia, pain, abdominal pain, cephalea, hypertension, diarrhea, nausea vomiting, anorexia, stomatitis, constipation, URTI, epistaxis, dyspnea, exfoliative dermatitis, and proteinuria. The *most severe events* detected on the same sample of patients include asthenia, pain, hypertension, diarrhea, and leukopenia. The *most serious and sometimes fatal events* include gastrointestinal perforation, would healing, hemorrhage (in particular pulmonary and intracranial hemorrhage, ATE, nephrotoxic syndrome, and RPLS. Other less common but serious toxicities includes non-GI fistula formation (trachea-esophageal, bronchopleural, biliary, vaginal, bladder), osteonecrosis of the jaw (ONJ), and nasal septum perforation.

GI perforations (GIP), serious and sometimes fatal, have been encountered during bevacizumab treatment. They also include dehiscence, fistula, abnormal healing, ischemic bowel, and abscess. Overall, their frequency range around 2 % (0.3–2.4 %) in bevacizumab-treated groups, when associated with standard irinotecan/fluorouracil/leucovorin (IFL) chemotherapy. In mCRC, rates tend to be slightly higher within this range. In a smaller group associated with FL chemotherapy, bevacizumab-induced GIP appeared to be higher (8 %), while they resulted less frequent when the biomedicine was associated to irinotecan alone (2 %). By contrast, frequencies encountered in control groups (chemotherapy alone) were 0.3 % or lower. However, a wide search for GIP associated with standard therapy (IFL) in databases and in the literature up to 2004, showed only four cases of GIP, and a definite casual relation to chemotherapy could not be ascertained [1, 13, 14]. A recent survey on 1,963 community-based valuable mCRC patients confirmed the rate of 2 % of GIP when bevacizumab was associated with standard therapy [15]. In a postmarketing study, 1.7 % of reports

referred to GIP (see below). These observations are relevant since they confirm data from clinical trials, relative to selected population of patients, in the far more heterogeneous population encountered in clinical care.

GIP has been reported in other tumor categories either included in official indications or used as off-label (NSCLC, GBM, mBC, EOC, and pancreatic cancer). Their overall frequencies were less relevant (≤1 %). However, a recent retrospective analysis reported higher GIP frequencies (3–8 %), except for GBM and for some off-label experiences, which will be discussed in the following paragraphs [16].

Non-GI perforations, serious and sometimes fatal, mainly generate fistulas at tracheo-esophageal, bronchopleural, biliary, vaginal, renal, and bladder level; they were reported as ≤0.3 %, mostly occurring within 6 months of therapy, and with higher frequency than in controls. In 2006, nasal septal perforation gained a FDA warning, and the package insert was consequently updated [1, 4].

Wound healing complications are more frequent during bevacizumab treatment and are often related to perforations. The overall frequency has been estimated as around 15 % after surgery in mCRC patients, and therefore treatment initiation was recommended after 28 days from surgery, due to the rather long half-life of bevacizumab (20 days). Other studies recommended its discontinuation at least 60 days before surgery [1, 13].

Controversial observations have been produced on ATE/VTE in relation to bevacizumab treatment. Official records report at present an average overall ATE of 2.6 %, and of serious VTE up to 15 %. ATEs are usually serious and include cerebral infarction, transient ischemic attacks, myocardial infarction, and angina. However, other observations have reported no substantial increase in the risk of ATE/VTE, while retrospective larger studies indicate an ATE incidence of 3.8 %. Part of these discrepancies may be explained by differences in the method for collecting and pooling data, but also in grading classification, and in the follow-up period [1, 13].

Severe or fatal *hemorrhage*, including hemoptysis, gastrointestinal bleeding, hematemesis, CNS hemorrhage, epistaxis, and vaginal bleeding occur at a rather low rate (3–5 %), but up to fivefold more frequently than in controls, while minor episodes, such as epistaxis lasting less than 5 min, are the most common occurring events. Premarketing studies reported serious bleeding events in 4 % of exposed subjects. *Pulmonary and cerebral hemorrhage* complications are particularly occurring at higher rates in the respective tumoral mass or nearby. Lung cancer studies on bevacizumab-treated patients indicate pulmonary hemorrhage at 9 % in NSCLC, with particularly high incidence in the squamous variant (31 %). Exclusion of this subgroup in other clinical studies may account for consistent differences in ratings of this life threatening event. The incidence of intracranial hemorrhage, either in GBM-treated patients (1–2.5 %) or in cerebral metastatic forms of other tumors, was not particularly increased with respect to control groups. However, data on this type of tumor could be underestimated because of their exclusion from selected trials.

GI-hemorrhage is the most frequent event (56 %) in mCRC, where overall serious hemorrhages ranged 3–6 % according to the tumor site location and the anti-coagulant additional treatments.

A particular association of AEs during bevacizumab therapy is related to the concomitant increased *risks of hemorrhage and thrombosis*, possibly related to the mechanism of action of this biomedicine [1, 7, 9].

All grades of *hypertension* (67 %), including hypertensive crisis and severe episodes (5–18 %), during bevacizumab treatment are dose-dependent. In particular, high dosage in mRCC increased frequency up to 36 %. However, this bevacizumab-induced AE is reversible and controllable by standard anti-hypertensive therapy [1, 13, 17].

Cardiac disorders, including CHF and LVEF were slightly increased at about the same rates (1 %) in bevacizumab-treated groups. However, resolution of these cardiac events in exposed patients was less pronounced. In mBC patients—a treatment indication not approved by FDA but endorsed by EMA—the incidence of severe CHF was increased (2.2–3.8 %) in subjects treated with bevacizumab plus paclitaxel [1, 13].

Proteinuria is rather frequent and potentially serious (3 %) during bevacizumab treatment. The overall frequency (21–63 %) is dose-dependent and is particularly severe in mRCC (7 %), with tendency to develop a *thrombotic microangiopathy* and a consequent irreversible renal damage. However, nephrotic syndrome is expected at rates lower than 1 %. Notably, renal and cardiovascular toxicities mutually synergize and affect the renal-vascular axis [16, 17].

Serious infections, usually neutropenic, occur in 4–5 % of cases, while overall severe neutropenia ranges from 21 to 26 %. Among them, pneumonia, wound, and catheter infections were predominant [1]. A recent aspect of *local infections* has been recently observed during off-label use of bevacizumab in AMD patients treated with intraocular injections.

Infusion reactions are not a major problem with bevacizumab. The rating is lower than 3 %, with severe episodes, usually occurring at first injections in 0.2 % of cases. Infusion-related hypersensitivity was reported in pivotal trials and is extremely infrequent, although potentially life threatening. Interestingly, this monoclonal—although containing approximately 10 % of murine protein—did not induce significant acute early reactions, even during short infusion regimens [1, 8, 13].

RPLS is a form of *brain capillary leak syndrome* related to hypertension, fluid retention, and cytotoxic effects of immunosuppressive agents, including bevacizumab, on the vascular endothelium. In clinical studies the observed overall incidence was <0.1 %. Two cases have been reported by postmarketing surveillance in mBC and mRCC [1, 18].

After some postmarketing spontaneous reporting of ONJ, this rare event was subsequently reported in six controlled studies starting from 2008, globally including 8 cases of ONJ after bevacizumab therapy in NSCLC, mCRC, mBC, BC, and mRCC. Overall, 55 cases of ONJ were signaled among a population of

800,000 patients treated with bevacizumab. The pathogenesis is not clear. Although ischemic damage is suspected, ONJ lesions exhibit intact vasculature at histological examination. Opportunistic local actinomycotic infections may represent an alternative pathogenesis, possibly due to potential impairment produced by VEGF inhibition on monocytes and macrophages defense mechanisms [19].

Taken together, AEs during bevacizumab treatment represent a serious concern.

10.4 Off-Label Experience

Some bevacizumab treatments for specific tumors are considered as off-label administrations in EU (e.g., GBM), but not in US, or they were considered off-label up to postmarketing extension approvals, and updates from 2004 to 2012.

Some of the AEs occurring in the off-label use may be of relevance because of their severity or potential novelty with respect to the general safety profile previously depicted.

GIPs have been detected in CRC, pancreatic cancer, LDBCL, EOC, and mBC (off-label for US) patients from 0.2 to 11 % of cases. Highest frequencies were observed in LDBCL (11 %), being about five fold over the highest frequencies reported in mCRC; in previously untreated patients, LVEF was decreased and CHF was significantly raised (10–11 %, respectively).

Interestingly, CRC was associated with one of the lowest incidence rates of perforation (1.3 %) after bevacizumab exposure, while higher rates were observed for ovarian, gastro-esophageal, and pancreatic cancers (respectively, 6, 5.3, and 5 %). Perforation occurred at the tumor site (38 %) and at anastomotic sites (17 %). GIPs in EOC were reported as <1 % or not different from controls. Recently, the incidence of bowel perforations in various tumor types during bevacizumab treatments has been reviewed [16], indicating that higher frequencies were observed in some studies on GBM (3–8 %) and ovarian cancer (3–11 %). Moreover, some predictive signs of GIP were identified (age, prior bowel surgery, obstruction/ileus, severe hypertension, carcinomatosis, heavy pretreatments). These conditions were indicated as additional risk factors, supporting the increased incidence of GIP in EOC compared to other solid tumors.

Ovarian failure was reported in adjuvant CRC therapy and reached 34 % of patients treated with bevacizumab in combination with chemotherapy, with a high relative risk (RR: 14) compared to women receiving chemotherapy alone. Notably, discontinuation of the biomedicine allowed recovery of the ovarian function in 22 % of cases [20–24].

Finally, limited experiences with bevacizumab were conducted on AML, prostatic cancers, melanoma, and multiple myeloma with no new AEs signals, yet with low tolerance and modest/poor efficacy.

10.5 AEs and Tumor Typology

The outlined overall picture of relevant AEs has showed some peculiarities of expression in single tumor categories, possibly related to their biospecificity and to the associated chemotherapy applied regimens. For example, increased incidences of the following events were observed: GI hemorrhage in mCRC, lung hemorrhage in NSCLC, intracranial hemorrhage in GBM, GI and abdominal hemorrhages in pancreatic and ovary cancer, possibly indicating a local enhanced (neo) vascular fragility due to the presence of the primary tumor.

Interestingly, in metastatic cerebral tumors, intracranial hemorrhage ratings did not increase, while in a different pulmonary tumor histotype (the squamous variant) the life threatening local hemorrhage sharply increased up to 31 %. Proteinuria increased (20 %) in mRCC, where overall serious events reached 31 % when bevacizumab was combined with IFL treatment. In mBC, the addition of bevacizumab to two standard chemotherapies, including paclitaxel and docetaxel, sharply increased the overall serious toxicity at about 20 %, and death rates at approximately 2 %. The safety worsening involved both AEs attributed to bevacizumab and unique reactions related to chemotherapy (such as palmar-plantar erythrodysesthesia and peripheral sensory neuropathy), thus suggesting the existence of reciprocal synergistic AEs-inducing effects. Surprisingly, GIP did not particularly increase in mCRC compared to other extra-intestinal neoplasms [25, 26].

10.6 The AMD Experience

The most interesting off-label administration of bevacizumab relates to its intravitreal (IVT) administration in wet AMD patients. In fact, it is still the most used monoclonal employed in this disease, due to its low cost compared to ranibizumab. This treatment was attempted also in proliferative diabetic retinopathy, even before large studies and trials had been conducted.

Previous studies were performed by standard intravenous administration, which induced a few systemic events, such as a transient hypertension. Results from the subsequent pivotal CATT trial reported serious systemic events occurring in 24 % (19 % with ranibizumab) of cases, while local serious events (uveitis, retinal detachment, ocular-vessel occlusion/embolism, retinal tear, vitreous hemorrhage) occurred in <1 % of cases. Recently, two additional studies have allowed a more stringent safety and efficacy comparison between bevacizumab and ranibizumab [26, 27]. Both direct (3 trials, 1,333 patients) and indirect comparisons (5 trials, 4,054 patients) were performed, including the pivotal CATT trial. Direct confrontation showed an increased relative risk of ocular AEs (RR: 2.8), serious infections, and GI disorders (RR: 1.3) with bevacizumab, while ATE events resulted equally distributed. Moreover, two significant signals came from hospitalization rates due to sepsis, pneumonia, or gastrointestinal disorders and possibly

from an increased risk of non-ocular hemorrhage following the intravitreal use of bevacizumab (RR: 1.3). Indirect comparison data showed that the absolute rate of ocular SAEs was lower (≤2 %) with ranibizumab, although the relative harm issue was raised and cumulative risk was expected to increase with repeated injections. However, a significant raise in non-ocular hemorrhage was also observed with ranibizumab (RR: 1.7). No significant differences between the two mAbs were recorded about deaths and ATE/VTE events, which were comparable to frequencies of the general AMD population. Another recent review on over 91,000 adults from a Canadian database, with a history of retinal diseases, and exposed to either or both anti-VEGF agents, focused on major vascular AEs and in particular to ischemic stroke, MI, CHF, and VTE as the events most potentially susceptible to increase after anti-angiogenic therapy. Incidences of these events ranged from 2 % to 3.6 %, which resulted to be not significantly higher than control groups. However, in diabetic proliferative retinopathy, in contrast with previous observations on small groups of patients, the large postmarketing analysis revealed a possible increase in MI in the bevacizumab-treated population [27].

10.7 Postmarketing Surveillance

Over 37,000 reports had been registered for bevacizumab by the end of 2012 in the FAERS database. Infections (6.2 % of reported events), GI signs (5.5 %), hematological (3.8 %), respiratory (3.5 %) disorders, and deaths (2.8 %) were the most common and relevant events. Among infections, pneumonia (430 reports) and sepsis (390) were the most frequently reported. Although it was not possible to individuate cases of off-label IVI administration within the reports, over 300 of them referred to endophthalmitis.

In the EUV database, 17,672 (99 % serious) reports had been registered up to 2012, including 33,562 AEs (1.9 AE/P). GI signs (20.4 % of events), neurologic signs (8 %), infections (5 %), hematologic disorders (4 %), malignancies (4 %), cutaneous disorders ((3 %), and renal disorders (2.5 %) were among the most frequent events. Interestingly, 1,569 reports included eye disorders (about 5 %), presumably subsequent to IVT administrations, including 107 uveitis, 63 retinal hemorrhage, and 60 retinal detachments. GI perforations were 333 cases (66 % in large intestine). Sepsis reports were 172, with 64 septic shock and 138 cases of pneumonia. Hypertension (597), ATE (26), and DVT (367) were the most frequently reported vascular events. Moreover, 243 cases of proteinuria, 133 cases of acute renal failure, 86 nephrotic syndromes, and 6 cases of bladder perforation were included.

Notably, 89 cases of anaphylactic shock, 39 cases of anaphylactoid reactions, and 2 cases of IgE mediated reactions were reported. Moreover, 136 cases of palmar-plantar erythrodysesthesia represented the most common and relevant event. Malignancies were mostly represented by disease progression.

10.8 Remarks

The systemic administration of bevacizumab carries serious and potentially life threatening toxicity risks. Its expanding use in combination with new chemotherapy and/or targeted therapies for oncological indications emphasizes the importance to have a clear understanding of the overall safety profile, of serious toxicities, and of potential toxic synergies with associated therapy. In addition, particular tumor types show a higher trend to develop individual toxicities with bevacizumab, which should be taken into consideration. For example, patients with squamous NSCLC and CRC have a higher risk of bleeding, patients with RCC have a higher risk of severe proteinuria, and patients with EOC, LDBCL, and CRC have a higher risk of GIP.

Some hypotheses on major AEs pathogenesis have been suggested. As for GIP, the reduction of blood supply to the tumor, induced by bevacizumab, is a necrotic factor not only for the tumor but also for the surrounding mucosal tissues. In fact, VEGF is also expressed on intestinal mucosa. As for the paradoxical increase of hemorrhage and thromboembolic events during some bevacizumab regimens, it has been postulated that VEGF inhibition reduces the viability and renewal capacity of endothelial cells; the interior vascular lining becomes altered, thus inducing bleeding and thrombotic events. As for wound healing, since new angiogenesis is crucial for tissue repair, VEGF inhibition by bevacizumab is expected to interfere with the process of healing. Hypertension and some renal dysfunctions may be related to the nitric oxide synthase reduction caused by VEGF inhibition. This decrease leads to vasoconstriction, which ultimately results in an elevation of blood pressure. VEGF is also constitutively expressed on renal podocytes, and its receptors are found on normal glomerular capillary endothelial cells. Therefore, VEGF apparently plays a pivotal role in blood pressure regulation and in glomerular capillary permeability. Consequently, its blockade by bevacizumab produces hypertension and proteinuria, respectively. Noteworthy, VEGF also stimulates angiotensin II receptors and therefore anti-VEGF agents seem to induce their rarefaction and secondary blood pressure elevation.

By contrast, some aspects of bevacizumab selective toxicity may offer suggestions for better therapeutic strategies. Usually, AEs related to bevacizumab differ from the typical toxicities of chemotherapeutic agents (myelosuppression, neuropathy, constitutional signs, etc.). When combined in therapy, they do not increase reciprocal toxicities, except for some associations experienced in mBC (paclitaxel, docetaxel), but seem to synergize in efficacy by combining anti-neoplastic and anti-angiogenic effects against the tumor. Second, different efficacy and toxicity of bevacizumab have been experienced in metastatic and non-metastatic tumors. A possible link between antiangiogenic therapy and increased metastatization has been postulated on the basis of experimental and clinical investigation [28].

An additional phenomenon observed in off-label ocular therapy with bevacizumab is non-cross reactive tachyphylaxis with ranibizumab, which may be relevant also in terms of risk/benefit evaluation. This rapidly decreasing

therapeutic effect seems to develop more frequently within the eye microenvironment, and at rates of about 2 % of cases with bevacizumab. Surprisingly, in one reported case of cystoid macular edema, the progressive reduction of response to bevacizumab did not affect the subsequent administration of ranibizumab, which showed to be fully effective [29].

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Brentuximab

11

Brentuximab vedotin (Adcetris™, Seattle Genetics; Takeda) is a chimeric IgG1k monoclonal antibody conjugated to the cytotoxic agent monomethyl auristatin E (MMAE). The complex (BV), targeting specifically CD30 (Ki-1 Ag; TNFRSF8), received an accelerated approval by FDA in 2011 for the treatment of relapsed Hodgkin's lymphoma (HL), after failure of autologous stem cells transplantation (ASCT) or after at least two unsuccessful multiagent chemotherapy regimens in patients who are not candidates for ASCT. BV was also indicated for systemic anaplastic large cell lymphoma (sALCL) in post-chemotherapy relapse.

BV was previously recognized as orphan drug for HL in 2007, for sALCL in 2008, and more recently (2012) for mycosis fungoides (MF). Meanwhile, EMEA in 2009 had recognized the orphan drug status for HL and NHL, and in January 2012 for the treatment of cutaneous TCL (cTCL). In July 2012, the European CHMP released a positive opinion for HL and sALCL indications, and conditional approval was released in October 2012 for the two following categories: (i) adult patients with relapsed/refractory CD30+ HL following ASCT, or after at least two therapies when ASCT or multiagent chemotherapy is not an option; (ii) adult patients with relapsed or refractory sALCL. In April 2013, the Swiss Authority (SATP) granted BV approval for the same two indications.

Two pivotal Phase II single-arm, single agent, multicenter trials (SG035003; SG35004) enrolling 102 HL and 58 sALCL patients respectively, collected efficacy and safety data on the whole cohort of patients. Supportive data on safety and efficacy came from two Phase I open-label trials (SG350001; SG350002) on additional 123 HL patients [1–4].

At present, initial favorable results have stimulated 37 trials, mostly ongoing and enrolling patients with HL, ALCL, cutaneous ALCL, sALCL, GVHD, stem cells transplanted hematologic malignancies, TCL, NKCL, DLBCL (EBV +), and various solid tumors.

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11.1 Mechanism of Action

CD30 is a 120-kDa cell surface glycoprotein. Together with its ligand (CD30L), they are members of the tumor necrosis factor (TNF) superfamily. The expression of CD30 is mostly restricted to a subset of activated T cells (Th2-type producing cytokine) and to virus–infected lymphocytes, while the ligand is present on activated T cells, B cells, granulocytes, and in the medulla of the thymus (epithelial cells and Hassall's corpuscles). Th2 cells produce IL-4 (having an autocrine effect), IL-5, and IL-13, which are active on B cell switching to synthesize IgE, and on effector cells (eosinophils, basophils, mast cells). Therefore, they are implicated in Type I allergic reactions.

The CD30/CD30L binding induces co-stimulatory signals, via TNF-associated factors (TRAF), which mediate transduction leading to NF-kB activation, promoting survival and proliferation, but also cell death via apoptosis. In animal studies, the signaling initially promotes cell survival and subsequently leave T cells more prone to apoptosis, possibly contributing to regulate the Th1/Th2 physiological balance. Moreover, the pathway inhibits potential autoreactive CD8+ effector T cells, thus acting against potential autoimmune aggression. Subsets of CD8+ memory cells, naive and regulatory CD4+ T cells expressing high levels of CD30 are present also during GVHD [5, 6]. CD30 can be cleaved from cell surface and released in low concentrations into circulation of normal individuals (sCD30 of 85 kDa). However, sCD30 is significantly increased in patients with GVHD, SLE, RA, in some viral infections and in lymphoproliferative disorders. HL and NHL cells, including Reed-Sternberg cells, overexpress this receptor (CD30hi) suggesting the existence of a common pathway involved in lymphomagenesis. In contrast with normal cell populations, CD30 expression on neoplastic cells promotes only survival and proliferation. Recently, the presence of CD30 on a wide series (875 patients) of solid tumors and nonlymphomatous malignancies has been investigated, showing that positivities are spread over different histotypes of neoplasms, with CD30 positivity ranging from 10 % to 80 % of malignant cells [7].

Brentuximab-vedotin (SGN-35) is an antibody-drug conjugate (ADC) made of a chimeric murine/human IgG1k specific for human CD30, conjugated by a protease-cleavable linker to the microtubule disrupting agent monomethyl auristatin E (MMAE). In particular, the original monoclonal antibody (cAC10, or SGN-30) is covalently linked to MMAE (SGD 1010) by a small linker (SGD-1006). An average of four MMAE molecules are thus linked to a single cAC10 antibody. After binding to CD30-expressing cells, the complex is internalized and within 4 h reaches lysosomes where MMAE is released, thus disrupting the microtubule network and subsequently inducing cell-cycle arrest and apoptosis [1, 3].

However, after intracellular release, free MMAE is found in circulation and therefore it may exert toxic effects on bystander cells. Moreover, spontaneous release of MMAE has been observed in CD30 negative animal models (rat). BV induces phagocytosis but not CDC or ADCC immune effector functions [3, 8–10].

11.2 Immunogenicity

The presence of transient HACA was detected in 30 % of cases (7 % of them had persistent levels). Positivity was related to neutralizing antibodies in 62 % of them. However, only 1 % of positive cases were associated with infusion reactions that caused discontinuation of therapy [1, 2, 10].

11.3 Adverse Events

AEs were observed in a relatively small number of patients (160) enrolled in the two pivotal trials and treated with BV as monotherapy, and in the two previously mentioned Phase I studies (123). A total of 261 patients received BV at the proposed doses and standard protocol. In particular, the majority of the AEs data derive from HL patients enrolled in Study SG035003. Data from sACLC were limited in number and typology, and therefore used as aid for potential identification of rare events. There were no substantial differences in the most common AEs between HL (102) and sALCL (58) cohorts of patients. The overall safety profile includes *peripheral neuropathy*, *infusion reactions*, *myelosuppression*, *tumor lysis syndrome* (TLS), *progressive multifocal* l*eukoencephalopathy* (PML), and *Stevens-Johnson syndrome* (SJS). In January 2012, a BBW for PML was included in the labeling information.

Most frequent *severe/serious reactions* (grade 3–4) *in HL* included neutropenia (21 %), anemia (10 %), thrombocytopenia (9 %), peripheral sensory (8 %) and motor neuropathy (4 %), fatigue (3 %), abdominal pain (3 %), pulmonary embolism (2 %), pneumonitis (2 %), dyspnea (2 %), pneumothorax (2 %), pyelonephritis (2 %), pyrexia (2 %), anxiety (2 %), dyspnea (1 %), and diarrhea (1 %). *In sALCL* these events were neutropenia (21 %), thrombocytopenia (10 %), peripheral sensory neuropathy (10 %), pain (5 %), pain in extremities (4 %), fatigue (4 %), motor neuropathy (3 %), septic shock (3 %), supraventricular arrhythmia (3 %), diarrhea (3 %), urinary tract infections (3 %), decreased appetite/weight (2–3 %), anemia (2 %), cephalea (2 %), pyrexia (2 %), musculoskeletal disorders (2 %), and nausea/vomiting/constipation/abdominal pain (2 % each).

A slight effect on cardiac ventricular repolarization (QT) was detected in all groups. One case of SJS was observed in the HL arm, although in concomitance with naproxene administration [1–4; 11].

Overall, in the two pivotal studies severe events were observed in approximately 55 % of cases, while serious events (SAEs) were 31 % and drug-related adverse events (DRAEs) were detected in 15 % of patients. These data are waiting for confirmation from other ongoing trials (NCT501100502 -AETHERA-, NCT01060904), and in particular after retreatment, and for the control of residual disease. In particular, interim results reported as most common adverse events peripheral sensory neuropathy (47 %), fatigue (46 %), nausea (42 %), upper

respiratory tract infection (37 %), diarrhea (36 %) and neutropenia (77 %). The most common grade 3 or 4 adverse events were neutropenia (74 %), febrile neutropenia (16–20 %), peripheral sensory neuropathy (8 %), thrombocytopenia (8 %), and anemia (6–13 %).

Peripheral neuropathy (52–55 %; 39 % sensory, 3 % motor, and 12 % mixed), mostly moderate, was the major cause of discontinuation, and 55 % of cases were judged as DRAEs together with a smaller cohort of functional nervous disorders (para/hypoesthesia, weakness). Major concerns are about the cumulative exposure to BV, and to substantial residual signs of neuropathy, which was infrequently severe yet recorded up to 35 weeks after treatment.

The second relevant group of AEs was related to *myelosuppression* (neutropenia, anemia, thrombocytopenia) occurring in 73 %, with severe ($\geq$3) cases ranging 9–21 %. This risk was not clearly dose-related.

Infections were frequent (64 %), albeit mild and limited to URTI (47 % in HL patients; 12 % in sALCL) and pneumonitis. Serious events ranged about 8 % of them.

Infusion reactions were usually mild/moderate in both groups and were induced in 12–14 % of patients. Two cases of anaphylaxis (one anaphylactic shock) were reported in a previous Phase I trial, but not in subsequent pivotal trials.

By June 2012, two additional cases of *PML* were experienced in the pivotal HL study, thus reaching a total of three cases among about 2,000 BV-treated subjects. One *TLS* and one *SJS* were reported, as well.

Due to the short experience so far accumulated, these data and additional cases of uncertain attribution (such as GI hemorrhage, pneumonitis, pulmonary embolism and hyperglycemia) are to be confirmed and should be considered with caution.

It must be noted that the median duration of treatment in pivotal studies was 20–27 weeks during which a median number of 6–9 cycles of therapy were performed.

11.4 Off-Label Experience

First-line treatment of HL, treatment of residual disease, and associations with other chemotherapy regimens are currently under investigation, but data are still limited. According to the interim data on 31 HL patients in first-line treatment, AEs included expected reactions such as peripheral neuropathy (48 %), fatigue (45 %), and neutropenia (77 %). Severe events were neutropenia (74 %), febrile neutropenia (16 %), and anemia (13 %). However, the BV administration associated with chemotherapy including bleomycin sharply increased pulmonary toxicity (dyspnea, interstitial lung disease) up to 28–40 %, causing a warning from FDA against such combination [11]. Treatments of other types of lymphoma, cutaneous lymphoma, leukemia, multiple myeloma, and number of nonlymphomatous malignancies are also ongoing and waiting for definitive reports [7].

A recent analysis was dedicated to the safety of brentuximab treatment in HL recurrence after allogenic stem cell transplant (alloSCT). This procedure is employed as second-line treatment after recurrence post-ASCT, with the aim of controlling the disease by inducing chronic GVHD versus lymphoma. All 25 enrolled patients experienced at least one AE.

Overall, the safety profile was similar, but in the post allo-SCT group some events showed a trend to higher frequencies over grade 3 [12].

In a similar approach, BV has been used as a bridge to stem cell transplant in HL [13]. In this case, the addition prior to allo-SCT did not adversely affect engraftment, insurgence of GVHD, or survival. Moreover, AEs showed a mild/moderate expression. The most common events (≥20 %) were mild renal toxicity (43 %), GI and hepatotoxicity (36 %), and stomatitis (43 %). Peripheral neuropathy was observed in 8 patients (32 %) before allo-SCT, and improved after transplant in all but one of them. No severe (≥grade 3) AEs were detected. EBV (11 %) and CMV (17 %) PCR reactivation was documented, without clinical manifestation of infection. Therefore, BV seemed to provide sufficient control of disease on selected patients, without producing relevant AEs, thus allowing allo-SCT. Since CD30 has recently been found increased during acute GVHD [6], one trial was launched on the effects of BV on steroid-resistant GVHD.

11.5 Postmarketing Surveillance

Up to the end of 2012, 316 reports on BV were registered in the FAERS database, including 128 cases of respiratory disorders, 131 infections, 65 neurological disorders (including 40 PNP), and 13 PML reports. No TLS or SJS cases were reported.

During the 5 months after EMEA approval, EUV received 130 reports about 28 infections, 34 respiratory disorders, 22 neurological disorders, including 7 PNP and one case of reversible posterior leukoencephalopathy syndrome (RPLS), being presumably the first case after BV treatment.

11.6 Remarks

AEs to BV treatment seem manageable and not of particular concern, although the short time experience suggests caution. Neutropenia, peripheral sensory neuropathy and PML are, so far, the major signals. The complex ADC expresses toxicities either related to the chimeric antibody or to the MMAE component, which is an antimitotic drug inhibiting tubulin polimerization. Upon internalization, primarily via clathrin-mediated endocytosis, and exposure to proteolytic enzymes in lysosomes, the ADC linker breaks down, releasing the cytotoxic MMAE from the monoclonal antibody. As for their respective class effects, the chimeric antibody is expected to produce infusion reactions and myelosuppression, while the

microtubule disruption-agent is known to induce neuropathy and myelosuppression [2, 3, 8, 9]. Moreover, the freed MMAE is toxic to cells regardless of CD30 expression status. Therefore, CD30 negative cells, including T and B resting cells, may be involved in adverse reactions. This "innocent bystander" effect may be enhanced by the fact that a small fraction of drug diffuses out of the targeted tumor cell and exerts cytotoxic effects on the surrounding tumor microenvironment, thus reaching circulation.

Another aspect of the potentially different toxicity expressed by the two components of this ADC is their half-life (measured in rat): 8–15 days that of monoclonal antibody, 5 h-3 days that of the linker (SGD1006)—MMAE (SGD 1010).

Despite the fact that the BV complex is rather stable (in Cynomolgus monkey lasts about 230 h) due to the presence of a dipeptide linker, eventually the unconjugated monoclonal component will compete with ADC, both in efficacy and in toxicity actions, in binding to CD30. Moreover, the amount of free MMAE, which is proportional to the injected dose of BV, contributes to expand additional asynchronous toxic effects (ADC half-life 4–6 days; MMAE 3–4 days).

Finally, the presence and the possible increase of soluble CD30 cleaved from disrupted cells surface during treatments competing with ADC, should be also considered in relation to efficacy and AEs insurgence and evolution.

So far, the low expression of CD30 on normal resting cells and its overexpression on a variety of malignant counterparts, together with the low immunogenicity and the selected intracellular killing produced by this ADC, are consistent safety advantages of BV, which deserves further investigation in a wider area of malignancies, including solid tumors. However, in some of these experiences, the alternative possibility of using only the "naked" anti CD30 mAbs should be further explored, possibly overcoming some of the mentioned limitations, perhaps by identifying cell-membrane specific CD30 epitopes or bispecific antibody fragments (diabodies). The latter forms, being of lower molecular weight, are expected to better penetrate the target and induce less adverse events [14].

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Canakinumab

12

Canakinumab (Ilaris®, Novartis) is a human IgG1k monoclonal anti-human antibody, which selectively binds to interleukin-1β (IL-1β) and neutralizes it.

It was approved by FDA in 2009 for the treatment of a rare group of cryopyrin-associated periodic syndromes (CAPS), known as inherited autoinflammatory diseases, resulting from an excess of IL-1β secretion. The same year, EMEA designated this biomedicine as orphan drug for the same indications. Health Canada released full approval in 2010. In particular, the official indications of all Agencies relate to Muckle-Wells syndrome (MWS) and familial cold autoinflammatory syndrome (FCAS). Health Canada also indicated the treatment of neonatal-onset multisystem inflammatory disease (NOMID), and of chronic infantile neurological, cutaneous, articular syndrome (CINCA). These indications were approved also by EMEA, together with the severe forms of familial cold urticaria (FCU) presenting signs and symptoms beyond cold-induced urticarial skin rash. All the mentioned syndromes are included in the CAPS group.

Pivotal study D2304 on 97 CAPS/MWS patients consisting in three parts (two uncontrolled arms, one placebo controlled double blind arm), and supportive open-label studies A2102 and D2306 on 95 FCAS/MWS/NOMID patients, was the basis for initial approval. All studies included adult and children subjects treated with SC injections (IV administration was performed in pharmacokinetic preliminary investigations).

CAPS are lifelong, multi-system, autoinflammatory diseases consequent to genetic mutations in the NLRP3/CIAS1 gene on chromosome 1q44 producing an excess of caspase-1 activity, resulting in increased production of IL-1β. The NLRP-3 gene encodes cryopyrin, a component of the inflammasome, which regulates the protease caspase-1 and controls the activation of IL-1β. Mutations in NLRP-3 result in an overactive inflammasome, and in a consequent excessive

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release of activated IL-1β that drives inflammation. Patients with CAPS exhibit overlapping phenotypes. They are inherited in an autosomal dominant pattern equally affecting both sexes, and usually appear in early childhood. NOMID/CINCA are the most severe and debilitating forms of CAPS, with symptoms appearing shortly after birth. Spontaneous remission does not occur in CAPS diseases. However, patients with the FCAS phenotype may experience improvement of symptoms during sustained warmer weather. Features common to all disorders include pyrexia, urticaria-like rash, arthralgia, myalgia, fatigue, and conjunctivitis. The most serious clinical outcomes include visual and auditory impairment, deafness, amyloidosis, kidney failure, growth failure, and intellectual disability [1–4].

12.1 Mechanism of Action

The group of IL-1 cytokines (11 proteins, two classes: IL-1α and IL-1β) are involved in the inflammatory response, being also identified as endogenous pyrogens, and as inducers of prostaglandin and collagenase release. The two IL-1 classes have different structure yet similar functions, and share the same cell receptors. These cytokines increase the expression of adhesion molecules on endothelial cells, enhance transmigration of leukocytes, induce vasodilatation (via prostaglandins stimulation), and pyrexia. Inflammation is influenced by the relative amounts of IL-1 bound to Type I receptor (IL-1RI) or to a Type II receptor antagonist (IL-1Ra), acting as a competitor and regulator of IL-1 signaling. Dysregulation of such mechanism may induce autoimmune and autoinflammatory disorders.

IL-1β is a pro-inflammatory cytokine produced by various cell types, including activated macrophages, mast cells, endothelia, synovial cells, keratinocytes, fibroblasts, microglia and astrocytes, neuronal and Schwann cells. IL-1β stimulates thymocytes and T lymphocyte proliferation by inducing IL-2 release, maturation and proliferation of B cells and dendritic cells, and induces mobilization of neutrophils and platelets from the bone marrow. The synthesis and release of IL-1β requires two distinct signals (for synthesis and assembly), which are normally initiated by pathogen-associated molecular patterns (bacterial RNA, lipopolysaccharides), but also by endogenous triggers (uric acid, heat shock proteins). Therefore, this cytokine is implicated not only in inflammatory processes after injury, stress, and infections, but also in acute and chronic autoimmune diseases, diabetes, pain, and neurological disorders.

In CAPS there is an over-secretion of IL-1β (up to five fold higher than in healthy subjects) and an increased expression of IL-1Ra, which apparently is not sufficient to counteract the IL-1β excess and activity.

Canakinumab is a high-affinity fully human IgG1k mAb generated in transgenic mice immunized with a recombinant form of human IL-1β, and subsequently expanded in the SP2/0 murine cell line. The light-chain transgene encodes for

nearly half of the human Vκ region, while the heavy-chain transgene encodes for human μ and γ constant regions. Canakinumab binds to human IL-1β blocking the interaction with its receptors and thereby functionally neutralizing its bioactivity. Although the epitope appears to be outside the IL-1β/IL-1RI interface, the canakinumab/IL-1β complex is unable to attach at the cell surface receptor, and therefore does not allow IL-1β signaling. Noteworthy, this mAb does not bind IL-1α or IL-1Ra. The complex is eliminated at a much slower rate than free IL-1β, which leads to an elevation of total IL-1β (free plus complex) following the administration of canakinumab, and to a decrease of IL-1β-induced downstream mediators including free IL-1β itself, the production of IL-2 and of acute phase proteins, such as serum Amyloid A (SAA) and C-Reactive Protein (CRP). CRP and SAA are known indicators of inflammatory activity and are elevated in CAPS; following treatment, their levels tend to normalize within 8 days. Elevated SAA has been also associated with the development of systemic amyloidosis in CAPS patients. Finally, there is evidence that canakinumab down-regulates the production of IL-1β [5–9].

12.2 Immunogenicity

The presence of anti-canakinumab antibodies was tested in 60 CAPS patients, with negative results. In subsequent various studies and trials such antibodies could not be detected, even when measured by sensitive and validated binding assays, thus indicating a low immunogenic potential of canakinumab [8]. Similarly, no antibodies could be detected in a number of off-label studies (RA, JIA).

12.3 Adverse Events

Initial pivotal D2304 trial, and two open-label studies (A2102 and D2306) on canakinumab safety refer to a small sample size of 78 adult and pediatric (15) patients with CAPS (63 MWS, 5 MWS/NOMID, 9 FCAS, and 1 NOMID). These data could not be pooled due to diversities among trials and to participation of some patients to more than one study. Because of the rarity of these diseases, additional safety data were collected from a mixed cohort of 57 RA (Study A2101) patients, and from 95 healthy subjects. In the following 2012 update report [4], the CAPS sample increased to 104 patients (72 MWS, 20 FCAS, 10 MWS/NOMID, 1 NOMID, 1 unspecified). Overall, by March 2012 a total of 833 subjects had been treated with canakinumab in clinical trials on CAPS and other diseases also enrolling healthy subjects. SAEs were reported in 1.8 % of cases. However, detailed safety data reported in the last FDA approved label refer to the 35 CAPS patients of the initial pivotal study [1–4].

From this experience a general warning was put about *serious infections, hypersensitivity*, *immunosuppression,* and *immunizations*. However, the latter two warnings were not based on direct canakinumab treatment experience, but on a

potential immunosuppressive activity of anti IL-1 therapy and of TNF-blocking drugs. Therefore, such association was not recommended; vaccinations with inactivated (killed) products were recommended (prior to canakinumab therapy initiation); live vaccine administrations were contraindicated.

Leukopenia and neutropenia were not observed in CAPS-treated patients. However, neutropenic patients had been excluded from these trials, but had been included among the RA patients in study treated with canakinumab (as off-label) [3].

According to the pivotal study including all the three parts, the most common AEs observed in 81 treated-patients were *infections*, as nasopharyngitis (11–34 %), viral infections (3–19 %), UTI (3–13 %), and URTI (3–7 %). Overall infections were 74 % in this study. Serious and opportunistic infections in CAPS-treated patients were reported, but their frequencies were not given.

Vertigo (6–10 %) and *injection site reactions* (3–13 %) were among the very common type of reactions. Other AEs collected only from part 1 of the pivotal study (35 treated patients) also include diarrhea (20 %), influenza and rhinitis (17 % each), nausea and cephalea (14 % each), bronchitis, pharyngitis, gastro-enteritis, musculoskeletal pain, and weight loss (11 % each). Noteworthy, most of these events were mild/moderate. Vertigo, a known complication in severe CAPS, was present only in MWS patients, classified as serious in two patients (3 %), and tended to resolve during treatment. No anaphylactic/anaphylactoid reactions were recorded. The pediatric AEs profile did not differ from the adult population, except for a moderate tendency to develop more infections.

Finally, on the basis of an observed *conversion of TB tests* (in 6/44 CAPS patients from D2034 and A2012; 4/48 in RA patients from A2101 studies) during treatment, caution was expressed on possible, yet not evidenced, risk of TB reactivation after canakinumab administration [1].

A recent open-label Phase III study conducted on 166 patients and extended for two years observations confirmed the general good tolerability of canakinumab [10]. About 90 % of treated subjects showed at least one AE of mild/moderate severity. Infections were the most common event in pediatric/adult patients (74/62 %). Noteworthy, in this trial higher doses of canakinumab were used, but the AE profile was not substantially changed. SAEs were more frequent in pediatric (13 %) than in adults (11 %), and mainly showing as URTI (11 %). Injection site reactions (8 %) and drug-related vertigo (5 %) were mild. No cases of TB, deaths or cluster signals were identified. Discontinuation rate was approximately 2 %.

12.4 Off-Label Experience

To date, the majority of off-label clinical studies with canakinumab have been performed in RA, systemic juvenile idiopathic arthritis (SJIA), and gout arthritis (GA). Minor studies include Type 2 diabetes, COPD, AMD, psoriasis, asthma, and Behçet's syndrome. Some of these attempts were dismissed due to poor efficacy, not because of AEs.

In a recent trial on RA, safety data were collected from 246 patients treated (SC) with canakinumab. Overall, AEs were observed in 53 % (range: 46–56 %) of cases and were mostly mild and dose-unrelated. SAEs were present in about 5 % of patents, mostly related to URTI. Injection site reactions were <8 %. No changes in hemato-biochemical parameters were observed, except for three patients receiving high doses of canakinumab (300–600 mg/kg given SC and/or IV), who showed a 3–6 fold transient increase of ALT/AST, although spontaneously decreasing to normal levels during treatment [11].

Preliminary data on SIJA have been recently collected from a dose escalation trial on 22 children treated with canakinumab after discontinuing other selected therapies including anakinra. At least one AE was observed in 96 % of patients, while drug-related SAEs were restricted to two cases (one EBV infection, and one gastroenteritis complicated by a coagulation disorder and syncope). Most frequent events included cough (39 %), pyrexia (35 %), and gastrointestinal disorders (26–35 %). URTI, including nasopharyngeal and tonsillitis ranged 13–17 %. Most events were mild to moderate. No AEs clusters, severe infusion reactions, MAS or drug-related deaths were observed during the study. Moreover, neither anti-mAb antibodies, nor dose–response effects were detected [12].

Experience in GA did not produce convincing evidence of efficacy. On the basis of eight studies—including the initial experience in trial A2212 on six patients [1], two pivotal trials and respective extensions on acute gout (on 660 patients), and one large Phase II trial on chronic gout—FDA denied approval to the extension to acute GA attacks, because of risk/benefits imbalance. Safety was assessed on about 700 patients from six trials, receiving 1–4 doses of canakinumab in comparison with triamcinolone or colchicine. SAEs were more frequent (7 %)—especially as infections (19 %) and serious infections (2 %)—than in controls, where serious infections were absent. Noteworthy, some AEs occurred even after a single dose of canakinumab and appeared to be associated with decline of renal function, hypertriglyceridemia, and serum uric acid elevation. These latter events occurred in a greater proportion of canakinumab-treated patients although of moderate level, but may be relevant for the gout patients' population [13].

A more recent analysis was conducted in 456 GA patients (225 treated with a single dose of canakinumab, 174 continued in extension studies) from two new Phase III trials (β-RELIEVED and β-RELIEVED-II). Canakinumab was either injected SC or IM upon new flare appearance in frequently flaring patients who were intolerant or unresponsive to non-steroidal anti-inflammatory drugs and/or colchicine, or to whom such drugs were contraindicated. AEs were reported as 66 % (severe 6 %), infections as 20 %, and serious infections as 2 % of canakinumab-treated patients, all being greater than controls. Serious infections included submandibular and limb abscess, pneumonia, and gastroenteritis, all encountered in the treated group. No opportunistic infections were detected. Interestingly, injection site events occurred in almost 1 % of SC, and 4 % of IM administrations. Cephalea, hypertension, GGT increase, arthralgia, and lumbalgia ranged 4–6 %. Notably, these studies confirmed increase of serum urate, imbalance in lipid parameters, and absence of anti-canakinumab antibodies. Moreover,

there was a decrease in platelets, neutrophils and WBC, although not associated to serious infections or bleeding. Importantly, retreatment with canakinumab did not increase the incidence of AEs or SAEs [14].

12.5 Postmarketing Surveillance

On 462 reports to FAERS, with an average of 4.9 AE/R, 199 related to infections (86 bacterial; 54 viral; 20 cases of sepsis), 131 to cutaneous reactions, GI, and respiratory disorders (about 100 reports each). Myelosuppression was reported in 32 cases. Urticaria was reported in 16 cases. Seven cases of vertigo and six cases of disseminated intravascular coagulation (DIC) were also indicated.

In the EUV database, 98 reports (96 containing serious AEs) were registered up to the end of 2012, including 43 infections, 30 respiratory disorders, 28 cutaneous reactions (10 rash, 5 urticaria), 24 GI disorders, and one case of vertigo. Interestingly, one case of CRS (cytokine storm) was also reported.

12.6 Remarks

Canakinumab has shown to be well tolerated in a number of inherited autoinflammatory diseases. However, the number of treated patients is limited due to their rarity, and long-term safety observations are still limited. The low discontinuation rates further suggest that canakinumab is equally well tolerated among adults and children. However, canakinumab is also associated with an increased risk of infection and a limited number of serious infections, although no opportunistic infections were encountered. Other notable adverse events include gastrointestinal disorders and vertigo. Overall, serious events are in the range of 2 %. No anti-canakinumab antibodies were detected in all the examined studies.

Since positive responses were experienced also in some off-label treatments, it is presumable that the use of this biomedicine will expand. With this prospect, some considerations appear worthy. The risk/benefit of canakinumab must be considered in the light of possible applications expected to primarily provide a symptomatic benefit versus some serious, albeit rare, AEs. The clinical controlled studies so far conducted mostly among the rare diseases had small sample sizes, especially for the pediatric population. Therefore, the potential risk for SAEs, such as systemic infections, may be underestimated in such small cohorts of patients. Potential cumulative risk of canakinumab in combination/sequence with other pharmaceutical agents, including other IL-1 blockers and TNF inhibitors, has not been investigated in controlled studies. Therefore, the safety profile in other diseases requiring long-term treatments, such as autoimmune diseases, may expose to unexpected risks, not predictable on the basis of the accumulated experience.

The long-lasting activity of this biomedicine may have double sward effects. Canakinumab has a long half-life and extended pharmacodynamic action. This may represent a real downside, until further and long-term studies investigate the role of the prolonged and profound neutralization of IL-1 and possible consequences on infections or on the whole immune response. The possibility to administer canakinumab as single dose or every 2 months with no implications for compliance, and minimal injection-site reactions, may be attractive. However, safety data suggest that adverse effects, even after a single subcutaneous injection, may be relevant and protracted.

The apparent absence of dose–response effect of canakinumab, both in efficacy and AEs induction, deserves some better explanations beyond the proposed "small sample cohort" bias.

In conclusion, if the dramatic role of canakinumab in the symptomatic treatment of CAPS has been well demonstrated, particularly in patients with FCAS and MWS, its application in other diseases offers more imbalanced risk/benefits, which need further careful evaluation. An interesting potential application for canakinumab, for safety consideration, is the possibility of decreasing/avoiding drugs with a higher risk of AEs by introducing low-reactive biomedicines in combined therapy, as for steroids vs. canakinumab in SJIA [12]. Finally, some patients, as experienced in CAPS, may not respond to canakinumab, which exclusively would expose them to risks of these costly treatments. Comparative efficacy and safety of similar biomedicines, such as canakinumab, rilonacept and anakinra, acting on IL-1 blockade with different mechanism of actions, should be better explored to select sensitive targets and complementary therapies [15].

A recent encouraging response in the adult Schnitzler's syndrome with canakinumab has showed that by blocking only IL-1β instead of both classes of IL-1 (as with rilonacept and anakinra), a remarkable clinical improvement could be reached, with low levels of induced AEs and a more acceptable therapeutic schedule [16].

Predictive biomarkers are needed in addition to old valuable CRP and ESR for better addressing therapy (e.g., those envisioned in MRP8 and MRP 14 released by activated phagocytes) [17].

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Catumaxomab

13

Catumaxomab (Removab®, Fresenius) is a trifunctional rat-mouse hybrid monoclonal antibody co-binding to the epithelial cell adhesion molecule (EpCAM) and to CD3 antigen. The double epitope specificity allows in forcing the interaction between cancer cells expressing EpCAM, and activated T cells. The third binding via the Fc-region of catumaxomab with Fcγ receptors allows direct involvement of immune accessory cells in killing the target.

Monospecific anti-EpCAM monoclonal antibodies, such as edrecolomab, adecatumumab, and other experimental prototypes were previously used for the same purpose with modest or absent clinical efficacy, possibly due to their low ADCC/CDC capacity. However, attempts to improve the cytotoxic efficiency through different structural modifications of the edrecolomab Fc terminal portion produced a sharp increase of serious AEs, in contrast with the safety demonstrated by the original molecule in clinical trials (see edrecolomab, Chap. 19).

Due to previous disappointing results, approval of catumaxomab was complex and only partially successful. The first dose-finding study commenced in November 2001. In 2004, EMEA granted orphan status for the treatment of ovarian cancer. Two years later, the same status was extended to gastric cancer, while FDA designated it for treating ovarian cancer. Finally, in 2009, while EMEA granted full approval to catumaxomab only for the treatment of malignant ascites in EpCAM-positive carcinomas, where standard therapy was not available or no longer feasible, FDA only extended the orphan status designation to the treatment of gastric carcinoma.

Overall, the clinical development of catumaxomab via intraperitoneal (IP) administration comprises 16 studies on malignant ascites, peritoneal carcinomatosis, ovarian cancer, and gastric cancer, including one dose-finding study (STP-REM-01) on 23 patients, a PK/PD study on 13 patients (IP-REM-PK-01-EU) and

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one pivotal two-arm, randomized, open-label trial (IP-REM-AC-01) enrolling 258 patients (170 treated with catumaxomab) with symptomatic malignant ascites, due to EpCAM-positive carcinomas. Supportive studies included one Phase I study (IP-REM-PC-01-DE) conducted in 24 patients with peritoneal carcinomatosis due to gastrointestinal cancers; one Phase IIa (AGO-OVAR-2.10) study on 41 patients with ovarian cancer, and one Phase I (IP-REM-GC-01) study on 12 patients with intra-abdominal epithelial tumors.

A total of 270 patients were treated in all completed studies. Additional Phase II trials were conducted on minimal residual disease setting with focus on gastric- and ovarian cancer. Further routes of administration were tested, including intrapleural and intravenous. Intrapleural infusion was clinically investigated in a Phase I study in patients with malignant pleural effusion, and the intravenous route was experienced in a Phase I study in patients with non-small cell lung cancer [1–4].

13.1 Mechanism of Action

EpCAM (EGP-2; CD326; 17-1A, etc.) is a Type I, transmembrane, 39–42 kDa glycoprotein initially identified as homophilic, epithelial-specific intercellular cell adhesion molecule. The extracellular portion comprises an epidermal growth factor (EGF)-like domain and a putative thyroglobulin (TY) domain. EpCAM, initially found on human colon adenocarcinomas, is one of the first defined tumor-associated antigens. Actually EpCAM is a pleiotropic molecule involved in cell signaling, migration, proliferation, and differentiation of epithelial cells, possibly acting as a proto-oncogene by upregulation of c-Myc. EpCAM also promotes tumor growth and metastasis. These peculiarities and the frequent overexpression of EpCAM on a number of epithelial tumors (GI tract, bladder, breast, cervix/endometrium, esophagus, head and neck, kidney, liver, lung, ovary, pancreas, prostate) and on cancer stem cells, raised the possibility that anti-EpCAM antibodies might interfere with the proliferative signal transduction cascade initiated by EpCAM in a number of epithelial tumors. Moreover, overexpression of EpCAM has been associated with advanced stages of the neoplastic disease, and with worse overall survival. Notably, EpCAM is not expressed on mesenchymal or neuroendocrine tissues, in cells of lymphoid origin, nor in melanoma. The only extra-epithelial positivity was found in esthesioneuroblastoma. Noteworthy, the peritoneum is of mesothelial origin and therefore lacks EpCAM expression. On this basis, catumaxomab was first introduced as orphan drug for cancer and ovarian cancer, and then approved for the treatment of malignant ascites.

Catumaxomab is a novel trifunctional rat-mouse hybrid monoclonal antibody co-binding to the epithelial cell adhesion molecule EpCAM and to CD3 antigen. The antibody consists of a mouse kappa light chain, a rat lambda light chain, a mouse IgG2a heavy chain, and a rat IgG2b heavy chain. Mouse IgG2a and rat IgG2b represent highly homologous IgG subclasses. In particular, the rat Fab fragment binds to human CD3, the mouse Fab binds to human EpCAM, and the

hybrid Fc-region binds to FcγRI (CD64), FcγRIIA (CD32), and Fcγ/RIIIA (CD16a) receptors on accessory cells (mononuclear cells, macrophages, DCs), and NK cells. EpCAM is overexpressed on most carcinomas. CD3 is expressed on mature T cells as a component of the T cell receptor (TCR). The double epitope specificity allows forcing interaction between cancer cells expressing EpCAM and activated T cells, while the third Fc-mediated binding involves cytotoxic immune cell effector functions. Thereby, a localized concerted immunoreaction against single tumor cells was realized. It involved T cell activation and killing through perforin or granzyme B-driven mechanisms; antibody-dependent cell-mediated cytotoxicity (ADCC); complement-dependent cytotoxicity (CDC); and phagocytosis via activation of FcγR positive accessory cells [2, 5, 6].

13.2 Immunogenicity

Being catumaxomab a rodent hybrid monoclonal antibody, the development of anti-mouse/anti-rat antibodies (HAMA/HARA) is expected. This was confirmed in several studies by identification of neutralizing antibodies in plasma, usually after the fourth infusion, and within days or weeks after treatment. In pivotal studies positivity for HAMA was detected in 5.6 % of the 127 tested subjects within the first 4 infusions, and in 94 % of them one month after the last administration. Interestingly, at baseline patients were HAMA negative.

The presence of these antibodies is not associated with any major safety issues. In fact, a retrospective analysis of pivotal trials revealed that HAMA/HARA are associated with prolonged survival compared to antibody-negative patients, especially when antibodies appeared within 8 days after the fourth infusion.

Similar differences were seen in the ovarian, non-ovarian, and gastric cancer populations [4, 7, 8].

13.3 Adverse Events

The overall safety profile of catumaxomab is characterized by the *cytokine-release syndrome* (CRS), *systemic inflammatory response syndrome (SIRS*), *gastrointestinal reactions*, and *constitutional disorders*.

CRS is characterized by an early violent release of pro-inflammatory and cytotoxic cytokines, expressed by a series of symptoms including pyrexia, nausea, vomiting, and chills. Less frequently they are accompanied by dyspnea and hypo/hypertension (see Chap 3). These symptoms reflect the mechanism of action of catumaxomab and are generally moderate and fully reversible. However, they may appear in spite of conventional premedications, even as severe and life threatening.

SIRS occurs less frequently after catumaxomab administration, usually within 24 h after infusion, showing pyrexia, tachycardia, tachypnea, and leukocytosis that can be controlled by premedication and symptomatic therapy.

Gastrointestinal reactions included abdominal pain, nausea, vomiting, and diarrhea, usually reported as mild to moderate. Noteworthy, there is no consistent correlation between infusion period, dose or serum cytokine values, and frequency/severity of these reactions.

Overall, the most common drug-related AEs encountered in pivotal studies were constitutional disorders (96 %, severe 4 %) and gastrointestinal disorders (70 % severe 13 %), usually occurring within 24 h from treatment initiation.

In particular, pyrexia (60–83 % severe 6 %), abdominal pain (43 % severe 10 %), nausea/vomiting (30–60 % severe 2–3 %), and lymphopenia (14 % severe 7 %) were the most representative. SAEs (15 %) mainly consisted in tumor progression (25 %), abdominal pain (12 %), hypertension (9 %), and lymphopenia (9 %). Other serious events included skin and catheter-related infections, extravasation, hemorrhagic erosive gastritis, bowel obstruction/ileus, and rash. Main laboratory abnormalities included CRP (17 %) and GGT (13 %) increase, followed by AST/ALT (7–9 %) increase. Usually, lymphopenia was reversible and tended to resume in one week, and notably no concurrent infections were observed. This transient effect may indicate a temporary shift of the lymphocytes into another compartment, more than a cytotoxic drug-induced effect. Noteworthy, there were no relevant cases of infections during catumaxomab experiences. However, GGT and hypertension showed a tendency to accumulation after the fourth infusion. Hepatic/hepatobiliary disorders accounted for 27 % and were usually asymptomatic. Discontinuation rates were low (<1 %) in the study arm. Overall, the majority of events were mild/moderate ($\leq$ grade 3) and there was no distinctive pattern of AEs corresponding for specific infusion regimens [5, 7–9].

13.4 Postmarketing Surveillance

In the EUV database 66 reports refer to catumaxomab up to the end of 2012. They essentially include constitutional signs (37), gastro-intestinal disorders (35), infections (11), cutaneous disorders (11), and respiratory disorders (8) as the most frequent events. Most common infections were reported as sepsis (3).

Interestingly, 8 SIRS, 2 cases of extravasation, and 1 FLS were included among the general disorders.

13.5 Remarks

When dealing with the AEs induced by a biomedicine used for palliative treatment, the health-related quality life of patients (HRQL) deserves particular attention. Unfortunately, this kind of evaluation is not frequent when considering biomedicines' safety.

Catumaxomab therapy can be generally considered as well tolerated, but some concerns deserve attention. CRS is one of the prominent adverse events to be evaluated. In fact, the "cytokine storm" can become serious and is also referred as a potent inducer of hepatotoxicity. CRS does not seem to be related to particular tumor cytotypes in frequency and severity. In addition, the experience regarding the magnitude of systemic exposure following intraperitoneal administration of biomedicines is limited, although in the case of catumaxomab is reassuring.

The most frequently observed AEs during catumaxomab were constitutional and GI disorders, some of which may be attributed to early and delayed effects of CRS. The increased levels of hepatic functional parameters, as well as the imbalance produced on arterial blood pressure, indicated that catumaxomab enters circulation and reaches extraperitoneal tissues. The binding to bile duct epithelium may explain some cases of cholangitis encountered during catumaxomab administration. In fact, despite the massive presence of malignant cells expressing EpCAM during malignant ascites, interaction of catumaxomab with EpCAM-positive normal tissues cannot be ruled out. On the other hand, a reverted flux of cells also occurs during catumaxomab therapy. It was shown that a dramatic increase of the CD45+ leukocytes occurred in the peritoneal cavity, resulting in a reversion of the tumor cell/leukocyte ratio, a condition that can be considered favorable as a potential anti-tumor adjuvant, but also an additional local CRS inducer. However, CRS signs are also regarded as an indicator of efficacy, since the tumor cell killing is related also to the activation of FcγR-mediated accessory cells, besides direct activity of T cells, leading to additional secretion of cytokines [5, 6].

In terms of HRQL, catumaxomab induced a considerable reduction of ascites, thus lowering the rate of paracentesis, which increases the risk of infection and cachexia.

The potential helper effect of HAMA/HARA on catumaxomab-induced prolonged survival is more difficult to explain. Similar results were obtained with a bispecific (F(ab)2 OC/TR) antibody on ovarian cancer, where high titers of HAMA were associated with longer median survival, suggesting a possible superior general immune reactivity of HAMA-positive patients.

Overall, AEs during catumaxomab therapy have been limited in intensity and resulted manageable and reversible within days or weeks. HRQL is ameliorated by catumaxomab treatment, and reduces the intervention of other deteriorating procedures (repeated paracentesis, intraperitoneal chemotherapy, peritoneal venous shunting, and catheter drainage) [8–10].

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Certolizumab

14

Certolizumab-pegol (Cimzia®, UCB) is a recombinant humanized Fab fragment composed of a single light and heavy chain derived from a murine IgG2a antibody, conjugated to polyethylene glycol (PEG2MAL40 K). This Fab, produced in E. Coli and subsequently linked to PEG, is directed to soluble and membrane-bound human TNFα, and inhibits its binding to p55 (TNFR1) and p75 (TNFR2) receptors, but it does not neutralize TNFβ.

Patients with Crohn's disease (CD) or rheumatoid arthritis (RA) have elevated levels of TNFα, which is considered a crucial pathogenetic factor, both in inducing and maintaining typical intestinal and joint-destructive lesions.

The first approval for this biomedicine was granted by Switzerland in 2007, but no official distribution has apparently occurred. Following the refusal by EMEA in 2007 and 2008 due to concerns on efficacy and safety, FDA approved certolizumab for the treatment of CD during 2008. In fact, since 2003 there have been preliminary agreements, and FDA had replicated requests to the manufacturer for additional information. This brought to the first original submission to FDA in 2006, granted with standard review request and followed by approval in 2008, after additional safety data presentation.

The following year EMEA approved the use of certolizumab in RA patients, and maintained the refusal for CD mainly for safety concerns, while FDA extended the approval to RA. Within the same year Health Canada granted approval for RA.

Pivotal studies include two Phase II (CDP870-004; CDP870-005) on 618 (545 exposed) patients, and two Phase III CDP870-031, PRECiSE 1; CDP870-032, PRECiSE 2) on 1,088 (547 exposed) CD patients; two long-term safety studies (CDP870-033; CDP870-034) on 905 CD patients from the same Phase III trials including the withdrawn group, contributed with interim data to safety analysis.

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The major outcomes concerned infections and malignancies. The risk of bleeding, which mainly caused the EMEA refusal, was subsequently removed by this Agency, but this was not sufficient to receive approval for CD, and therefore the two main Agencies maintained their different opinions on the same material, while the two pivotal trials C870-027 and C870-050 (RAPID1, RAPID2) on 1,601 patients, followed by Study C870-011 (FAST4WARD) on 220 patients, submitted for RA application and extension, received a general consensus.

At present, over 75 trials are completed or ongoing on RA and on some aspects of CD, AS, PsA, JIA, SLE, axial and peripheral spondylitis, and on pediatric inflammatory bowel diseases (IBD) [1–5].

14.1 Mechanism of Action

The tumor necrosis factor (TNF) family is a group of 19 cytokines mainly involved in apoptosis, including TNFα and lymphotoxins (LTα, previously TNF β, and LT-β). Their structures consist of homotrimeric (the former) or heterotrimetric (the latter) chain associations recognizing specific receptors. TNFα (also identified as TNF, being the pivotal molecule of the group) is expressed at the cell surface, mainly on activated macrophages and T lymphocytes, and can be cleaved by a TNFα converting enzyme (TACE) in a soluble form, which is considered the mature expression of this cytokine. However, the transmembrane precursor (tmTNF, 26 kDa) acts also as a bipolar molecule that transmits signals both as ligand and receptor in a cell-to-cell contact fashion, while the soluble form (sTNF, 17 kDa) acts also at distance by interacting with the same receptors. However, sTNF binds to TNFR1 with a 30-fold higher dissociation rate than to TNFR2. Therefore, much of the sTNF linked to the TNRF2 is promptly released and possibly captured by TNFR1. Moreover, shedding of both receptors, mediated by TACE, is capable of neutralizing TNF in solution, thus acting as potential natural TNF antagonists. This effect is controlled by TACE inhibitors via metallopeptidase-3 (MMP3).

TNFR1 is ubiquitous (except for RBC) and constitutionally expressed, whereas TNFR2 is generally inducible and preferentially expressed on endothelial and hematopoietic cells. Macrophages, T and B cells, NK cells, neutrophils, endothelial cells, smooth muscle cells, osteoclasts, and fibroblasts produce TNF as a result of innate and adaptive immune responses induced by exogenous molecules from bacteria and viruses, but also by immune complexes, hypoxia, and trauma. However, the primary source of TNF in immuno-inflammatory processes is the monocyte/macrophage lineage. TNF release, in turn, stimulates the secretion of cytokines (IFNγ, IL-1, 6, 8,17, G-CSF), chemokines (MCP-1), adhesion molecules (ICAM-1, E-selectin), and inflammatory proteins (MIP-1 and 2), acting also on leukocyte activation/mobility and on endothelial permeability. The production of TNF is regulated by feedback loops initiated by TNF-induced factors. In particular, IL-1, IFNγ, and IL-2 induce TNF production, while IL-10, prostaglandins, and corticosteroids downregulate their production by inhibiting transcription of

TNF mRNA. Therefore, TNF is a key pro-inflammatory cytokine with a central role in inflammatory processes. TNF plays a crucial role also in granuloma formation and maintenance.

In healthy humans, circulating TNF is hardly detectable. However, in patients with acute infections, septic shock, chronic inflammatory, and autoimmune diseases such as CD and RA, TNF levels are rapidly and consistently increased, being detectable also in serum, stools, and synovial fluid. TNFRs or TNF antagonists can bind to tmTNF at cell surface. This binding induces reverse signaling, which in turn triggers cell activation, cytokine suppression, or apoptosis of the tmTNF-bearing cells. This peculiarity may be also responsible of AEs induction [6–8].

Certolizumab-pegol binds with high affinity and neutralizes soluble and membrane TNFα, thus inhibiting their physiological interaction with TNF-R1 and TNF-R2 receptors. This effect is dose-dependent. Pegylation increases the half-life of the Fab fragment to levels of the whole antibody molecule. Certolizumab-pegol also inhibits LPS-induced cytokine release (IL-1β, TNFα), but does not interfere with TNFβ (LTα) secretion and function. Certolizumab has a unique structure compared to other anti-TNFα biomedicines such as infliximab, adalimumab, and the fusion protein etanercept, since it does not contain the Fc portion of the antibody and therefore cannot activate complement and induce CDC or ADCC. The overall experience with TNF antagonists in a number of inflammatory/autoimmune diseases represents also a proof of concept on the role of the TNF family in their pathogenesis. As previously mentioned, their action is complex and involves cell activation and proliferation, cytokines and chemokines release, cell recruitment, immune response modulation, angiogenesis, and extracellular matrix degradation, all acting as partners and competitors in inflammation and autoimmunity. However, not all TNF antagonists have identical mechanisms of action; these differences may in part explain diversities in AEs induction and in clinical response, including cases of resistance/intolerance to one TNF antagonist and good response to another [6–8]. Recently, it was shown that certolizumab interferes also with TNF-dependent leukocyte adhesion and chemotaxis, and inhibits two independent pathways promoting angiogenesis, thus adding new knowledge on therapeutic effects and on AEs pathogenesis as well [9].

14.2 Immunogenicity and Immunity

The incidence of anti-drug antibodies (ADA) in CD and RA controlled studies was 8 % and 7 % respectively, including a proportion of neutralizing antibodies (6 and 3 %). Other concomitant immunosuppressive therapies decrease these rates (2–3 %). The presence of ADA is associated with some AEs, such as arthralgia, pain, peripheral edema, injection site pain, and URTI since they occurred at a higher frequency (>3 %) than in ADA-negative patients. Interestingly, antibody formation was associated with a lowered plasma concentration of certolizumab; in fact, ADA-positive patients showed an about 4-fold increase in its clearance.

As for autoantibodies, new overall insurgence of ANA was detected in about 4 % of treated CD and RA patients. In some controlled studies, ANA were increased up to the 17 % of RA-treated patients versus 12 % in placebo; anti-dsDNA antibodies were present in 2.2 versus 1 %, respectively. Cases of lupus-like-syndrome (LLS) were also rarely detected (0.1–0.2 %). In particular, on 1,564 CD treated patients LLS frequency was reported as 0.064 %, indicating that only a minor portion of ANA-positive subjects developed this syndrome.

Finally, the response to some conventional no-live vaccines (pneumococcal, influenza) and antigens (LPS) resulted similar in RA and placebo controls, although other concomitant immunosuppressive treatments reduced the level of specific antibodies.

14.3 Adverse Events

Most safety data on certolizumab come from the experience in RA and CD patients treated with SC injections [1–5]. The basic safety profile mainly derives from the mentioned pivotal controlled trials on about 620 CD patients and on 640 RA patients.

When considering also uncontrolled studies the overall safety sample reached 1,500 patients. Safety features based on such initial data were adjusted on the basis of subsequent experiences on larger cohorts of patients.

In premarketing controlled trials of all CD and RA combined populations, the most common adverse reactions ($\geq$8 %) included *infections* and *cutaneous reactions*, mainly represented by URTI (18 %), UTI (8 %), and rash (9 %), respectively. Discontinuation rates were 8 % for CD and 5 % for RA. Most common causes of discontinuation were intestinal disorders (0.4 %) in CD patients, and TB (0.5 %), pneumonia, pyrexia, and rash/urticaria (0.3 % each) in both groups of patients. Additional data on RA come from an EGR Report reviewing 31 clinical studies on about 4,000 P/Y followed for almost 2 years [4]. On this basis, the major AEs categories were *serious infections* and *malignancies* followed by *hypersensitivity reactions*, *heart failure*, *HBV reactivation*, *neurological* and *hematological disorders*.

The most peculiar event of this class of biomedicines is the risk of serious infections, and in particular of *granulomatous infections*. Tuberculosis, new or reactivated, pulmonary or diffuse, together with some opportunistic infections, can be fatal. The overall relative frequency, estimated in over 5,100 CD and RA patients, ranged 0.3–0.5 per 100 P/Y, with a trend to be higher in RA (0.9 per 100 P/Y). Reactivation also may occur for HBV. Overall incidence of infections in controlled CD trials was 38 %, mainly as URTI (20 %) and UTI (7 %). In RA (15 %) most infections related to URTI (6 %) and were associated with hypertension (5 %), pyrexia, and rash (3 % each). Serious infections occurred in 3–10 % of CD cases. Opportunistic infections from a variety of microbial agents preferably appeared as disseminated serious events.

Malignancies were not more frequent in certolizumab-treated groups than in controls. Rates obtained from 4,650 CD patients were 0.5 per 100 P/Y (0.6 per 100 P/Y 1319 controls). However, a general alert was issued also for certolizumab on the basis of experiences with other anti-TNF biomedicines, since the limited time of observation and the smaller number of controls in certolizumab initial studies suggested caution. Approximately 50 % of malignancies in these studies were *Hodgkin* (HL) and *non-Hodgkin* (NHL) *lymphomas*. In particular, one case occurred in CD (0.03 % on 2657 patients) and 3 cases in RA (0.1 % on 2,367 patients) in controlled studies. The latter rate was about 2-fold higher than in the general population. However, it is known that patients with active RA are at higher risk for these neoplasms (see adalimumab, Chap. 6).

Local and systemic *hypersensitivity reactions,* including anaphylaxis, were registered as rare events (<0.1 %) during these studies, although severe in some instances.

Neurological disorders, including seizures, optic neuritis, and peripheral neuropathies were also reported as rare events in patients treated with certolizumab, while other central and peripheral demyelinating disorders, such as MS and GBS, were reported as experienced during treatment with other TNF blockers.

Cardiac failures and *hematological disorders* were also reported as infrequent events.

Additional information on CD has been provided by a large multicenter international trial. The safety population consisted of 438 patients (223 treated with certolizumab for 6 weeks). Any AEs occurred in 51 % of treated patients and were considered as drug-related in 18 % of cases. Any infections were encountered in 16 % of treated patients. SAEs were 5 % and serious infections approximately 1 %. Although these frequencies were comparable to controls, a significant increase in Candida and Herpes infections was recorded in the certolizumab group. One case of malignancy (metastatic adenocarcinoma 28 days after treatment initiation) and no TB cases were registered. New onset of ANA was detected in one patient. Injection site reactions (5 %) were significantly higher than controls. Overall, safety data were in line with previous experiences and confirmed a rather low rate of AEs in CD [10].

Recent data came from a broad group of RA patients either in monotherapy or in conventional multi-therapy, including patients resistant at least to one DMARD enrolled in the REALISTIC Phase IIIb trial. This group was considered more closely resembling patients in routine clinical care, than selected patients in previous trials [11]. Safety data were obtained from 1,065 RA patients (846 receiving certolizumab) after 12 weeks of treatment. AEs were observed in 67.5 % (serious 6 %) of the treated group, and were mostly infections (29 %, URTI 13 %). Injection/infusion site reactions reached about 6 %. The majority of events were mild/moderate. Serious events (6 %) were LRTI (0.8 %) and UTI (0.6 %). Two opportunistic infections were detected. Malignancies consisted in four cases (0.5 %) of solid tumors. Overall, this more heterogeneous population of active RA patients showed a safety profile similar to that of previous studies, such as RAPID 2 and other pivotal trials conducted on selected cohorts.

Long-term observations on RA patients of RAPID 1 trial after 2 years of treatment with certolizumab in association with MTX, and its extension study (OLE), provided safety data also from patients withdrawn from the official trial. AEs were reported in about 88 % of the safety population (958 patients), and SAEs experienced by 23 % of them. As expected, the most common events were infections (63 %), mostly as URTI and UTI (10 % each). Injection site reactions occurred in 10 % of cases. Serious infections (10 %) pertained to LRTI and TB (2–2.5 %). Malignancies (1 %) included solid tumors (9 cases) in various organs and one B cell lymphoma of MALT type. Mortality rates were similar to controls and comparable with previous studies.

Overall data, including the OLE group, showed that the incidence of serious infections and malignancies did not increase over time and with prolonged exposure to certolizumab. It was also noted that TB cases were registered in patients from geographic areas with high background incidence of this infection.

Finally, no new or increased signals were observed, but it must be noted that the 2 years observation time and the sample size cannot be considered fully adequate to detect rare/very rare events in this study [12].

Another study explored the possibility of treating active RA with certolizumab (124 patients), injected every 4 weeks for 2 years, in addition to continued MTX therapy. The overall safety profile did not change (total AEs 78 %, SAEs 13 %, serious infections 2.4 %) [13].

Finally, a recent comparative meta-analysis on certolizumab efficacy and safety was performed by The Cochrane Collaboration in five selected trials on 2,094 adult RA patients for safety. Their analysis registered 5–10 % of SAEs including serious infections and TB. There was no difference in AEs severity between the two standard doses of certolizumab, 200 and 400 mg. However, a significant increase in serious infections, including LRTI and TB cases, was confirmed in both groups. In contrast with other analyses, Cochrane found the risk of TB homogenously distributed among countries with different incidences of this infection. Overall, an increased risk of total AEs, and in particular of serious infections, was confirmed. A possible, but not confirmed higher risk of mortality was suggested, while a major incidence of malignancies and in particular of lymphomas, could not be confirmed [14].

14.4 Off-Label Experience

Certolizumab is used in a wide range of off-label conditions; some of these experiences are included in controlled trials on IBD, UC, Ps, PsA, and JIA patients. A peculiar situation concerns CD that is considered off-label in Europe, where 22 trials are still collecting data in various treatment conditions.

Small studies and postmarketing reporting include cases of polyarthropathy, spinal osteo-arthropathy, myositis, sarcoidosis, Still's disease, reactive arthritis, seronegative arthritis, and autoinflammatory diseases treated with certolizumab.

So far, no new safety signals have been raised from such experiences.

However, there are some concerns about uveitis, often associated with a number of autoimmune diseases—including JIA, IBD, sarcoidosis, seronegative spondyloarthropathy, Wegener's granulomatosis, and Behçet's disease (recently authorized in Japan)—and therefore treated with certolizumab. These observations are mostly small and provided by non-controlled studies or case series, yet some of them have reported a recrudescence of the ocular disease [15].

Two recent studies on the treatment of PsA (RAPID trial enrolling 409 patients) and severe plaque Ps (176 patients) have showed promising efficacy and a limited incidence of AEs. In particular, the most common adverse events (≥5 %) in PsA, both in treated and placebo groups were nasopharyngitis and URTI, and the most common serious adverse events (≥1 %) were infections and infestations [16].

In the first Phase II study on severe plaque Ps, 123 out of 175 patients included for safety studies were treated with certolizumab, and 71 of them were retreated after relapsing. No significant differences were observed between treated and control groups. Most AEs were mild to moderate, and consisted in nasopharyngitis, cephalea, and pruritus. Serious events (3–5 %) were all observed during the first treatment with certolizumab, and included one disseminated TB reactivation, two gastroenteritises, one UTI, and one psoriasis reactivation. Discontinuation rate was similar to controls. No deaths or new safety signals were registered during the study. ADAs were positive in 4-5 % of treated patients, with no apparent dose relation after the first treatment cycle (12 weeks). However, at the last follow-up visit they raised to 18–25 %, in relation with the administered dose. No ADAs were reported in the retreatment group. Interestingly, patients reported fewer TEAEs during the re-treatment period than during the first treatment period [17].

14.5 Postmarketing Surveillance

Up to April 2013, 14,800 reports were collected as cimzia/certolizumab in the FAERS database. Infections (7.2 %), GI signs (9.4 %), cutaneous reactions (5.0 %), and injection site reactions (4.6 %) were the most common cited events.

In particular, 79 TB cases (65 pulmonary, 24 disseminated, 29 extrapulmonary, 16 latent) were recorded. Nasopharyngitis (362), pneumonia (425), UTI (298), and URTI (178) were among the most frequent infections. Hypersensitivity reactions were reported as rash (511), urticaria (172), anaphylactic reactions (40), and anaphylactic shock (16). Malignancies (39) included lymphoma (25; 5 NHL), lung neoplasms (34), and melanoma (20) as the most frequently reported. Among neurologic disorders, 41 cases of PNP were reported.

Up to the end of 2012, the EUV database received 2,797 reports (99 % on serious events) concerning certolizumab administration. Gastrointestinal signs (18.5 %), infections (14 %), cutaneous disorders (8 %), nervous disorders (6 %), and musculoskeletal disorders (5 %) were the most frequently reported. Pneumonia (99), UTI (57), sepsis (42), abscess (36), nasopharyngitis (33), cellulitis (29)

clostridial infections (21), and URTI (13) were among the most frequent infectious expressions. TB was reported in 15 cases (6 extrapulmonary, 5 pulmonary, 2 disseminated, 2 latent). Among neurologic disorders, 12 cases of PNP and six cases of optic neuritis were observed. Hypersensitivity reactions included rash (80), urticaria (44), 21 cases of anaphylaxis (8 shock), and two anaphylactoid reactions. Six cases of unspecified malignancies, 11 breast cancers, six lymphomas, five B cell lymphomas, and five melanomas were also reported.

14.6 Remarks

Due to the functional differences of TNF factors and to the heterogeneity of diseases treated with their antagonists, the overall picture of AEs among members of the same drug class is expected to be complex. However, the existence of different biomedicines in the same class allows comparative evaluations of some relevance.

The overall safety profile of certolizumab appears rather selective compared to other TNF antagonists and inhibitors. Major safety issues concern serious infections (including TB and opportunistic infections), and with a lesser extent demyelinating disorders, hepatotoxicity, lymphoma, and other malignancies. Additional interesting differences emerge from comparisons among TNF-inhibitors with respect to granulomatous inflammation and selective resistance to therapy. Both safety and efficiency show different profiles with adalimumab, infliximab, and certolizumab in RA and CD, as well as in other off-label experiences such as in other IBD and psoriasis. Possibly, some of these differences relate to their mechanisms of action. For example, these three monoclonals equally neutralize membrane-bound TNF via its two receptors, but certolizumab is two fold more potent in neutralizing soluble TNF signaling via either receptor compared to infliximab and adalimumab. Moreover, certolizumab did not show increases in particular AEs, although the response of different autoimmune diseases was variable (see also adalimumab and infliximab, Chap. 6, 24). Notably, infliximab and adalimumab induce CDC and ADCC, while certolizumab does not interfere with both immune effector systems, being deprived of the Fc portion [18–21].

These observations are also a proof of concept that these differences, and in particular the presence and functions of the Fc fragment, are not determinant in the induction of some AEs. By contrast, they may be more relevant in limiting TB infectivity and dissemination.

An in-deep comparative analysis among five biologic antirheumatic drugs has been recently performed [22]. Although such confrontation shows limitations in trial's design directed to investigate efficacy more than safety, the analysis has revealed some interesting hierarchy in AEs expression. As for certolizumab, a meta-analysis on four trials revealed a high risk of serious infections compared to four other biomedicines (adalimumab, golimumab, etanercept, and infliximab) and

to controls. Similar data indicated an increased risk of TB in RA patients compared to standard DMARDs therapy.

Overall data were insufficient to determine an increased risk of all products with respect to malignancies. An indication of increased mortality observed in some studies on certolizumab was not sufficient to confirm the issue, due to the small sample of patients involved. Similarly, it has not been confirmed that the higher risk of demyelinating diseases is certolizumab-related.

Another related challenging question is why most patients who either fail to respond, loose response, or are intolerant to one TNF antagonist, respond well when switched to another TNF antagonist. This opportunity, together with an accurate evaluation of possible cumulative safety problems, may stimulate research toward more personalized complementary therapies with anti-TNF agents, instead of racing for the absolute best product in the field. Therefore, when comparing different treatments, safety should always be considered in relation to efficacy, since these two parameters often do not correlate.

Finally, a further area of concern refers to some paradoxical and new adverse effects, such as exacerbation of the original disease (RA, CD, psoriasis, etc.) or development of new onset psoriasis and uveitis during these treatments, which still need to be clarified [8, 18–20].

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Cetuximab

15

Cetuximab (Erbitux®, Eli Lilly) is a recombinant, human-murine chimeric monoclonal IgG1k antibody that specifically binds to the extracellular domain of the human epidermal growth factor receptor (EGFR). EGFR expression and upregulation occurs in 60–80 % of colorectal carcinoma (CRC).

The request for approval originally submitted to FDA by ImClone Systems in 2001 was not accepted, due to procedural and safety reasons. The following request was submitted in 2003 on the basis of a pivotal trial (EMR 62 202–007—BOND) on 329 metastatic CRC (mCRC) patients treated with cetuximab, either in monotherapy or in association with irinotecan, and approved in February 2004. Additional supportive investigations included two Phase II trials IMCL-CP02-9923 on 138 (134 exposed, 4 monotherapy) patients, and IMCL-CP02-0141 on 57 exposed patients; additional safety data from 111 patients in monotherapy. Confirmatory studies Phase III CA225006 and CA225014 were required for accelerated approval. All trials included patients with EGFR-expressing metastatic colorectal cancer (mCRC), whose disease had progressed after receiving irinotecan. Overall, the sample for safety evaluation database included 1,123 patients treated with cetuximab during the global development program. In June 2004, EMEA granted approval for mCRC.

In 2005, the same sponsor in collaboration with Merck KGaA successfully submitted a supplemental application to FDA, EMEA, and Swiss Authority to extend the indication of cetuximab to squamous cell carcinoma head and neck (SCCHN) on the basis of a new Phase II (EMR62 202–016) and a Phase III (IMCL-CP02–9815) trials including 538 patients with advanced, recurrent and/or metastatic tumors in combination with irradiation, or with platinum-based chemotherapy, or as monotherapy in recurrent-resistant cases.

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Additional evaluations on SCCHN were conducted in Study EMR 62 202-006 comparing cetuximab associated with radiotherapy (211 treated subjects) to radiotherapy alone (213 patients), and in Phase III Study EMR 62 202–002 (EXTREME) comparing cetuximab associated with chemotherapy (222 treated patients) to chemotherapy only (220 patients).

A request for the extension to first-line colorectal cancer was submitted in 2007, followed by another to first-line SCCHN treatment in 2008. By the end of 2011, FDA approved the SCCHN first-line indication in combination with platinum-based chemotherapy on the basis of the EXTREME Study, using the EU-approved version of cetuximab, which provided 22 % less exposure than the US version of this biomedicine.

Finally, in 2012 the indication for mCRC was specifically directed to KRAS mutation negative EGFR-positive tumors, either as monotherapy or in association with irinotecan alone, or together with FOLFIRI (5-FU, leucovorin, irinotecan) in the FDA approval, or with FOLFOX (5-FU, leucovorin, and oxaliplatin) in the EMEA approval. In late 2012, the sponsor withdrew the extension request for SCCHN from EMEA.

The KRAS status was identified as predictive factor in four studies (EMR 62 202–013, EMR 62 202–047—OPUS, CA225006, and CA225025) in 2,072 patients. The association with FOLFIRI was evaluated in Study EMR 62 202.013 (CRYSTAL) including 599 mCRC patients treated with cetuximab. The association with FOLFOX was analyzed by another study (EMR 62 202.047) including 169 patients also receiving cetuximab.

Additional studies on mCRC treated with alternative chemotherapies were COIN, CA225006, and EMR 62 202–007, which respectively included 659, 648, and 218 patients, receiving chemotherapy in association with cetuximab. Monotherapy in mCRC was evaluated in a separate study (CA225025) enrolling 287 patients. The vast majority of these patients and the relative control groups were followed also for safety evaluation [1–5].

Finally, a group of studies analyzed the combination of cetuximab with bevacizumab (a VEGF antagonist), with or without irinotecan, in order to evaluate the potential synergistic action of the two mAbs in mCRC. In particular, the mentioned therapeutic combination was evaluated in Phase II BOND-2 Study enrolling 83 patients, and in Study BOND 2.5 on 33 patients, all with irinotecan-refractory CRC. Similarly, the ongoing Phase III KRK-0306 (NCT00433927) Study is comparing cetuximab and bevacizumab in association with standard FOLFIRI as mCRC first-line treatment [6–8].

At present, over 580 trials have investigated cetuximab on a variety of solid tumors, and some of them are still ongoing or recruiting patients. This indicates the great interest in searching for better administration strategies and in evaluating possible extensions of the therapeutic uses of such biomedicine. In particular, among 300 trials on a wide variety of carcinomas, 290 concern digestive tract neoplasms, 128 bronchial neoplasms, 122 adenocarcinomas (mainly bronchopulmonary), and 64 studies involve bronchogenic carcinoma. Part of these consistent groups relate to the in-deep exploration of CRC and SCCHN tumors and also

include extensions of previous trials; other investigations are performed on laryngeal (45), pancreatic (30), breast (30), endocrine, and skin (24 each) tumors.

15.1 Mechanisms of Action

EGFR (cErbB-1, HER 1 in humans) is a transmembrane protein of a subgroup of Type 1 receptor tyrosine kinase (RTK), the ErbB family, which includes EGFR, HER2, HER 3, and HER 4. EGFR is constitutively expressed in many epithelial tissues, including skin and hair follicles, as well as in epithelial cancer cells. There are 11 known natural ligands to these receptors, including TGFα, HB-EGF, EGF, epigen, betacellulin, AREG (amphiregulin), and EREG (epiregulin), which interact with EGFR. However, HB-EFG, betacellulin and EREG also interact with HER 4, yet not with the other two ligands of the subgroup.

Upon interaction, EGFR forms homo- or heterodimers with other ErbB receptors, a step related to activation of the receptor/ligand complex, via the intracellular tyrosine kinase pathway. The signaling essentially produces DNA synthesis, cell cycle progression, migration, adhesion, and proliferation of cells expressing EGFR. Therefore, this pathway is crucial for the homeostasis of epithelia, for innate immunity, and also as a downregulator of myelin regeneration. EGFR is usually overexpressed on neoplastic cells of epithelial origin—and in particular on CRC, lung carcinoma, SCCHN, and GBM—due to gene mutations/overactivity leading to uncontrolled cell division, angiogenesis, cell migration, and cellular invasion/metastasis [1–5].

Cetuximab (formerly C225; ICM-C225) is a human/mouse chimeric monoclonal composed of the Fv regions of a murine anti-EGFR antibody with human IgG1 heavy and kappa light chain constant regions. Cetuximab specifically binds to EGFR on normal and neoplastic cells with high specificity and with a higher affinity (5–10 folds) than the natural ligands, thus blocking ligand-induced phosphorylation of EGFR and subsequent activation of the tyrosine kinase pathway.

In vitro, the binding blocks phosphorylation/activation of receptor-associated kinases, resulting in the inhibition of cell growth, induction of apoptosis, decrease of metalloproteinases, and VEGF production. EGFR is present in the majority of CRC and SCCHN cells. Signal transduction through the EGFR also results in the activation of wild-type KRAS protein, which contributes to EGFR-mediated cell proliferation and survival, and to the production of proangiogenic factors. However, in cells with activating KRAS somatic mutations, the mutated KRAS protein is continuously active and appears independent from EGFR regulation, thus making these tumor variants resistant to the action of cetuximab.

In vitro, cetuximab can mediate ADCC against EGFR-expressing tumor cells. In addition to blocking the proliferative signals to cells, cetuximab can also decrease the expression of target antigens (HER2 and EGF-R) through internalization and/or shedding, thus decreasing receptors' signaling capability. In vivo, cetuximab inhibits the expression of angiogenic factors by tumoral cells and

causes a reduction in tumor neovascularization and metastasis. However, not all of these mechanisms are demonstrated to be active in vivo; neither efficacy nor safety can be fully predicted by in vitro testing [3, 9].

The response of different tumors to cetuximab is heterogeneous. In fact, other factors can influence efficacy and safety of cetuximab, such as resistance of some KRAS mutations and cross-suppression of AREF/EREG ligands inducing selection of cell clones resistant to cetuximab [10].

In addition, the role of VEGF inhibition may not be secondary in the antitumor activity of cetuximab. In fact, some antineoplastic activity of this monoclonal has been observed also against EGFR-negative CRC [11]. Sixty percent of these patients had VEGF serum levels lowered of more than 50 % during treatment with cetuximab, while about 40 % subjects had stable levels. Only the former group seemed to have a partial benefit from treatment. Therefore, the mechanism of action of cetuximab appears more heterogeneous than expected, in consequence of concomitant actions of receptor blocking, ADCC effects and of antiangiogenic activity due to a partial inhibition of VEGF activity. All these factors, other than KRAS mutations, may influence the final response and the insurgence of AEs to cetuximab.

Another mechanism of resistance derives from an interaction between EGFR signals and activation of transcription 3 (STAT3), which plays a role in the regulation of gene transcription. In fact, STAT3 is often constitutively activated in SCCHN and is associated with decreased survival. EGFR activates STAT3, which stimulates cell proliferation and can be inhibited by cetuximab. However, STAT3 can be activated independently from EFGR, and thereby may play a role in resistance to EGFR inhibitors [5].

As for the capacity of cetuximab to induce AEs, an interesting finding came from the identification of preexisting IgE antibodies in 76 cancer patients treated with cetuximab, and in a consistent cohort (462) of healthy controls [12]. This is a relevant aspect for cetuximab, since a consistent rate of serious infusion reactions were experienced, leading to the insertion of a boxed warning.

Hypersensitivity reactions in the treated group were detected in about 33 % of patients during the first infusion. The majority (68 %) had IgE, directed against galactose-α-1,3-galactose and present before treatment, but recognized the same epitope present on the Fab-murine part of cetuximab. Noteworthy, the murine Sp 2/0 cell line, in which cetuximab is produced, expresses the gene for α-1,3-galactosyl-transferase. IgG directed to this oligosaccharide is present in all humans, and are related to similar structures present in the ABO blood groups. However, only a minor part of individuals produces also IgE against the same antigen, which in this study was found to correlate with typical Type I hypersensitive reactions at first injection.

In addition, the presence of a murine-derived sialic acid (N-glycolylneuraminic acid) was also found on cetuximab, as a consequence of the manufacturing process. Most healthy individuals have antisialic antibodies as well, which may interfere with cetuximab activity and clearance, due to the formation of immune complexes [13].

This paradigmatic observation indicates that preexisting antibodies cross-linking with nonhuman structures present on the biomedicine may determine early reactions to monoclonals. More interestingly, this reaction is rather unusual since it is directed against a saccharide, while Type I hypersensitivity is mostly directed to the proteic component of antigens. In this case, glycosylation of the recombinant monoclonal derives from the supportive Sp 2/0 murine cell line, while other cell lines (CHO) are lacking of α-1,3-galactosyl transferase.

However, other mechanisms may produce cross-linking anti-cetuximab antibodies, such as infectious and infestation organisms that frequently invade these patients, and even pollen and plant antigens. This may explain the clustering of some hypersensitivity reactions encountered in some US areas [14].

Finally, apparent immune-related events, such as part of rash eruptions, may be driven by completely different mechanisms. In the case of cetuximab or other EGFR inhibitors, rash is more likely to depend from cetuximab interaction with EFGR, which is particularly expressed on epidermis.

Overall, the cetuximab experience is a good example of the complexity of mechanisms behind activity and reactivity occurring and overlapping during treatment with a biomedicine.

Recently, in a new in vitro essay, colon cancer cells were exposed to 5-FU followed by cetuximab, a known drug combination used in vivo [15]. These drug treatments have been found to increase expression of EGFR, CEA, TS antigens, and HSP90. Furthermore, they promote phagocytosis and activate dendritic cells and cytotoxic T cells effect on cancer cells.

Therefore the accumulating evidences on the existence of synergistic mechanisms between standard chemotherapeutics and new biomedicines may be important also for the understanding of AEs genesis, and for the individuation of new chemo-immuno therapeutic combinations.

Panitumumab is a successor of cetuximab showing a higher receptor affinity and a consequent stronger cytotoxic action on EGFR-positive tumor cells. A comparative analysis revealed two large, partially overlapping, functional epitopes consisting of 17 critical amino acid positions. Four of them were selectively targeted by cetuximab, and other four were selectively recognized by panitumumab. Saccharides present on the cetuximab molecule, driving IgE-mediated responses, are absent on panitumumab. Moreover, some EGFR negative tumors showing resistant to panitumumab are sensitive to the action of cetuximab (see panitumumab, Chap. 32).

15.2 Immunogenicity

Cetuximab induces a low immune response compared to other therapeutic proteins [16]. Nonetheless, it triggers a rather consistent number of hypersensitivity reactions, mostly related to first infusion reactions episodes.

In official labels, data on HACA have reported the presence of nonneutralizing antibodies in 5 % of the about 1,000 tested patients. Previous data indicated that 4 % had a positive antibody response to cetuximab, which in some cases appeared to be preexisting [4, 17]. These may cross-react with cetuximab structures and induce hypersensitivity reactions (reported in about 13 % of patients) [12].

Moreover, the presence on cetuximab of murine-derived N-glycolylneuraminic acid—which can cross-react with preexisting antibodies present in most individuals—has been demonstrated [13].

15.3 Adverse Events

The safety profile of cetuximab is based on studies conducted in CRC and SCCHN patients and appears similar in the two groups, allowing to preliminary depict a common framework, followed by additional peculiarities summarized in the subsequent paragraphs.

Safety data examined for initial applications related to 19 Phase I–II studies, with 1,123 enrolled patients. Detailed information came from over 900 patients of these trials and from additional ones. It must be noted that the majority of these subjects had mCRC, and some groups were treated with EU-approved cetuximab, which is known to produce a 22 % lower exposure than the US-approved cetuximab. Therefore the observed AEs in these studies appeared reduced in incidence and severity, compared to patients receiving the US analog product [1–5].

In November 2011, a BBW about *serious infusion reactions* and *sudden death/cardiopulmonary arrest* has been inserted in the US product information. Additional general warnings refer to *pulmonary* and *dermatologic toxicity*, and to *electrolyte abnormalities* (mainly hypomagnesemia).

Overall, the *most serious* adverse reactions associated with cetuximab across all studies were *infusion reactions, cardiopulmonary arrest*, *dermatologic toxicity* and *radiation dermatitis*, *sepsis*, *renal failure*, *interstitial lung disease* (ILD), and *pulmonary embolism.*

The *most common* adverse reactions associated with cetuximab ($\geq$25 %) were *cutaneous reactions* (including rash, pruritus, and nail changes), *cephalea*, *diarrhe*a, and *infections*.

Overall rates of *infusion reactions* ranged 15–21 % across studies, with serious involvement in 2–5 % of them and one fatality, as reported in the 2012 updated official label. Approximately 90 % of severe reactions occurred during the first infusion, despite standard prophylaxis. As previously reported, some cases of cetuximab-induced early reactions were associated with the presence of preexisting IgE antibodies directed to galactose-α-1,3-galactose and cross-reacting with similar murine-derived structures situated in the Fab fragment [12].

Cardio-pulmonary arrest/sudden death events were observed in a subgroup of SCCHN patients treated with cetuximab in association with irradiation (208) or with platinum-based therapy (219). Rates of sudden death (2–3 %) were mostly

related to cardiac failures in patients with history of coronary artery disease, congestive heart failure, or arrhythmias.

Dermatologic toxicity including acneiform rash, skin drying and fissuring, paronychial inflammation, infectious sequelae, and hypertrichosis were frequently observed in a large sample (1373) of CRC and SCCHN patients. The major event consisted in acneiform eruptions (76–88 %), which became serious in 1–17 % of cases. Eruptions usually appeared within 2 weeks of therapy and lasted approximately one month in about 50 % of patients. It must be noted that these events are common to other EGFR blockers and may also involve mucosal tissues with life threatening consequences [18].

Pulmonary toxicity evidenced as ILD was observed in less than 0.5 % of 1,570 CRC/SCCHN patients.

Late radiation toxicity in SCCHN resulted to be higher when combined with cetuximab, and involved salivary glands (65 %), larynx (52 %), subcutaneous tissue (49 %), mucous membrane (48 %) esophagus (44 %), and skin (42 %). All frequencies were over 9 % higher than in controls, except for subcutaneous lesions (4 %).

Electrolyte abnormalities mostly occurred as reduced serum levels of calcium, potassium, and magnesium, the latter being the most frequent, occurring in about 55 % of cases (severe 6–17 %) receiving cetuximab. In a group of patients receiving the EU-cetuximab formulation associated with platinum-based therapy, hypo-magnesemia was present in 14 % (severe 7 %).

Overall *infections* ranged from 13 to 35 % (severe 8–11 %) in CRC and SCCHN. Sepsis occurred in 1–4 % of cases.

Within this framework, some peculiarities emerged in the two types of treated neoplasms, apparently determined by different therapeutic approaches and possibly by different tumor typologies, KRAS status, response to therapy, and possibly by the anatomical localization of the neoplasms.

15.3.1 AEs in SCCHN

The most frequent adverse events seen in SCCHN patients receiving cetuximab associated with radiation therapy ($n = 208$) versus radiation alone ($n = 212$) were acneiform rash (87 % vs. 10 %), weight loss (84 % vs. 72 %), and asthenia (56 % vs. 49 %). Radiation dermatitis resulted higher in controls (86 % vs. 90 %). However, severe AEs ($\geq$10 %) were higher in cetuximab-treated patients, and included radiation dermatitis (23 % vs. 18 %), acneiform rash (17 % vs. 1 %), and weight loss (11 % vs. 7 %). Infections were less frequent (13 % vs. 9 %) and severe forms were low and equivalent to controls (1 % in both groups).

The most frequent AEs for the EU-approved cetuximab associated with platinum-based therapy and 5-FU combination therapy (CT) in 219 patients versus CT alone (215) were: acneiform rash (70 %/2 %), nausea (54 %/47 %), and infections

(44 %/27 %). Severe grades of AEs in cetuximab with CT versus CT alone included infection (11 %/8 %) and acneiform rash (9 % vs. 0 %) [4, 5].

A further association of cetuximab with cisplatin was experienced in 30 cases of naso-pharyngeal carcinoma, which usually overexpress EGFR in >80 % of cases. Acute drug-related overall toxicity was considered mild. However, lymphopenia was registered as severe/serious in all reports on over 80 % of patients. The most common cetuximab-related event was acneiform rash (93 %, severe 10 %). Moreover, an exacerbation of local radiotherapy skin and mucosal lesions in the cetuximab-associated group was suspected, and in spite of consistency with previous reports it was not demonstrated. No severe infections were observed. As for the known cetuximab-related hypomagnesemia and other constitutional and GI disorders, they were all around 3 %, in line with previous reports [19].

15.3.2 AEs in mCRC

This subset of data refers to the EU-approved cetuximab, administered in KRAS mutation negative patients who were pooled according to the associated therapy (FOLFIRI, in 317 patients; irinotecan in 354 patients). An additional study group of 242 patients in monotherapy with cetuximab associated with basic supportive care (BSC) allowed the evaluation of potential differences with the AEs observed in associated therapies.

Overall, the most frequent events seen in patients with KRAS mutation-negative mCRC, treated with EU-approved cetuximab + FOLFIRI (317 patients) versus FOLFIRI alone (350) were acneiform rash (86 % vs. 13 %) and diarrhea (66 % vs. 60 %). The most severe (≥10 %) included: neutropenia (31 % vs. 24 %), acneiform rash (18 % vs. < 1 %), and diarrhea (16 % vs. 10 %).

The most frequent events seen in patients with KRAS mutation-negative mCRC, treated with cetuximab monotherapy and BSC (n = 118) versus BSC alone (n = 124), were rash/desquamation (95 % vs. 21 %), fatigue (91 % vs. 79 %), nausea (64 % vs. 50 %), dry skin (57 % vs. 15 %), pain general (59 % vs. 37 %), and constipation (53 % vs. 38 %). Severe events (≥10 %) included: fatigue (31 % vs. 29 %), pain general (18 % vs. 10 %), rash/desquamation (16 % vs. 1 %), dyspnea (16 % vs. 13 %), gastrointestinal signs (12 % vs. 5 %), and infection without neutropenia (11 % vs. 5 %).

The most frequent adverse events seen in patients with mCRC (354), treated with cetuximab only in combination with irinotecan in clinical trials, were acneiform rash (88 %), asthenia/malaise (73 %), diarrhea (72 %), and nausea (55 %). The most common severe adverse events (≥10 %) included: diarrhea (22 %), leukopenia (17 %), asthenia/malaise (16 %), and acneiform rash (14 %).

A recent study examined the efficacy of cetuximab-irinotecan association, in EGFR- negative ($EGFR^-$) patients with mCRC, as second-line therapy in irinotecan-resistant cases. This attempt was suggested by the observation that efficacy of cetuximab was similar in some EGFR negative and positive mCRC patients.

This opportunity allowed also to investigate for the first time AEs in a different cohort of mCRC patients receiving biweekly repeated cycles of cetuximab, and followed for 21 months [20].

Severe events were observed in 40 % of $EGFR^+$ and in 35 % of $EGFR^-$ patients. Among hematologic severe events, neutropenia was present in 5 % of $EGFR^+$ and in 20 % of $EGFR^-$ subjects. No other severe hematologic events were registered. Among nonhematological events, skin toxicity was present as mild/moderate in all patients (severe 10 %) and was frequently associated with nail abnormalities. Diarrhea was present in both groups, with a slight prevalence in $EGFR^+$ patients. Two patients (5 %) experienced severe toxicity to the anesthetic, and one ($EGFR^+$) had a severe allergic reaction. There were no treatment-related deaths. Reactions causing therapy delay/modifications were mainly related to neutropenia. Although the small dimension of the study group did not allow conclusive remarks, the overall rate of severe toxicity (37.5 %) appeared similar to the 40 % rate observed in previous trials, where patients had weekly received cetuximab/irinotecan.

All in all, neither patients receiving biweekly administration nor the $EGFR^-$ cohort showed increased levels of AEs.

Cetuximab was also evaluated in combination with bevacizumab, with or without chemotherapy, in order to evaluate the potential synergistic action of the two mAbs in mCRC. In the Phase II BOND-2 Study enrolling 83 patients and in Study BOND 2.5 on 33 patients, the concurrent monoclonal association, with or without irinotecan, was experienced in irinotecan-refractory CRC.

Overall, in BOND-2 Study toxicities occurred as expectable from the single employed agents. Serious events included intestinal perforation, enterococcal endocarditis (fatal), ATE, nonperforated duodenal ulcer (all in triple therapy), perirectal fistula, and myocardial infarction in diabetes (concurrent mAbs). In BOND-2.5, patients treated with the same regimen had previously received cetuximab and chemotherapy, but the disease progressed. However, the safety profile was similar to that of BOND-2 study. Severe events included acneiform rash (18 %), neutropenia (6 %), diarrhea (6 %), hypomagnesemia, and hypophosphatemia (6 % each). One patient had neutropenic fever, and one had a hypersensitivity reaction to cetuximab [6, 7].

Finally, a recently started Phase III study (AIO KRK-0306) is comparing cetuximab and bevacizumab in association with standard FOLFIRI as mCRC first-line treatment [8]. The study is testing these combinations, also in a limited subgroup of KRAS mutated mCRC patients (admitted up to 2008). This offers the opportunity of evaluating AEs in patients usually excluded from trials due to KRAS mutation (codons 12 and 13) acquired resistance.

Cetuximab induced a significant rate of acneiform rash (20 %) that was absent in bevacizumab-treated group. By contrast, severe hypertension was more frequent in patients receiving bevacizumab (22 % vs. 8 %, $P = 0.082$). There were no other major imbalances between the two groups, and no gastrointestinal perforation was observed in any arm.

15.4 Off-Label Experience

As previously reported, cetuximab-associated therapies is being experienced in a number of solid tumors, in trials and in clinical care, including nonmetastatic CRC, squamous cell carcinomas, pancreatic, gastro-esophageal carcinomas, and glioblastoma multiforme (GBM).

An early study on 40 patients with a variety of solid tumors (mostly CRC, breast, ovary, pancreas, prostate, cholangiocarcinoma, SCLC, and NSCLC) treated with cetuximab monotherapy confirmed a nonlinear pharmacokinetics of cetuximab, and a significant association between insurgence of rash (mostly acneiform) and treatment-induced disease stability [17]. In fact, this and other studies experiencing different anti-ECFR agents obtained better clinical course and survival rate in patients with various solid tumors, suggesting the possibility of using rash as an efficacy predictor and dose titration for this drug class.

Additional preliminary studies reporting AEs encountered in other solid tumors treated with cetuximab and dasatinib (a multi TYKs inhibitor) have been recently published [21]. In the small cohort there were 31 cases of mesothelioma, sarcoma and salivary gland, esophageal, and bladder tumors, including some cases of in-label forms of SCCHN and mCRC.

Therapy discontinuation was frequent and mostly related to AEs, which included three cases of severe hypersensitivity (12 %), moderate/severe dyspnea (16 %), gastro-intestinal, and constitutional signs (8–12 %) in the group of treated patients (25). These events were considered as drug-related and led to dosing modifications, but could not be strictly attributed to each drug component. However, cephalea was the most common event. It started at first injection of cetuximab and could be also observed prior to dasatinib association. Noteworthy, only mild skin toxicity (rash, xeroderma) was observed with this association.

Overall, these data showed a high incidence of severe events compared to cetuximab monotherapy experienced in the previous study, in which two cases of grade 3 hypersensitivity (5 %) and one case of rash (2.5 %) were detected.

Cetuximab combined with irinotecan was experienced also as second-line therapy in 63 patients with gastro-esophageal cancer resistant to platinum chemotherapy. Overall, the combination was well tolerated. Severe AEs were limited to neutropenia (11 %, febrile 2 %), fatigue (5 %), and GI signs (3–6 %). Most common additional events included skin lesions (rash 73 %, alopecia 50 %, nail toxicity 36 %), and GI signs (33–54 %). One patient had an allergic serious reaction during the first cetuximab infusion and was withdrawn from the study [22].

A retrospective review evaluated overall data from 6 Phase II trials in which cetuximab, either alone or combined with chemotherapy, was associated with radiotherapy in 359 patients with nonsmall cell lung cancer (NSCLC). Although direct comparisons were not possible, two trials reported high rates of treatment-related deaths and adverse events compared to all the other studies where no concomitant chemotherapy was given. This indicated that a consistent additional risk was coming from concomitant therapy regimens. Serious events in the study

group ranged from 17 to 70 %, involving hematological and nonhematological toxicities [23].

A wide review of controlled trials on NSCLC indicated contrasting effects on efficacy and safety of add-on chemotherapy regimens, combined or not with EGFR inhibitors. Overall, these regimens tend to favor AEs, while statistically significant clinical improvements were not so evident. These data indicate the existence of cytotoxic cumulative actions on AEs induction, which may imbalance the risk/benefit ratio in multi-drug therapeutic protocols [24].

The association of cetuximab and bevacizumab was experienced in solid tumors, in the attempt of synergizing the simultaneous inhibition of EGFR and VEGF blockers with a relative low toxicity, with a limited use of chemotherapeutics or without them. In the group of 29 patients with pancreatic cancer treated with both monoclonals, in the absence of standard gemcitabine administration, severe AEs were limited to constitutional and gastrointestinal signs (fatigue, nausea/vomiting, and abdominal pain) ranging from 7 to 21 %. Acneiform rash was 3.4 % compared to over 10 % in the group treated also with gemcitabine. Severe hematologic events, dyspnea, and cephalea were absent in the biomedicine-treated group, while present in 10–17 % of cases in the gemcitabine-associated group [25].

Another recent report compared data on irinotecan and bevacizumab in combination with cetuximab in recurrent primary GBM. EGFR is usually present in 35–45 % of these tumors and correlates with a poor prognosis. EGFR expression, tested in over 90 % of enrolled patients (41), was highly variable (<10 % to >50 %), but had no correlation with efficacy. However, overexpression was rather limited (37 %). Three patients (7 %) experienced severe allergic reactions during the first cetuximab injection and could not continue this therapy. Cetuximab-related skin reactions were frequent (67 %), and severe in 5 % of cases, often leading to dose modification and discontinuation. Infections (35 %) and vascular thrombotic complications had also a high frequency of severe events (14 % and 9 % respectively) compared to controls. Overall discontinuations occurred in about 14 % of patients and the safety profile was considered acceptable, except for skin toxicity. Noteworthy, diarrhea was moderate (7 % severe) and did not cause discontinuation or dose reduction, while in the alternative combination of irinotecan with anti-TYKs inhibitors (erlotinib, sunitinib) the intestinal disorder represents a relevant limiting factor [26].

15.5 Postmarketing Surveillance

The only postmarketing event reported in official labels is aseptic meningitis.

In 14,000 spontaneous reports within the FAERS database were registered about 47,000 AEs (3.3 AE/R). Most frequent reports (≥4 %) included GI signs, infections, dermatological reactions, respiratory disorders, and electrolyte imbalance. Pneumonia (443 reports) was the most frequent infectious event followed by

febrile neutropenia (364) and sepsis (293). Hypersensitivity reactions (88) included anaphylactic reactions (66), rash (162), and urticaria (22). Cutaneous disorders included dermatitis acneiform (88) and signs of skin toxicity (39).

Interestingly, 3 cases of aseptic meningitis and 2 cases of progressive multifocal leukoencephalopathy (PML) were reported.

In the EUV database, 5,996 reports (98 % including serious events) included 13,656 events (AEs/R 4.8; SAEs/R 2.3). Infections (3.65 %) included 50 cases of pneumonia. GI signs (8 %), cutaneous reactions (16 %) including rash (415), urticaria (193), and dermatitis acneiform (186) were among the frequent relevant events. Noteworthy, 997 infusion-related reactions, 585 hypersensitivity reactions, 354 anaphylactic reactions (353 with shock), 38 cases of anaphylactoid reactions, and 36 cases of rash (pustular) were also reported. Aseptic meningitis was included in 19 reports.

Interestingly, no cases of TB and two cases of PML were reported.

In Japan, after cetuximab approval (July 2008) a postmarketing surveillance was conducted on all treated patients. Up to January 2010, the safety population included 2,006 of the 2,126 registered patients. Positivity for EGFR was ascertained in 98 % of tumors affecting colon (61.6 %), rectum (38.6 %), and other sites (0.2 %). Total AEs (90 %) included skin reactions ((84 %), GI disorders (23 %), hypomagnesemia (11.5 %), and infusion reactions (6 %).

15.6 Remarks

Although this chimeric monoclonal shows a low immunogenicity, the frequency of acute and early hypersensitivity reactions is relevant, possibly due to cross-reacting preexisting antibodies directed to nonproteic antigenic determinants located on its Fab fragment. At least two saccharides have been identified as targets against which a wide portion of healthy population, if not all, develops antibodies including IgE, which are more likely to trigger severe anaphylactic reactions at first administration of cetuximab [12, 13]. These reactions are not fully controlled by routine premedications, and therefore further efforts should be directed to the elimination of both glycosylated structures from the proteic backbone of cetuximab (see also panitumumab, Chap. 32).

A second concern is related to skin toxicity, presumably due to the presence of EGFR on epidermal and follicular cells and to the consequent induction of acneiform rash in almost 90 % of cases, frequently associated with xeroderma and paronychial inflammation after interaction with infused cetuximab [18]. The rash can be severe up to 17 % of cases and usually appears after 2 weeks of treatment. However, this event has been associated with an improved tumor regression in some studies, and has been proposed as a possible marker of positive response to cetuximab treatment, although no specific investigations have been conducted on this important aspect.

Therefore, acneiform rash seems to be strictly related to the mechanism of action of cetuximab. Due to its early appearance, it may represent a selective and predictive parameter to be used in association with KRAS mutations, AREG, and EREG expression [10, 22]. Finally, nail and paronychial alterations are frequent events, although not serious, that seem to be common to all EGFR blockers and appear also in monotherapy experiences. Overall incidence of all grade AEs examined in over 2,000 patients in 22 trials was approximately 17 %, with no significant difference among cetuximab, panitumumab, and erlotinib [27].

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Daclizumab

16

Daclizumab (Zenapax®, Roche) is a humanized murine monoclonal IgG1k antibody specifically binding to the high affinity interleukin-2 (IL-2R) expressed on human lymphocytes. At the end of 2009 its production was discontinued and the supply was announced to last up to 2011. However, some trials are exploring new indications, and long-term observations are still collecting data, which are relevant for safety evaluation of chronic exposures to this class of biomedicines.

The initial therapeutic indication was restricted to prophylaxis of acute kidney allograft rejection, as part of an immunosuppressive regimen including cyclosporine (CsA) and corticosteroids.

In 1999, FDA first licensed the biomedicine, followed by Switzerland (1998), EMEA (1999) and many other countries. Trials presented for the initial approval included two Phase I studies (NO14392; NO15301) on 95 subjects, and two pivotal Phase III trials (NO14874; NO14393) enrolling 535 patients in US, EU, Australia, Canada, and Sweden. In the latter study, 267 patients received daclizumab associated with the mentioned immunosuppressive medications (plus a subgroup of NO14393 treated with azathioprine). The follow-up lasted one year before BLA presentation, and was planned for 3 years posttransplant.

The safety profile was based on observations obtained from a larger cohort of 877 patients, including 630 kidney allograft recipients and 247 adult patients with graft-versus-host disease (GVHD) and IL-2R+ tumors. In particular, 336 kidney allograft recipients and 182 additional patients (GVHD and tumors) received daclizumab associated with additional immunosuppressive regimens.

At present, over 100 trials have been performed with daclizumab. They include studies on solid organ transplants, such as kidney (23), pancreatic islet (14), liver (4), pediatric kidney or heart (5), and thymus (1). Non-transplant studies involve HL/NHL and leukemia (15), MS (9), uveitis (7); melanoma, GVHD, GBM, and Ps (2 each); BC, MDS, Behçet's syndrome, HIV infection, AA, TCP, asthma, Wegener granulomatosis, UC, JIA, lung transplant, AMD, and ovarian cancer (1 each). Most of them are completed. Ten studies are still recruiting patients [1–6].

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16.1 Mechanism of Action

Daclizumab is a recombinant humanized IgG1k monoclonal antibody binding to the α-subunit (Tac, p55a, CD25) of the high affinity IL-2R complex, acting as a receptor antagonist.

The human sequences (90 %) derive from the C domains of human IgG1 and from the V framework of the Eu myeloma antibody. The murine sequences derive from an anti-CD25 antibody complementarity-determining regions (CDR), originally developed at NIH to inhibit the proliferation of virus (HTLV-1) transformed T cells.

IL-2R is composed of three subunits (α, β, γ). Only after T cell activation the subunits assemble to form either the intermediate-affinity IL-2R (β, γ_c dimer) or the high-affinity IL-2R (α, β, γ trimer). After activation, T cells express the α chain (CD25), which only enters in the high affinity receptor assembly. IL-2/IL-2R binding elicits T cell proliferation and differentiation, cytokine production, cytotoxicity, and B cell help. The epitope targeted by daclizumab is located on the extracellular domain of CD25, and overlaps with basiliximab binding site. At the recommended dosage regimen, daclizumab saturates CD25 for approximately 90–120 days. Blocking of this ligand-receptor interaction interferes specifically with the T cell effector response, a critical pathway of the cellular immune response. The pathogenetic counterpart is involved in GVHD, T cell-mediated autoimmune disease, and possibly in the insurgence of EBV-driven posttransplant hematologic malignancies (PTLD). Noteworthy, by masking IL-2 binding sites daclizumab neither lead to complement fixation, nor to significant CDC, ADCC, or CD25 modulation, nor to signal-induced events [5, 26].

Recently, other functions and mechanisms of action have been elucidated, such as the inhibition of CD40L expression interfering with dendritic cell activity, and the enhancing effects on $CD56^{bright}$ NK cells. Interestingly, some of these mechanisms have been further clarified after off-label experiences with daclizumab, such as in non-infectious uveitis and in MS, where they seem to play a role both in drug efficacy and in mitigation of immune-mediated events. At structural level, the recognized epitope in CD25 was identified and found to be the same target of basilliximab and daclizumab [7].

Finally, a new daclizumab preparation called daclizumab high-yield process (DAC HYP) has been recently developed. The molecule has an identical amino-acid sequence of daclizumab, but has a different glycosylation pattern affecting the binding to Fc receptors, resulting in decreased CDC and ADCC activity. Therefore, DAC HYP, indicated for subcutaneous administration, is expected to evoke less AEs relating to these effector mechanisms. At present, this mAb is not commercially available and is investigated in Phase IIb (SELECT) and Phase III (SELECTION, DECIDE, OBSERVE) clinical trials for MS, planned for long-term treatment [8, 9].

16.2 Immunogenicity

Daclizumab has showed a notable immunogenic potential. HACA developed in approximately 14 % (adult) and 34 % (pediatric) of treated subjects, but did not appear to affect efficacy, safety, and serum drug levels.

No gross changes to circulating lymphocyte numbers or cell phenotypes were observed, except for the expected transient decrease in CD25+ cells. However, in pre-clinical investigations on monkeys daclizumab produced antibodies against the murine and the human part of the molecule. In particular, anti-murine antibodies consisted in a mixture of anti-isotype and anti-idiotype antibodies, while anti-human response was mainly anti-idiotype, and was directed to the H1, H2 and L3 CDR regions of the molecule. Some monkeys experienced anaphylactoid reactions after rechallenge.

Interestingly, immunogenicity was suppressed when daclizumab was administered in combination with CsA, while combinations with deoxyspergualin or with an anti-IL-2Rβ antibody increased the immunogenic capacity of daclizumab in these animals, thus indicating that the reactivity to these humanized antibodies can be affected by co-administration of other immunosuppressive agents in humans [5, 10].

16.3 Adverse Events

Since the initial pivotal studies on renal transplant recipients, it was clear that there was no relevant difference between the AEs profiles of daclizumab-treated patients and controls. Some events were registered as more frequent in the treated group, albeit with no statistical significance, possibly depending on the concomitant elevated frequency of adverse events in controls (over 95 %). Some serious events, such as infections and death rates, were even slightly lower in the treated groups. However, among 434 cardiac transplant recipients, *mortality* and *infections* were found to be higher in study patients than in placebo (6.5 % vs. 1.9 %) at one year after transplant. This led to the exclusion of heart transplants from official treatment indications for daclizumab [4, 5].

Episodes of hypersensitivity during initial exposure or after re-treatment with daclizumab were also observed in selected studies and registered in the post-marketing experience. Therefore, FDA raised a warning about cardiac events and hypersensitivity in 2003 [11].

Hemato-chemical laboratory abnormalities were equally distributed, except for fasting hyperglycemia, which was found to be two fold higher in patients administered with daclizumab. The profile in *pediatric patients*, evaluated in different trials, was comparable to that of adult patients, yet with a *higher trend* in *diarrhea*, *post-operative pain*, *pyrexia*, *emesis* (all >30 %), and *hypertension*, *pruritus*, *URTI*, *UTI* (all >18 %) [3, 5].

More recent data confirmed that the majority of AEs were mild to moderate, and serious cases remained anecdotal.

In conclusion, except for the cardiac transplant experience, daclizumab in the area of official indications was rather safe and well tolerated, with possible bias due to the relative short-term observations and to the high background level of AEs in this type of patients.

16.4 Off-Label Experience

Since daclizumab was not withdrawn due to safety reasons, a number of additional trials have continued to investigate different pathologies, leading to some interesting outcomes. At present, it has been mainly experienced in non-renal organ transplants (liver, pancreas, lung), malignancies (lymphoma, leukemia, melanoma, breast cancer), demyelinating diseases (MS), and localized or systemic inflammatory/autoimmune disorders (Uveitis, AMD, Behçet's, Ps, JIA, UC, AA, ITCP) [12].

As additional information accumulates, it becomes more evident that these studies are relevant both for exploring daclizumab efficacy in other conditions and for better understanding its mechanism of action, which appears to be more complex than expected. Moreover, in cohorts of patients where controls do not have the high background levels—usually encountered in kidney and other solid organ transplants—the analysis of drug-related AEs has showed a good tolerability of daclizumab compared to other similar products.

Since latent/chronic viral infections are frequent in some off-label applications, such as in liver transplant recipients, the additional immunosuppressive effect of this biotherapy on potential viral reactivation could also be evaluated.

Initial experiences in a number of *non-renal solid organ transplants*, mainly on liver transplant (LT) recipients, reported a few side effects to daclizumab, both in adult and pediatric use [3]. In addition, daclizumab association did not increase the incidence of AEs, allowing the reduction of conventional immunosuppressive therapy and its consequent undesirable effects.

In non-renal transplanted patients with impaired renal function, daclizumab-based induction therapy did not increase the incidence of infection, including CMV infection, compared to no-induction groups [13]. In a subsequent trial enrolling 148 patients (72 on daclizumab) receiving LT, only one patient in the investigational arm was withdrawn for an unspecified adverse event. However, SAEs including CMV infections (40 %), neurological (11 %), respiratory (7 %), and hematological (8 %) events resulted 4–8 % higher in the study group. Other infections, as well as gastrointestinal, cardiac, and respiratory events were equal or lower than controls. Noteworthy, neoplastic events (benign and malignant) were 1.4 % in daclizumab-treated patients versus 0 % in controls [14].

Since most LT are performed in HCV+ patients, the possibility of HCV-reactivation induced by daclizumab had been postulated. Daclizumab did not have a real impact on HCV viral reactivation and load, nor on graft rejection [15, 16].

An additional study on 157 LT (79 on daclizumab) showed that the induction treatment with daclizumab did not increase AEs, although a significantly greater proportion of patients experienced mild/moderate leukopenia (32–35 %), and diarrhea (18–23 %). SAEs were in the range of 1 % in both groups. After 2-year follow-up, any drug-related AEs were similar in both groups (77–78 %). The most common additional events in daclizumab-treated patients included anemia (14–31 %), thrombocytopenia (6–13 %), cholestasis (8–17 %), and hyperglycemia (23–27 %). Recently, the safety and efficacy of steroid-free immunosuppression (IS), with or without daclizumab induction, was compared to 2 standard IS regimens. After 2 years observation, there were no differences in HCV recurrence, patient survival, or graft survival rates. The side effects of IS did not differ, except for a trend toward less diabetes in the steroid-free group [17].

Experience with daclizumab in other solid organ transplants is more limited, and is substantially in line with safety data collected in renal and liver transplants. In fact, infectious disorders, including CMV infection, fungal infections, pneumonia, and local infections have been reported in daclizumab recipients, but their incidence was not different from that of patients not receiving induction therapy. Moreover, non-randomized studies indicated that daclizumab was associated with a lower incidence of infectious complications than OKT3 in recipients of various solid organ transplants, and that daclizumab tolerability in transplanted children was similar to that of adult recipients [3, 18].

A peculiar type of complication on the native kidney after non-renal solid organ transplantation is attributed to the calcineurin inhibitor toxicity. It has been shown that induction therapy with daclizumab was renal sparing, reduced corticosteroid exposure, allowed a delayed initiation of calcineurin inhibitors, and reduced steroid-related AEs.

A recent review has evaluated the impact of various immunosuppressive agents, including daclizumab and basiliximab, in pediatric solid organ transplantation, showing that these monoclonals had the lowest AEs impact among a number of other immunosuppressive agents, such as tacrolimus, sirolimus, everolimus, MMF, and also among other depleting T and B cell mAbs (OKT3, alemtuzumab) [19].

Since 1999, a series of studies focused on the possibility of controlling *uveitis* by daclizumab administration, with promising results and low rates of AEs [20].

Subsequent experiences obtained partial success, compared to other biomedicines and standard therapies, in a number of ocular inflammatory diseases, especially when associated with Behçet's syndrome, [21].

As expected, serious forms of posterior uveitis, panuveitis, and other non-infectious forms associated with systemic syndromes were the most investigated.

In another small study, daclizumab was employed at IV dosage in uveitis [22]. The infusion was well tolerated. Moderate AEs were registered in 3 out of 5 patients, and one developed lobar pneumonia. However, occurred additional reactions including follicular conjunctivitis, URTI, cephalea, tremor, dyspnea, anxiety, gastralgia, pruritus, and rash, were judged as non-related to treatment,

A recent review on biotherapy of posterior uveitis and panuveitis examined the alternate effects of five anti-TNF agents (infliximab, adalimumab, certolizumab, golimumab, and etanercept), three lymphocyte inhibitors (basiliximab, rituximab, and abatacept), and two cytokine receptor antagonists (anakinra and alefacept), evidencing the more peculiar AEs of each biomedicine. In the case of daclizumab they were hypersensitivity reactions, cephalea, and GI signs. This study warned against the indiscriminate use of biotherapy, because of risk/benefit imbalance and cost [23].

In the only long-term study (11 years follow-up), including high-dose IV and SC administration in noninfectious ocular inflammatory diseases, the side effect profile remained acceptable. However, malignancies raised some concern, since 4 patients developed cancer (RCC, cSCC, esophageal, vulvar) [24]. Cutaneous reactions were the most common drug-related AEs, and included drug eruption, eczema, fibrosis, psoriasis, and folliculitis. Other side effects possibly attributable to daclizumab included liver functional tests (LFT) abnormalities, lower extremity edema, herpes zoster skin infection, upper extremity neuralgia, lymphadenopathy, isolated chest pain (with normal EKG), UTI, GI infections, lethargia (1 patient), and cramping. Overall, the safety profile remained in the known framework, but discontinuation rates were more consistent (46 %) and related to various reasons, including the development of neoplasms.

Anti-IL-2R immunotherapy is being experienced in *myasthenia gravis* (MS) on the basis of the response obtained in EAE following the blockade of autoreactive activated T cells, and of consequent decrease in inflammatory damage [25]. A major concern in using daclizumab in MS was about the lack of long-term safety information on this biomedicine. In fact, most of the experience in renal transplantation was limited to the induction phase of immunosuppression.

Preliminary data on MS, from four open-label studies (53 patients) and from the first larger controlled trial (CHOICE) enrolling 230 patients, showed similar infection rates in daclizumab-treated and control groups. Skin rashes were seen in 13 % of daclizumab subjects and in 8 % of placebo. Higher frequencies of cutaneous infections, URTI, and UTI were detected in the study groups, together with anecdotal cases of transient lymphadenopathy, photosensitive rash, paresthesia, and constitutional signs including pyrexia. Minor and transient elevations of hepatic enzymes and bilirubin were also present. However, no SAEs were registered [25, 26].

Subsequent reports on five completed trials and preliminary data on six ongoing studies have been recently released [27]. Treatment with daclizumab was given IV or SC and was reported as well tolerated up to 2 years follow-up, not only compared to other immunosuppressive/modulatory conventional drugs, but also to other five monoclonal antibodies. No serious events or deaths were reported. Mild/moderate and anecdotal events included cephalea, constipation, paresthesia, depression (1 case), lymphadenopathy, URTI, UTI, cytopenia, transaminase or bilirubin elevation, transient TCP, presence of autoantibodies, granuloma annulare, and photosensitivity rash.

More recently, studies were addressed to test the DAC HYP modified daclizumab molecule in monotherapy, by monthly SC administration. Interim data from the SELECT trial (621 patients treated for 52 weeks) reported SAEs in 6–8 %

in the DAC HYP groups (5 % in placebo), including infections (2 %) and cutaneous reactions (1 %) that were absent in controls. LFT abnormalities (5-fold the maximal normal range) were present in 4 % of the study group (<1 % in placebo).

However, some concern was raised from 2 deaths; the first was observed in SELECT in one patient recovering from a serious rash, due to an infectious complication (psoas abscess); the second was registered in an ongoing extension study due to possible autoimmune hepatitis. A contributory role of DAC HYP in these events could not be excluded [28].

Most recently, a complete report on 412 MS patients of the SELECT study, after 1-year treatment with two doses of DAC HYP (208 with 150 mg; 209 with 300 mg), was released [9]. AEs occurred in a similar proportion in all study groups, and serious events resulted lower in patients receiving the mAb. Serious infections were 2 % in treated groups and 0 % in placebo. One of these patients discontinued the treatment, and six interrupted the administration only temporarily. A total of four malignancies were observed: two melanomas in the high-dose group, and two cervical carcinomas in the 150 mg group and placebo group, (1 each). Five patients had serious cutaneous events (rash, atopic dermatitis, allergic dermatitis, exfoliative dermatitis, erythema nodosum), while none was found in the placebo group. The only death occurring during the study was the serious rash case, complicated by a psoas abscess, mentioned by the interim report. Noteworthy, four cases of immune-mediated serious disorders (autoimmune thyroiditis, CD, hypersensitivity, lymphadenopathy) only occurred in the high-dose recipient group.

Finally, hepatic enzymes abnormalities (over 5-fold the ULN level) occurred rather late (median onset 208 days) and were more frequent in the study group. Neutralizing antibodies were observed in 2 % of cases in treated patients, being neutralizing in two subjects. There was a moderate increase of NK cells and a decrease of T cells.

Overall, these results indicated an increased risk of infections, and of cutaneous and hepatic events with a suspected immune-mediated cause. The concern about long-term therapy remained, mainly for the death-related infection in SELECT, the possible induction/reactivation of autoimmune disorders also observed in the SELECTION ongoing trial (autoimmune hepatitis), and for malignancies [9, 28, 29].

A general concern was underlined in a recent meta-analysis by the Cochrane Collaboration, which examined over 470 references on daclizumab and considered inadequate a consistent number of studies and trials, also due to the absence of proper controls. The analysis was performed before the release of the 1-year results of SELECT trial [9, 30].

However, the Cochrane analysis showed no significant difference in AEs across all the groups, which had at least one adverse event/patient in 97 % of cases. SAEs (13 % vs. 5 %) were mainly infections (5 % vs. 1 %). The most frequent severe drug-related AEs were infections and infestations (7 % vs. 3 % in controls). There were no opportunistic infections, and all infections resolved with standard therapy. The Cochrane conclusion was that better designed, controlled, and crossover-controlled trials are required to evaluate the efficacy and safety of daclizumab. The mentioned ongoing SELECT and SELECTION trials, along with the new

DECIDE and OBSERVE studies—planned to enroll 1,880 and 150 patients administered with subcutaneous DAC HYP for 3–4 years—are expected to bring enough data for drawing a definitive conclusion.

16.5 Postmarketing Surveillance

At present, 4.95 AEs/Report were observed in over 1,500 FAERS postmarketing records. Infections (13.5 %) were the most common event. About 50 % of them were viral, and 2 % had fungal origin. CMV infections (306), UTI (161), sepsis (160), and pneumonia (108) were the most frequently reported. Immune disorders occurred in about 3 %, including about 20 cases of rash and three cases of anaphylactic shock.

In the EUV database, 248 reports included 789 AEs (3.2 AEs/R). Most frequent events were infections (159) and immune reactions (109). Among the former 12 cases of sepsis, 8 CMV infections and six cases of pneumonia were registered. Immune-related events mostly concerned GVHD. Seven cases of anaphylaxis (4 with shock) were also reported. One case of capillary leak syndrome (CLS) was reported. Among 22 reports of cardiac disorders, three cases of cardio-respiratory arrest, two cases of cardiac arrest, and one CHF were registered.

16.6 Remarks

Daclizumab is a moderate inducer of AEs, not only compared to conventional immunosuppressive treatments, but also to other biomedicines targeting T lymphocytes and the IL-2 pathway.

Noteworthy, daclizumab does not lead to CDC, ADCC, or CD25 modulation, or to signal-induced events, all known to enhance cytotoxicity. The mild safety profile observed in some off-label non-transplant indications may open to further assessment of efficacy in other clinical conditions. Although the original monoclonal production was discontinued, the modified DAC HYP is under investigation, with encouraging result.

As for the involved mechanisms of action, recent observations have widened their understanding. With this respect, two related issues seem particularly relevant: the daclizumab-induced marked expansion of $CD56^{bright}$ NK cells, and the killing mechanism of these cells via the granzyme-mediated apoptosis. These effects are possibly related to the increased availability of free IL-2, as a consequence of the daclizumab-dependent IL-2R blockade and the subsequent increased stimulation of intermediate-affinity IL-2R (not affected by daclizumab) expressed on NK cells. These cells are able to kill, among other cells, CD4+ lymphocytes, via granzyme-mediated apoptosis [31]. Therefore, this mechanism may be more important for both efficacy and safety, compared to the inhibiting effect on activated T cells, since these lymphocytes only show a moderate reduction in the presence of daclizumab, both in vitro and in vivo. Furthermore, the granzyme mechanism appears to be less active in producing AEs, compared to other blockers

of the IL-2 pathway strongly acting on the T cell lineage and on their cytokine release cascades.

However, cutaneous SAEs were observed even with DAC HYP—a molecule modified in its capacity of Fc-related effector activity—suggesting the existence of alternative pathways, such as the inhibition of FoxP3 in T-regulatory cells, or the mentioned granzyme-mediated processes as inducers of such adverse events. The latter pathway may be also implicated in liver function abnormalities observed with daclizumab, since $CD56^{bright}$ NK cells are a relevant immune cell population in the liver. By contrast, other studies indicate $CD56^{bright}$ NK cells acting more as down-regulators of immune-mediated tissue damage, and of latent/persistent infections, suggesting a more protective effect of this and other related innate immune effector mechanisms [8, 32]. This conclusion is supported by repeated in vivo observations in transplants, in which patients receiving daclizumab (even in addition with standard immunosuppressive therapy) had lower infectious complications, including CMV and other opportunistic infections.

Noteworthy, NK cell deficiency is associated with autoimmune diseases including MS, thus suggesting a potential new role of daclizumab in this area, as an NK cell stimulator. In addition to the low rate of induced AEs, daclizumab has allowed to lower dose regimens of a number of immunosuppressive drugs, usually more prone to generate severe adverse events, leading to a global reduction of drug-related AEs.

However, the issues on long-term effects of daclizumab remain. Although experience with daclizumab covers about 15 years observation, most treatments were limited in time and adequate follow-up studies are still lacking, mostly in the new off-label applications. On this side, particularly in MS, data are still incomplete, and have raised some concerns about the possibility of an increase in malignancies and autoimmune processes.

Finally, caution was expressed against the abrupt suspension of daclizumab after one case of cerebellar herniation in one patient with Behçet's disease one month after cessation of the monoclonal [24].

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Denosumab

17

Denosumab (Prolia®, Xgeva®, Amgen) is a human recombinant IgG2k antibody directed to receptor activator of nuclear factor kappa-B ligand (RANKL), and thus inhibiting its interaction with the specific RANK receptor. The blocking interferes with formation, function, and survival of osteoclasts, which are essential for bone resorption.

After the original application (IND 9837) presented in 2001, Amgen submitted Prolia® to FDA four BLA in December 2008 for four separate indications: treatment, and prevention of postmenopausal osteoporosis (PMO); treatment, and prevention of bone loss associated with hormone ablation therapy for breast and prostate cancer.

However, during the initial evaluation, hypocalcemia, serious infections (including serious skin ones), development of new malignancies, potential for tumor progression, dermatologic AEs, pancreatitis, and oversuppression of bone turnover raised significant concern in the reviewers. Consequently, further safety data were requested, and were examined in 2009 [1].

The first approval by FDA and EMEA was granted during 2010, only for the treatment of postmenopausal women with osteoporosis at high risk of fracture. The same product with different dosing regimens, under the name of Xgeva®, was approved during the same year (in 2011 by EMEA) for prevention of skeletal-related events (SRE) in patients with bone metastases from solid tumors. On the basis of supplemental data, the following year Prolia® indications were extended to the treatment for increasing bone mass in men at high risk of fracture receiving androgen deprivation therapy for non-metastatic prostate cancer, and to the treatment for increasing bone mass in women at high risk of fracture receiving adjuvant aromatase inhibitor therapy for breast cancer.

In June 2013, FDA approved the treatment of unresectable giant cell tumors in adults and mature adolescents on the basis of two single arm trials on 304 patients (204 exposed up to 3 years) [2–5].

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Pivotal primary studies included four Phase III trials for the different indications. In particular, Study 20030216 (FREEDOM) on 7,808 (3,886 exposed) patients, and Study 20040132 on 329 (164 exposed) patients related to PMO, The third trial (20040135) on 252 (127 exposed) enrolled patients with BC, and the fourth study (20040138) included 1,468 (734 exposed) 384 patients with prostate cancer.

Additional safety data came from one Phase II trial 20010223 on 412 patients and five related extension studies. Further safety studies, including three Phase III trials (20060289, 20050233, 20060232) and one Phase II study (20080287), were requested on over 5,600 patients, and ongoing up to 2015. Initial trials on breast and prostate cancer patients did not meet the criteria for priority review [1].

Three subsequent pivotal studies, which supported the extension of indication of both denosumab commercial forms, included one trial (20050136) on 2,046 breast cancer patient; one study (20050103) on 1,904 prostate cancer patients, and one (20050244) on 1,776 patients with other advanced solid tumors [4–8]. However, requests for the extension to myeloma and to SRE-free prostate cancer patients were rejected due to imbalance in the risk/benefit ratio.

Finally, approval for the indication of denosumab for the treatment of bone loss in man with osteoporosis (OP) at high risk of fractures was granted in 2013 on the basis of a Phase III trial (20080098, ADAMO) additional on 242 patients, while the extension to prevention of bone metastases in prostate cancer was rejected [9].

At present, a total of 56 trials are completed or ongoing, including 21 studies on PMO, 8 on osteoporosis, 7 on BC, 7 on PC, 2 on myeloma, 2 on other advanced cancers, 2 on RA, and 1 on hyperparathyroidism.

17.1 Mechanism of Action

RANKL (TNFSF11) is a cytokine of the TNF family expressed at the surface of osteoblasts and bone marrow stromal cells, and then released by the same cells and by activated CD4+ T cells. However, some epithelial cells (prostate, mammary gland, keratinocytes), T cells, and at a lower level B cells, can also express both soluble and membrane-bound forms of RANKL, while osteoclasts, dendritic cells and Langerhans cells express its receptor RANK.

Two of the three known isoforms of RANKL have a transmembrane domain with a biologically active carboxy-terminus in the extracellular part. RANKL2 is a shorter alternative splicing variant of RANKL1. Both variants can be proteolytically cleaved into soluble forms, with osteoclasts stimulating activities within their TNF-homology domains. RANK is the unique receptor for RANKL molecules, and their interaction activates formation, adherence, function, and survival of osteoclasts, the sole cell type responsible for physiological bone resorption, bone metastatic destruction, and myeloma osteolytic localizations. RANK is also expressed on monocytes/macrophages, dendritic cells and T cells, thus acting in a paracrine circuitry of immune modulation. RANKL/RANK interaction involves the extracellular receptor binding domain of RANKL and the extracellular cysteine-rich domains of RANK. This interaction causes oligomerization of RANK

and the subsequent activation of several protein kinase pathways. The catabolic effect of RANK activation is balanced by competition with osteoprotegerin (OPG), the RANKL decoy receptor, which functions as its natural antagonist. OPG is another receptor of the TNF family produced by the same cells releasing RANKL, including B lymphocytes.

Vitamin D3, parathyroid hormone, prostaglandin E2, IL-1, IL-6, TNF, prolactin, and corticosteroids increase the expression of RANKL. By contrast, estrogens, calcitonin, TGF, PGF, and calcium increase the expression of OGP, thereby inhibiting osteoclastogenesis and resorption. Therefore, osteoclasts homeostasis is controlled by a number of cytokine pathways and depends mainly on the local RANK/OPG competition for the same ligand. Prostate and breast cancer cells in bone metastases express and upregulate RANKL of osteoblasts and bone marrow stromal cells, thus inducing osteoclastogenesis. Interestingly, RANK signaling is involved in lactation development during pregnancy. Furthermore, RANKL promotes migration of epithelial cells and may act as a bone-specific factor for migration of cancer cells.

Denosumab is a fully human monoclonal antibody (IgG2k) that binds with high affinity and specificity to primate soluble and membrane-bound RANKL, preventing the RANKL/RANK cell surface interaction. This lowers osteoclasts' number and functions, thereby decreasing bone resorption and cancer-induced bone destruction. Denosumab also prevents steroid-induced bone loss through a pronounced antiresorptive effect. Moreover, in the absence of this ligand, osteoclast numbers decline dramatically due to loss of precursors and apoptosis of the existing osteoclasts.

Denosumab and OPG have similar mechanisms of action. The blockade of RANKL inhibits also T and B cells, thus increasing the risk of infection. However, denosumab neither binds to TNF-related apoptosis-inducing ligand (TRAIL) nor to other TNF family members including TNF-α, TNF-β, and CD40 ligand. At present, no neutralizing antibodies against denosumab have been detected. By contrast both TRAIL binding and induction of neutralizing antibodies against OPG have been shown.

Denosumab can be detected, primarily in circulation, for several weeks (half-life: 26 weeks) [10–13].

17.2 Immunogenicity

The low immunogenicity of denosumab is reassuring. This fully human antibody induced HAHA in < 1 % of the 13,000 tested patients with PMO, OP, or bone loss due to hormone ablation therapy (HALT). Neutralizing antibodies were virtually absent.

The incidence of adverse drug reactions potentially associated with hypersensitivity (1.3 %) was equal in denosumab-treated and control groups. The most common adverse event potentially associated with hypersensitivity was urticaria (0.7 % in both groups). No data have been reported in the information label of

Xgeva product. Therefore, no evidence of an increased risk of hypersensitivity, drug hypersensitivity, or drug allergy reactions to denosumab has emerged in clinical trials [3, 14, 15].

17.3 Adverse Events

Official Prolia® indications (60 mg of denosumab SC every 6 months) for women are limited to the treatment of PMO, or to subsequent aromatase inhibitors administration for breast cancer (BC), when at high risk of fractures. In men, indications are for increasing bone mass in OP, or for patients at high risk of fractures receiving androgen deprivation therapy. Warnings for Prolia-induced AEs are: *hypocalcemia* and mineral metabolism, *serious infections*, *dermatologic toxicity*, *osteonecrosis of the jaw* (ONJ), *atypical fractures* and *suppression of bone turnover*, and *hypersensitivity* reactions including *anaphylaxis*.

The indication for Xgeva ® (120 mg of denosumab SC every 4 weeks) is limited to prevention of skeletal-related events (SRE) in patients with bone metastases of solid tumors, and quite recently it was extended to treatment of giant cell tumors of bone. Warnings for Xgeva-induced AEs are *hypocalcemia*, *ONJ*, and *embryo-fetal harm*.

The safety profile of Prolia in-label indications is based on over 10,000 exposed PMO patients in controlled studies, receiving denosumab mainly for 1–5 years and up to 8 years.

Exposure to Xgeva for SRE prevention in controlled studies included over 4,500 patients with solid tumors, mainly represented by BC and PC. Additional recent updates on PMO and on male OP treatments have been also provided [9, 15–18]. Therefore, the safety profile of denosumab is well documented in these diseases, and administration is usually well tolerated and stable within the indicated dosages and time intervals. In particular, the adverse events profile at 6–8 years is similar to that reported after 1–4 years of drug exposure, with no report of new signals emerging over time. Most AEs were similar in study and control groups, both in frequency and typology. However, some events were increased in denosumab-treated patients, and a number of safety issues, mainly concerning high dose and prolonged treatments, are still to be solved, and more consistent data need to be collected.

In the attempt of providing a common safety profile of denosumab, the two Prolia and Xgeva formulations, administered in different pathologies, have been respectively considered as the low and high dosages of the same mAb, taking into account differences depending on the underlying treated diseases.

Hypocalcemia was an early and expected event strictly related to the mechanism of action of denosumab. It is also a typical contraindication of the class of drugs interfering with bone resorption and bone remodeling. Levels lower than the minimum normal range (< 8.5 mg/dL) were approximately 2 % (< 0.5 % in controls) during initial treatment of PMO with Prolia, but reached about 18 % (9 % controls) in cancer patients treated with higher doses of denosumab (Xgeva).

In the latter group, severe hypocalcemia (<7 mg/dL) involved over 3 % of treated patients (1.3 % in controls). The risk was higher in patients with severe renal impairment. However, the imbalance was usually transitory (within one month), and followed by spontaneous resolution without sequelae.

Overall, recent long-term results showed no specific, unique, or worsened findings related to hypocalcemia usually occurring in PMO patients and enhanced by anti-resorptive treatments. In overall analyses comparing the most common AEs to denosumab with those to the alternative treatment with zoledronic acid, hypocalcemia, and toothache resulted predominantly in the former treatment [6, 16].

Severe *hypophosphatemia* (serum levels less than 2 mg/dL) was not reported after Prolia treatment, while it occurred in 15.4 % of patients treated with Xgeva (7.4 % in controls treated with zoledronic acid) [1, 3, 5].

Osteonecrosis of the jaw (ONJ) and *atypical fractures* may occur after dental disease and surgery, cancer, anti-cancer therapy, and steroid administration, but are usually related to anti-resorptive treatments, including denosumab, especially when associated with HALT. Initial reports on long-term treatment with bisphosphonates reported the development of ONJ and of atypical fractures (subtrochanteric femur). Observations on PMO patients treated with denosumab also reported anecdotal cases of ONJ and of atypical fractures. However, subsequent treatments with higher dosage (Xgeva) registered 2.2 % of ONJ in neoplastic patients—especially in PC patients (5 %)—receiving also HALT [5, 19].

A recent concern was issued due to the observation of an increased risk in study 200502147. In fact, data suggested that the more increased the exposure to denosumab as Xgeva, the more increased the risk of ONJ, and a continued long-term exposure could raise the ONJ rate to a level that off-sets the risk–benefit profile for the SRE indication in patients with prostate cancer. Indeed, the 120–day safety update to the BLA reported additional six patients with ONJ events out of 100 patients who had received denosumab, thus raising the frequency of ONJ to 5.4 %, a level considered relevant for a population of patients with non-metastatic prostate cancer.

Additional concerns related to the possibility that ONJ frequency registered in clinical trials could underestimate the risk in the clinical care population, and that denosumab, as a bone targeted agent, could shift the pattern of metastatic disease to non-bony sites. On this basis, FDA rejected the requested extension of denosumab therapy to prevention of metastasis. However, the incidence of adverse events other than ONJ and hypocalcemia, including severe and fatal adverse events, were similar to those of the placebo group [20]. Noteworthy, toothache, which is among the AEs at higher frequency in denosumab-treated patients, was not associated with the development of ONJ [21].

There were no instances of hypocalcemia, and ONJ complications (or for fracture healing, or atypical femoral fractures) during the first year treatment of OP in men [9, 20].

Infections as an expression of collateral immunosuppressive effect of denosumab were expected, since lymphocytes and dendritic cells also express RANKL and RANK molecules, thus establishing a link to immune and inflammatory defense. Overall, infective events were frequent (over 54 %) but not significantly

higher than in controls (53 %). Interestingly, serious nonfatal infections were limited in frequency (4–12 %) and typology, involving mainly the urinary tract (6–12 %), the upper respiratory tract (5–6 %, mainly nasopharyngitis), and the skin (0.4–0.9), the latter being similar to controls in frequency, and comprising erysipelas (0.2 %) and cellulitis (0.2 %). Similarly, opportunistic infections (0.1 %) and infection-related deaths (0.2 %) were low and comparable to placebo groups. However, there were more hospitalizations in denosumab-treated patients due to skin infections (0.4 %) than in controls (< 0.1 %); this may be due to a particular susceptibility of some cutaneous cells to RANKL inhibitors.

Moreover, a recent meta-analysis on PMO trials using a model of fixed effects has revealed a significantly higher risk of serious infections in women treated with denosumab than in controls (RR: 1.26; CI: 1.01–1.57, $p = 0.04$). The risk seems to be mostly involving a subgroup of patients with non-metastatic breast cancer included in one trial. However, in another recent overall meta-analysis, infectious AEs were found similar in denosumab-treated patients and in the control arm (RR; 1.01; CI: 0.95–1.07), and no new or differential signals could be found for serious infections. Nonetheless, an increased risk for infectious disease could not be fully excluded in long-term exposure [3, 13, 15, 18, 21, 22].

Dermatologic toxicity was present in a significantly high number of patients treated with denosumab. Epidermal and dermal adverse events in over 7,800 PMO patients were significantly higher (10.8 % vs. 8.2 % of placebo; $p < 0.0001$), and included rash (2.5 % vs. 2 %) and eczema (1.3 % vs. 0.6 %). Initial records of the applicant reported 15 % vs. 13 % of cutaneous events on over 9,000 patients (550 events on 4,550 patients in denosumab therapy), and included bullous conditions, exfoliative conditions, dermatitis, eczema, photosensitivity, eruptions, rash, and exanthema. However, no convincing evidence related the four cases of toxic skin eruptions (one serious) to denosumab. As for bullous conditions, 3/11 cases could not be ruled out to be denosumab-dependent, although the difference with controls was statistically non-significant [1]. Therefore, some concern remained for long-term treatments, and for older subjects, or therapy-immunosuppressed patients that might be treated with denosumab [20].

The overall incidence of *new malignancies* in PMO was 4.8 % in the denosumab group (Prolia) and 4.3 % in the placebo groups. The overall incidence of new primary malignancies in cancer patients with bone metastases was 0.99 % in the denosumab (Xgeva) group and 0.63 % in the zoledronic acid control group. In the large PMO experience the most common malignancies related to breast (0.9 % vs. 0.7 % in controls), reproductive system (0.5 %. vs. 0.2 % in controls), and gastrointestinal system (0.9 % vs. 0.6 % in controls). In the latter group the highest incidence was observed in the prostate cancer trial (1.9 % in the Xgeva group vs. 1.1 % in controls). However, a causal relationship to drug exposure has so far not been established nor excluded.

Overall, the most common events related to denosumab therapy were arthralgia, cephalea, nausea, lumbalgia, fatigue, and pain in extremity. The most serious events (0.7 %) included ONJ, osteomyelitis, and tooth abscess.

The safety profile in patients with giant cell tumors was similar.

17.4 Off-Label Experience

The most common off-label use of denosumab, either as Prolia or Xgeva, is for treating bone and cancer diseases.

In particular, Xgeva has been used for the prevention of PMO and OP, and for prevention of bone metastases in prostate cancer, as well as for the treatment of RCC. As for Prolia, a large number of reports (23 %) in FAERS databases are for "Product used for unknown indication", followed by off-label osteopenia (1 %) and anecdotal cases, including prophylaxis of OP, osteoarthritis, and RA.

17.5 Postmarketing Surveillance

Among over 5,200 reports in the FAERS database (3,809 as Prolia, 796 as Xgeva, 639 as denosumab), the most common AEs (4–9 %) to Prolia are related to dermatological conditions, musculo-skeletal disorders, infections, neurological and GI signs. Calcium and bone disorders, GI signs, respiratory disorders, and dermatological conditions are the most reported (4–14 %) for Xgeva. The respective reported fatalities are 0.87 % and 2.2 %. Infections are more frequently reported for Prolia.

Severe-serious events (grade 3, 4) events included musculo-skeletal disorders, dermatological conditions, infections, GI signs, and neurological disorders for Prolia. Serious reports for Xgeva included bone and calcium disorders, GI signs, musculo-skeletal signs, and dermatological conditions.

Overall, no new signals have emerged from these reports. However, a recent preliminary analysis on FDA records draw attention on the consistent presence of cutaneous adverse events (40 cases; 33 in 1 year) related to overall Prolia administration (63 %). Serious events occurred in 82 % of cases. Proportional reporting ratio (PRR: 2.77) was significant for skin rash and dry skin, suggesting the convenience of a closer monitoring of denosumab-associated dermatologic toxicity (C. Bankhead, www.medpagetoday.com; May 11, 2012).

In the EUV database, 2,455 (2,375 on serious events) reports (3.1 AEs/R) were registered up to the end of 2012, including GI disorders (8,7 %), skin reactions (7,3 %), infections (7 %), respiratory disorders (3 %), malignancy (2.8 %), and immune disorders (1.3 %).

Among infections, pneumonia (61), UTI (43), cellulitis (42), and sepsis (21) were the most relevant and frequent registered events. Among immune reactions, hypersensitivity (53), anaphylactic reactions (8), anaphylactic shock (3) were reported. Cutaneous reactions as rash (69), erythema (38), and urticaria (29) were predominant.

17.6 Remarks

The overall long-term safety profile of denosumab, mainly in the official indications as Prolia, appears safe and stable, with an exposure extended over 5 years.

This fully human antibody pertains to the IgG2 subclass, which is known to be relatively inactive in eliciting Fc effector functions [23]. Experience with Xgeva showed a similar trend, although the time of observation is still limited. So far, most AEs after prolonged cycles of therapy remain generally mild/moderate.

At present, no specific, unique, or worsened findings with respect to adverse events, including hypocalcemia, ONJ, infections, malignancies, hypersensitivity, and increased immunogenicity have become more evident. In particular, the initial imbalance of some SAEs—such as pancreatitis, diverticulitis, UTI, URTI, new and atypical fractures, fracture repairs, and laboratory findings (hypocalcemia, HAHA levels)—have not increased, or have not been subsequently confirmed in larger cohort of treated patients. This suggests that duration of exposure has no major impact on the safety profile with denosumab. No new signals appeared over time. However, an increased risk for infectious disease cannot be fully excluded, and some concerns remain at least about some initial signals: three cases of pneumonia developed in healthy volunteers after a single dose of denosumab, three cases of endocarditis, seven cutaneous SAEs, and one infective arthritis in subsequent treated patients, virtually absent in controls [1]. Pneumonia, cellulitis, and UTI remain the most serious infectious events during this therapy, yet without significant increased frequency compared to alternative therapies. However, opportunistic infections were unusual and equally distributed among treated and control groups.

There is no direct evidence that denosumab is broadly immunosuppressive, although the RANKL pathway involves also pivotal cells of the immune system, such as dendritic cells in lymph nodes and skin, and also lymphocytes yet possibly at lower extent.

Nonetheless, because of the existence of multiple pathways for immune regulations, the RANK pathway may explain a secondary effect on protective immune reactivity. For example, in experimental models RANKL is abundantly expressed on CD4+ T cells, while OPG (the alternative decoy receptor which blocks RANKL) is markedly expressed by B lymphocytes (B cells express also RANK at lower density). RANKL does not increase the production of cytokines, indicating that it has low impact on mature T cells. However, it generates co-stimulatory signals for dendritic cells, which in turn activate T cells after the CD40L/CD40 primary signal; this suggests a possible accessory role of the RANKL pathway in these processes. Interestingly, CD4+ T cells and total lymphocytes are not severely decreased ($\leq$ grade 3) during administration of denosumab.

Taken together, these data may explain the low impact of denosumab on infections, except for localized areas (skin) where the co-stimulatory signaling may become more prominent, albeit not exclusive. As previously mentioned, the RANKL/RANK signaling system is expressed at cutaneous level, involving dendritic cells, Langerhans cells (cutaneous specialized dendritic cells), keratinocytes,

and circulating T and B cells. Moreover, in experimental models, RANKL enhances DC survival, antigen presentation, and production of pro-inflammatory cytokines; in contrast, inflamed keratinocytes increase Treg cells, which down-regulate cutaneous inflammation and local allergic responses. Therefore, RANKL inhibitors might amplify cutaneous allergic and inflammatory responses rather than increasing susceptibility to infection itself [13]. However, the low number of injection-site reactions and the low rate of acute-phase hypersensitivity encountered during denosumab SC therapy do not support this hypothesis.

Alternatively, the inhibition of the local RANKL/RANK circuitry might affect the protective and allergic capacity of immune reactions, thus combining a lower rate of hypersensitivity reactions (injections site reactions <1 %, all events 1.3 % as in placebo) with an increased susceptibility to infections. Because of the existence of multiple pathways involved in immune regulation, the outcomes may become rare and unclear.

With this respect, some safety aspects deserve particular attention. As previously mentioned, dermatologic conditions should be followed to better understand whether or not local mechanisms expose to higher long-term risks. Although experimental and clinical evidences neither support an immunosuppressive effect nor a cancer-promoting activity of denosumab, further observations are needed, especially in immunosuppressed and immunocompromised subjects. In fact, their number may increase in the future due to the extension of denosumab indication, at higher doses, in cancer patients.

Although no single event of malignancy could be specifically referred to denosumab, they will continue to be monitored, with particular attention to the typologies in which RANK/RANKL circuitry may be of particular relevance, such as in breast or prostate cancer. Interestingly, RANK signaling also plays a role in the development of breast epithelium and on some intestinal cells [3–5, 15, 18, 22], as well as in angiogenesis and endothelial permeability, which may play a role in the inflammatory and neoplastic genesis.

Breast cancer cells have been shown not only to express RANK, but also to upregulate RANKL expression by osteoblasts and bone marrow stromal cells. Prostate cancer cells can also upregulate RANKL expression in osteoblasts [11]. Therefore, the homeostatic balance of this system, modified by RANKL inhibitors, is complex a may generate opposite inflammatory and proliferative responses that deserve major attention.

Consequently, the risk assessment of denosumab withdrawal seems important, for the possible rebound of inflammatory/immune responses, and for the insurgence of potential tumorigenic stimuli due to the removal of RANK inhibition.

The immunogenicity of denosumab is low, as expected from a fully human antibody. HAHA response and hypersensitivity reactions, mainly of the acute phase, were negligible, as for injection-site reactions. As an antibody, its clearance does not affect the renal function, being catabolized by the reticulo-endothelial system, which is also reassuring for patients with renal insufficiency in need of high dose anti-metastatic bone therapy [24].

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Eculizumab

18

Eculizumab (Soliris®, Alexion) is an IgG2/4 k monoclonal antibody that binds to the C5 complement factor, thereby inhibiting the formation and activity of the terminal components of complement. The orphan drug status was designated by FDA in 2000 for the treatment of dermatomyositis, in 2001 for idiopathic membranous glomerular nephropathy, in 2003 for paroxysmal nocturnal hemoglobinuria (PNH), in 2009 for atypical hemolytic uremic syndrome (aHUS), and in 2011 for Shiga toxin-induced HUS. EMEA recognized such status in 2003 for the treatment of PNH, in 2009 for aHUS, and in 2012 for infection-associated hemolytic uremic syndrome (HUS).

Trials and studies on RA, pemphigus, polymyositis, and CHF followed after 2006, the latter by employing a single chain version of eculizumab called pexelizumab. However, most results were not encouraging and attention focused on the treatment of PNH, which was approved by FDA and EMEA in 2007, and by Canadian and Australian Authorities in 2009. Approvals were based on a pivotal trial (TRIUMPH, C04-001) enrolling 87 patients with PNH (43 treated with eculizumab). Additional data came from a single arm trial (SHEPHERD, C04-002) conducted on 97 treated patients. During 2011, further studies were submitted to both Agencies for extending the indication to aHUS. They included two randomized (Study C08-002A/B and Study C08-003A/B) trials on 40 (37 exposed) adult and adolescent patients with aHUS, and a retrospective data collection study (Study C09-001r) on 30 exposed patients. Approval was granted within the same year by both Agencies.

At present, there are 45 trials—completed, ongoing, or recruiting—on PNH (13), aHUS (8), antibody-mediated rejection of kidney transplant (8), Shiga toxin-related HUS, or STEC-HUS (2), and on asthma, antiphospholipid antibody syndrome, dermatomyositis, CD59 (MAC-IP) deficiency, cold agglutinin disease, vasculitis, AMD, NMO, and MG (1 each)[1–7].

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18.1 Mechanism of Action

Eculizumab is a humanized IgG2/4 k monoclonal antibody derived from the murine anti human C5 antibody. The complementary-determining regions (CDR) of m5G1.1 were inserted into a germline framework of human IgG2 (including the hinge region), which does not bind to Fc receptors, and the CH2-CH3 domains from human IgG4, which are unable to activate complement. Therefore, the IgG2/IgG4 hybrid lacks both functions. These modifications minimize immunogenicity and prevent pro-inflammatory responses mediated by the IgG Fc portion.

Eculizumab binds to the human C5 complement protein with high affinity, thereby inhibiting its cleavage to C5a and C5b, and thus preventing the generation of the terminal membrane attack complement complex C5b-9 (MAC). The binding site of eculizumab is located at the contact interface between C5 and C5 convertase, thus blocking the downstream complement action. The biological consequence is the blockade of pro-inflammatory, pro-thrombotic, and final lytic action of the complement, which resembles the congenital C5 deficiency syndrome. In fact, these patients suffer from recurrent infection episodes, particularly meningitis caused by Neisseria species and other encapsulated bacteria. This "experiment of Nature" indicated the pivotal role of the complement in contrasting such life-threatening infection, and eculizumab confirmed the importance and selectivity of this immuno-mediated defense mechanism.

Eculizumab binds also to human tissues, including smooth and striated muscle fibers, as well as to renal proximal epithelium. In animal studies, this mAb was found to cross the placental barrier showing fetal morbidity and mortality. However, in humans, the poor transplacental transfer of IgG2 may account for the low detection of the mAb in cord blood samples harvested in pregnant women with PNH, who were receiving eculizumab at the time of delivery. A plasma serum concentration over 35 μg/ml of eculizumab is sufficient to block C5 cleavage and consequent hemolysis.

In PNH patients, uncontrolled terminal complement activation is responsible for the lysis of PNH RBCs, due to the lack of cell surface terminal complement inhibitor CD59 exerting a potent protective effect on MAC.

In aHUS patients, overactivation of the alternative complement pathway produces uncontrolled MAC formation and activity, resulting in endothelial cell damage, which triggers platelet recruitment and thrombus formation in the kidney (thrombotic microangiopathy). Also in the case of aHUS, the pathogenesis seems to be linked to an inefficient protection of the endothelium from MAC attack.

In some C3-mediated glomerulopathies, in which eculizumab is also experienced, the pathogenetic mechanisms is located upwards the complement cascade, and consists of C3 deregulation leading to consistent release of C3 split products (C3a) in the bloodstream and to the consequent deposition into the renal glomerulus.

Eculizumab is being experienced in other diseases on the basis of a possible role of complement activation in their pathogenesis, such as in solid organ rejection and in some autoimmune diseases. Studies on kidney transplant

recipients have shown that activation of the terminal complement pathway is necessary for the development of acute antibody-mediated rejection. In myasthenia gravis (MG), complement seems to play a relevant role in the disease. Immunocytochemical and ultrastructural studies in the human disease have identified dense MAC deposits at the neuromuscular junction. On this basis, anecdotal cases of MG have been treated with eculizumab, and one trial has been completed.

Finally, studies on neuromyelitis optica (NMO) are still ongoing [7–11].

18.2 Immunogenicity

Low titers of antibodies (HAHA) to eculizumab were detected in 2 % of PNH and in 2.7 % of aHUS patients by the ECL assay. Similar antibody responses have been found in patients across all other studies, including some placebo groups, possibly determined by previous sensitizations. Neutralizing antibodies were absent in aHUS patients, and 1.2 % in PNH. However, in the TRIUMPH study, a larger proportion of patients reported early (within 24 h) and retarded (48 h) post-infusion AEs at higher rates (79–86 %) than in placebo (66–70 %), although usually occurring as mild/moderate. Late events were rare and moderate. The immunogenicity of this IgG2/IgG4 monoclonal antibody is expected to be low and relatively inactive in expressing Fc-mediated effector responses, such as ADCC and CDC, the latter being also inhibited by the specific action of eculizumab on the common arm of complement cascade. However, the proportion of murine components inserted by CDR-grafting is small (about 10 %) yet immunogenic, although no data on HAMA have been reported. Finally, the poor transplacental transfer of IgG2 subclass may account for the low detection of eculizumab in cord blood samples of eculizumab-treated pregnant women with PNH [2, 4, 8].

18.3 Adverse Events

Since the first label, a BBW for serious and *fatal meningococcal infections* was issued, leading to a consequent restriction of eculizumab use through a REMS program. However, such risk was expected on the basis of its mechanism of action.

In PNH clinical studies, 3 cases of *Neisseria infections* and 2 cases of *meningococcal sepsis* occurred. Most frequent (>5 %) and prominent AEs in eculizumab-treated patients included cephalea (44 vs. 27 %), nasopharyngitis (23 vs. 18 %), lumbalgia (19 vs. 9 %), nausea (16 vs. 11 %), fatigue (12 vs. 2 %), sinusitis (7 vs. 0 %), respiratory tract infections (7 vs. 2 %), constipation (7 vs. 5 %), myalgia (7 vs. 2 %), pain in extremity (7 vs. 2 %) and flu-like syndrome (5 vs. 2 %). Herpes simplex infections were detected at 7 % and were considered as drug-related in 25 % of cases.

However, in the TRIUMPH Study on 43 patients SAEs were higher in controls (20 %) than in the study group (9 %), and none was considered as drug-related or had sequelae. In the single arm study (SHEPHERD) on 97 patients, 44 SAEs were

reported (16 %), including viral infection (2 %), cephalea (2 %), anemia (2 %), pyrexia (2 %), and renal impairment (1 %). In overall uncontrolled studies on 193 PHN patients, serious events were viral infections (2.6 %), cephalea (2.1 %), anemia (1.6 %), pyrexia (1.6 %), and hemolysis (1 %).

In clinical trials and extension studies of eculizumab in PNH, the *Neisseria infection* rate was 4.2 cases per 1,000/PY, and 2/474/PY for *meningococcal sepsis.*

Infections also occurred in subjects that had previously received a meningococcal vaccine, yet one unvaccinated patient had serious complications (pneumonia, pulmonary embolus, amputations). An increased risk of developing serious infections due to *Streptococcus pneumoniae* and *Haemophilus influenzae* (Hib) was also reported in children.

In aHUS pivotal studies, almost all patients (37/40) received eculizumab. SAEs were frequent in the study group (54 %) mainly as hypertension (16 %) and infections (14 %). The latter were mainly represented by URTI (35 %) and UTI (16 %). Most common events (>15 %) included pyrexia (47 %), GI disorders (53 %), URTI (32 %), cough (26 %), nasal congestion (21 %), and tachycardia (21 %).

Overall, at least one AE was observed in 98 % of patients treated with eculizumab (91 % placebo). However, in 72.5 % of cases, events were reported as unrelated to treatment (0.2 % related; 28 % possibly/probably related). No major differences emerged between adult and pediatric patients, and between prospective and retrospective analyses [1, 4–8].

18.4 Off-Label Experience

As previously mentioned, a small number of trials were launched to investigate the effect of eculizumab in extra RBC lytic disorders, including MG, NMO, and renal allograft rejection. Moreover, despite not indicated in official records, some *Shiga toxins Escherichia coli (Stxs)-related hemolytic uremic syndrome* (STEC-HUS) were also treated, and only recently have been included in the FDA list of orphan drugs for this disease. Among these, particular attention is given to *MG* on the basis of a potential role of complement in causing damage at the motor end plate. In fact, early findings evidenced MAC deposits at this site in some patients. In experimental models of EAMG, which mimics some pathognomonic signs of human MG, the blocking of C5 complement factor produced relevant beneficial effects encouraging clinical attempts with eculizumab. In a recent preliminary communication concerning a small Phase II study authorized by FDA on 14 women with MG, eculizumab appeared well tolerated, showing mild constitutional signs (nausea, cephalea, lumbalgia), and two unspecified SAEs in one patient. No discontinuation cases were recorded [12]. Similarly, in a small open trial with 14 NMO patients, all vaccinated with tetravalent meningococcal vaccine, eculizumab was administered for 48 weeks. One case of meningococcal sepsis, and one UTI associated with a transient reduction of visual acuity were

observed. Other mild events included cephalea, nausea, dizziness, cough, diarrhea, lumbalgia, abdominal pain, and rash. The patient with meningococcal sepsis recovered and resumed eculizumab treatment. Approximately 4 months after eculizumab treatment completion, one patient died of myocardial infarction, which was classified as unrelated to therapy [13].

The activation of the terminal complement pathway seems also involved in the development of *acute antibody-mediated rejection* in recipients of kidney allograft with high titer of donor-specific antibodies [14]. In a few published cases, no new safety signals were observed. Noteworthy, in these patients antibody-mediated rejection was inhibited, in contrast with 60 % of controls, showing the same antigraft antibody titers.

Finally, attempts to treat some *cardiac vascular disorders* with eculizumab and with its single chain version—pexelizumab—have showed similar rates of infections (28 vs. 27 % in placebo) and allergic reactions (0.7 vs. 0.2 % in placebo). The most frequent AEs related to cardiac events, yet no relevant clinical differences were observed between the two groups, except for a significant reduction in the prevalence of postoperative sepsis in the treated group (4.5 placebo vs. 3.0 % pexelizumab).

The rational behind these studies was the pro-inflammatory role of C5a as promoter of cardiac damage, associated with the cytolytic action of MAC, which could be prevented by the action of eculizumab or pexelizumab. However, no beneficial effects could be demonstrated. The production of pexelizumab was discontinued after testing in over 15,000 patients [15, 16].

Recent studies analyzed the possibility of extending the anti-complementary effect of eculizumab to STEC-HUS, which represents about 90 % of typical HUS, on the assumption of a potential involvement of the complement cascade in aggravating the toxic effects culminating in microvascular thrombosis.

In fact, in severe STEC infections there is a reduction of C3, along with increased levels of breakdown products of the complement alternative pathway and of MAC. Notably, all these parameters rapidly normalized after treatment and resolution of the acute episode, thus proving the causative role of complement in this disease. No particular AEs were signaled after these preliminary observations. Another trial has been recently launched in Germany [17].

The administration of eculizumab in C3 glomerulonephritis, mediated by a dysregulation of the complement cascade at a higher level than C5, showed clinical improvement in some patients, but also large deposits of MAC complex at glomerular level, with a positivity for IgG2, IgG4, and k chain, indicating the deposition of eculizumab directly at the same site along with the tubular basement membrane, and at vessel walls, without apparent functional harm [18].

Quite recently, preliminary data were released on a Phase II study of eculizumab in 14 NMO women treated with weekly IV infusions for 12 months. Cephalea, nausea, and dizziness were the most common observed AEs. One patient developed meningococcal sepsis and resumed treatment after infection resolution [19].

18.5 Postmarketing Surveillance

Over 7,571 reports in FAERS indicated infections, hematological disorders, gastrointestinal disorders, respiratory signs, and cephalea as the most common reported events (4–8 %). The average AEs/report was 5.6.

Among 3,027 reports on infections, 30 % were bacterial and 22 % viral. Meningococcal sepsis (63), and meningitis (29) were reported. Neisseria infections were ascertained in 9 cases, while streptococcal sepsis was ascertained in 8 cases, streptococcal pneumonia in 16 cases, and Haemophilus pneumoniae in 2 cases, on 384 total reports of pneumonia. Hypersensitivity reactions (78) and drug-related hypersensitivity (11), one case of anaphylactoid reaction, and no cases of anaphylaxis were registered. Moreover, 7 cases of progressive multifocal encephalopathy (PML), one case of systemic inflammatory response syndrome (SIRS), 102 cases of flu-like syndrome (FLS), and 49 cases of infusion reactions were enclosed.

In the EUV database, 1,768 reports were registered up to the end of 2012, for a total of 6,775 events (3.8 AEs/R). Infections (14, 5 %), gastrointestinal signs (13.6 %), and hematological disorders (7, 9 %) were the most frequently reported.

Among 986 reports on infections, there were 90 pneumonia, 56 UTI, 29 viral infections, 77 cases of sepsis, and 28 septic shock. Meningococcal infections included 20 sepsis, eight cases of meningitis, and six bacteriemia. Neisseria was identified in four of the above cases and in one septic arthritis. Three cases of streptococcal pneumonia, two Haemophilus infections, and one Haemophilus pneumonia were also reported.

Hypersensitivity reactions were included in 12 reports, and in three of them they were considered as drug-related. Four cases of anaphylactic reactions, one anaphylactic shock, two anaphylactoid reactions, and two cases of serum sickness were registered. Infusion reactions were observed in 15 cases.

Notably, one case of disseminated TB, two cases of SIRS, one PML, and seven FLS were also reported.

18.6 Remarks

The eculizumab safety profile seems to be reasonably defined and safe in PNH and in aHUS. The major concerns—which had been expected due to its mechanism of action—remain infections due to Neisseria and other encapsulated bacteria. However, the cases observed in controlled studies are limited because of patients' selection and vaccination, although this procedure proved to be not fully protective.

As for off-label investigations, overall safety data from 11 clinical studies (including 716 patients exposed to eculizumab in six disease populations, other than PNH and aHUS) reported only one case of meningococcal meningitis in an unvaccinated subject. However, a few additional cases and some new warning signals appeared in the postmarketing surveillance databases, such as PML and SIRS.

Long-term observations are therefore needed to better assess the risk of further signals or increasing of infectious events, such Neisseria gonorrhea, pneumococcal infection, and Haemophilus influenzae.

Unexpected observations, such as the deposition of eculizumab at glomerular level, deserve further investigation, since prolonged eculizumab administration may represent a potential harm over time.

Due to the rarity of these diseases, a Registry (NCT01374360) for eculizumab has been established in order to facilitate data collection [3, 11].

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Edrecolomab

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Edrecolomab (Panorex®, GSK) is a murine IgG2a monoclonal antibody derived from the original 17-1A murine mAb, and directed to the epithelial cell adhesion molecule EpCAM (EGP-2; CD326; 17-1A). Five anti-EpCAM monoclonal antibodies have been tested in cancer patients: murine edrecolomab, its chimeric IgG1 antibody version, the humanized and the fully human IgG1 antibodies (3622W94 and ING-1, respectively), and adecatumumab (MT201). None of them have shown to be effective as anti-neoplastic biomedicines. EpCAM is one of the first identified tumor-associated antigens expressed on human colon adenocarcinoma. However, EpCAM is also expressed by pancreatic, prostate, breast, kidney, lung, and ovarian tumors. It has also been selected as target of antitumoral vaccines.

As for edrecolomab, its clinical evaluation has been mainly investigated in patients with colorectal cancer. As single agent, edrecolomab exerted minimal antitumor activity, also as chimeric variant. Positive responses were initially found in a randomized small trial (166 eligible patients) conducted in Germany, but subsequent larger studies in Europe and the USA did not confirm edrecolomab clinical activity as adjuvant therapy in various settings. Therefore, the manufacturer withdrew edrecolomab after the preliminary registration in Germany in 1995 [1, 2].

The major AE observed with edrecolomab monotherapy was diarrhea (32 %; severe 2–4 %). In a cohort of 823 patients, severe events were reported in about 29 % of cases and serious reactions in approximately 5 % of patients. Anaphylactic reactions, flushing/erythema, and other minor gastrointestinal symptoms, including abdominal pain, nausea, and vomiting were also reported. Recently, a better understanding of possible causes of clinical failure have risen new interest in this class of monoclonals, including new insight in their mechanism of action [3, 4].

Edrecolomab is a low-affinity antibody and therefore the overexpression of EpCAM on tumoral cells may be crucial for its efficacy. None of the previous trials did prospectively or retrospectively analyze levels of EpCAM expression in patients.

The main effector arms of edrecolomab are ADCC and CDC, which mainly rely on glycosylation of the antibody molecule, a variable feature even on the same antibody in relation to production procedures. Since edrecolomab proved to be

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safe, albeit with limited efficacy, attempts to improve its effector capacity were experienced in various directions. The building of the chimeric version of edrecolomab was based on the IgG1 isotype framework, which had high ADCC/CDC effector capacity compared to the original IgG2 molecule.

However, investigations with this antibody were very limited. Other attempts to improve clinical efficacy by enhancing affinity up to 100-fold produced serious clinical toxicity, including pancreatitis. Overall, these aspects may explain modest results and clinical discrepancies.

The recent finding that EpCAM is a proto-oncogene and signal transducer has raised the possibility that anti-EpCAM antibodies may be able to interfere with the proliferative signal transduction cascade initiated by EpCAM in a number of other tumors. A novel approach has brought to the production of an anti-EpCAM trispecific antibody called catumaxomab (Chap 13) [5].

This class of antibodies may be revisited, hopefully with more stringent clinical approaches.

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Efalizumab

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Efalizumab (Raptiva®, Genentec) is an immunosuppressive recombinant humanized IgG1k monoclonal antibody that binds the CD11a component of the lymphocyte function-associated antigen-1 (LFA-1) receptor expressed on all human leukocytes. Approval by FDA was given in 2003, followed by EMEA in 2004, for the treatment of adult patients with chronic moderate to severe plaque psoriasis (Ps), who are candidates for systemic therapy or phototherapy. However, FDA issued a warning in October 2008 after three cases of fatal progressive multifocal leukoencephalopathy (PML) following treatment with efalizumab. In December 2008 Health Canada warned for serious infections, including PML. In February 2009 EMEA recommended the suspension of marketing the product for serious safety concerns, including the occurrence of PML. In April 2009, although efalizumab showed high efficacy in controlling Ps, the producer spontaneously withdrew the product from the US market, and in May 2009 from the EU area [1–6].

Even if this biomedicine is no more in use, the history of efalizumab and in particular the analysis of major safety issues of a highly effective drug is instructive for the purpose of this monograph.

Initial application for efalizumab was presented in December 2002 (STN BLA125075/0). Two sponsors (Xoma Ltd. and Genentech Inc.) participated in the development of efalizumab. The former produced efalizumab for most Phase I (4/5 studies, excluding ACD2142g) and Phase II (HUPS252) clinical studies, while the latter manufactured the product for most of the Phase III trials (ACD2243g, ACD2390g, ACD2391g, ACD2600g) and one Phase I study (ACD2142g). A total of 2,762 patients with Ps, including preliminary studies, received efalizumab (1219 from Xoma; 1543 from Genentech). Additional long-term exposures were programmed for three of the previous Phase III studies, up to 24 (ACD2058, ACD2059g) and 48 weeks (ACD2243g). The total database for safety consisted in over 2,500 exposed patients. However, in pre-admission analysis FDA expressed concerns about the comparability of efalizumab produced by the two companies and recommended a PK additional study (ACD2389) on healthy volunteers. This study confirmed that the two products were not pharmacokinetically equivalent, i.e. they had different bioavailability and clearance. Therefore, an additional Phase

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III (ACD2390g) trial was requested on the Genentec product, which completed the initial package of studies for the application. Overall, there were four randomized Phase III trials (two for each manufacture, one for SC injection) and two open-label Phase III studies contributing in part with interim data. On this basis, FDA, EMEA, and other Agencies granted their approval. A subsequent assessment report from the EMEA CHMP Committee in 2008 re-evaluated efficacy and safety, and performed a meta-analysis on the basis of final data from previous trials and new data from Phase IV studies [7]. Overall, 29 trials were examined and safety database included over 7,000 patients (over 6,500 Ps patients; over 6,000 treated with efalizumab; 2,800/PY exposure). Additional relevant information came from long-term treatment (over 3 years). On this basis, changes in product information were demanded, including also a warning for treatment discontinuation after disease exacerbation, which was not avoided by gradual reduction of administration. However, the final risk/benefit evaluation was still in favor of the latter. Adverse events were mostly mild to moderate, and remained acceptable within the 3 years, with no increase in serious events and no unexpected common new events. In particular, the incidence of serious infections and malignancies was evaluated in 29 studies, from 12 to 144 weeks of treatment (4,709 patients over 12 w). There was no evidence of increased risk of malignancies with time, except for NMSC. However, in February 2009 the same Committee recommended the suspension of efalizumab because of modest benefits in the treatment of psoriasis, with a risk of serious side effects including the occurrence of PML. In particular, the conclusion of the Scientific Commission was: *The new safety signals that have emerged (especially PML) together with the known risk of opportunistic infections do compromise the benefit/risk ratio. Since the grant of the Marketing Authorization, the safety issues have arisen leading to the addition of a number of warnings into the SPC such as aseptic meningitis, immune mediated hemolytic anemia, antibody development with vaccinations, interstitial pneumonitis, arthritis, erythema multiforme, inflammatory polyradiculoneuropathy, Miller Fisher syndrome, facial palsy and Bells palsy and severe infections, malignancies during long-term use, including serious (fatal) events such as opportunistic infections and Guillain Barré syndrome (GBS). In addition the MAH recently notified the EMEA about three cases of encephalopathy and five cases of encephalitis*. In 12 May 2009, the European marketing authorization holder notified the European Commission the voluntarily withdrawal of the marketing authorization for the product, as it did not intend to conduct the clinical trials necessary to fulfill the requirements for lifting the suspension. In June 2009 EMEA halted the marketing authorization [8]. Similar procedural steps were taken by other agencies.

20.1 Mechanism of Action

Efalizumab is a humanized IgG1k monoclonal antibody containing murine complementarity determining regions (CDRs) in a human framework (approx. 90 %) that binds to the CD11a α-chain of leukocyte function antigen-1 (LFA-1), which is

a $\alpha L\beta 2$ integrin expressed on all leukocytes. The binding blocks the interaction of LFA-1 of T cells with its ligand intercellular adhesion molecules (ICAM-1,2,3) expressed by keratinocytes, endothelia and antigen-presenting cells (APC), thus inhibiting the activation of T lymphocytes via cell-to-cell contact favored by LFA-1/ICAM adherence. This interaction is also important for T cell trafficking and interaction with keratinocytes, and for other immune functions mediated by NK cells, monocytes, and granulocytes. In addition, saturating doses of efalizumab rapidly downmodulates CD11a on leukocytes, leading to impairment of intercellular adhesion with long-lasting effects (weeks). Therefore, leukocytes tend to accumulate in the circulation because of the inhibiting action on their capacity of extravasation. The effect is similar when induced by IV or SC administration, with 50 % reduced bioavailability for the latter.

ICAM-1 is overexpressed on keratinocytes and endothelia within the psoriasic plaque. Therefore, efalizumab has the capacity of interfering with the activation, adhesion, migration, and function of a number of cells other than T lymphocytes, which explains the wide range of immunosuppressive effects experienced with this biomedicine [2, 3].

In animal models (chimpanzee) efalizumab induced atrophy, reduction of CD3+ cells infiltration of neutrophils, and hyperplasia of reticulo-endothelial cells in the paracortical area of lymph nodes. These animals showed also a reduced antigen-induced (tetanus toxoid) immune response. Overall, efalizumab mostly acts via the blockade of lymphocyte trafficking, and by inducing a peculiar hyporesponsiveness of T cells (e.g. against JVC) associated with a reduced capacity of induction of specific antibody response to a neoantigen. These effects are reversible and may also explain the exacerbation of psoriasis after efalizumab upon cessation of therapy, possibly caused by immediate cell release from circulation into the skin, a mechanism called “release of breaks,” after efalizumab blockade [3, 8, 9].

20.2 Immunogenicity

Some chimpanzees developed antibodies to efalizumab [1]. Moreover, the presence of HAHA was detected in 6.3 % of cases in a cohort of 1,063 patients at low titer. In previous studies there was a slight difference between early (Xoma) and late (Genentech) preparations (6.4 % vs. 6.9 % respectively). However, the sample of tested subjects was greater in the Xoma preparation (623 vs. 173 of the Genentech formulation). In this study six subjects with local injection-site reactions were tested, resulting positive for HAHA. These adverse events classified as consequences of injection-site mass, hypersensitivity or inflammation, and resolved despite efalizumab therapy had continued. Therefore, a potential relationship between the presence of HAHA and local cutaneous reactions was suggested. Interestingly, mean values of eosinophils were increased by 50 % in about 10 % of efalizumab-treated patients (3 % in placebo). Hypersensitivity reactions, reported

as associated symptoms, were slightly higher in treated patients (8 % vs. 7 % in placebo), and included also urticaria (1 % vs. 0.4 % in placebo), erythema multiforme, asthma, and allergic drug eruption. Noteworthy, inflammatory processes also seem to be enhanced (e.g., inflammatory arthritis, 0.4 %). Interestingly, the association of arthritic and other inflammatory adverse events with HAHA positivity was considered as underestimated in initial drug evaluation *given that some of the patients discontinued the study prematurely due to their adverse event and thus, have missing data with regard to anti-efalizumab antibody screening*[1]. Moreover, the response to specific antigens (tetanus toxoid) was reduced, and almost abolished at higher doses of efalizumab. Among these events, some "first dose reactions" were enucleated (cephalea, pyrexia, nausea, vomiting) since they were dose-related in incidence and severity (including one case of aseptic meningitis) and could be reduced by administering a lower conditioning dose for therapy initiation [3, 7]. Overall, efalizumab is moderately immunogenic, and the presence of HAHA may be associated with local and general hypersensitivity and inflammatory reactions, which may be also related to the relative increase of the eosinophils in circulation. However, no data are available on IgE specific induction.

20.3 Adverse Events

AEs listed in the first 2003 label information included *serious infections* (0.4 % vs. 0.1 % in placebo), *malignancies*, *thrombocytopenia*, and *Ps exacerbation* (0.7 %). Overall, safety analysis was performed on 2,762 adult Ps patients, including 2,400 patients exposed for 3 months, 904 for 6 months, and 218 exposed for 1 year or more. AEs data were collected from 1,928 patients (1,213 treaded with efalizumab; 715 controls). Events over 2 % higher than controls were cephalea (32 %), infections (29 %), some constitutional signs (nausea, chills, pain, myalgia (8–13 %), flu-like syndrome (7 %), lumbalgia (4 %), and acne (4 %). Serious infections were reported as 1.1 %, and included cellulitis, pneumonia, abscess, sepsis, sinusitis, bronchitis, gastroenteritis, aseptic meningitis, Legionnaire's disease, septic arthritis, and vertebral osteomyelitis. In controlled trials, the overall rate of infections in efalizumab-treated patients was 3 % higher than in placebo-treated patients. Overall malignancy rate was 1.1 %, and included 37 types of tumors in 31 patients, including NMSC, non-cutaneous solid tumors, HL, NHL, and melanoma. The majority regarded NMSC (0.7 %). The incidence of non-cutaneous solid tumors and malignant melanoma were referred as within the range of the general population, while a higher incidence of NMSC could not be excluded. Thrombocytopenia (0.3 %) was present only in the efalizumab-treated group and was diagnosed as immune-mediated, and brought to discontinuation of treatment and hospitalization (3/8 patients). Exacerbation of Ps was observed in 3.2 % of treated patients (1.4 in controls). SAEs occurred in 0.7 % of cases, and included pustular, erythrodermic, and guttate subtypes. However, most of them occurred after the therapy cycle completion (12 weeks), both in responsive and

non-responsive subjects. Abnormal laboratory tests included ALP increase and other liver functional parameters (3–4 % vs. 0.6–1.5 %in placebo), lymphocytosis (40 %, some atypical), and leukocytosis (26 %). Overall, the initial relevant signs were serious infections, malignancies, thrombocytopenia, and Ps worsening [10]. Finally, adverse events mostly resulting in discontinuation of efalizumab treatment were psoriasis (0.6 %), pain (0.4 %), arthritis (0.4 %), and arthralgia (0.3 %).

The official label update of 2005 confirmed the above data. However, *hemolytic anemia* and *arthritis* events were added to previous relevant AEs. Postmarketing additional information included serious *necrotizing fascitis*, *tuberculous pneumonia*, *neutropenic severe pneumonia*, *bacterial sepsis*, *worsening of infection* e.g. *(cellulitis, pneumonia), severe thrombocytopenia*, and *immune-mediated hemolytic anemia* (some severe). Worsening of some Ps cases was confirmed, and a clear warning was included for *severe cases of arthritis and psoriasic arthritis* already observed in trials and in the postmarketing reporting. Thrombocytopenia rates were also confirmed, and their immune-mediated origin was better specified. As for hemolytic anemia, 2/5 cases were observed in clinical trials; the remaining came from postmarketing observations, all as late events (4–6 months after efalizumab initiation), and two of them were serious. Among immune-mediated inflammatory disorders, previously included in the first original label, *myositis,* and *eosinophilic pneumonia* were added as postmarketing signals. Finally, *epidermal necrolysis* and *photosensitivity reactions* were added as reported from the same source. Overall, the initial list of relevant AEs including four classes of events (*infections, malignancies, thrombocytopenia and Ps worsening*) was incremented to six classes (including *anemia* and *arthritis*) [11].

In the last official label (2009) a *boxed warning* on risk of *PML* and other *serious infections* was included, on the basis of three postmarketing cases of fatal PML. It was calculated that 46,000 patients were exposed to efalizumab at that time, with long-term observation up to 2 years (5,100 subjects) and 3 years (1,900 subjects).

A possibility that additional cases of PML could have been misdiagnosed and/or not reported was also prospected.

The postmarketing experience signaled *serious bacterial, viral, fungal, and opportunistic infections, including pneumonia, sepsis, meningitis, and encephalitis.* Some of these infections were fatal, and included *CMV infections; blastomyces, cryptococcal and tuberculous pneumonia; serious herpes infection; severe pneumonia with neutropenia sepsis with seeding of distant sites; necrotizing fasciitis; and worsening of infection* e.g. *(cellulitis, pneumonia) despite antimicrobial treatment.* Consequently, a new class of serious events (*neurologic events)* was included in the warnings. One case of *transverse myelitis,* observed among the initial cohort of 2,762 patients enrolled in pivotal trials, was mentioned; *GBS, PNP, facial palsy,* and additional cases of transverse myelitis, which had been reported in the postmarketing setting, were also signaled. Finally, additional cases of unspecified *lymphoma* were recorded [3].

As previously noted, the EMEA assessment report of April 2008 (and similar documents of other Agencies and product information) did not include new relevant events, neither among serious infections nor in the neurologic events. The report included information over 3 years (319 patients) and concurrent postmarketing experience. In February 2009 the European CHPM asked for suspension of marketing authorization due to the new AEs outcomes. All neurologic events, including PML, and other serious infections, seemingly occurred after April 2008, most of them after 3 years of treatment/observations.

20.4 The PML case and Postmarketing Surveillance

As previously mentioned, FDA received the first adverse event report of PML associated with efalizumab from a postmarketing safety registry in September 2008. Based on this case, the efalizumab label was updated in October 2008 to include a boxed warning on PML. Additional information was added to the the BBW in March 2009, in March 2009, following receipt of two additional confirmed cases of PML in October 2008 and January 2009. In July 2009, efalizumab was voluntarily withdrawn from the US market because of the risk of PML [3–6].

It became clear that long-term safety monitoring over 3 years therapy was crucial, although the abrupt comparison of three cases within a limited time window was unexpected. A detailed description of these three paradigmatic cases of PML has been recently published [12]. This devastating encephalopathy is caused by JCV infection. However, the virus is widespread over the general population (about 80 %), and its neurotropic diffusion is related to immunodeficiency/immunosuppression states. The latter condition may be related to other specific infections, such as HIV, or to other biomedicines, such as adalimumab, etanercept, infliximab, and natalizumab. Recently, the possible pathogenetic mechanism after efalizumab treatment has been investigated in one of the PML reported cases [13]. It was shown that after onset of disease, T cells expressed lack of differentiation in their effector functions, and showed a dramatic impairment in their mobility. Clonal expansion was also blocked. However, after plasma exchange to remove efalizumab, these functions became immediately evident, although not completely restored, and in particular with persisting absence of reactivity against JCV. Moreover, efalizumab appeared to impair intrathecal APC antigen-mediated restimulation of T cells. Overall, efalizumab seems to impair mostly adaptive antiviral immunity, by inhibiting activation and proliferation of T cell, restimulation of memory T cells and T cell migration to sites of infection.

Since most of recent cases of PML are associated to natalizumab treatment (159 reported cases), the Authors analyzed possible differences between the two hypothetical mechanisms of action. Both natalizumab and efalizumab block and downregulate leukocyte derived integrins through which T cells cross the blood–brain barrier to eliminate pathogens and virus-infected cells. They also have similar pharmacokinetics. However, only natalizumab causes the decrease of cells

in the cerebrospinal fluid and releases CD34+ bone marrow progenitor cells into the circulation, possibly leading to the release of virus from a bone marrow site of nonpathogenic replication.

Interestingly, most PML cases (75 %) survive, while the three cases after efalizumab were fatal, which further indicates the existence of different pathways of immune impairment. These differences, which are not fully clarified yet, seem to be crucial for survival.

20.5 Remarks

In order to ensure the best safety evaluation of medical products the long-term surveillance and the adequate number of patients under clinical trials are crucial requirements. These two aspects are not alternative but both necessary. In the case of efalizumab, pivotal and supportive trials appeared sufficiently dimensioned (almost 3,000 evaluated patients in the application for safety study). However, most patients had a short-term therapy, compared to those who were exposed after the market authorization. In approval studies, among over 2,762 treated patients 87 % were treated for 12 weeks, 34 % for 24 weeks, and only 8 % received efalizumab for 1 year of continuous treatment.

The most serious neurological events, including PML, appeared after 3–4 years of treatment. Notably, these reports were provided to FAERS and other postmarketing settings, yet meanwhile the official re-assessment evaluation was performed mainly on the basis of initial controlled data from trials and their extensions.

The risk of exponential diffusion of a drug is obviously remarkable when efficacy is high. In the case of efalizumab its long-term continuous treatment is mandatory, while the premarketing observation is inevitably shorter. Therefore, only pharmacovigilance and early reporting from clinical care may help in detecting most rare, albeit dangerous, signals. In the case of efalizumab, new signals may still be coming. For example, the reactivation of other latent viruses, such as HPV [14] or HBV [15], after efalizumab long-term therapy has been observed and associated to the development of cervical cancer and hepatocellular carcinoma, respectively. In the former case, treatment with efalizumab was continued for 45 months on request of the patient, although cervical smearing revealed a high-grade squamous intraepithelial lesion (HGSIL) after 27 months of therapy. In the case of hepatocellular carcinoma, efalizumab was administered for over 8 months, while HBV reactivation with high viral load, together with the insurgence of neoplastic lesions, were all detected at 10 months. It is surprising that similar signals had not been caught before, due to the rather short duration of the treatment. Noteworthy, the reactivation of two different viruses, in addition to JVC, may indicate that this pathway is critical for anti-viral defense, more than expected. Finally, additional concerns may derive from the association of multiple immunosuppressive therapies, including associated biomedicines in “hit and run”

therapeutic approaches, where infliximab and efalizumab were combined to treat Ps [16]. In addition, one case of DLBCL developed in a similar situation where efalizumab was administered for more than 4 years [17].

Overall, the efalizumab experience further stresses the importance of dimensioning premarketing pivotal trials in relation to potential long-term treatments, and the crucial role of a long-term constant postmarketing safety surveillance.

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Gemtuzumab

21

Gemtuzumab-ozogamicin (Mylotarg®, Pfizer, Wyeth) is a humanized IgG4k monoclonal antibody conjugated to a cytotoxic agent, binding to CD33 antigen. This antigen is expressed on normal and leukemic myeloid cells of more than 80 % of patients with acute myeloid leukemia (AML). In 2000, FDA granted Gemtuzumab-ozogamicin (hereinafter "GO") an accelerated approval for the treatment of AML patients 60 years of age or older at first relapse, when cytotoxic therapy was not indicated. The approval was granted on the basis of interim data provided by three studies conducted on 142 patients. Gemtuzumab was the first monoclonal antibody approved for treating acute leukemia. The same year EMEA designated the product as an orphan drug. An application for marketing authorization was presented to EMEA later in 2005, and was rejected two years later. During 2007, the application was submitted for re-examination to the European Agency, which in 2008 confirmed the negative response. In 2010, FDA announced the withdrawal from the US market, as a spontaneous decision of the manufacturer after the failure of the post-approval trial (SWOGS0106), which had been discontinued due to clinical inefficacy and safety concerns [1–6]. A following trial (E1900) performed in UK collected similar results. The Japanese regulatory Agency approved GO as an orphan drug in 2005, and the approval was reaffirmed in 2010 on condition that postmarketing surveillance had been strengthened. Recently, a French study (ALFA) provided data establishing the positive effects of GO administration in combination with daunorubicin and cytarabine. Other following studies confirmed that a well-defined subset of AML patients could benefit from the addition of GO to their therapies [7–11]. Therefore, the proposal for a re-evaluation of GO with a more appropriate and restricted indication was raised [12, 13].

The safety profile analysis of this monoclonal becomes particularly interesting due to different policies adopted by agencies in the risk/benefit evaluation of GO, and also in the light of recent re-evaluation proposals.

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21.1 Mechanism of Action

CD33 is a 67 kDa transmembrane glycosylated adhesion protein of the Ig-like lectin family, expressed on immature normal cells of myelomonocytic lineage, and on committed myelomonocytic and erythroid progenitor cells. CD33 binds to most myeloid and monocytic leukemic cells. It is not expressed on pluripotent hemopoietic stem cells, mature granulocytes, lymphoid cells, and nonhematopoietic cells. The intracytoplasmatic tail of CD33 is similar to the immunoreceptor tyrosine-based inhibitory motif (ITIM), suggesting that CD33 may act as an inhibitory receptor within the myeloid compartment, suppressing signals generated by receptor systems containing immunoreceptor activation motif (ITAM). The binding to ITAM high affinity FcγRI (CD64) generates signals, leading to activation of underlying myelomonocytic cells and to phosphorylation of CD33, resulting in inhibition of the CD64-mediated signals.

Moreover, CD33 activation interferes with internalization of antibody-bound molecules. During differentiation of the myeloid cell line, CD33 is downregulated and mature cells become more responsive to FcγRI-mediated and other activation signals. The CD33 antigen is expressed in more than 80 % of patients with AML, but not on normal precursor hematopoietic cells.

Gemtuzumab ozogamicin (CMA-676) is a humanized IgG4k monoclonal antibody against the CD33 antigen (hP67.6), conjugated to a semi-synthetic cytotoxic agent N-acetyl gamma calicheamicin dimethyl hydrazide (NAc-gamma calicheamicin DMH) via the bifunctional acid-labile AcBut linker. Constant and framework regions contain human sequences (98.3 %) while the complementarity-determining regions (CDR-grafted) are derived from a murine antibody (p67.6) that binds CD33. This antibody is not cytotoxic; it binds to the CD33 antigen and when internalized delivers the calicheamicin derivative to the inside of the leukemic cell. GO is internalized into lysosomes, where acidification releases the NAc-gamma calicheamicin DMH moiety. Noteworthy, the GO complex has approximately 50 % of the antibody loaded with 4–6 mol calicheamicin per mole of antibody. The remaining 50 % of the antibody is not linked to the calicheamicin derivative. Calicheamicin is a potent anti-tumor antibiotic, about 1,000 times more potent than doxorubicin; it binds to the minor groove of DNA and produces site-specific double-strand breaks resulting in cell death. Gemtuzumab has been engineered as the IgG4 isotype to avoid direct immune effector functions mediated by FcyRI receptors, and thereby allowing only the post-internalization cytotoxic effect of calicheamicin. Despite this subclass of antibodies is incapable of triggering ADCC or CDC reactions, a substantial level of binding capacity for FcyRI is still retained. This may be relevant also for the induction of AEs [3, 5, 6, 14, 15].

21.2 Immunogenicity

Two patients (5 %) in the initial Phase I Study 0903A1-101 developed antibody titers against the calicheamicin/calicheamicin-linker portion of GO after the second and third doses. One of them experienced transient fever, hypotension and dyspnea. Antibody formation to the calicheamicin/calicheamicin-linker portion appeared to be dose independent. However, none of the other 277 patients of Phase II trials developed antibody responses to the hP67.6 monoclonal antibody or to the calicheamicin-linker portion of the GO molecule. Noteworthy, 20 of these patients received multiple courses of the drug [1, 16].

21.3 Adverse Events

The original safety profile was based on three open-label Phase II studies (0903A1-201, 202, 203) presented for the accelerated approval, enrolling 142 patients and was subsequently reviewed because of serious signals soon appearing in the postmarketing setting [1, 3, 4].

The *most common* AEs reported were fever (85 %), chills (73 %), nausea (70 %), vomiting (63 %), thrombocytopenia (59 %), leukopenia (54 %), asthenia (44 %), diarrhea (38 %), abdominal pain (37 %), cephalea (35 %), stomatitis (32 %), dyspnea (32 %), epistaxis (31 %), and hypokalemia (31 %). Despite prophylactic premedication, *infusion reactions* (5 %), mainly expressed as chills (62 % severe 11 %), pyrexia (61 % severe 7 %), and hypotension (11 % severe 4 %) were observed, yet were usually well tolerated. The incidence of severe (grade 3–4) reactions was 34 % after the first infusion, and 12 % after the second administration. Their etiology was attributed to *cytokine release syndrome (CRS)* and mostly related to first infusions. *Tumor lysis syndrome (TLS*), one fatal, occurred in about 3 % of patients.

Severe reactions occurred in 33 % of cases and included pyrexia (15 %), nausea/vomiting (11 %), dyspnea (9 %), AP imbalance (8–9 %), and infections (28 %) including sepsis (16 %), pneumonia (7 %), and neutropenic pyrexia (7 %). About 2 % of cases suffered severe hypoxia.

Laboratory abnormalities were very common and severe. They were mainly of hematological origin (thrombocytopenia 58 %, neutropenia 53 %, anemia 15 %) and consequence of hepatotoxicity in 23 % of cases (hyperbilirubinemia 23 %, and AST/ALT increase 17 %).

Bleeding (15 %) was of major concern, with fatal cases (4 %) of intracranial (1), intracerebral (4) and intraperitoneal hemorrhage (1), occurring within 24 h and up to 30 days after treatment. Mucositis (35 %) and severe stomatitis (4 %) were frequent. *Hepatotoxicity* was usually transient, except for two cases of fatal hepatic failure during *TLS*, one of them occurring five months after treatment. Hepatic veno-occlusive disease (VOD) developed in 5 % of patients. Interestingly, VOD occurred only among patients receiving stem-cell transplants (HSCT) after GO treatment, but not before it.

Overall *death rate* was 13 %. It was held that in the absence of randomized studies, definitive conclusions regarding GO-related toxicities compared to conventional chemotherapy could not be made. However, the rates of hematological toxicity and treatment-related mortality appeared to be similar to the rates reported in studies with conventional chemotherapy [2]. On this basis, the FDA accelerated approval was granted.

Major concern came afterward from another randomized trial (SWOG-S0106) comparing induction chemotherapy alone and associated with GO. These data were only included in one abstract and the study was halted after an interim analysis showed an *increase in mortality* in the GO arm (5.8 % vs. 0.8 %). On this basis marketing authorization of GO was withdrawn in the USA, due to concerns about safety and lack of efficacy [11].

However, a number of subsequent trials started to observe more reassuring data, both on safety and efficacy. Cumulative analyses on these studies have been recently published [7–11, 13, 16]. In the ALFA0701 trial [8], duration of treatment-induced neutropenia and thrombocytopenia were significantly longer in the GO arm (140 patients). Severe and persistent thrombocytopenia was found in 16 % of cases (3 % in controls). Similarly, severe hemorrhages were higher in the GO group, yet the difference did not reach statistical significance (9 % vs. 3 %). Three patients (2 %, one fatal) in the GO arm developed *VOD*. Overall, severe hematologic and non-hematologic AEs were increased in the study group. However, the incidences of cardiac events, infectious events, transfers to intensive care unit, and to toxic-related *deaths* did not differ significantly between the two groups (6 % vs. 4 % in controls*)*. Severe toxicity was mainly reported in the context of sepsis in both groups. These more favorable safety data were accompanied by better efficacy rates.

In AML14 and AML16 trials, where GO was added to standard chemotherapy, overall severe AEs were limited ($\leq$6 %). There was a significant difference in the GO arm only for nausea/vomiting (4–6 % vs 13 % in controls), gastrointestinal events (diarrhea 4 %) and liver toxicity (4 %). There were no reported cases of VOD. In another AML16 arm, where GO was associated with an induction therapy, the safety profile was similar. No major increase in toxicity was found with GO. However, severe nausea/vomiting were significantly higher in the GO arm than in controls (9 vs. 4 % respectively), and to a minor extent, liver functional tests, oral and GI disorders were higher as well. Levels of efficacy rates were confirmed being better than in the SWOG studies [9, 10]. Moreover, another review of a Phase II study on 277 patients with relapsed AML noted a response rate of 26 %, essentially identical to the one reported in the studies that led to the FDA approval.

Overall, GO had less GI toxicity than chemotherapy, but was associated with hepatic VOD (also called sinusoid obstructive syndrome or SOS), mainly in HSCT patients [13]. Further studies experienced low doses of GO in combination therapies, mainly in elderly patients (>60 years). In one of these studies GO treatment was associated with severe neutropenia and thrombocytopenia in all patients, as expected. No liver toxicity or VOD was observed. One patient died (sepsis) during

induction therapy [17]. Similar results were obtained in over 70-year-old patients [18]. The toxicity profile was also manageable. The mortality rate was 6.25 %, which is relatively low in this high-risk group.

However, the addition of GO (low dose) to conventional chemotherapies in younger (<60 years) patients also proved to be safe and beneficial. In particular, no excess hematologic or nonhematologic toxicity occurred within 120 days, even in HSCT transplanted patients [19].

GO has been used in *pediatric treatment* of refractory relapsed AML. In a study on 29 children infusion reactions (28 % severe) were associated with low-grade chills (55 %), vomiting (41 %), pyrexia (35 %), nausea (28 %), tachycardia (14 %), cephalea (10 %), pain (10 %), sweating (7 %), and hypo/hypertension (7 %). There were no instances of anaphylaxis, anaphylactoid reactions, or delayed hypotension. Almost all patients had transient and reversible elevations in liver function test results (AST/ALT, bilirubin). Severe elevations were observed in 28 % of cases. The most common severe adverse events were leukopenia (48 %), thrombocytopenia (35 %), sepsis (24 %), pyrexia (24 %), hypokalemia (21 %), hypochromic anemia (17 %), pleural effusion (17 %), and pneumonia (17 %). VOD was ascertained in seven patients undergoing HSCT, except for one [20]. In a subsequent study (NOPHO-AML 2004), safety data were collected from 53 patients receiving two cycles of GO as postconsolidation monotherapy after chemotherapy and HSCT. No major events were reported in relation to the infusions. Only a minor decrease in Hb was observed after each GO infusion. Severe leukopenia was seen in 81 % of cases after first GO infusion, and in 67 % after the second one. Severe neutropenia was almost universal 94–96 % after first and second GO treatment. Febrile neutropenia occurred in about 40 % of cases, as seen in overall pediatric relapsing AML patients. None of the infectious episodes were serious. Thrombocytopenia was moderate but increasing after first (15 %) and second (39 %) GO course. Liver toxicity was also moderate. No VOD were observed. Notably, two cases of myelodysplastic syndrome (MDS) occurred and were related to GO treatment, which is unusual since second malignant neoplasms are rare in pediatric AML (2 %), and have not been described before in relation to GO administration. Moreover, no patients had severe hyperbilirubinemia or VOD in contrast to the 23 % with severe liver toxicity in adults. Overall, the toxicity profile showed good tolerability with some differences with respect to similar treatments in adults [21].

21.4 Postmarketing Surveillance

By the end of 2012 the FAERS database received 2,805 reports on gemtuzumab/Mylotarg including approximately 15,500 AEs with about 5 AEs per patient. The most frequent events included hematologic disorders (12 %), infections (7 %), and hepatobiliary disorders (5 %). Febrile neutropenia (485 cases), sepsis (441) were the most common and serious events. Moreover, 45 cases of TLS were included.

21.5 Remarks

Approval for Gemtuzumab ozogamicin has not been granted in EU. After an accelerated approval, GO was voluntarily withdrawn in the US in June 2010, mainly due to ensuing safety concern about mortality (6 %) related to GO treatment, and before the results of other randomized trials had been available. A number of subsequent data showed that the overall profile of GO was within the range of similar treatments and, in particular, induction death rates from 5 to 7 % are features of most induction therapies in AML patients of that age. By contrast, the mortality rate of the control arm treated with chemotherapy alone was unexpectedly low (0.8 %). Thus, the difference resulted much heightened with respect to average AML cohorts treated with chemotherapy alone.

These differences were not observed in subsequent large experiences on thousands of randomly assigned patients [10, 12, 13, 17]. Since data on efficacy were also reassuring, a call for re-evaluation was put forward and seems appropriate. It is now known that AML is an heterogeneous disease at morphologic, cytogenetic, and molecular levels. On this basis, reproducible methods are available to select patients likely to benefit of specific treatments. Age and dose differences have shown also that by selecting proper subclasses of patients efficacy may be improved in the presence of moderate, reversible and manageable AEs. The toxicity profile remained manageable in patients older than 70 years with good performance status. The possibility of combining therapies deserves further investigation, given the proved absence of additional GO-related major toxicity.

Finally, the original and persistent difference between regulatory Agencies evaluations in the case of GO, also need to be considered. Recently, this issue has been explicitly raised [22], particularly stressing that the lack of coordination among the agencies, as showed in the GO case, causes confusion among healthcare providers and patients worldwide, and has a heavy impact on daily clinical practice and public health. Perhaps GO has further lessons to give.

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Golimumab

22

Golimumab (Simponi®, Janssen) is a human IgG1k monoclonal antibody directed to human tumor necrosis factor alpha (TNF-α), preventing the binding of TNF-α to its receptors. In 2009, FDA approved golimumab for the treatment of the following pathologies: (i) moderately to severely active rheumatoid arthritis (RA) in adults, in combination with methotrexate (MTX); (ii) active psoriatic arthritis (PsA) in adults, alone or in combination with MTX; (iii) active ankylosing spondylitis in adults (AS). In 2009 also EMEA and Health Canada granted approval for the same indications. The national Japanese Agency PMDA approved the marketing on September 2011. Three multicenter, randomized, double blind, controlled trials (studies RA-1, RA-2, and RA-3) enrolling 1542 RA patients were pivotal for initial approvals. Primary endpoints were fixed at weeks 14 (RA-2) and 24. In particular, the GO-FORWARD (RA-1) trial evaluated 444 patients that already were on a stable MTX dose, but had not been previously treated with other anti-TNF agents. The GO-AFTER (RA-2) study evaluated 445 patients, previously treated with one or more of the anti-TNF agents (adalimumab, etanercept, or infliximab). These patients were allowed to continue concomitant DMARD therapies. An extension of this study was conducted up to week 160. The GO-BEFORE (RA-3) trial evaluated 637 patients with active RA, who were MTX-naïve and had not previously been treated with an anti-TNF agent. In all three pivotal trials, patients received golimumab monthly up to week 24. In a Phase II exploratory study, 231 patients with severe asthma were treated with golimumab at higher doses. Separate additional studies were performed on 405 PsA (GO-REVEAL) patients (primary endpoint at week 14), and on 356 AS (GO-RAISE) patients with the same primary endpoint. An extension of the latter trial was conducted up to 104 weeks of observation. The safety of golimumab was further supported by clinical data beyond 24 weeks in the five Phase III studies, together with safety data from Phase I and II studies in RA patients, completed and ongoing

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studies on other indications, and Phase I studies in healthy subjects. In Japan, approval was based on an additional Phase II trial (GO-FORTH) enrolling 269 RA patients with a primary endpoint at week 24. A subsequent study (GO-MONO) experienced golimumab monotherapy in 316 RA patients at 24 weeks [1–8].

22.1 Mechanism of Action

The tumor necrosis factor (TNF) family is a group of 19 cytokines mainly involved in apoptosis, including TNF-α and lymphotoxins (LT-α, previously TNF β, and LT-β). Their structures are homotrimeric (the former) or heterotrimetric (the latter), and are recognized by specific receptors (TNF-R1; TNF-R2). TNF-α (also identified as TNF, being the pivotal molecule of the group) is expressed at the cell surface, mainly on activated macrophages and T lymphocytes, and can be cleaved by a TNF-α converting enzyme (TACE) in a soluble form, which is considered the mature expression of this cytokine. However, the transmembrane precursor (tmTNF, 26 kDa) acts also as a bipolar molecule that transmits signals both as a ligand and as a receptor in a cell-to-cell contact fashion, while the soluble form (sTNF, 17 kDa) acts also at distance by interacting with the same receptors. However, sTNF binds to TNF-R1 with a 30-fold higher dissociation rate than to TNF-R2. Therefore, much of the sTNF linked to the TNRF2 is promptly released and possibly captured by TNF-R1. After shedding, mediated by TACE, both receptors are capable of neutralizing TNF in solution, thus acting as potential natural TNF antagonists. This effect is controlled by TACE inhibitors via metallopeptidase-3 (MMP3). TNF-R1 is ubiquitous (except for RBC) and constitutionally expressed, whereas TNF-R2 is generally inducible and preferentially expressed on endothelial and hematopoietic cells. Macrophages, T and B cells, NK cells, neutrophils, endothelial cells, smooth muscle cells, osteoclasts, and fibroblasts produce TNF as a result of innate and adaptive immune responses induced by exogenous molecules from bacteria, viruses, but also by immune complexes, hypoxia and trauma. However, the primary source of TNF in immuno-inflammatory processes is the monocyte/macrophage lineage. TNF release, in turn, stimulates the secretion of cytokines (IFNγ, IL-1, 6, 8,17, G-CSF), chemokines (MCP-1), adhesion molecules (ICAM-1, E-selectin), and inflammatory proteins (MIP-1 and 2), acting also on leukocyte activation/mobility and on endothelial permeability. The production of TNF is regulated by feedback loops initiated by TNF-induced factors. IL-1, IFNγ and IL-2 induce TNF production, while IL-10, prostaglandins and corticosteroids downregulate their production by inhibiting transcription of TNF mRNA. Therefore, TNF is a key pro-inflammatory cytokine with a central role in inflammatory processes. TNF plays a crucial role also in granuloma formation and maintenance.

In healthy humans, circulating TNF is hardly detectable. However, in patients with acute infections, septic shock, or chronic inflammatory and autoimmune diseases, TNF levels are rapidly and consistently increased, being detectable also in serum, stools and synovial fluid. TNFRs, or TNF antagonists, can bind to

tmTNF at cell surface. This binding induces reverse signaling, which in turn triggers cell activation, cytokine suppression, or apoptosis of the tmTNF-bearing cells. This peculiarity may be also responsible of some AEs induction.

Golimumab is a human IgG1k monoclonal antibody binding with high affinity to both sTNF and tmTNF. This interaction inhibits the biological activity of this proinflammatory cytokine. Golimumab does no bind to other TNF super-family ligands, such as human lymphotoxins (LTs). The binding of human TNF neutralizes TNF-α-induced cell-surface expression of the adhesion molecules E-selectin, vascular cell adhesion molecule (VCAM)-1, and intercellular adhesion molecule (ICAM)-1 by human endothelial cells. In vitro, TNF-induced secretion of IL-6, IL-8, G-CSF, and GM-CSF are inhibited by golimumab. In vivo, it induces a significant reduction of serum levels of IL-6, ICAM-1, MMP3, and vascular endothelial growth factor (VEGF). In addition, golimumab administration reduces levels of TNF-α in RA and AS patients, and levels of IL-8 in PsA patients. Golimumab modulates complement-dependent cell lysis (CDC) and antibody-dependent cell cytotoxicity (ADCC) [9, 10].

22.2 Immunogenicity

Antibodies to golimumab (HAHA) were detected in 5 % of over 1,300 RA, PsA and AS patients in the Phase III studies up to week 52. The majority (98 %) showed neutralizing anti-drug antibodies (ADA) activity in vitro. Similar rates were shown across rheumatologic indications. Treatment with concomitant MTX resulted in a low number of patients with antibodies to golimumab compared to patients receiving golimumab without MTX — approximately 3 % (41/1262) versus 8 % (64/853), respectively. Antibodies to golimumab were dose-dependent and peaked at week 16. The presence of ADA may increase the risk of injection site reactions, and interfere with response rates to golimumab treatment. This feature seems to be common to other anti-TNF agents, and is related to the presence of neutralizing antibodies [3, 4].

22.3 Adverse Events

Original evaluation of golimumab safety was essentially based on pivotal clinical studies and on additional data from Phase II and III clinical studies. Altogether, safety data were obtained from 2,522 patients including 1,544 with RA, 394 with PsA, 353 with AS, and 231 with severe persistent asthma [1–6]. Overall, the most important AEs observed after 24 weeks of treatment were *serious infections* including *sepsis, tuberculosis* (TB), *invasive fungal infections, and other opportunistic infections.* However, in all studies treatment with golimumab was generally well tolerated. The most common AEs were URTI (7.2 % vs. 5.8 %), while the major causes of therapy discontinuation were infections in RA patients, malignancies in PsA, and investigation SOC for AS.

Overall, the safety subgroup analysis showed comparable data in RA, PsA and AS in terms of frequency and typology. Notably, SAEs (5–6 %) were in the range of controls. However, serious infections (5 %) were higher than in controls and in the general RA population treated with conventional therapy (DMARDs), although in the range of other anti-TNF inhibitors.

Malignancies (0.3 % vs. 0 % in controls) were estimated within the range of general population, except for lymphomas (3.8 %). However, these neoplasms have higher rates in the RA cohort. Notably, 5/8 malignancies appeared in the group treated with highest doses of golimumab. In a safety group of 2,057 patients exposed to golimumab for 24 (289 subjects) or 52 (1,768 subjects) weeks, *mortality* (0.7 %) was higher in treated patients and highest in those receiving highest doses, and included malignancies (3), cardiac events, and sepsis (2) [1–4].

Updates on previous pivotal trial have been published and add further knowledge on safety after longer treatments and prolonged observations. Data on the GO-FORWARD cohort of RA patients treated up to 52 weeks reported AEs highest rates at 86 %, and SAEs at 18 %. Infections (53 %) and serious infections (8 %) including sepsis (5, 1 fatal), sinusitis (5), cellulitis (3), UTI (3), pneumonia (2), GI disorders, TB pleurisy (1), bronchitis (1), and acute pneumopathy (1), resulted more frequent than in controls at the higher dose employed (100 mg). Notably, none of them was present in the placebo group. In addition to three treated patients showing malignancies within week 24, four more patients had BSC and SCC to week 52. Injection site reactions (5–12 %) were mild/moderate and consisted mostly of injection-site erythema and bruising. Overall, the safety profile was similar in nature and frequency to those observed at 24 weeks of the study [11].

Additional long-term data on the GO-AFTER trial were reported on 51 % of 459 patients treated for 160 weeks.

Overall, the long-term treatment experience confirmed the typology of encountered AEs, with no new signals registered. The first short-term safety profile after 16 weeks of treatment was comparable to placebo, but the frequency of total AEs, SAEs, and infections increased with time and at higher doses of golimumab (4–6 times the placebo group follow-up; 3–10 times the initial dose at first endpoint). Moreover, the incidence of serious infection, malignancy/lymphoma and death was higher in the highest dose group, indicating a potential progressive dose–effect of golimumab. [12, 13].

An update of the GO-REVEAL trial reported safety data on 394 PsA patients, who continued treatment up to week 52. The most common AEs in golimumab treated patients were URTI and nasopharyngitis, both in low and high dose groups. Infections (51 %) and serious AEs (4.6 %) were frequent. However, serious infections, excluding opportunistic infections and TB (0.8 %), were uncommon and were represented by abscess, superficial thrombophlebitis, and one sepsis in acute cholecystitis. Malignancies (1,5 %) included BCC (2) and CC, PC, SCLC (1 each). Lymphopenia and eosinophilia were observed in 0.8 % of cases. Overall, about 4 % of the golimumab-treated patients discontinued study agent because of an AE. Liver functional abnormalities (AST/ALT, bilirubinemia) were reported in about

1 % of patients, and hyperglicemia in <1 %. The incidence of HAHA remained low through week 52 (4.9 %), and showed (95 %) neutralizing activity in vitro. As expected, 90 % of HAHA positive patients did not receive concomitant MTX. Injection-site reactions were present in about 5 % of cases, mostly as erythema (2–10 %). However, no severe/serious events were observed, nor events resulting in treatment discontinuation. No patient experienced anaphylactic or serum sickness–like reactions [14]. An update of the GO-RAISE trial reported safety data in AS patients up to week 104. The number of subjects with at least one adverse event (94 %) generally increased with longer average duration of follow-up. Serious events (11 %), infections (68 %) and serious infections (3 %) also increased with golimumab increasing dosage. Serious infections included urosepsis (1), tonsillitis (1), anal abscess (2 events in 1 patient), bursitis, cellulitis, Lyme disease and pulmonary TB (one each), and pelvic inflammation in endometriosis. The overall safety profile did not change with time from week 23 to week 104.

One opportunistic infection (coccidiomycosis) was reported after week 104. No additional malignancies were observed after week 24. Injection-site reactions occurred as mild/moderate in 11 % of cases. Liver enzyme elevations (1–3 %) were more common at higher doses (5 %) of golimumab, reaching 2–3 times baseline levels of AST/ALT and bilirubin. Notably, 50 % of patients with positive HAHA became negative by week 24. Overall, golimumab was still well tolerated up to 104 weeks of treatment. The study will continue to be followed for up to 5 years, to provide a long-term analysis of the effects of golimumab in patients with AS [15]. Additional data came also from two studies for golimumab approval performed in Japan [7, 8]. In the GO-FORTH study, data were collected on 269 Japanese patients with active RA for 24 weeks. Global AEs were 77–82 %. SAEs (2–3 %) included one case of ileus, herpes, intervertebral disk and tendon disorders, and on case of aortic dissection. Infections were the most common (36–40 %) in all treated groups, including rhinopharyngitis (19 %). No deaths, malignancies, or TB infections were observed during the study; two neoplasms (1 %) appeared between weeks 16-24, and included one benign breast lesion and one low-grade hemangioendothelioma. Injection site reactions (7–10 %) were usually mild. No cases of anaphylactic reaction nor serum sickness-like reactions were observed. In the GO-MONO trial golimumab was experienced as monotherapy in 316 Japanese patients with active RA up to 24 weeks. AEs (71 %), SAEs (4 %), infections (33 %), and serious infections (1 %) occurred in the study groups and were not related to dosage. Infections included one case of pneumonia, one atypical mycobacterial infection, and one case of cellulitis. As expected, the most common infection was nasopharyngitis (16 %). Liver functional tests were elevated (4 %) only in the high dose treatment. However, injection site reactions (10–12 %) appeared not related to administered dose of golimumab. Finally, malignancies (0.9 %) included BC, skin papilloma and ovarian cancer (1 each). No anaphylaxis, anaphylactoid reactions, or serum sickness-like events were observed. No TB or deaths occurred up to week 24.

No cases of anaphylaxis or anaphylactoid reactions were encountered during Phase III observations. However, urticaria (0.6 %) and rash (0.3 %) were observed at slightly higher rates than in controls. Finally, a suspected signal of autoimmune drug-induced disorders (1 case of SLE, 2 vasculitis, 6 pustular psoriasis) was raised in official reviews of original safety trials data. In subsequent studies and long-term treatments up to 160 weeks, the presence of HAHA remained within the initial range (5 %) and confirmed their predominant typology as neutralizing (95 %). Moreover, their presence was much pronounced in monotherapy, and was approximately 30 % lower in patients treated with MTX. Interestingly, about 50 % of positive cases converted into negative in the long run (about 104 weeks). No reports of anaphylaxis, anaphylactoid, nor serum sickness-like events were observed. However, comparative studies of anti-TNF biologics have shown that each drug has a different sustained efficacy profile depending on immunogenicity [16].

22.4 Off-Label Experience

Most off-label uses of golimumab are experienced in autoimmune and inflammatory disorders, including SLE, refractory psoriasis, spondylitis, seronegative arthritis, Crohn's disease, colitis ulcerative, sarcoidosis, spondyloarthropathy, and leukocyte antigen B-27 positive conditions, and immune system disorders. Particular attention is also given to autoimmune and inflammatory eye disorders, such as Behçet's disease, other forms of vasculitis refractory inflammatory eye, and chronic uveitis. Moreover, in postmarketing spontaneous database records a relevant number of treated conditions are reported as unknown. The potential application of golimumab or other TNF inhibitors to the treatment of most of these diseases is based on the assumption that inflammation is also involved in many pathological immune-mediated and autoimmune diseases. TNF-α plays a key role in the pathogenesis of immune-mediated diseases, including SLE. In fact, SLE pathogenesis is due to a "galaxy" of concatenated events, including autoantibody production, cytokine imbalance, immune-complex formation, CDC and ADCC, all influenced by these biomedicines. However, treatment of SLE and related states with TNF inhibitors raises concerns, not much about AEs-induced additional risks, but mainly due to some severe SLE and other autoimmune disorders reported during standard therapy for approved indications. Despite some patients experienced increased levels of anti-dsDNA antibodies during treatment with TNF inhibitors, no lupus flares were observed [17]. Recently, considerable and long-lasting improvements have been observed in lupus nephritis. In contrast, one case of subacute cutaneous lupus erythematosus after treatment with golimumab has been reported [18]. Overall, although the safety profile of golimumab appears acceptable for short-term use, longer-term treatments needed in most of these off-label disorders may represent an additional risk [19].

Recently, two cases of uveitis treated with golimumab have been reported, without remarkable side effects, after a medium follow-up time of 27 weeks. One patient with JIA was treated with other alternate TNF inhibitors, and both arthritis and uveitis improved after golimumab, without apparent AEs. The second case was diagnosed as idiopathic retinal vasculitis and was treated similarly, with no consequences [20]. Three additional cases of uveitis associated to JIA have been recently published. Interestingly, two patients reported AEs (one severe) to another TNF inhibitor (infliximab), having no reactions to golimumab [21]. It should be emphasized that in ophthalmic disorders the monthly golimumab subcutaneous administration, instead of intravenous or intravitreal, is preferable both for patient's compliance and safety.

Recently, final data of the study on 231 patients with severe asthma, administered with doses of golimumab as high as 200 mg, have been published [22]. The study was discontinued due to serious safety concern, when approximately 50 % of patients had completed the 52 weeks control. SAEs occurred more frequently in the study groups (28–32 % vs. 20.5 % in controls), and included serious infections (13–19 % vs. 9 % in controls), pneumonia (6–10 % vs. 5 %), cellulitis (1–3 % vs. 0 %), sepsis (1–3 % vs. 0 %), and chest pain (1–3 % vs. 0 %). One case of TB was observed in an aged patient living in an endemic area for tuberculosis. Eight malignancies (1.3–6.4 % according to dosage, vs. 0 % in controls) were reported in the study arm, and included BC in the low dose cohort, BCL, MM, CC (stage 0), RCC, and two BCC in the highest dosage group. Overall, in the asthmatic patient golimumab produced malignancies and respiratory SAEs, including pneumonia, at rates that were not observed in anti-TNF-a trials in other diseases.

No other controlled trials have been so far published and the number of patients treated is limited in all these off-label diseases. However, at present most of the over 43 trials on golimumab-treated patients are ongoing, and include patients with spondylopathy (44), joint disease (33), autoimmune disease (23), connective tissue disorders (23), bone disease (14), spinal disease (14), skin disorders (12), anchylosis spondylitis (9), psoriasis (6), colitis/ulcerative colitis (6), gastrointestinal disorders (6), and sarcoidosis (2). A number of trials still investigate peculiar aspects of on-label rheumatic diseases and other minor pathologies, in the aim of assessing long-term safety and efficacy. Hopefully, these investigations will better clarify the border from uses and misuses of this biomedicine.

22.5 Postmarketing Surveillance

In the FAERS database 2,125 reports included an average of 3.3 AEs/Report and most frequent events were infections (18 %; bacterial 5 %), respiratory, neurological, and dermatological disorders (about 3% each). GI (1.4 %), and skin (1.1 %) tumors were prevalent among reported malignancies.

In the EUV database, 937 reports included 2,219 AEs (SAEs/R 2.4) up to the end of 2012. Infections (15 %), constitutional signs (14 %), neurological (9.5 %), dermatological (8 %), muscular (7 %), GI (7 %), and respiratory disorders (6 %) were the most commonly registered. Among infections, 41 cases of pneumonia, 12 sepsis, and 7 TB including one spleen and one liver localization were collected. Three cases of anaphylaxis, and one anaphylactic shock were also reported.

22.6 Remarks

From the safety point of view, golimumab is an interesting anti-TNF inhibitor, since it is a low-rate, low-grade inducer of AEs for unknown reasons. Major concerns remain about infections and malignancies, although recent observations over 3 years are reassuring. One possible explanation of drug- induced malignancies may be the inhibition of the natural TNF "tumor necrosis effect" acting on tumor vasculature. Such activity synergizes with the "immunosuppressive" capacity of these TNF-inhibitors, thus enhancing oncogenesis. However, a recent analysis on the risk of malignancies after anti-TNF therapy, including golimumab, has held that present literature is insufficient and full of discrepancies, complicating factors, and biases for being accepted as conclusive. As for golimumab, data did not indicate a risk of malignancy higher than placebo, except for lymphoma in psoriasis. However, the risk of lymphoma is increased per se in psoriatic patients. Therefore, the establishment of registries of treated patients was proposed, with the aim of providing a prospective surveillance to better evaluate cancer risk [23]. A recent meta-analysis on the risk of malignancies in RA patients treated with biologic therapy, including golimumab, has showed that no significant association with an increased risk of malignancy was present, compared to DMARDs or with placebo [24]. Another meta-analysis on safety and efficacy of TNF inhibitors, including golimumab, pointed at etanercept and golimumab as safer alternatives among others, while certolizumab, possibly more effective than golimumab, was associated with a higher risk of SAEs [25]. In fact, the question of safety should also be considered taking into account other similar treatments for the same diseases, that seem to share serious infections and malignancies as the most concerning AEs. A serious limitation of these studies is the short comparative observation of placebo groups (usually interrupted after 24 weeks) compared to treated groups (lasting up to 160 weeks). The shorter observation period is explained by the necessity to avoid keeping patients under ineffective therapies for a long time. The presence of antibodies against golimumab did not correlate with a loss in response, and patients who at baseline were on methotrexate did not form significant levels of anti-golimumab antibodies.

Efficacy and safety of this class of biomedicines may be related to different mechanisms of action. For example, TNF-inhibitors show different affinity and different capacity of binding to sTNF and tmTNF. In particular, the affinity of various antagonists for tmTFN ranges from 20- to 1400- fold, in relation to sTNF

[10]. This difference may be relevant, since mutant mice that show only tmTFN are resistant to infections, suggesting that sTNF targeting may be a higher inducer of infectious AEs. Moreover, different affinities for tmTNF may spare immune cells expressing lower levels of this surface molecule, thus allowing better protective immune responses. Finally, the stability of these biomedicines may play another important role, since they allow a lower number of injections and pre-filled syringes ready to be used directly by the patient.

Taken together, these observations suggest the possibility in future to isolate distinctive mechanisms providing more efficient therapy with reduced safety risk.

Meanwhile, further long-term monitoring of this monoclonal is required for better assessing its safety, possibly remodeling therapeutic strategies.

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Ibritumomab

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Ibritumomab-tiuxetan-^{90}Yttrium (Zevalin®, Idec, Baxter-Shering) is a CD20-directed radiotherapeutic IgG1k indicated for relapsed or refractory, low-grade or follicular B cell non-Hodgkin lymphoma (NHL), and for previously untreated follicular NHL who achieve a partial or complete response to first-line chemotherapy. Ibritumomab is the parent anti-CD20 antibody from which rituximab was generated.

Marketing accelerated approval was granted in 2002 by FDA, and in 2004 by EMEA for the first therapeutic indication. In 2008, the approval was extended by the European Agency to consolidation therapy of follicular NHLs after remission induction in previously untreated patients (Study 304820). In 2009, FDA extended approval to previously untreated follicular NHL achieving partial or complete response to first-line chemotherapy, on the basis of the same study. Health Canada approved the use of ibritumomab-tiuxetan in 2005 for patients with relapsed or refractory, low-grade or follicular CD20+ B cell NHL, including those with rituximab-refractory follicular NHL.

Safety and efficacy were initially evaluated in one Phase I (106-01), two Phase I/II (106-03; 106-05) on 80 patients, and two Phase III (106-04, 106-06) multicenter trials enrolling a total of 348 subjects for safety analysis, and on preliminary information coming from 138 patients of an ongoing (106-98) expanded access trial.

Treatment with Y2B8 is preceded by two doses of rituximab and a bioscan with ^{111}Indium-labeled ibritumomab (^{111} In2B8) to control its biodistribution. However, in November 2011 FDA approved the removal of the bioscan preliminary checking. At present, about 100 trials have been completed, or are still ongoing [1–5].

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23.1 Mechanism of Action

Ibritumomab (IDEC-2B8) is a recombinant murine IgG1k monoclonal antibody specific for human B lymphocyte-restricted differentiation Bp35 antigen (CD20), located at the surface of normal human and primate B lymphocytes and on human malignant B cells. During B cell maturation, CD20 is first expressed on pre-B cell, but is lost during the final stage of B cell maturation to plasma cells, but it is not shed from the cell surface and it is not internalized. CD20 is expressed on a subpopulation of precursor-B cells, on mature B lymphocytes, and on follicular dendritic reticulum cells. CD20 is also expressed by low grade B cell NHL, precursor B cell neoplasms, precursor B-Lymphoblastic leukemia/lymphoma (B-LL), HCL, B-CLL (weak), and by B-PLL. CD20 is a tetra-spanning membrane protein with a possible role in B cell activation/proliferation, enabling optimal B-cell immune response against T-cell independent antigens. CD20 is supposed to be a receptor, but no known natural ligand has been so far identified. Ibritumomab is covalently linked to the chelator tiuxetan (MX-DTPA) radiolabeled with Y-90 for therapy (^{90}Y-2B8-MX-DTPA, shortly Y2B8) or with In-111 for imaging (^{111}In-2B8-MX-DTPA, or In2B8). Ibritumomab is the parent anti-CD20 antibody of rituximab, which is also used in advance to reduce the number of leukemic B cell. Treatment with [90Y]-radiolabeled monoclonal also leads to further depletion of the leukemic burden as well as of normal CD20+ B cells. This effect is transitory, but recovery of normal B cells tends to normalize within 9 months after treatment. The aim of radioimmunotherapy is to target ionizing radiation at radiosensitive tumors through the use of monoclonal antibodies. In the case of ibritumomab, its intrinsic apoptotic effect on B cells is potentiated by the radiation emission of Y-90, which causes the formation of free radicals damaging target and surrounding cells located up to 5 mm away from the antibody. Therefore, the double stage therapy consisting in rituximab followed by ibritumomab-tiuxetan-Y90 seems the most appropriate to destroy circulating B cells, in order to more specifically deliver to the bulk of lymphoma B cells the radio-immuno conjugate, which seems also to circumvent the residual disease and the metastatic diffusion. Prior to treatment, an imaging biodistribution screening is performed with In2B8, since this gamma emitter is more suitable for imaging purposes than Y-90, and has no therapeutic emission. The median biologic half-life of the fully murine ibritumomab is much shorter than the chimeric rituximab (<2 days vs. 7 days respectively), presumably due to a lesser affinity of the murine Fc domain for the human FcRn receptor. However, in this case, a rapid clearance of the conjugate is desirable to avoid excessive exposure to internal radiation. While serum IgG and IgA have median normal levels during therapy, IgM decrease after treatment and slowly recover within 6 months. The ibritumomab regimen is similar to tositumomab-Iodine^{131} tositumomab regimen, although linked to a different emitting isotope [6, 7; see also rituximab and tositumomab, Chap. 35, 37].

23.2 Immunogenicity

Hypersensitivity reactions, mainly as early infusion reactions after Y2B8 administration, are reported as common (1–10 %), while severe reactions are <1 %. Nonetheless, HAMA/HACA antibodies have been observed in about 4 % of cases, although they do not seem to be strictly associated with allergic events. Some patients (about 2 %) may have previous positivity for these antibodies, but evidence of immunogenicity may also be masked by preceding immunosuppressive treatments.

However, another important implication of anti-drug antibody presence is related to the possibility of altering its biodistribution in different organs. This may improve local induction of AEs, especially at bone marrow level, more than altering the therapeutic efficacy. In fact, the more lymphomatous cells engulf bone marrow, the higher is the accumulation of the radiolabeled antibody at local level, and greater is the cytotoxic damage to residual healthy marrow. Moreover, an increased frequency of GI adverse events has been associated to localization of the radiolabeled antibody in the bowel, thus increasing the level of local radiation damage. However, in most cases, HAMA/HACA titers resolve spontaneously and do not increase with time, even in case of long-term follow-up. Similarly, immune reconstitution after initial B cell depletion, induced by Y2B8 and IgM decline (IgG and IgA usually remain within normal range), levels to normal standards in 3–6 months.

Interestingly, a recent case of unsuspected pneumonia was detected due to an abnormal lung uptake of In2B8. Such test was performed to confirm the normal biodistribution before treatment with Y2B8, and kept from further potential damage at pulmonary level [3–5, 8, 9].

23.3 Adverse Events

A BBW on *fatal infusions reactions* and *severe cytopenias* was inserted in the initial official label of Y2B8, which in 2005 was integrated with *severe cutaneous* and *mucocutaneous reactions*.

Basic safety information on Y2B8 comes from the mentioned five studies submitted for application: two pivotal trials (106-04 and 106-06), and three supportive studies (106-03, 106-05, and 106-98) on 419 subjects. Of these patients, 349 were treated with Y2B8, and 70 were treated with rituximab as a control therapy. Of the 349 subjects treated with Y2B8, 182 received one dose of In2B8 for imaging investigation, followed by one dose of Y2B8. Overall, the safety profile is characterized by hematologic *cytopenias* (57 %), including *severe neutropenia* (55 %), *thrombocytopenia* (57 %), *anemia* (17 %) and *hemorrhage* (33 %) while thrombocytopenic, some severe (0.8 %) and fatal (two cases of intracranial hemorrage). Nonhematologic AEs (100 %; 6.7 AEs/patient) were mostly mild (>90 %). Severe nonhematologic toxicities (10 %) included *serious infections* (mostly bacterial), and *hypersensitivity reactions* (bronchospasm and

angioedema). The most common drug-related nonhematologic toxicities were *GI signs* (nausea, vomiting, abdominal pain, and diarrhea) occurring as mostly mild in 57 % of cases versus 34 % in the control arm. Additional AEs observed at a higher rate in Y2B8 arms included *constitutional and respiratory symptoms* (cough, dyspnea, dizziness, arthralgia, anorexia, and anxiety). *Malignancies/dysplasias* (1.7 %) pertained to the myeloid compartment and consisted of fatal AML (3) and MDS (2). One case of meningioma was also observed. Cumulative annualized rate was 0.79 %.

Overall, infections were common (37 %, 1.6/patient), yet mostly mild/moderate (78 %), including febrile neutropenia (2.5 %), pneumonia (2 %), sepsis (1.6 %), UTI (1.6 %) and others (3 %). As for their typology, they were bacterial (13 %), viral (5 %), and fungal (4 %) infections.

Potential additional effects related to In2B8 imaging inspections were investigated in 348 patients, showing a general increase in overall frequency of AEs. However, the incidence of severe hematologic and non-hematologic reactions remained similar, whether or not therapy included In2B8.

Since the treatment in study consists of a combination of rituximab followed by Y2B8, the safety of each component was also evaluated in trial 106-04. Overall, the incidence of non-hematologic AE was similar during treatment (96 % vs. 99 %, respectively) and follow-up (27 % vs. 34 %), most events being known as characteristic of rituximab. Some mild constitutional (nausea), respiratory (throat irritation) and GI (vomiting) symptoms appeared more frequent after Y2B8 administration. However, major difference between the two regimens concerns hematologic toxicity: relevant dissimilarities were perceived in reported cases of severe neutropenia (32 % vs. 0 %), thrombocytopenia (5.5 % vs. 0 %), and anemia (83 % vs. 0 %), which indicated a more pronounced myelosuppression related to Y2B8. Interestingly, infections were more frequent after the combined therapy (41 % vs. 18 % in rituximab monotherapy), but equalized during follow-up (8 %).

Overall, additional AEs related to Y2B8 included GI signs (nausea, vomiting, and anorexia), respiratory symptoms/complications (cough, bronchospasm, infection), and hematologic toxicity (cytopenia), which has a prolonged yet transient effect, possibly related to radiation damage caused to hemopoietic precursor cells. In fact, consequent infectious events increased during treatment but leveled during follow-up, showing the effective recovery of initial neutropenia/lymphocytopenia [1–3].

When the request for Y2B8 extension to consolidation therapy after remission induction in previously untreated patients with follicular NHL was submitted to EMEA, an additional Phase III study (304820) was demanded. This study enrolled 414 patients (208 receiving Y2B8, 206 untreated controls after first-line chemotherapy) with follicular NHL (stage III–IV), followed for 2–5 years. Both hematologic (72.5 % vs. 15 % in controls) and non-hematologic (95 % vs. 80 %) events were more frequent in the study group. The most frequent events included infections/infestations (61 % vs. 38 %), constitutional signs (58 % vs. 30 %), GI disorders (47 % vs. 25 %), musculoskeletal disorders (47 % vs. 33 %), dermatological reactions (37 % vs. 16 %), thoracic/respiratory disorders (32 % vs. 17 %),

and nervous disorders (29 % vs. 22 %). Noteworthy, 119 patients (58 %) versus only one patient (0.5 %) in controls had drug-related AEs. Similarly, drug-related infections, GI disorders, and dermatological reactions (13–36 %) including injection site reactions (36 %) were prevalent in the study group. As for severe (grade 3–4) hematologic events such as thrombocytopenia (>60 % vs. 0 % in controls) and neutropenia (66 % vs. 2.5 %), they all appeared more frequent in the study group. Overall, this study confirmed that myelotoxicity is the most prominent event related to Y2B8 administration as consolidation therapy after standard chemotherapy, and that safety profile was similar to previous trials. As expected, this toxicity was mostly serious yet transitory and manageable. [4, 5, 10].

By combining stem cell transplants (ASCT, HSCT) with chemo- and radioimmunotherapy, myelotoxicity could be lowered. In a retrospective study on 71 patients receiving chemotherapy followed by ASCT, AEs were similar to controls. Febrile neutropenia was the most representative (95 %) event. One case of sepsis (resolved) and one VOD (fatal) were also observed [11]. In another study based on Y2B8-delayed therapy after stem cell transplant, myelotoxicity could be also controlled. In particular, 6/9 patients only suffered mild hematological AEs when receiving the radiolabeled antibody 18 months after transplant [12]. Additional long-term (27 months) data on safety have been recently reported in a review on 65 patients treated in a single institution from 2005 to 2012. They received the double step conventional therapy (as in study 304820) of follicular NHL after chemotherapy as front line consolidation support. Asthenia (50 %) was the most frequent nonhematological AE. Severe thrombocytopenia (36 %), and neutropenia (19 %) were the most frequent hematological disorders observed, followed by spontaneous recovery in about 2 weeks. Four malignancies were present at time of treatment and one (PC) developed 4 years after treatment. One patient suffered mild mucositis. Overall, AEs remained within the expected profile even in heavily treated patients. The protocol was judged as well tolerated and manageable even for outpatient administration, and suitable for elderly patients [13].

An interesting comparison between Y2B8 and ^{131}I-tositumomab—an analogue anti-CD20 radiolabeled monoclonal indicated as alternative treatment—was recently reported. Toxicities of the latter are similar to Y2B8, yet milder, being the predominant adverse effect hematological toxicity resulting in anemia, neutropenia, and thrombocytopenia. However, thyroid toxicity related to ^{131}Iodine represented an additional risk of ^{131}I-tositumomab, given that tositumomab also induces a much higher HAMA response (10–50 % of patients, mostly without prior chemotherapy) and an increasing incidence of AML/MDS (10 %) during the 27–39 months follow-up [14].

23.4 Off-Label Experience

Mostly reported off-label usage of ibritumomab-tiuxetan is in the range of other CD20+ B cell lymphomas, such as DLBCL, FCL, and MCL in association with stem cells transplant, and in bone marrow conditioning regimens. Furthermore, the

NCCN recognizes ibritumomab tiuxetan in the treatment of gastric mucosal-associated lymphoid tissue (MALT) lymphoma, non-gastric MALT lymphoma, nodal marginal zone lymphoma, primary cutaneous B cell lymphoma, and splenic marginal zone lymphoma. In the attempt to reduce hematological AEs, short-term chemotherapy followed by only one dose of rituximab and Y2B8 was used in MCL consolidation treatment. Severe neutropenia (54 %) and lymphopenia (43 %) were the most representative AEs, but no patient had delayed recovery of blood counts [15]. Moreover, delayed Y2B8 administration (over 18 months) after HSCT or ASCT resulted also in reduced rates of hematological adverse events [12]. Therefore, it seems that adjusting proper strategies these AEs could be better managed. Overall, treatment was reported as well tolerated, with safety profile similar to on-label basic experience. Severe events were mostly hematologic, including severe neutropenia, gastrointestinal, including mucositis and nausea/vomiting, and flu-like syndrome.

23.5 Postmarketing Surveillance

The most important signals coming from postmarketing experience were hemorrhage, including fatal cerebral hemorrhage, severe infections, some with fatal outcome, severe cutaneous and mucocutaneous reactions, some of them fatal, including erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis, bullous dermatitis, and exfoliative dermatitis. Signals appeared between 2004 and 2008 contributed to the extension of BBWs in official labels. Additional information came also from a Postmarketing Registry designed to collect biodistribution images, which reported 1.3 % of cases of altered biodistribution in a cohort of 953 patients. Finally, additional postmarketing data on 746 patients with relapsed refractory NHL reported 19 cases of AML/MDS (2.6 %), including 535 patients of an expanded access program registering a frequency of about 1.5 %.

Recently, an interesting report searched in one major database (WHO Drug Monitoring AE databank) and in current literature for Progressive Multifocal Leukoencephalopathy (PML) in patients who had received therapy with various monoclonal antibodies, including ibritumomab and rituximab. Search in the database retrieved 182 cases of PML, including 114 patients treated with rituximab and five treated with ibritumomab. Search in the literature detected 95 cases of PML, none after therapy with these two monoclonals. The Authors indicated as a possible cause of discrepancy, among others, the preference of medical journal editors not to publish single or small case series reports related to known adverse reactions. This finding indicates once again the importance of safety spontaneous data collection in addition to published reports, for attempting estimations of AEs in clinical practice and off-label usage, possibly via well designed, mandatory registries on rare/very rare and long lasting relevant events such as PML [16].

The most reported AEs in FAERS (over 1,350 records) and EUV (about 350 records) databases remain hematological disorders (7-30 %), including myelodepression, anemia and cytopenia, infections (6-11 %), GI (3-4 %), and dermatological (2-3 %) signs.

23.6 Remarks

Among AEs to Y2B8 treatment, either as monotherapy (considering also when associated to rituximab) or mostly as consolidation treatment after various chemotherapies, the most relevant is myelotoxicity. The time for marrow recovery is slightly prolonged in the consolidation setting compared to monotherapy for refractory patients. However, with proper strategies and stem cells infusions they seem to be manageable. The overall safety profile seems so far stable over time and no new signals have been evidenced. Additional concerns recently evidenced by postmarketing reporting come from malignancies and possibly from other rare long-term life threatening pathologies, such as PML. Despite a direct comparison between Y2B8 and ^{111} I-tositumomab to evaluate their differences has not been performed in proper trials yet, respective AEs have similar profiles. However, additional thyroid toxicity, a stronger HAMA response and a possible higher incidence of malignancies have been documented for the latter radiotherapeutic. Differences in energy and type of emission may suggest different indications (tumor mass over 5 cm indicated for Y2B8; lower marrow toxicity expected from ^{111} I-tositumomab), more than supremacies.

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Infliximab

24

Infliximab (Remicade ®, Janssen) is an IgG1k chimeric human-murine monoclonal antibody that specifically binds with high affinity to TNFα, and is indicated for active adult and pediatric Crohn's disease (CD), adult and pediatric ulcerative colitis (UC), rheumatoid arthritis (RA), ankylosing spondylitis (AS), plaque psoriasis (Ps), and psoriasic arthritis (PsA). Initial approval from FDA was granted in 1998 for active CD and fistulizing CD. The following year EMEA approved infliximab for the same indications under 'exceptional circumstances', while FDA extended authorization to active RA in association with MTX on the basis of an additional multicenter study. Initial approval from Health Canada was given in 2001 for RA and CD. FDA extended approval in 2002 for maintaining clinical remission in CD, in 2004 for AS, in 2005 for PsA and UC, in 2006 for pediatric CD and Ps, and in 2011 for pediatric UC. Meanwhile, alerts were issued by FDA on fungal infections (2008), and on increased risk of lymphoma and other cancers associated with the use of TNF blockers (including infliximab) in children and adolescents (2009). Additional safety surveillance, consisting of in-depth follow-up of malignancy cases reports, and expedited malignancy reporting for pediatric and young adult patients (2011) were provided. Proposal of extensions were also submitted to EMEA for the same additional indications during the aforesaid period. However, due to safety concerns and limited efficacy, the initial indications for the treatment of CD were restricted in 2002, while the indication for RA (2000) remained unchanged, and a patient alert card was introduced. Since then, additional new data from clinical studies and postmarketing data have been submitted, and the therapeutic indications have been updated to include AS (2003), PsA (2004), Ps (2005), UC (2006), pediatric CD (2007), MTX- naive RA (2007), pediatric Ps (2008), and moderate RA (2011). Finally, in 2012 infliximab was granted by EMEA for the treatment of moderately to severely active UC in

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pediatric patients with inadequate response to conventional therapy, or intolerant/contraindicated for such therapies. During 2012, the first two infliximab biosimilar monoclonals were presented for approval to EMEA.

Major pivotal studies for the mentioned progressive approvals include ACCENT I (545/573 treated patients), ACCENT II for fistulized CD (273/273), REACH (112/112) for pediatric CD, ACT-1 (243/364), ACT-2 (241/364) for adult UC, Study C0168T72 (60/60) for pediatric UC, ATTRACT (260/428) and ASPIRE (722/1004) for RA, P01522 (70/70), and ASSERT (202/279) for AS, IMPACT (104/104), and IMPACT 2 (200/200) for PsA, EXPRESS-I (301/378), EXPRESS-II (627/835), and SPIRIT (198/249) for Ps. Supporting safety data were provided from extensions of these studies and a number of additional trials. At present, over 276 trials are completed or ongoing on a number of on and off-label disorders [1–7].

24.1 Mechanism of Action

The tumor necrosis factor (TNF) family is a group of 19 cytokines mainly involved in apoptosis, including TNFα and lymphotoxins (LTα, previously TNFβ, and LT-β). Their structures are homotrimeric (the former) or heterotrimetric (the latter), and are recognized by specific receptors (TNF-R1; TNF-R2). TNFα (also identified as TNF, being the pivotal molecule of the group) is expressed at the cell surface, mainly on activated macrophages and T lymphocytes, and can be cleaved by a TNFα converting enzyme (TACE) in a soluble form, which is considered the mature expression of this cytokine. However, the transmembrane precursor (tmTNF, 26 kDa) acts also as a bipolar molecule that transmits signals both as a ligand and as a receptor in a cell-to-cell contact fashion, while the soluble form (sTNF, 17 kDa) acts also at distance by interacting with its receptors. Both soluble and transmembrane TNF can bind to TNFR1 and TNFR2, and are bioactive. However, sTNF binds to TNFR1 with a 30-fold higher dissociation rate compared to TNFR2. Therefore, much of the sTNF linked to TNRF2 is promptly released and possibly captured by TNFR1. Moreover, shedding of both receptors, mediated by TACE, is capable of neutralizing TNF in solution and acts as potential natural TNF antagonists. This effect is controlled by TACE inhibitors active on metallopeptidase-3. TNFR1 is ubiquitous (except for RBC) and constitutionally expressed, whereas TNFR2 is generally inducible and preferentially expressed on endothelial and hematopoietic cells. Macrophages, T and B cells, NK cells, neutrophils, endothelial cells, smooth muscle cells, osteoclasts, and fibroblasts produce TNF as a result of innate and adaptive immune responses. However, the primary source of TNF in immuno-inflammatory processes is the monocyte/macrophage lineage. TNF release, in turn, stimulates the secretion of cytokines (IFNγ, IL-1, 6, 8,17, G-CSF), chemokines (MCP-1), adhesion molecules (ICAM-1, E-selectin), and inflammatory proteins (MIP-1 and 2), acting also on leukocyte activation/mobility and on endothelial permeability. The production of TNF by cells is regulated by feedback loops initiated by TNF-induced factors. IL-1, IFNγ,

and IL-2 induce TNF production, while IL-10, prostaglandins, and corticosteroids downregulate their production by inhibiting transcription of TNF mRNA. Exogenous molecules from bacteria, viruses, immune complexes, hypoxia, and trauma can activate these cells. Therefore, TNF is a key pro-inflammatory cytokine with a central role in inflammatory processes. TNF plays a vital role also in granuloma formation and maintenance.

In healthy humans, circulating TNF is hardly detectable. However, in patients with acute infections, septic shock, or chronic inflammatory diseases (RA, CD), TNF levels are rapidly and consistently increased, being detectable also in serum, stools, and synovial fluid. TNFRs, or TNF antagonists, can bind to tmTNF at cell surface. This binding induces reverse signaling, which in turn triggers cell activation, cytokine suppression, or apoptosis of the tmTNF-bearing cells. This peculiarity may be also responsible of some AEs induction [8, 9].

Infliximab (cA2) is a recombinant IgG1k chimeric human-murine monoclonal antibody that specifically binds with high affinity to sTNF and tmTNF, thus inhibiting the binding with their natural receptors. It is composed of human (75 %) constant and murine (25 %) variable regions. The binding induces apoptosis on monocytes and activated T cells, and inhibition of integrin expression on endothelia. It also reduces the production in vitro of TNF, IFNγ, and GM-CSF by intestinal and circulating T cells, and inhibits IL-1β release from monocytes. Moreover, infliximab interferes with CD40/CD40L linkage in lymphocytes leading to an anti-inflammatory effect, and reduces interactions between the intestinal microvasculature and T cells.

The selective action of infliximab on activated T cells via tmTNF seems crucial for the induction of cell death. It is known that Bcl (B-cell lymphoma) proteins regulate apoptosis of T cells. In fact, Bcl-2 prevents apoptosis of resting T cells (passive cell death), which is opposed by the pro-apoptotic effect of BAX. Quiescent T lymphocytes exposed to infliximab do not change BAX/Bcl-2 levels, but infliximab raises BAX on activated T cells, thus accelerating their selective death. Interestingly, MTX and infliximab synergize for apoptosis. Overall, these infliximab-induced mechanisms seem to have a long-term response (up to 3 months).

The first anti-inflammatory activity of infliximab is expressed in circulation, while effects at intestinal level appears after 20 h from infusion. In fact, a single injection of infliximab in CD patients rapidly reduces CRP to normal levels (in 2 weeks); the effect lasts up to 8 weeks. IL-6 is reduced and remains low. However, further mechanisms might be involved, since TNF neutralization is not sufficient to provide such beneficial effects at intestinal level. For example, etanercept—the recombinant human soluble TNF receptor—is not effective in CD despite binding to both tmTNF and sTNF, and induction of apoptosis.

Specifically, monocytes and *lamina propria* T lymphocytes are induced to apoptosis when exposed to infliximab. The action of infliximab at tmTNF level seems crucial for local intestinal efficacy, possibly through reverse signaling activation on NFkB pathways in leukocytes. Cells expressing tmTNF-bound by infliximab can be lysed in vitro by complement (ADC) or effector cells (ADCC).

Finally, infliximab induces collagen deposition and thereby facilitates intestinal wound healing.

In RA, treatment with infliximab reduces infiltration of inflammatory cells into active areas of the joint, as well as expression of adhesion molecules, chemotaxis, and tissue degradation. After infliximab treatment, patients show decreased levels of IL-6 and CRP.

In Ps patients, treatment with infliximab reduces epidermal inflammation and induces normalization of keratinocytes in psoriatic plaques. In PsA, short-term treatment reduces T cells and neovascularization in the synovium and in psoriatic skin.

In CD, infliximab reduces the infiltration of inflammatory cells into affected intestinal areas and of inflammation markers at these sites, together with a substantial reduction in local TNF, IFNγ, a substantial reduction of CRP, and evidence of mucosal healing [10–12].

Infliximab and etanercept are largely unable to penetrate the blood–brain barrier, and this limits their use at CNS level.

24.2 Immunogenicity and Related Events

The *incidence of HACA* after a standard induction and maintenance treatment with infliximab is about 10 %. However, consistent differences have been observed among studies on various underlying diseases, as well as in relation with concomitant supportive immunosuppressant treatments. In particular, higher levels of HACA occur in adolescent and young adults suffering CD, and UC (about 15–20 %), and in Ps/PsA patients (20–50 %), especially when in monotherapy. HACA were detected in 75 % of cases within a small group of AS patients. Interestingly, their presence was significantly associated with the lack of HLA-B27 marker, while their absence correlated with a better response to therapy. Although the presence of HACA raises the overall risk of infusion reactions, their level does not increase the severity of reactions. In fact, serious events remain below 1 %. When searched, HACA isotypes were mostly IgG1 and IgG4. As in the majority of cases during treatment with biomedicines, early hypersensitivity reactions, including infusion reactions, are not related with an increase of serum IgE although they were not frequently object of search in such studies. HACA usually interfere with the circulating concentration of infliximab, and shorten duration of response, although their relevance in terms of global efficacy is not clear. In fact, dose escalation investigations in small cohorts did not reach conclusive evidence. As previously mentioned, the concomitant administration of other immunosuppressive drugs, such as MTX, reduces HACA presence and infusion reactions, but presumably increases the risk of serious infections and malignancies in the long term. Less frequently, a severe *serum-sickness-like reaction* (SSLR) was reported as associated with episodic treatment and re-administration. Finally, it must be noted that HACA may persist for a rather long time into circulation, at least after

up to one year after last infusion [5, 6, 13, 14]. Less frequently, anti-nuclear antibodies (ANA, dsDNA) are also produced during infliximab treatment.

Infusion reactions, mostly as early manifestations, were present in about 20 % of cases (5 % in controls). Twenty-seven percent of patients who had an early reaction also showed delayed episodes during maintenance therapy, while 9 % of patients only had a new delayed reaction (mostly CD patients). Serious reactions were <1 % and were accompanied by hypersensitivity/anaphylactic signs. Overall, these reactions tended to be mild and stable during treatment and/or discontinuation and re-administration. In HACA-positive patients, the rate of infusion reactions was increased (2- to 3-fold), while concomitant immunosuppression lowered their appearance. Less frequently, a SSLR occurred (1–2 %), usually correlated with the presence of HACA, and delayed. Interestingly, the rate of infusion reactions and HACA positivity were higher within the low-dose (3 mg/kg) infliximab cohort, as for ANA and anti-dsDNA autoantibodies. The latter are usually of the IgM subtype (IgG in SLE), and are rarely accompanied by a SLE-like reaction (0.18 % on 11,000 treated patients). Overall, infusion reactions and the presence of HACA represent a concern for treatment efficacy, rather than for safety, since their presence correlates with the drop of circulating infliximab and of clinical performance [11, 13, 15].

24.3 Adverse Events

In the label issued in 1998, warnings included only *hypersensitivity reactions* and *autoimmunity*. Precautions were recommended since immunosuppressive secondary effects could possibly determine an increase in infections and malignancies, as an expected consequence of the chronic evolution of CD during immunosuppressive therapies. Another mentioned possible secondary effect was the induction of HACA in 13 % of patients. This safety profile came from one trial on 108 patients with moderate and severe CD, and on 94 patients with fistulizing CD.

Starting from 2002, these alerts have been better identified in a subsequent BBW as *serious and fatal infections*, including *TB (reactivation and new), sepsis*, invasive *fungal* pathogens, other *bacterial*, *viral*, and *opportunistic infections*. From 2006 also *hepato-splenic T cell lymphoma* (HSTCL) and other *malignancies* mainly *lymphoma* in younger patients (from 2010), were subsequently added. This more complete profile emerged from a number of additional trials and postmarketing observations on CD, pediatric CD, RA, adult and pediatric UC, AS, PsA, and Ps, which were progressively added to the initial official indications [5].

The following depicted profile represents a general framework, and differences among diseases were mainly related to their incidence rather than to typology, as subsequently underlined [6, 7, 10–23].

Serious infections, some of them fatal, include opportunistic infections due to bacterial, mycobacterial, invasive fungal, viral, or parasitic organisms, with particular concern about reactivated and new TB (0.08 % in about 400,000 treated

patients). Cases of reactivated or new infection were reported even in patients receiving treatment for latent or active tuberculosis. A twofold increase of granulomatous non-TB, fungal and bacterial infections was reported in treated patients. They tended to suffer disseminated rather than localized infections/infestations. HBV reactivation in chronic carriers generating serious and fatal hepatitis was also observed. Similarly, HPV, HZV, and VZP (9 % in children) reactivation are known. In contrast, there was no increased risk of HCV reactivation.

Overall, the risk of serious infections was twofold higher in patients administered with infliximab and seemed to be dose-dependent. Interestingly, the risk of cytopenia remained low.

Malignancies have been observed at all ages. About 50 % of them were lymphomas and skin carcinomas. Overall rates ranged 0.08–0.1 % (median appearance within one year in 5,707 patients). These rates were over threefold the incidence in the general population. The risk increases with higher dosage ($\geq$ 6 mg/kg/8 weeks). No cases were observed in enrolled controls, albeit the observation period was shorter (0.4 years). These neoplasms were mostly observed in younger patients with CD, yet also in active RA and Ps disease. Two cases of AML (in AS) and CLL (in CD) were reported in the literature. Moreover, cases of CLL have been observed mostly in RA in postmarketing settings, which identified also HSTCL in CD and UC, mostly in adolescent and young adult males. In particular, 10 HSTCL (0.02 %) were detected in over 37.000 patients receiving infliximab and concomitant therapies (AZT, MP), up to 2006. More recently, another study reports 22 cases of HSTCL in CD patients with a median age of 22 years. At present, of the approximate 200 cases of HSTCL reported in the literature, 28 (14 %) were identified in CD patients [24]. Other malignancies included mostly solid tumors, such as breast cancer, melanoma, and NMSC, the latter being more frequent in psoriasic patients, at rates within the general population range, albeit higher than in control groups of the studies. However, the rate of lung cancer was increased in a subgroup of treated patients with COPD. Overall, in a meta-analysis by FDA on RA patients total rate of malignancy was 0.65/100/PY in the infliximab cohort and 0.13/100/PY in controls. Postmarketing data on 5.155 RA patients reported a lymphoma rate of 0.017/100/PY.

Other AEs in the general profile include hepatotoxicity (icterus, cholestasis, hepatitis, liver failure), autoimmune hepatitis, in some cases with fatal development, heart failure (worsening and new), and occasional severe/fatal cytopenias and thrombocytopenias [25]. Rare cases of vasculitis and demyelinating disorders (MS, GBS, ON) were also reported. One case of PML in RA was reported in USA. As for vasculitis, about 80 % cases were females, with cutaneous (42 %) main involvement (purpura, ulcers, maculopapular, nodular). In refractory Takayasu's arteritis, on 79 cases studied 80 % was treated with infliximab, with a good response. AEs were present in 21 % of cases, mainly showing infections (50 %), viral reactivations (33 %), histoplasmosis (15 %), skin infections, URTI, and TB (1 case each) [26]. Finally, a Phase II trial on 150 patients with congestive heart failure, supported by benefits obtained in animal models, and subsequent postmarketing signals reported serious exacerbation and new CHF cases after

infliximab treatment. The trial was halted and a contraindication issue was added to product label.

24.3.1 AEs Peculiarities

Despite the general safety profile similar in all diseases, some differences among them are discernible, possibly related to the underlying pathologies and additional therapies.

In RA patients, infliximab is often associated with MTX. About 40 % of lymphomas were observed in patients under combined therapy (0.08/PY). However, it must be noted that the risk of developing these neoplasms is about twofold higher in RA population than in the general population. Yet when infliximab is administered in other pathologies, such lymphomas occurred more frequently as well.

The risk of developing HACA and related AEs is lowered by concomitant immunosuppressant therapy, including MTX. However, patients in long-term treatment with negative HACA have lower rates of clinical response, and undetectable infliximab serum concentrations.

Neurological AEs are more frequently reported in people with RA. In a recent study, 24/33 cases were observed in RA patients, including demyelinating disorders, MS, Lewis-Summer syndrome, multi-motor and sensory neuropathies [22].

In CD patients, abdominal pain was particularly frequent and severe, occurring in 26 % of treated subjects, while HACA positivity was lower, possibly in relation to concomitant therapy. However, possible associations of HACA and antinuclear antibodies with rare SLE-like reactions (0.2 %) were also reported. Neurological events were reported in 5/33 cases after infliximab treatment and 19 cases were reported in the postmarketing setting [22].

In pediatric CD, infections were reported in 56 % of cases (50 % in adults), being pneumonia and abscess the most serious events. Similarly, anemia (11 %), leukopenia (9 %), flushing (9 %), viral infection (8 %), neutropenia (7 %), bone fracture (7 %), bacterial infection (6 %), and respiratory tract allergic reaction (6 %) were more frequent in pediatric CD than in adults receiving similar treatment.

In UC patients, infections were lower than in CD (27 % vs. 18 % in controls), but with similar typology. TB and serious/fatal opportunistic infections were occasionally present. In a pediatric UC trial, as in pediatric CD, infections were more frequent (52 %) than in adult UC, and the most common AEs were URTI and abdominal pain. Similarly, infusion reactions tended to be higher (13 %) than in adults. Moreover, SAEs frequency was higher (40 % vs. 18 %) in the younger age group. During treatment lupus-like reactions and neurologic disorders were slightly higher among patients treated with infliximab.

All encountered HSTLC developed in young treated CD or UC patients. Therefore, this rare form of T cell lymphoma seems to be associated with IBD and/or with concomitant therapy. Moreover, in a large series (over 20,000 patients) of

IBD, including CD and UC, a higher incidence of demyelinating diseases (MS, GBS, ON) was detected.

In Ps patients NMSC were more frequent. These patients could also experience serious infusion reaction (4 %) and a delayed serum-sickness-like syndrome (1 %), following to the re-administration of infliximab. Positivity to HACA was particularly frequent (51 %). However, infusion reactions tended to be within the general range and stable. Signs of hepatotoxicity were more frequent in these patients than in the IBD groups. Two cases of TB were observed. In PsA patients, URTI were not increased, being lower than in controls. It must be noted that Ps, either exacerbated or new, could appear during treatment with infliximab. In a review on 200 cases, Ps occurred in patients with RA (43 %), SpA (26 %), and CD (20 %) as underlying diseases, respectively. However, over 50 % of cases showed palmoplantar pustular Ps, which is considered a new disorder, both for epidemiological and genetic findings, instead of a Ps exacerbation [27].

In AS patients, signs of hepatotoxicity were more frequent (ALT 51 %) and more severe (14 %) than in IBD patients. They were also higher, yet similar in typology, than those encountered in Ps/PsA patients. The presence of HACA was relevant and increased with infliximab dosage, being inversely associated to HLA-B27.

Overall, infliximab induces a number of AEs mostly mild and manageable. The main concerns are about serious infections, such as reactivation or new TB insurgence, reactivation of HBV, and induction of malignancies including lymphoma and HSTCL. However, underlying diseases or concomitant immunosuppressive therapies might per se raise the risk of such events, as previously mentioned for lymphoma in RA patients.

The effect of long-term therapy on the development of malignancies is not known.

Finally, a series of paradoxical manifestations during infliximab therapy need to be considered among drug-induced adverse events. These are exacerbations of the underlying disease or appearance of "new" diseases, including non-TB granulomatous disorders, that usually are responsive to infliximab therapy. Among underlying diseases there are exacerbations of CD and RA, while new diseases include cases of IBD appearance (0.8/100/PY) during treatment of AS or JIA. Two cases of granuloma annulare, three cases of interstitial granuloma dermatitis and reports on sarcoidosis were also observed in RA and AS patients treated with infliximab. The mentioned issue of palmoplantar pustular eruption remains to be defined [5, 7, 13–23].

24.4 Off-Label Experience

The off-label use of infliximab has been rapidly growing, as well as other TNF antagonists.

Of 276 trials with infliximab, completed or ongoing, 121 are investigating arthritis, 120 musculoskeletal and joint disorders, 92 autoimmune diseases, 87 GI inflammatory disorders, 84 connective tissue diseases, 54 skin disorders, 44 bone disorders, show that a wide spectrum of potential new indications is under investigation. Incoming results seem more promising in some of these areas. For example, up to 2005, a retrospective search in the literature found infliximab used in sarcoidosis (7 cases), hidradenitis suppurativa (10), extraintestinal CD (23), Behçet's disease (6, mostly ocular), pyoderma gangrenosum (45, 1 associated to CLL), Sneddon–Wilkinson disease (1), SAPHO syndrome (3), pityriasis rubra pilaris (3), and eosinophilic fasciitis, panniculitis, necrobiosis lipoidica diabeticorum, dermatomyositis, scleroderma (1 each). A number of additional repors include a variety of off-label IBD, uveitis, and attempts to control posttransplant GHVD. The vast majority of them consisted in case reports or small series of patients, and no new AEs signals were identified [12, 28–30]. *Initial attempts in the treatment of JRA* analyzed in one multicenter randomized trials and followed by an extension observation up to 44 weeks, were unsuccessful. They showed, among others, a higher rate of immunogenicity compared to adult RA-treated patients, accompanied by 35 % of infusion reactions (10 % serious, 7 % anaphylactic), HACA (up to 38 %), and infections (68 %) in combination with MTX. Postmarketing reports on malignancies in JRA and in JIA, such as lymphomas and other types usually observed in children and adolescents, were also reported.

A cumbersome area of off-label application relates to *ocular-retinal disorders*. A recent review of the literature examined potential effects and safety on a series of case reports/small cohorts of patients treated with systemic or intravitreal injections of infliximab, with alternate results. Some of these cases were vasculitis, associated to Behçet's or CD, mostly noninfectious uveitis, AMD, and diabetic AMD. Major AEs after systemic administration of infliximab were severe uveitis (about 42 %), and vitreous opacities. Notably, even after intraocular low-dose treatment, HACA were detected in 3/4 patient's serum, indicating that a systemic immunogenic response could be triggered [30]. An interesting single case of B-27 anterior uveitis, which had appeared years before AS insurgence, was reported. Infliximab administered for AS, also cured uveitis with no reported AEs [31]. A number of studies have also experienced infliximab in JIA-associated uveitis with alternate success and concern [32].

Attempts in SS induced little effect on the skin lesions, and produced frequent infusion reactions, which further limited the efficacy evaluation [23].

A better response was detected in *GVHD after allo-HSCT* in 21 patients with hematological malignancies [29]. No patients had infusion, allergic, or other toxic reactions. However, bacterial (81 %), fungal (48), and viral (67 %) infections were observed. About 40 % were bacteriemias, followed by UTI (27 %) and respiratory (20 %) infections. Viral infections were mostly related to CMV reactivation (67 %). Further attempts showed a similar safety profile, yet efficacy was not apparent.

Finally, infliximab was experienced in SLE. Anti-DNA and anticardiolipin antibody titers increased, usually transiently, but no SLE flares were observed. The overall safety profile was judged satisfactory, except for patients under long-term treatment [23].

24.5 Postmarketing Surveillance

In the FAERS database, over 57,600 reports (AES/R 3.6) included infections (6 %), respiratory and dermatological disorders (about 4 % each), site reactions (3 %), GI and neurological signs (3 % each) as the most frequent events. Among infections, Mycobacteria were common (2.4%), including 1,963 TB (and disseminated 765). Among malignancies, skin tumors (0.9 %), GI neoplasms (0.8 %), and breast cancer (0.6 %) were the most frequently reported. Moreover, there were 321 cases of HSTCL. Among hypersensitivity reactions, there were 460 cases of anaphylaxis, and 201 cases of SSLR. Autoimmune disorders included 821 cases of SLE, and 1,460 LLS. Finally, 96 cases of PML, and 37 RPLS were registered.

In the EUV setting, over 47,300 (SAEs/R 2.6) were registered, with infections (13 %), constitutional signs (11 %), GI disorders (9 %), respiratory (8 %), nervous and dermatological events (about 7 % each) as tthe most frequent AE categories. Neoplasms were reported as slightly over 5%. Among them there were 429 BC, 333 BCC, 224 SCC (50 skin SCC), 227 lung malignancies, 223 MM, and 156 CC/CRC. Moreover, 63 cases of HSTCL and 49 cases of HLH/HPS were also registered. IRS (4,921) and hypersensitivity events (970) were filed. Finally, 35 PML and 22 RPLS were observed.

24.6 Remarks

Infliximab has been largely experienced in on- and off-label indications with a rather safe profile. Major concerns are about serious infections and long-term insurgence of drug-induced malignancies. Interestingly, hepatotoxicity, hematotoxicity, and hematological malignancies are not of particular concern. The experiences coming from off-label uncontrolled studies are mostly related to occasional case reports or small cohorts of very different types of patients and diseases, with a lack of comparator groups. This framework raises more concern than additional knowledge. Nonetheless, it is reassuring that a wide number of trials on most relevant pathologies are currently ongoing.

Most of AEs encountered with infliximab are similar to events detected during treatments with conventional therapy, and with other TNF antagonists [33]. However, some important differences may help in understanding their pathogenesis, and the potential relation to the respective mechanisms of action. For example, infliximab, adalimumab, certolizumab, and golimumab bind both to sTNF and tmTNF, while the fusion protein etanercept only binds to sTNF.

Etanercept induces a low number of infections compared to the above listed monoclonals, but has no effect at GI level, thus indicating different underlying mechanisms for AEs induction and local efficacy. Notably, in a recent meta-analysis, differences in severe infections between infliximab (8 %; 76.9/1000/PY) and etanercept (0 %; 34.5/1000/PY) were remarkable [34]. The capacity of inducing both CDC and apoptosis through tmTNF positive targets represents a key feature of infliximab, which seems particularly effective in the treatment of granulomatous diseases, such as CD. However, this aspects contrasts with the capacity of inducing reactivating latent TB, thus favoring diffuse infections due to inhibition of the granulomatous reaction. In fact, tmTNF plays a pivotal role in granuloma formation. In contrast, this risk is not relevantly increased with anti-sTNF fusion protein, possibly since it does not interfere with T cell-mediated IFNγ production, even at high doses. Additional paradoxical effects of infliximab are: exacerbation of underlying diseases, such as RA, SpA, and CD; no-TB granulomatous disorders, such as sarcoidosis and other cutaneous granulomatous diseases; new eruptions such as Ps, palmoplantar Ps, and similar disorders.

In the case of sarcoidosis, TNF inhibition was reported, mainly after etanercept treatment. However, infliximab proved to be effective in refractory sarcoidosis, severe pulmonary, and extrapulmonary manifestations (including skin reaction and joint manifestations), while etanercept was not effective in patients with chronic pulmonary sarcoidosis. This framework indicates different blocking pathways of the two TNF inhibitors and different pathogenetic mechanisms involved in this disease [12, 23, 28, 35].

Overall, the pathogenetic mechanisms underlying TNF antagonist-induced exacerbation/initiation of these diseases still remain obscure. The IFNα/TNFα imbalance in favor of the former during therapy has been indicated as a potential pathogenetic mechanism in genetically predisposed subjects [36].

Combination of infliximab (or other anti-TNF monoclonals) with immunosuppressive therapies provides a beneficial effect without a major increase of global frequency of AEs. This synergistic effect is presumably related with prevention of HACA formation, which allows higher levels of circulating free infliximab and reduction of hypersensitivity events.

Another relevant finding provided by long-term trials is that AEs tend to gradually decrease in time. For example, in case of treatment with most TNF blockers, infections tend to reduce their frequency in time.

It must be considered that the progressive selection of patients due to AEs occurrence during long-term studies represents a potential confounding bias.

Data provided by registries often report low frequencies of AEs compared to clinical trials. This suggests that dilution of data among more heterogeneous cohorts of patients makes rare signals less detectable, although registries are supposed to be closer to clinical care practice.

Since infliximab and other TNF antagonists are supposed to be of long-term use in chronic cyclic diseases, the information that discontinuation of infliximab seems safe is reassuring. In fact, no loss of efficacy or increase of relevant AEs was observed after delayed re-administration. This kind of approach might become a

standard of care in diseases with long-term induced or spontaneous remissions. Finally, when considering the global exposure of patients to combined drug-induced AEs, the association of infliximab was useful also for decreasing corticosteroid therapy and related adverse events.

In terms of AEs controls, premedications, and prophylactic measures are also crucial. This is the case of infusion reactions and TB infections. It has been calculated that over 80 % of TB infections have been reduced since the introduction of routine screening and specific prophylaxis.

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Ipilimumab

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Ipilimumab (Yervoy®, BMS), previously known as MDX-010 or BMS-734016, is a fully human IgG1k directed to the extracellular domain of CTLA-4 (cytotoxic T cell antigen-4, or CD152) present on activated T cells [1–5]. FDA licensed the product for the treatment of unresectable or metastatic melanoma (mMM) in 2011. During the same year, EMEA granted approval for the treatment of advanced (unresectable or metastatic) melanoma in adults who have received prior therapy. Meanwhile, TGA (Australia) granted approval for the treatment of patients with advanced melanoma in whom previous therapies failed or were not tolerated. However, at that time, NICE recommended the UK National Health Service against using ipilimumab, due to lack of biomarkers (the drug was found active only in part of the patients), and due to the severity of potential AEs. In 2012, Health Canada indicated ipilimumab for the treatment of patients with unresectable or metastatic melanoma in whom other systemic therapy for advanced disease failed or were not tolerated.

Pivotal trials for initial approval include three Phase I-II-III studies (one each) enrolling a total of 910 patients. Dose–response studies were performed in two preliminary trials (MDX10-15, CA184022) on 234 patients. However, the pivotal Phase III (MDX10-20) study raised some concerns about the categorization of AEs, and the FDA required additional information, which subsequently was provided by four studies (CA 184004, CA184007, CA184008, and CA184024), safety information included, which were based on 676 (540 exposed) patients of the Phase III trial and 644 patients of the four additional studies [1–5]. The EMEA Assessment Report examined three supportive studies (MDX10-08, CA184042, MDX10-28) in addition to the previous ones, for a total of 1,107 evaluated patients. Supplemental data came from 14 completed studies on safety conducted on 568 treated patients, investigating ipilimumab use for the treatment of

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metastatic melanoma and other cancers including prostate, renal, and breast. More recently, ipilimumab was evaluated for treatment of naive patients through a second randomized Phase III trial on 502 subjects (250 exposed) with mMM [6].

At present, there are over 100 ongoing trials concerning several specific aspects of neoplastic diseases of melanoma (70), neuroectodermal and germ cells tumors (72), carcinomas of various origins (41), nervous system tumors (72) lymphomas and other lymphoproliferative disorders (35), and urogenital tumors.

25.1 Mechanism of Action

CTLA-4 (CD152) is a member of the Ig superfamily expressed on activated CD4+ and CD8+ T lymphocytes. It has soluble (monomer) or membrane-bound (homodimer) isoforms and acts as a transmembrane receptor. The intracellular domain is similar to that of CD28, and contains YVKM sequences, which can bind to intracellular proteins and to protein complexes AP1 and AP2, to PP2A, SHP-2, PI3 K, and to a proline-rich motif. These structures are able to catch SH3 containing proteins acting on dephosphorylation of CD3 and LAT (linker activator for T cells) components of the T-cell receptor (TCR), thus inhibiting its function. CTLA-4 can also affect the co-stimulatory signal by competing with CD28 for B7 receptors (B7-1, or CD80; B7-2, or CD86) binding at the surface of APC cells. In fact, some B7 isoforms show dramatically increased binding avidity for CTLA-4 over CD28 (differential binding ratio 1:8) deeply affecting APC cell cycle and cytokine production. An important difference between CTLA-4 and CD28 is that the latter is constitutively expressed on almost all human CD4+ T cells and on about 50 % of CD8+ T cells, whereas CTLA-4 is expressed only after T-cell activation. CTLA-4 rapidly appears at the cell surface (in 2 days), and its presence is not durable (background levels in 4 days; clearance by clathrin-mediated endocytosis). In fact, it induces internalization and proteolysis of CD28 leading to consistent reduction of its surface expression. Therefore, the final input from CTLA-4 is a potent inhibitory signal on the whole T-cell compartment, which balances activating signals from CD28/B7 interaction only *after T-cell activation*, giving these inhibiting signals an important homeostatic role without interfering with initial activating signals, possibly modulating an overactivity of the T-cell compartment, and maintaining tolerance to self antigens.

Further, important effects of CTLA-4 activation include a decreased expression of IL2 and of IL2R presence on thymocytes and B cells, which might potentially be involved in still unknown protective mechanisms or in the induction of adverse events. Noteworthy, CTLA-4 expression, which also depends on Wnt (Wingless-Type MMTV Integration Site Family) growth factors, has been found on various tumor cells, including melanoma. When expressed on nonlymphoid cells, CTLA-4 may exploit important additional functions in relation to tumor/microenvironment interactions. In fact, stimulation of CTLA-4 positive tumor cells induces signals which may play a key role in tumor's escape from anti-tumoral immune responses,

including a direct inhibition of cytotoxic CD8+ tumor-specific effector T cells, causing increased clonal tumoral expansion, together with a raise of tumor-induced regulatory CD4+ immunosuppressive T cells (Treg) blocking the immune response to tumor-associated antigens via cytokine production (IL-10). However, it must be noted, also for safety evaluations, that complete knockout of CTLA-4 is rapidly lethal in animal models, and induces massive and rapid polyclonal lymphoproliferation and diffuse parenchymal infiltration of T cells, ending in organ destruction.

Ipilimumab, is a fully humanized IgG1k monoclonal antibody produced by recombinant DNA technology in a CHO mammalian cell expression system, binding with high affinity to the extracellular domain of human (and Cynomolgus monkey) CTLA-4, and acting as an inhibitor of its complex functions. This essentially results in T-cell activation and proliferation, and in lymphocyte infiltration leading to tumor cell death. However, the enhancement of T effector cell function, combined with the inhibition of CD4+ Treg and CD8+ suppressive cell types, are considered essential for mediating the full therapeutic effects of ipilimumab.

The binding to activated CD4+ T cells was found at its peak at day 3 and declining by day 7. Ipilimumab does not have CDC activity in vitro, yet mediates low to moderate ADCC at higher concentrations on activated (but not on resting) T cells, with a stronger binding to FcγRI (CD64) than to FcγRII (CD32) or FcγRIII (CD16) receptors for the Fc portion of IgG. In peripheral blood of patients with melanoma, a mean increase of activated CD8+ (statistically significant) and of CD4+ T cells, together with a mean decrease in naive CD4+ and CD8+ T cells, was observed after treatment with ipilimumab. These effects are consistent with its mechanism of action. However, Tregs are reduced at local tumor level, being the ratio with T-effector cells shifted in favor of the latter, with potential enhancement of tumor aggression. Overall, no total depleting effects on the T-cell compartment were observed in nonclinical and clinical studies. As expected, ipilimumab enhances specific immune response to conventional and anti-tumor multipeptide vaccines (tyrosinase, gp100, MART-1, MAGE-A4, SSX2NY-ESO-1), up to fivefold baseline levels [5, 7–10].

25.2 Immunogenicity

The presence of HAHA was tested in over 1,000 subjects treated with ipilimumab, and was found positive for nonneutralizing antibodies in 1–7 % of cases, under different administration conditions. No infusion related or peri-infusional hypersensitivity or anaphylactic reactions were observed. Neutralizing antibodies against ipilimumab were not detected. Overall, no apparent association was observed between antibody development and adverse reactions [4].

25.3 Adverse Events

From the safety point of view, ipilimumab generates unusual adverse events compared to the majority of other mAbs or fusion proteins. In fact, these events derive from an enhanced activity of the immune aggression, instead of being a consequence of immunosuppression. Furthermore, the majority of immune-related (mediated) adverse events (IrAEs or IMAEs) with ipilimumab are related to its mechanism of action, while in case of other biomedicines such events are a consequence of immunogenicity, or a secondary effect of immunosuppression. Ipilimumab boosting of immune responses, due to one single blocking of a natural inhibiting signal, is also instructive for understanding the role of this pathway in the homeostatic regulation of the whole immune system.

A BBW, issued since the first label, includes *fatal immune-mediated adverse reactions* due to T-cell activation and proliferation in any organ system, and in particular *enterocolitis*, *hepatitis*, *dermatitis* (including toxic epidermal necrolysis), *neuropathy*, and *endocrinopathy*. These warnings remained unchanged after the October 2012 label update, based on 647 patients enrolled in the main trial (MDX10-20), including 131 treated with the indicated dose of 3 mg/kg ipilimumab, 380 with the same dose associated to an investigational vaccine (gp100) in incomplete Freund's adjuvant, and 136 controls receiving only gp100.

Gastrointestinal reactions, mainly represented by *enterocolitis,* were present in 12 % of cases, including severe (7 %) conditions, intestinal perforation (1 %), and related mortality of 0.8 %. The majority of these events (90 %) responded to therapy (74 % of severe grade; 79 % moderate). *Hepatitis*, moderate (2.5 %), or severe (5 %; 0.2 % fatal) also occurred; the respective underlying pathology was not ascertained in all patients, but included immune-mediated hepatitis. *Dermatitis,* either moderate (12 %) or severe (2.5 %; 0.2 % fatal toxic epidermal necrolysis) was observed, with resolution rates of 70–80 % of cases. *Endocrinopathies* (2.3 % moderate) consisted in hypothyroidism, adrenal insufficiency, hypopituitarism, and one case each of hyperthyroidism and Cushing's syndrome. Severe cases (1.8 %) were all showing hypopituitarism, in some cases with concomitant adrenal insufficiency, hypogonadism, and hypothyroidism. *Neuropathies*, represented by one fatal GBS, and one PNP (motor and sensory) also occurred (0.2 % each). A minor number of *additional immune-mediated AEs* (<1 %) included nephritis, pneumonitis, meningitis, pericarditis, uveitis, iritis, and hemolytic anemia. The most common adverse reactions were fatigue (15 %, severe 2.3 %), diarrhea (13.5 %, severe 1.8 %), pruritus (10 %, severe <15), rash (11 %, severe 0.85), and colitis (2.5 %, severe 1.6 %). Overall, the severe to fatal immune-related events represented 5.2 % of cases, being enterocolitis the most frequent, followed by endocrinopathies, dermatitis, and hepatotoxic conditions. Other severe reactions, such as pneumonia, meningitis, nephritis, pericarditis, and eosinophilia were $\leq$1 %.

Safety data reported in the EMEA Assessment Report were based on 1,107 patients enrolled in six of the major studies, including the MDX10-20 trials, and on supplemental safety data from 568 patients treated in 14 completed studies. The studies investigated ipilimumab use for the treatment of metastatic melanoma or other cancers, including prostate, kidney, and breast cancer. In most instances, tabulated data refer to 622 patients treated with 3 mg/kg, and 353 treated with 10 mg/kg. Overall, AEs of any grade were present in >96 % of melanoma patients, with the same profile encountered in the pivotal trial. However, fatigue (23–26 %), diarrhea (25–36 %), pruritus (16–28 %), rash (26 %), colitis (5–11 %) were all found at higher rates in studies where higher doses (10 mg/kg) of ipilimumab were employed, thus indicating a dose response effect. No correlation was found with pyrexia (4–12 %) and neurological disorders (7–14 %), including fatal GBS and MG-like syndrome (< 1 % each), or with injection site reactions, absent in the high dose groups and mostly concentrated in the MDX10-20 Study (28 %). As for drug-related endocrinopathies, all grade hypopituitarism was reported in 4 % of cases. Adrenal insufficiency, hyperthyroidism, and hypothyroidism of any severity were reported as mild/moderate, in 2 % of patients each. Other less frequent AEs included uveitis, eosinophilia, lipase elevation, and glomerulonephritis (<2 %), with occasional cases of iritis, hemolytic anemia, amylase elevations, multiorgan failure, and pneumonitis.

Overall, SAEs (13–29 %) showed increased frequencies at higher doses of ipilimumab, mainly as colitis (3–7 %) and diarrhea (3–11 %). Both were not reported as treatment-related SAEs in the gp100 vaccine group. However, treatment-related deaths for the entire study duration ranged 2–3 % (1.5 % in controls with vaccine only), while immune-related deaths were 1.3–1.5 %, with no dose-related differences. WBC counts remained mostly within baseline levels, with moderate neutrophil absolute number decrease in <1 %, but with mild/moderate Hb level decrease in about 50 % of cases (<2 % severe). No major differences were reported at higher doses of ipilimumab, as well. Similarly, liver enzymes encountered mild/moderate imbalance in 5–10 % of cases for ALT/AST and 10–20 % for ALP (severe <2 and 2–3 % respectively). In the high dose pooled studies, severe enzyme imbalance reached 7 % and was consistent with the higher rate of hepatic immune-related AEs in the pooled 10 mg/kg groups. As for kidney function, levels of creatinine remained normal, except for one case (0.2 %) in the 10 mg/kg group, showing severe elevations. As for exocrine pancreatic function, lipases resulted unbalanced in about 18–20 % in all groups, including controls, and severe abnormalities ranged 4–10 % [2, 4, 5].

The dose response effect of infliximab as monotherapy on immune-related AEs was previously examined in two Phase II trials with a similar profile. No severe reactions (grade 4), or serious reactions (5 %) related to gastrointestinal events were reported in patients administered with 10 mg/kg dose. Therefore, this dose was suggested as induction-maintenance regimen in patients with pretreated advanced melanoma, although this was not indicated in subsequent label information [4, 11].

In a recent survey, on most relevant trials with ipilimumab, either as monotherapy or associated with supportive treatments, the question of dose and regimen of ipilimumab was still debated, since the increase of AEs counterbalances a potential increased benefit of the high dosage. Moreover, the development of IrAEs showed a possible correlation with disease response: 9/11 patients without autoimmune symptoms had a relapse, while 3/8 patients experienced AEs. Notably, all individuals who had a drug-induced tumor response had rash, and most of them reported gastrointestinal reactions. However, 25 % of patients receiving the 10 mg/kg dose had severe AEs, including intestinal perforation [12]. According to a recent retrospective safety review on 14 completed Phase I–III trials of ipilimumab in 1,498 patients, IrAEs occurred in 64.2 % of cases, and resulted in death in <1 % of patients, confirming the overall profile previously depicted. Rash was reported as the most common event. Notably, patients treated with high dose of ipilimumab showing colitis reported significant higher IL-17 serum levels than patients without colitis. These levels paralleled the course of the inflammation. It must be noted that after the implementation of guidelines for this treatment [5], there was a 50 % reduction in most severe GI-related complications (perforation or colectomy rate), in spite of the higher dose of ipilimumab.

An interesting observation on 62 patients suggests the possibility that unique patterns of radiologic response and toxicity related to ipilimumab therapy could be identified before the symptomatic appearance of IrAEs, such as colitis mimicking IBD patterns, and hypophysitis revealed sellar enlargement, which may help in preventing major damage [13]. However, the immune response generated during the first few weeks of therapy may be wrongly interpreted on radiological imaging as a progressing disease. In this instance, T-cell infiltration (CD8 and CD4 T cells) and inflammation cause an increase in tumor size on radiological images [8]. In a series of 7 adrenal insufficiencies related to ipilimumab, the enhanced immune response seems to have a selective predilection for corticotroph and possibly for thyrotroph cells [14].

Quite recently, a case of associated *autoimmune alveolitis* following treatment with ipilimumab (3 mg/kg) was reported [15]. The patient developed skin rash, but no other signs of toxicity appeared after the first infusion; bilateral alveolitis was observed after the second infusion and resolved after 5 days of corticosteroids and 36 h of antibiotics, while completing 4 cycles of ipilimumab without additional complications. A previous case of pulmonary toxicity related to ipilimumab was observed in a hematologic malignancy after HSCT. This is the first report of pulmonary alveolitis clearly related to an IrAE.

When combined with supportive therapies, such as dacarbazine [6], the AEs profile was somewhat modified, most probably due to interactions between the two drugs. The *chemotherapy association* seemed also to enhance the antitumor activity of ipilimumab through a massive release of tumor antigens, which may further boost the immune response. With the aim of reducing synergizing effects on IrAEs and increasing the transfer through the blood–brain barrier, infliximab was associated with fotemustine. However, total IrAEs (71 %, severe 28 %) were not reduced. The detected typical ipilimumab-induced reactions were

dermatological (47 % severe 2 %) and gastrointestinal (26 %; severe 5 %) disorders, thyroiditis (2 %), exocrine pancreatic abnormalities (21 %; severe 6 %), and hepatotoxicity (38 %; severe 10-14 %). In addition, myelotoxicity (76 %; severe 43 %), a typical fotemustine-induced reaction, occurred. No cases of hypophysitis were registered [16].

The insurgence of hypophysitis after ipilimumab treatment deserves more attention, because it seems the unique IrAEs potentially irreversible, and probably not as infrequent as estimated in initial studies. This event has not been reported yet with other classes of anticancer drugs. The incidence of hypophysitis (0–17 %) is highly variable in different studies on melanoma patients, and seems to be dose-dependent. It also occurs in patients with solid tumors of various types, including kidney and prostate cancer [17]. Notably, no cases of hypophysitis were reported when ipilimumab was associated with dacarbazine. Moreover, the IrAE-type of hypophysitis occurs mostly in males, in contrast with other spontaneous forms of this disease.

25.4 Off-Label Experience

Limited data on other experiences with ipilimumab, including treatment of prostate cancer, NSCLC, lymphoma, renal cancer, and ovarian cancer are available. Particular attention is given to the treatment of ocular melanoma. However, as previously mentioned, several trials on neuroectodermal and germ-cell tumors, nervous system tumors, lymphomas and lymphoproliferative disorders, urogenital tumors, and carcinomas of various origins are currently ongoing. This could bring new insights for a wider spectrum of indications and on respective safety profiles.

Initial experience on transgenic animal models, showing promising results with no major evidence of IrAEs besides prostatitis and vitiligo, encouraged the first study on *metastatic prostate cancer* (mPC) in 14 patients with or without orchiectomy [18]. The most frequent AEs were mild and included arthralgia (50 %), malaise (43 %), bone pain (43 %), pallor (36 %), lumbalgia (36 %), constipation (29 %), fatigue (29 %), and decreased appetite (19 %). Severe reactions were limited to rash/pruritus, asthenia/fatigue, dizziness and pain (1 case each). In this study, investigations for potential IrAEs endocrinopathies were not performed. In addition, one possible Clostridium intestinal infection occurred, while no laboratory abnormalities were detected. A second injection of ipilimumab induced less arrhythmias and mild cutaneous events (rash, pruritus). Overall, the profile of AEs was acceptable and manageable.

Additional information is coming from ipilimumab *associations with antigen-specific vaccines,* in which the monoclonal is meant to boost the immune response of the vaccine against antigen-positive tumor cells. In a recent Phase I trial on 30 patients with mPC, escalating doses (1–10 mg/kg) of ipilimumab were associated with a fixed dose of the PSA-Tricom vaccine [19]. No dose-limiting toxic effects were reported during the two weeks observation. Mild/moderate injection site reactions (97 %) were common. Among IrAEs, rash (33 %) was the most common

and appeared to be dose-dependent, as for endocrinopathies (37 %), which included hypophysitis (4 cases), hypothyroidism (4), and adrenal insufficiency (3). However, colitis/diarrhea (27 %) of all grades was present at all doses, starting from the standard 3 mg/kg indication. Minor and mostly mild liver enzymes imbalance, neutro/leukopenia, and one case of thrombocytopenia were also encountered, although not strictly related to therapy. This study confirmed that the majority and most severe of IrAEs were detected with the 10 mg/kg dose. Overall, the combination of a vaccine with ipilimumab did not increase the frequency and severity of IrAEs. This safety profile was similar to another concerning the combination of ipilimumab with a tumor vaccine (GVAX). Injection reactions were present in 100 % of cases, together with rash (39 %, severe 4 %), fatigue (79 %), pyrexia (57 %), and flu-like syndrome (43 %). Among IrAEs, hypophysitis (25 %) and consequent adrenal insufficiency/hypothyroidism (18 %) occurred. Colitis (11 %), GI-disorders (21–29 %), leukopenia (4 %), together with the known mild hepatotoxic enzymatic signs also occurred. One case of uncommented alveolitis was also reported. Overall, the recorded IrAEs were considered higher in the combined ipilimumab-vaccine treatment than in ipilimumab or vaccine monotherapy [20].

A number of studies are ongoing on *NSCLC*. In the first prospective Phase II randomized study, 204 patients were treated with repeated doses (10 mg/kg) of ipilimumab and paclitaxel/carboplatin therapy [21]. Severe treatment-related AEs were similar across arms (39–41 % vs. 37 % in controls). These included typical AEs related to chemotherapy, such as nonhematologic (15 %) and hematologic (40–90 %; 35–90 % in controls) laboratory abnormalities, which were similar across all arms. As expected, more typical IrAEs were increased in the ipilimumab arms and included mild/moderate rash (13–28 vs. 10 % in controls), pruritus (8–17 % vs. 5 %), and diarrhea (24–30 % vs. 18 %). The overall incidence of severe IrAEs was 6 % for the control arm, and 15–20 % for ipilimumab arms. However, only one case of hypophysitis/hypopituitarism occurred after ipilimumab treatment. Interestingly, three cases of hypersensitivity, including one severe anaphylactic reaction, not encountered in other studies, were observed. One fatal septic shock, secondary to toxic epidermal necrolysis was registered in the infliximab group. Overall, ipilimumab did not potentiate the toxicities of the chemotherapy, but associated IrAEs of moderate intensity.

Studies on *ocular melanoma*, as metastatic uveal melanoma, have been recently initiated. Ipilimumab has been administered in 13 patients with unresectable melanoma receiving four induction doses of 10 mg/kg, followed by delayed maintenance with equal doses [22]. AEs (77 %) were all mild, while IrAEs were present in all patients (1.4/patient), and included three (23 %) cases of severe thrombocytopenia, diarrhea and hepatotoxicity (one case each). The only endocrine dysfunction was limited to one case of mild thyroiditis.

All these data are very preliminary and should be evaluated with caution. However, the general safety profile, and particularly the IrAEs profile, seem superimposable to previous experiences from cutaneous metastatic melanoma.

25.5 Postmarketing Surveillance

About 1,600 reports on FAERS quoted GI signs (mostly diarrhea and colitis) and infections as the most frequent signaled cases (5–8 %) by the end on 2012. The AEs/P ratio was about 1.5. Among infections, sepsis (70), pneumonia (52), and UTI (36) were most frequently reported. Intestinal perforations were <1 %. Only one case of anaphylactic shock and 1 CRS were recorded.

EUV database includes 1,027 reports (99 % on serious events) by the end of 2012, enlisting 2,026 events (1.97 AEs/P). Gastrointestinal disorders (21.6 %), neoplasms (15.7 %, mostly tumor progression, one case of T-cell lymphoma), nervous disorders (6.2 %, manly cephalea/dizziness), skin disorders (5 %) including rash (30 cases) and one case of toxic epidermal necrolysis, and endocrine disorders (4.1 %) were the most frequent reported events. Hematologic disorders were mainly represented by anemia, thrombocytopenia and neutropenia; five cases of lymphoadenopathy, two cases of blast proliferation, and two cases of DIC were also observed. Most prominent GI signs were colitis, diarrhea (over 100 cases), and most serious was intestinal perforation (20),). Signs of hepatotoxicity were reported in about 10 cases. Infectious serious disorders were represented by sepsis (8) and septic shock (2). Eleven cases of pancreatitis were also referred.

Endocrine abnormalities included 28 cases of hypophysitis, 17 cases of hypopituitarism, hyper (4) and hypothyroidism (5). Interestingly, one case of anaphylaxis, one anaphylactic shock and one CRS were also reported.

25.6 Remarks

The experience with ipilimumab has enlarged the typology of AEs by including the new class of immune-related adverse events (IrAEs), which are exclusive of its mechanism of action. These events can be divided into two subclasses: acute and delayed IrAEs. The former include dermatologic (rash, dermatitis) and hepatotoxic abnormalities (mostly limited to enzymatic) and GI signs (colitis, diarrhea), which can appear rapidly, even after one single dose. In the case of ipilimumab, these reactions are not generally attributable to classical toxic, infective, or allergic damage in different organs/systems, but to a precise immune attack to these targets. However, when rapidly identified and cured, avoiding serious complications such as life-threatening intestinal perforation, these reactions are usually manageable. The endocrine system seems more vulnerable to ipilimumab than to other biomedicines, which rarely involve endocrine organs. In fact, delayed IrAEs mainly affect the endocrine system, particularly the hypophysis (predominantly the anterior portion), the adrenal and thyroid gland, which are also considered secondary to hypopituitarism. More rare targets of IrAEs are joints and kidney. At all levels, one of the major effects of IrAEs is the local parenchymal infiltration of lymphocytes (CD8 and CD4 T cells) and neutrophils. The infiltration has disruptive effects on the neoplastic tissue, albeit causing autoimmune/inflammatory

aggression of normal tissues. An important aspect of IrAEs induction is their possible correlation with clinical benefits. It is still a matter of debate whether IrAEs beneficial effects could be provided without risks. However, the fine balance among various mechanisms triggered by CTLA-4 blockade is still to be clarified. Examples are the role of Tregs, the new subset of T-17 helper lymphocytes (producing IL-17, IL-22), the role of IL-10 immunosuppressive action, and more in general the over-boosting of immune reactions versus more specific anti-tumor immune aggression [9, 15]. Nonetheless, IrAEs do not enhance common hypersensitivity-allergic anaphylactic reactions. Another peculiar aspect of the ipilimumab over-boosting on immune response is the absence of clear signs of cytokine release syndrome, which might be expected given the possible release of overwhelming quantities of cytokines during such events. However, during preclinical studies on animals, infusion reactions, possibly due to acute cytokine release resulting from a rapid injection rate, were reported [1].

In fact, a better understanding of these features is crucial for the still unknown long-term IrAEs, as well as for long-term efficacy of ipilimumab, since the production of IL-17 and IL-22 cytokines has been related to some relapses observed in clinical trials. Another potential delayed risk concerns Tregs migration, which may repopulate residual tumors and hence compromise long-term therapeutic effects. IL-17 secretion is also linked to the development of colitis. Furthermore, unbalanced IL-17 versus Tregs might play a role in the pathogenesis of delayed endocrine aggression. If a better dissection of these contrasting actions were operated, either better biomedicines or more efficient therapeutic combinations could unbalance the current risk/benefit ratio in favor of the latter.

A consistent development of new biomedicines, and of new stimulatory combined strategies, such as associations with anti-tumor vaccines can be expected in the near future. Therefore, better definitions for immune-related response criteria (IrRC) should be defined and used in order to ensure early recognition and timely treatment. In addition, HRLQ evaluations should be demanded in oncoming trials, as part of future investigation parameters [15, 17, 23–26].

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Muromonab

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Muromonab (muromonab-CD3, orthoclone-OKT3), is the first monoclonal antibody commercialized for human therapy. It was initially approved by FDA in 1986, and subsequently almost everywhere, but was discontinued in 2010 due to the availability of other treatments with similar efficacy and fewer side effects, and consequent declining sales. OKT3 is a fully murine IgG2a directed to the T3 antigen (CD3) expressed on human T cells. It was indicated and largely used to control acute allograft rejection in renal, cardiac and hepatic transplant patients. The mechanism of action consisted in the total blocking of all human T lymphocytes, including the subsets involved in graft rejection, by preventing from the association of the CD3 molecule with the remaining part of the T cell surface receptor (TCR), which is essential for antigen recognition, transmission of the transduction signal to the underlying lymphocyte, and its subsequent activation. However, the binding of OKT3 to CD3 generates violent signals of T cell activation, which produce the so called "cytokine storm" or the cytokine release syndrome (CRS), along with a profound decrease of all CD3+ T cell subsets. After the OKT3 binding, CD3 positive cells are opsonized and removed from circulation in the liver and spleen by the reticulo-endothelial system. Cytokines are not produced in advance, but are synthesized ex novo and then released after T cell activation by OKT3. However, the binding effect is reversible in about one week, and usually followed by a rebound of circulating T cell, together with a rapid increase of neutralizing anti-OKT3 antibodies effectively interfering with the OKT3/CD3 binding. These antibodies occurred with an incidence of 21 % for IgM, 86 % for IgG, and 29 % for IgE, as early as 10–20 days from therapy initiation. Subsequent administrations increased the level of HAMA by 50–100 % in recipients reaching titer of 1:10,000 or more, thus completely blocking the monoclonal therapeutic action. OKT3 administration induces leukocytes migration also in cerebrospinal and peritoneal fluids, a capacity possibly determined by membrane permeability disruption due to the CRS.

The known panorama of alloantibody-related AEs is almost complete and includes primarily CRS consequences as shock-like reactions, with cardiovascular and CNS manifestations, and a wide spectrum of symptoms, such as pyrexia,

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chills, cephalea, tremor, nausea/vomiting, diarrhea, abdominal pain, malaise and muscle/joint aches and pains, and generalized weakness. Serious and fatal events include acute cardio-respiratory disorders (dyspnea, shortness of breath, bronchospasm/wheezing, tachypnea, respiratory arrest/failure/distress, cardiovascular collapse, cardiac arrest, angina/myocardial infarction, chest pain/tightness, tachycardia, hypertension, hemodynamic instability, hypotension including profound shock, heart failure, pulmonary edema, ARDS, hypoxemia, apnea, arrhythmias and neuro-psychiatric events. Severe pulmonary edema has been observed in euvolemic patients or in patients with fluid overload. Within days there is an acute decline in the kidney function with reduction of GFR, resulting in the increase of serum creatinine. CRS is considered responsible of acute or delayed renal allograft functionality. Similarly, hepatotoxicity signs are developed. Neuropsychiatric events are also impressive, and include cephalea, seizures, aseptic meningitis, encephalopathy and cerebral edema/herniation. Manifestations of encephalopathy may include impaired cognition, confusion, obtundation, altered mental status, auditory/visual hallucinations, psychosis/delirium, paranoia, mood changes/mania, agitation, diffuse hypotonus, hyperreflexia, myoclonus, tremor, asterixis, involuntary movements, major motor seizures, lethargy/stupor/coma and diffuse weakness. Encephalopathy may be associated with aseptic meningitis. Cerebral edema and other signs of increased permeability (otitis media, nasal and ear stuffiness), irreversible blindness, impaired vision, quadri-or paraparesis/plegia, cerebrovascular accident, aphasia, transient ischemic attacks, subarachnoid hemorrhage, palsy of the VI cranial nerve, and hearing loss have been observed, along with post-therapy encephalopathy, meningitis, CNS lymphoproliferative disorders and infection.

Serious immediate and fatal anaphylactic reactions have been reported, as well as hypersensitivity reactions with rash and pruritus, urticaria, serum sickness, arthritis, allergic interstitial nephritis, immune complex deposition resulting in glomerulonephritis, vasculitis, temporal arteritis, and eosinophilia.

A wide spectrum of infections has been also experienced, including systemic bacterial, fungal and viral dissemination, pneumonia, and sepsis. Viral infections are particularly related to the Herpes family (HSV, CMV, EBV), including reactivation, causing pyrexia, pneumonia, viremia, hepatitis, liver/renal dysfunction, gastritis or gastrointestinal ulcerations, pancreatitis, chorioretinitis, leukopenia and thrombocytopenia.

Malignancies mainly relate to lymphoproliferative disorders (from benign polyclonal B cell hyperplasia to more frequent monoclonal B cell lymphoproliferation), lymphomas and skin cancers. In particular, lymphomas include B cell, large cell, polyclonal, non-Hodgkin's, lymphocytic, T cell and Burkitt's, occurring also early after treatment initiation. Skin carcinomas included basal cell, squamous cell, Kaposi's sarcoma, melanoma, and keratoacanthoma. Hematopoietic and vascular severe disorders include intravascular thrombosis, pancytopaenia, aplastic anaemia, neutropenia, leukopenia, leukocytosis, thrombocytopenia, lymphopaenia and disturbances of coagulation.

Being the fully murine antibody OKT3 able to block the whole T cell compartment, and to exploit a strong immunogenicity, the entire framework of the safety profile of this drug class is now clear. However, OKT3 has been fundamental for the control of allograft rejection for decades. A 1996 review on the first ten-years of therapy with OKT3 states that "OKT3 has proven to be the most highly effective drug for both the prevention and treatment of acute allograft rejection in solid organ transplantation. No other drug is comparable as an anti-rejection agent". This review also anticipated that genetic engineering of "humanized" monoclonal antibodies was also expected to decrease anti-OKT3 IgG antibody formation, and to lower the development of T cell subset-specific antibodies that disarm the immune response to allograft antigens, yet leaving immune defenses against infectious pathogens intact [1].

After almost 30 years experience in the production and therapy with monoclonals, the list of dramatic AEs, taken from an early label of OKT3 [2], is now important from an historical point of view. In fact, looking back to such risk/benefit balance, the extraordinary progress achieved in this type of biomedicines stands out clearly.

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Natalizumab

27

Natalizumab (Tysabri®, Elan, Biogen) is a recombinant humanized IgG4 murine antibody binding to $\alpha 4\beta 1$ and $\alpha 4\beta 7$ integrins. In November 2004, FDA granted approval for treatment of relapsing forms of multiple sclerosis (MS). However, as soon as February 2005 the Agency suspended the marketing due to two cases of progressive multifocal leukoencephalopathy (PML) in MS-treated patients, but readmitted the biomedicine in 2006 with a mandatory restriction of participation to a RiskMAP (TOUCH) distribution program, to assess and minimize the risk of PML and other serious AEs. During 2006, EMEA examined the requests for approval in MS and in Crohn's disease (CD), and granted approval for rapidly evolving severe relapsing-remitting MS and for highly active relapsing-remitting MS resistant to IFNβ, but rejected the CD indication, which was re-examined in 2008 with confirmed refusal. In 2006, Health Canada approved natalizumab for treatment of MS in patients with inadequate response or intolerance to other therapies for that disease. The Japanese PMDA approved natalizumab in MS patients in 2008. In May 2013 the manufacturer announced the withdrawal of the indication for MS without high disease activity and with negative anti-JVC antibodies.

Pivotal studies for MS include two Phase III trials, S1801 (AFFIRM) and S1802 (SENTINEL), including 942 patients (627 treated with natalizumab as monotherapy) and 1,171 patients (589 treated in combination with IFNβ), respectively. The latter trial was stopped approximately one month early due to PML reporting. Studies for CD included two pivotal trials, CD301 (induction study) and CD303 (maintenance study), with 905 patients (724 exposed) and 428 (171 exposed), respectively. Five additional supportive studies (CD 201, 251, 305, 351, 352) were assessed for safety and efficacy, and CD202 for efficacy. Overall, evaluation of efficacy was performed in 1,295 patients, while over 3,600 patients supported safety analysis for the two diseases [1–7].

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At present, natalizumab is approved in over 60 countries, and about 65 trials are completed (about 50 %) or ongoing, mostly investigating MS (50), CD (8), and other diseases (4).

27.1 Mechanism of Action

Natalizumab (Tysabri®, formerly Atregen) is a recombinant humanized IgG4 murine antibody binding to the α4 portion of α4β1 and α4β7 integrins, expressed on the surface of all T and B cells, NK cells, the majority of monocytes and macrophages, and granulocytes (except mature neutrophils). In particular, the crucial binding seems to occur on the very late antigen A (VLA-4, or CD49d), a member of the α4β1 group of integrins, known to permit the transmigration of lymphocytes across the blood–brain-barrier (BBB). VLA-4 is primarily expressed on T cells and monocytes, and to a lesser extent on granulocytes (eosinophils, basophils). Natalizumab also interferes with the binding of both classes of integrins with cell adhesion molecules (CAM) — namely VCAM-1, interacting with α4β1 on endothelia — and with MAdCAM-1 — interacting with α4β7 on leukocytes — thus inhibiting the adherence of these cells to the endothelial wall and the subsequent extravasation. Notably, VCAM-1 is expressed on inflamed cerebrovascular endothelial cells and upregulated, together with MAdCAM-1 (mucosal.vascular addressin), on intestinal endothelia in CD. Therefore, natalizumab prevents autoreactive leukocytes adhesion and migration through vessels toward extra and intra-BBB inflamed tissues, which is considered the basis of its potential therapeutic action in MS and CD. However, additional underlying mechanisms are expected to further modulate therapeutic and adverse effects. For example, natalizumab may affect the expression of differentiation genes of leukocytes, and suppress ongoing inflammatory reactions in extravascular and parenchymal spaces.

Overall, natalizumab is expected to inhibit leukocyte migration, suppress inflammatory activity at disease site, and interfere with further recruitment of immune cells into inflamed tissues. However, the exact mechanism of natalizumab on MS and CD lesions is not known. In MS, plaque formation may be related to BBB entrance of activated T lymphocytes and consequent inflammatory damage. In this case natalizumab may be beneficial, because of the inhibiting effect on cell migration. Its action may also be extended to other cell–cell interactions, such as with nervous parenchymal cells expressing CS-1 and osteopontin, since the antibody also binds to these molecules in vitro. Notably, natalizumab reduces leukocyte migration and plaque formation in EAE.

In CD, the main interaction of the antibody is related to MAdCAM-1, which is overexpressed in this disease, and seems crucial for T cells migration to Peyer's patches [7–9].

Therefore, at present, direct and indirect hypothetical mechanisms are considered to be the basis of the therapeutic actions, while even less information is available on their responsibility in the induction of AEs. As of direct effects,

natalizumab inhibits the migration of immune cells into CNS, and the turnover of local APC in the CNS. However, this monoclonal mobilizes bone marrow cells (pre-B, CD34+ lympho/myeloid progenitor cells), which may host JCV, with still unknown implications in its bioactivity. As of indirect consequent effects, natalizumab decreases antigen presentation and access to extracerebral effector cells by stabilizing BBB [7–10].

27.2 Immunogenicity

About 10 % of treated patients revealed neutralizing anti-drug-antibodies (ADA). Among the adolescent CD patients, 8 % developed ADA. Hypersensitivity reactions (1–1.5 %) including anaphylaxis (<1 %) occurred in patients of both groups receiving natalizumab, usually within 2 h from first infusion. Acute (4 %) and delayed (5 %) hypersensitivity reactions increased in MS (4 %) during monotherapy, compared to CD (0.5–2 %). Generally, these reactions are associated with HACA/HAMA positivity, and may reduce efficiency. Similarly, infusion reactions were higher in MS patients (24 %) than in CD patients (11 %), both being higher than in controls (18 and 7 %, respectively).

27.3 Adverse Events

In May 2006, after readmission of natalizumab to the market following the alert of FDA on the first two cases of leukoencephalopathy, a BBW on the *serious risk of PML* in MS patients was inserted in the official label. Meanwhile, a third case of PML occurred in one CD patient of the 1,043 who were receiving natalizumab as off-label treatment. Therefore, the access to the medicine was concomitantly restricted under the TOUCH Prescribing Program for MS (MS TOUCH) and CD (CD TOUCH) patients, well before the treatment was extended to CD. This program is still operative. As of February 2012, more than 100,000 patients worldwide have been treated with this biomedicine.

The general profile of relevant AEs in these patients comprises *infusion* and *hypersensitivity*, including *anaphylaxis*, *infections* as consequence of the immunosuppression, *hepatotoxicity*, and the rare yet serious, sometimes fatal, *PML.* Moreover, after rapid discontinuation of therapy, cases of *Immune Reconstitution Inflammatory syndrome (IRIS)* were observed, mainly in association with PML and plasma exchange procedures, to reduce the circulating levels of natalizumab.

The overall experience coming from pivotal clinical trials is based on 1,617 MS patients and 1,563 CD patients [1–9, 11].

Infections in MS patients include UTI (21 %), LRTI (17 %), gastroenteritis (11 %) and other localizations (7–10 %), and were ≥1 % higher than in controls. Serious infections in MS (3.2 %) include UTI (0.8 % vs. 0.3 % in controls), pneumonia (0.6 % vs. 0 %), and appendicitis (0.8 % vs. 0.2 %).

Infections in CD patients after short-term exposure to natalizumab (<3 months) include URTI (22 %), UTI (3 %) and other localizations (3–4 %), being all ≥1 % higher than in controls. In maintenance observations (11 months) CD-treated patients suffered influenza (12 %), sinusitis (8 %), and other localizations (8 %). In all groups vaginal infections ranged 4–10 % of treated females. Serious infections in all CD groups ranged 2.1–3.3 %. However, overall rates of infections were 1.5/PY for MS, and 1.7/PY for CD treated patients, not dissimilar to respective controls. Notably, two serious non-bacterial meningitis and few opportunistic infections (<1 %) were observed in the whole cohort of enrolled patients.

PML, as previously mentioned, in February 2005 caused the suspension of natalizumab manufacturing and administration. The two MS patients received the antibody for more than 2 years, receiving 29 and 37 doses of natalizumab in association with IFNβ1a. A third case was a CD patient (Study CD351) receiving 8 monthly doses as monotherapy, with an interval of 9 months between the first three doses and the remaining five administered, because of a relapse of the disease. After reintroduction of natalizumab treatment within the TOUCH restricted program, three more cases of confirmed PML in MS patients were identified by 2008, all on natalizumab monotherapy for less than 17 months. As of May 2012, a total of 242 PML cases on approximately 99,600 treated patients were reported.

PML is a rare, lethal and untreatable opportunistic infection caused by reactivation of human polyoma JC virus (JCV), mainly occurring in HIV-induced immunodeficiency and during immunosuppressive treatments. PML was initially associated with B cell lymphoproliferative disorders (CLL, HL). About 60–80 % of subjects are symptomless carriers, retaining resting viruses, mainly in kidney and bone marrow (CD43+ progenitor cells), which upon reactivation reach the CNS presumably transferred by infected B lymphocytes. In particular, subsets of cells expressing the transcriptional factor Spi-B (in dendritic cells, CD34+ progenitors and B cells, but not on T cells and granulocytes) increase JCV transcription, and express more Sp1-B during natalizumab treatment. After an accurate retrospective analysis on the safety data of the TOUCH cohort, associate risk factors have been identified in previous immunosuppressive therapy, along with the presence of pathogenic forms of JCV (rearrangement in NCCR, with or without gene point mutation in VP1) and the presence of anti-JC antibodies, which are now tested before starting natalizumab administration, as recommended by FDA from January 2012. The risk of PML due to previous immunosuppression has been estimated in three to fourfold (TYGRIS trial). The overall risk of PML has been evaluated in 2.63/1000 patients. However, a stratification study in the natalizumab-treated population indicates the highest (1/90 subjects) risk to develop PML in JCV positive subjects who were previously undergoing immunosuppressive therapies and receiving natalizumab for over 2 years. JCV negative patients have a risk of 1/11.000 subject. Notably, positivity to JCV is about 50 % in MS patients, with seroconversion rates around 3 %/year, while almost all (98 %) of MS patients developing PML were positive. Recently, plasma exchange

procedures were adopted to rapidly remove natalizumab in patients with suspected diagnosis of PML, which drop two to three-fold anti-JCV antibody titers, and seem to reduce severity and related fatalities. At present, mortality of the 242 PML patients is high (225). Nonetheless, the number of PML during natalizumab treatment is low, compared to over 100,000 patients already receiving this monoclonal. This indicates the complexity of mechanisms inducing the disease.

As of JCV, potential requirements for the progression from latent to replicating stage are: (i) the induced unbalance of the immune homeostasis; (ii) the gene rearrangements in JCV; (iii) the migration of infected cells to CNS; (iv) the adequate cerebral microenvironment, where different actions may upregulate/downregulate the viral replication and the inflammatory response of parenchymal cells (mainly olygodendrocytes, which lyse and spread JCV).

Interestingly, JCV drug-related reactivation may occur also in non-permissive environments, such as epithelial intestinal cells, and therefore they may be crucial in CD, either for potential local carcinogenic capacity of JCV or for induction of PML developing, albeit less frequently, in CD patients [12, 13].

Experience on natalizumab rapid discontinuation, mainly on PML patients, showed a frequent *insurgence of IRIS*, mostly after plasma exchange to eliminate the circulating biomedicine. Shortly after discontinuation, IRIS produces a rapid and serious decline of patient's neurological conditions, and death. As mentioned elsewhere (Chap 3), IRIS was originally observed in AIDS patients after highly active antiviral therapy (HAART), and was related to mycobacterial, cryptococcal and viral secondary infections. The pathogenesis consists in rapid and exuberant inflammatory response of the host, freed from inhibitory actions of natalizumab, directed to resident microbial agents, or in an aspecific non-infective homeostatic rebound, causing a consistent increase of CD8+ T cells, macrophage infiltration and necrosis. In MS patients developing PML, IRIS may show early or late signs in relation to treatment discontinuation, and may induce worsening of the underlying disease before benefit. In fact, galloping immune reconstitution remains a major risk and can lead to permanent damage or death. This evolution seems more common and severe in natalizumab-associated PML than in HIV-associated PML.

IRIS may develop also in non-PML patients after natalizumab discontinuation, even in the absence of plasma exchange procedure, with neurological clinical evolution in part consisting of MS relapsing signs, and possibly of PML-like signs, albeit milder and in the absence of JCV. Overall, mortality is evaluated around 30 % of IRIS cases [14–16]. Recently, a Registry is being established at NIH/NINDS (USA) for monitoring PML in patients with various underlying diseases and under various treatments.

Additional information on natalizumab, either as monotherapy or in association with other treatments, is coming from various studies. Among these, a particular importance from the safety point of view is the GLANCE trial, enrolling 110 equally randomized patients. This trial associated glatiramer acetate (GA) with the monoclonal in study. In spite of the expected favorable synergistic effects of such combination, the trial was later discontinued due to emergence of new PML cases

during the extension study. In fact, GA requires cellular entry beyond BBB and induces a shift toward Th2-biased immune response. Therefore, natalizumab blockade could interfere with efficacy and induce increased levels of hypersensitivity/immunogenicity in relation to migration of unbalanced subsets of T cells. Notably, this is one of the few trials primarily designed to investigate potential AEs synergism, although primarily assessing potential efficacy loss in combination therapy. Total AEs/patient were similar in the study and control (GA alone) groups (91% and 93 %, respectively). Overall, common AEs, such as URTI (16 % vs. 9 %), lumbalgia/arthralgia (9–16 % vs. 2–7 %), and flushing (11 % vs. 2 %) were higher than in controls, while other constitutional signs, and infections (60 % vs. 65 %) were equal or inferior. Infusion reactions occurred in 11 % of patients in the study group, and in 13 % in controls. No opportunistic infections were detected. Serious events were anecdotal and similarly distributed, and included one case of rigors in the study group and one case of anaphylaxis in the control group. WBC variations remained within normal ranges. The overall safety profile was comparable within the study, as well as with previous natalizumab monotherapy and in combination with IFNβ [17].

Recently, new interim data have been provided by a long-term safety and efficacy study (TOP) enrolling 3,976 MS patients on natalizumab, after 2-year freedom from clinical disease activity.

Overall, about 6 % of patients suffered at least one SAE, including infections (1.5 %) and hypersensitivity reactions (0.6 %), which are within the known safety profile of this biomedicine. However, nine cases of PML occurred, in spite of the demanded adopted precautions [18]. In the attempt to reduce the risk of PML and frequency of major AEs, new strategies of treatment based on early initiation, delayed dose intervals and patients selection, have been recently proposed [19].

Data on CD are limited, and controlled studies are ongoing. In a previous short-term study on 38 adolescents, total AEs (84 %) included constitutional signs (cephalea 26 %, pyrexia 21 %), and infections (39 %). SAEs (21 %) were mostly related to CD exacerbations (24 %), although not considered to be drug-related. Infusion reactions (13 %) were mild/moderate, and seemed not related to the presence of ADA (8 %) [20]. More recent data on CD experience raise less expectation in terms of efficacy, while adverse events appear to be moderate. In a small group of 30 patients, all experienced at least one adverse event. However, none of the 13 patients in whom natalizumab administration was stopped (43 %), discontinued treatment due to adverse events. Five patients had infusions held for infection. No patient developed PML [21]. In another recent interim presentation on 69 CD patients treated with natalizumab outside clinical trials, about 9 % had allergic reactions. Overall, the most frequent AE (23 %) were the mentioned allergic reactions, cephalea, pyrexia and infections (herpes zoster, sore throat, perianal and abdominal abscess). No case of PML was observed [22].

Finally, a recent case report has focused on another relevant AEs in the first two CD refractory patients who entered a Phase II trial on natalizumab and developed *pulmonary and hylar sarcoidosis* after 15–17 monthly doses. This report is intriguing, since both CD and sarcoidosis are granulomatous diseases sharing

genetic susceptibilities, and should be sensible to this therapy. The Authors have suggested a possible trafficking deviation of migrating inflammatory cells toward extra-intestinal locations, including the respiratory district [23].

27.4 Postmarketing Surveillance

In the postmarketing, 34 cases (0.06 %) of PML were observed in 61,177 CD patients, including 11 cases reported in 2011 and eight cases reported in 2012 up until November. In the FAERS database, over 93,000 reports, most common AEs were neurological (>7 %), infections (7 %), and demyelinating disorders (5 %). Reported off-label treatments included neuromyelitis optica, RA, and a number of treatments during pregnancy.

Cases of CNS herpetic infections, including HSV encephalitis, HSV meningitis, and HZV meningitis, were recorded.

In the EUV database, 7,638 reports (96 % serious) included 2.9 SAEs/R, with neurologic (18.4 %), Infectious (16 %), and GI signs (5.5 %), among the most frequent reported events.

27.5 Remarks

While waiting for additional results, the safety profile of natalizumab raises serious concerns essentially for PML, being the majority of other events manageable. IRIS, mainly as consequence of immediate discontinuation and natalizumab abatement via plasma exchange procedures, raises also concerns, although predictable and more controllable. These two serious events are faces of the same medal: IRIS, albeit capable of producing life-threatening damage in CNS, is the result of the monoclonal efficacy in contrasting immune events in MS. Likely, PML is not caused by the same mechanism of action of natalizumab on cell transmigration, rather being an effect of the monoclonal capacity to induce overexpression of the transcriptional factor Spi-B in JCV infected cells (B cells and glial cells), thus helping its replication. The potential splitting of these two mechanisms may represent an advantage for the development of new biomedicines and/or therapeutic strategies. Since basic defensive immune mechanisms are not impaired by natalizumab, it is also expected that this agent shall not raise major concerns on infective complications, and possibly on anti-tumoral immunosurveillance, although long-term data are still unavailable. Different therapeutic strategies, varying in dosage and/or interval extension, might mitigate these dramatic events.

There is evidence that natalizumab over time accumulates in the serum of patients treated with monthly regimen, possibly inducing more AEs without gaining in efficiency. Therefore, the administration might be more appropriate in MS patients than in CD, mostly in patients refractory to alternative treatments.

In fact, safety profiles of natalizumab and anti-TNF biomedicines used in the same pathology do not overlap, given that the latter show increased risk of infections, including TB reactivation, while the former produces predominant neurological damage. By contrast, in most cases, treatment with natalizumab demands suspension of other immunosuppressive treatments, which may be an additional limiting factor, mostly for CD patients, although not only in relation to natalizumab administrations. Moreover, in pediatric CD the limited experience and the risk of PML should inspire extreme caution in attempting uncontrolled studies. However, the analysis of patient's risk stratification of developing PML in MS indicates the use in JCV-negative subjects is the best strategy for this biomedicine, provided that efficacy data are confirmed and implemented, and keeping in mind the lessons learned [24–26].

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Nimotuzumab

28

Nimotuzumab (TheraCIM, Theraloc, CIMAher, BIOMAb EGFR, YM, CIMYM Biosciences) is an IgG1k humanized monoclonal antibody binding to epithelial growth factor (EGFR), thus inhibiting the tyrosine kinase (TYK) pathways activation. At present, it is approved in over 35 countries,—US, EU, Canada, and Japan not included—for the treatment of adult and pediatric glioma, head and neck carcinoma (HNC), nasopharyngeal carcinoma (NPC), non-small cell lung cancer (NSCLL), cervical and breast cancer, esophageal, colorectal, pancreatic, and prostatic tumors. In 2004, nimotuzumab was designated as orphan drug for the treatment of glioma by FDA and EMEA. However, in 2008 nimotuzumab (under the name of Theraloc) was spontaneously withdrawn from European market for this indication and the same year designated as orphan drug for pancreatic cancer by EMEA, under the name of TheraCIM. In 2004, FDA granted permission to conduct a trial on pediatric glioma, which was extended to other solid tumors in 2009. Nimotuzumab was developed at the Center of Molecular Immunology (CIM), Havana, Cuba and is produced and marketed by YM Biosciences through a number of out-licencees and a consortium of eight companies around the world. Between 2005 and 2006 nimotuzumab was approved in Cuba, Argentina, Colombia, India and China.

Phase II, some Phase III trials, and small studies have been developing in different countries including US, EU and Canada, and interim data have been published on diffuse intrinsic pontine glioma (DIPG), malignant gliomas and astrocytomas, glioblastoma multiforme (GBM), squamous cell carcinoma head and neck (SCCHN), NSCLC, esophageal tumors, and NPC. However, in May 2011 YM halted two Phase II trials on NSCLC in North America due to slow rates of patient accrual and the lack of financial support to complete the studies. It has been calculated that about 15,500 patients have received nimotuzumab by mid 2011 [1, 2].

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At present 35 trials are officially registered, including esophageal cancer (7), SCCHN (6), NSCLC (5), DIPG (2), glioma/GBM (2), and gastric carcinoma, pediatric high-grade glioma, pharyngeal carcinomas, pancreatic carcinoma, and other solid tumors.

28.1 Mechanism of Action

EGFR is a cell membrane protein receptor, providing signal transduction and epithelial cell growth. It is a member of the Her or Erb-B family of Type I receptor tyrosine kinases. A huge family of ligands (about 34) can interact with EGFR at cell surface, including EGF and TGFα (transforming growth factor). EGFR is expressed in an inactive (monomeric or tethered) form, which is transitioned by ligands to an active homodimeric (Her1) and/or heterodimeric (extended) forms by pairing with another similar receptor (Her2). At homeostatic equilibrium, over 85 % of EGFR is in the tethered conformation. Activation switches the TYK pathway leading to induction of cell proliferation, adhesion and migration. EGFR is overexpressed in a number (30 %) of epithelial and glial tumors, due to gene mutations or disregulation, leading to permanent activation and uncontrolled cell proliferation and cancer.

Nimotuzumab (h-R3) is an IgG1k humanized monoclonal antibody that binds to the extracellular domain of EGFR, thus inhibiting interaction with the natural ligands. It contains about 5 % of murine IgG2a monoclonal (ior egf/r3) CDR in 95 % of human framework. The anti-tumor activity is complex, and includes inhibition of cell proliferation (five fold increase of apoptotic index), cytotoxicity (via CDC and ADCC), and anti-angiogenetic activity, the latter being caused by a dose-dependent inhibition of VEGF expression. Nimotuzumab has an intermediate affinity for EGFR (10^{-9} M) with respect to other monoclonals, and in particular to other anti EGFR monoclonals, such as cetuximab (10^{-10} M), and panitumumab (10^{-11} M). Moreover, these monoclonals, together with others under study, bind to different sites of the EGFR molecule, leading either to the blocking of the ligand's binding or to a sterical interference with the active molecular conformation. In particular, nimotuzumab blocks the receptor without inhibiting the active EGRF conformation. Overall, these aspects are considered important for the different efficacy and safety profiles among anti-EGFR monoclonals [3–5].

28.2 Adverse Events

Although available studies are not sufficient to draw definitive conclusions on nimotuzumab efficacy, the considerably safe profile of this monoclonal appears to exclude severe AEs, in particular severe skin events, typical of other anti-EGFR biomedicines.

In one of the first Phase IIb (BEST) studies on SCCHN, 92 Indian and Asian patients were equally assigned to chemoradiotherapy or radiotherapy only, subsequently receiving 6 weekly doses of nimotuzumab (23 patients per group).

Overall, recorded DRAEs included mild/moderate *asthenia, dizziness, microscopic hematuria, vomiting and loose stools, pyrexia, chills, pruritus, rash, urticaria, cephalea, blood pressure hypertension and fluctuation.* Four cases (5 %) in the study groups suffered skin reactions including rash, urticaria and pruritus. One SAE consisted in an early, drug-related infusion reaction [4]. The Authors also reported preliminary data concerning adult and pediatric glioma patients collected in a Cuban study (99 patients) and in other ongoing and completed trails on different tumors conducted in Canada, China, Germany, and Korea with similar results (no specific figures were reported).

A subsequent retrospective analysis on NSCLC and other neoplasms, reviewing data on about 9,000 patients, confirmed the *lack of a severe skin reaction*, the absence of rash, and other adverse reactions [6]. Moreover, in a Canadian dose-escalation study, an excellent tolerability of up to 800 mg infusions was observed. Nimotuzumab profile also showed mild *extra-skin reactions*, such as hypomagnesemia and GI signs seen in the same class of drugs. In a more recent study on esophageal cancer, where safety was chosen as the primary end-point, all DRAEs were mild/moderate and included cephalea, deglutition pain and phlebitis (22 % each), nausea, pyrexia, and hypertension (11 % each). None of the nimotuzumab treated patients had allergic reactions or skin rash. Severe events, including death, were not attributed to the drug in study [7]. According to a recent review on efficacy of nimotuzumab on malignant gliomas, the antibody might be associated with other drugs for the treatment of certain tumors, such as MGMT-negative, EGFR-amplified, not completely resected gliomas of adulthood and juvenile DIPG, due to its low rate of toxicity [8]. Along this line, an encouraging case report of NPC was treated with external beam therapy, which induced a complete response. However, multiple large lung metastasis occurring 18 months later were treated with chemotherapy associated with nimotuzumab, achieving a sustained resolution and prolonged survival [9]. The patient reported a severe persistent dermatological event (*palmoplantar dysesthesia*) related to capecitabine administration leading to therapy discontinuation with no signs of tumor recurrence up to the observation period. Recently, data on about 9,000 patients were reviewed confirming the reassuring safety profile of nimotuzumab, particularly on the mildness of cutaneous AEs bioptically analyzed in a Korean trial [6,10]. These data have encouraged in attempting new radioimmunotherapy approaches by using nimotuzumab as carrier of Lu177 against tumors overexpressing EGFR [11].

Data on postmarketing experience are lacking, except for those of the Cuban postmarketing surveillance [4], including mild/moderate pyrexia, chills, nausea, vomiting, mucositis, pruritus, and hyperpigmentation. No severe/serious events were reported.

28.3 Remarks

Nimotuzumab is reported as the only affinity optimized™ anti-EGFR monoclonal antibody and apparently shows a superior safety profile among the class of anti-EGFR agents, with no striking differences in terms of relative efficacy when

compared to other biomedicines of the same class. Therefore, for what concerns nimotuzumab, it is possible to separate anti-tumor effects from the induction of AEs. The remarkable low rate of AE encountered in different clinical situations, mostly as dermatological serious events, has been related to nimotuzumab peculiar binding affinity, with respect to other biomedicines of the same drug class. In particular, the intermediate affinity, lower as 1–2 logs than other anti-EGFR monoclonals, is considered to target mainly cancer cells overexpressing EGFR rather than normal epithelial cells. Secondly, the binding of nimotuzumab does not seem to interfere with a minimum level of EGFR presence on normal epithelial cells, thus assuring their survival and normal functioning. In fact, nimotuzumab blocks the afferent natural ligands to EGFR, but does not interfere with transition from tethered to dimerized unveiled form of active receptor, which may continue to receive liminal signals for normal epithelial cell survival.

Given the confirmed efficiency of this monoclonal in controlling some neoplastic forms, a question raises: when seeking for best affinity, are we looking for the proper monoclonals for clinical use, when in the presence of overexpressed cell targets?

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Ofatumumab

29

Ofatumumab (Arzerra®, GSK) is a humanized IgG1k monoclonal antibody directed to the CD20 surface antigen expressed on B lymphocytes. In 2008, EMEA designated this monoclonal as orphan drug for the treatment of B cell chronic lymphocytic leukemia (CLL). In 2009, FDA granted orphan drug designation for the same treatment, and subsequently granted accelerated approval, restricted to CLL double refractory patients to fludarabine and alemtuzumab (DR). In 2010, EMEA released a conditional marketing authorization with the same indication. Within the same year, the Australian TGA granted approval with the same indication. In March 2012, Health Canada approved ofatumumab for CLL treatment with the same restriction. Approvals were mainly based on one pivotal clinical trial Hx-CD20-406 (Study 1) on 154 patients and a supportive study Hx-CD20-402 (Study 2) on 33 patients, for safety and efficacy of ofatumumab monotherapy enrolled 187 CLL patients. An additional ongoing study (Hx-CD20-407) evaluated the monoclonal in association with chemotherapy (Fludarabine + Cyclophosphamide) on 28 CLL patients. Studies on non-CLL patients were also submitted, namely in 147 FL (Hx-CD20–001, -405, -409), in 5 DLBCL (GEN45) subjects, and in non-neoplastic patients, as 277 RA (Hx-CD20-403, GEN410, -411, -413) and 5 CODP (Hx-CD20-408) patients [1–10].

29.1 Mechanism of Action

CD20 (Bp35) is a tetraspanning transmembrane human B lymphocyte-restricted differentiation antigen, located at the surface of normal and primate B lymphocytes and on human malignant B cells. During B cell maturation this phosphoprotein is first expressed on pre-B cell, but is lost during the final stage of B cell maturation

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to plasma cells. In particular, CD20 is expressed on a subpopulation (about 30 %) of precursor-B cells, on mature B lymphocytes, and on follicular dendritic reticulum cells. CD20 is also expressed by low-grade B cell NHL, precursor B cell neoplasms, precursor B lymphoblastic leukemia/lymphoma (B-LBL), HCL, B-CLL (weak), and by B-PLL. As for its presumed receptor function, CD20 has a possible role in B cell activation/proliferation, through the Src family tyrosine kinases, and enables optimal B cell immune response against T cell independent antigens. However, no natural ligands are known, and the receptor may act as a calcium ion channel.

Ofatumumab is a glycosylated (2 %) IgG1k human monoclonal antibody produced in a murine transfectoma NSO cell line, recognizing both large and small extracellular loops of CD20 antigen expressed on B lymphocytes (mostly non-memory cells). The proposed mechanisms of action are based on the capacity of Fc fragment to induce antibody-dependent cell cytotoxicity (ADCC), and/or complement-dependent cytotoxicity (CDC). In fact, the binding to the membrane proximal CD20 epitope recruits and activates the complement pathway at the cell surface, leading to CDC and resulting in lysis of tumor cells. In addition, the binding of ofatumumab induces also cell death through ADCC. Ofatumumab has been shown to induce lysis in cells both with high and low CD20 expression, such as CLL cells, including cells with high expression levels of complement regulatory/inhibitory molecules. The binding site of ofatumumab is different from the epitope recognized by another anti-CD20 monoclonal antibody, rituximab. In particular, the former seems to be located in a position (binding to the small loop) closer to the cell membrane, compared to rituximab, thereby suggesting an enhanced cytotoxic effect on the targeted cell, by a closer binding of C1q to the cell membrane. In fact, ofatumumab lyses in vitro 100 % of cells expressing over 60×10^3 molecules, and about 18 % of cells expressing as few as 4,500 CD20 molecules per cell. Rituximab did not reach maximal lysis against clones expressing the highest levels of CD20 and showed CDC activity toward cells expressing at least 30×10^3 CD20 mol/cell. However, this enhanced property may be also a major risk for the killing of normal B lymphocytes. In particular, ofatumumab shows a higher CDC activity and an equivalent ADCC cytotoxicity as compared to rituximab, being able to lyse also rituximab-resistant cells, although some subsets of leukemic cells are resistant to both monoclonals. In addition to the mentioned cytolytic effects, the binding of ofatumumab is able to recruit also NK cells. Notably, after binding CD20 it is not dissociated or internalized, and NK cells are not affected by the presence of ofatumumab [8, 11–13]; see Chap. 23, 35, 37].

29.2 Immunogenicity

HAHA were tested in 274 subjects in Study Hx-CD20-001 and in the two main studies (Study 1, and 2), resulting in only two (0.7 %) positive cases. In particular, HAHA were negative in 82 patients who had sufficiently low circulating ofatumumab concentrations to allow a significant detection. Two more cases were

identified in FL and RA patients (1 each) enrolled in preliminary supportive studies.

It must be noted, in addition to the known interference of circulating ofatumumab in the testing, that the assay was restricted to detection of one antibody isotype (IgG1k) and was not capable of detecting antibodies directed to CH2 and CH3 domains. Although affecting the B cell compartment, ofatumumab did not significantly affect the circulating Ig serum concentrations, except for a mild and limited decrease of IgM. The immune response capacity to conventional antigens was not tested in humans. In the animal model (monkey), a lower response to KHL and normal DH response were reported. Moreover, hemolytic HAHA occurred in few animals. Overall, the immunogenicity of ofatumumab appears to be low [3, 4].

29.3 Adverse Events

Initial safety analysis at the time of BLA application was performed on a database of 648 patients, further extended to 1,138 patients from 17 studies including the previous trials at the time of safety update (April 2009). The former database included additional studies performed in other diseases, namely FL (3 studies on 147 patients), DLBCL (1 study on 4 patients), COPD (1 study in 5 patients), and RA (3 studies in 76 patients). However, these patients received lower doses of ofatumumab and the 154 DR patients enrolled in Study 1 (Hx-CD20-406) were the only subjects receiving ofatumumab at the doses and schedule related to the requested approval. Therefore, most official analyses of AEs are referred to this study and to the supportive monotherapy Study 2 (Hx-CD20-402) [1–10].

The general safety profile of ofatumumab includes *infusion reactions*, *cytopenias*, *infections*, *HBV reactivation, intestinal obstruction,* and *PML*. However, no BBWs are included in official labeling. Pooled AEs of any grade from the two main studies were frequent (82–95 %), either estimated as drug-related (DRAEs) in over 60 % of cases, or classified as severe (≥grade 3) in 30–60 % of cases. An average of 9 AEs/patients was recorded. Drug-related discontinuations were 14 % (10 % due to infections). SAEs occurred in 30–54 % of patients and fatalities in 16 % of cases. Noteworthy, most AE, and most of the severe ones, were observed in the 27 patients treated with the highest dose of ofatumumab in Study 2, although the number of patients treated with lower doses was very small.

Infusion reactions (40–60 %) were usually mild and occurred more frequently during the first two infusions (about 50–60 and 30 %, respectively). These could be severe (20 %) or serious (5 %). Anaphylactoid events included 5 patients with CRS, mostly during the first infusion. Drug-related events were observed in 47 % of patients. No confirmed mucocutaneous reactions were observed, as noticed in other similar monoclonals (rituximab). During infusions, 2 cases of CRL and 8 cases of anaphylactic/anaphylactoid reactions were observed. In a comparative analysis with supportive studies concerning non-neoplastic diseases, the rate of infusion reactions was higher in RA patients.

Bacterial, viral, and fungal infections (50–85 %) were severe (20 %), serious (12–17 %), and fatal (5 %), and included mainly URTI (30 %), pneumonia (16 %), and septic complication. About 16 % infections were considered as opportunistic. SAEs included pneumonia (1 fatal case), HZV infections, and interstitial lung disease (fatal). Due to cytolytic hepatitis, one patient was withdrawn after the first infusion. DRAEs were estimated as 21 %, but none of the fatal cases were considered as drug-related. The rate of infections decreased over time (median time 83 days). One case of PML was observed in Study 1, in a patient receiving ofatumumab for about 5 months, previously treated with various chemotherapies and alemtuzumab.

In the extended follow-up, additional infective fatalities (9 %) were observed

Hematotoxicities included *neutropenia* (16 %), mostly as severe/serious (60 % of them), and *thrombocytopenia* (9 %), classified as DRAEs in over 80 % of cases.

In addition, other common AEs included pyrexia (20 %, 6 % severe), cough (19 %), diarrhea (18 %), and anemia (16 %, including 4 cases of hemolytic anemia, fatigue (15 %), and dyspnea (14 %).

Two cases of *intestinal obstruction* of unknown origin were reported in the Study 1.

Overall, due to the typology of the studies, the safety profile was not considered sufficiently delineated, and concerns about infections and fatalities were raised. Nonetheless, this framework was considered acceptable for the group of patients double resistant to cytarabine and alemtuzumab.

Being the pivotal studies organized as single arm trials, it is difficult to evaluate the increased risk related to treatment. For example, neutropenia is also caused by CLL, and therefore the role of ofatumumab in this event was difficult to evaluate. However, in a subgroup of patients (46) with normal cellular levels at baseline, the neutropenic effect could be individuated as grade 4 in about 43 % of patients, which is higher than the average reported for CLL in official labels. Similarly, a real estimation of the HBV reactivation risk, which is included in the label warnings, could not be assessed, because patients with active hepatitis B were excluded from the trial, and the safety database contained a low number (2 %) of HBV positive patients. Therefore, this warning was included mainly on the basis of experience with the analog rituximab. One case of fatal HBV hepatitis in a RA patient was added in the safety update warning of July 2009.

The most common causes of *death* were disease progression and infections. However, the adopted criteria assessing a link between infections and deaths (17 %) raised concerns. Overall, no safety signals were identified in laboratory tests, except for the mentioned cytopenias.

As for secondary *malignancies*, no evaluation was possible, due to short-term treatment/follow-up and to the underlying disease. In fact, CLL has a higher risk (about twofold) to develop secondary solid tumors and lymphomas (3 %). However, 4 cases of solid tumors (2 after safety update) and 3 lymphomas were observed in the pivotal studies, and additional 6 solid tumors were reported in supportive studies on other diseases (FL and RA).

In a subsequent Phase II study, safety and efficacy of ofatumumab were evaluated in *patients resistant to rituximab*, on the basis of potential different CD20 targeting of the two monoclonals. As compared to previous rituximab-related AEs, ofatumumab increased the incidence of mild/moderate infusion reactions, but left unchanged the rates of severe (≥grade 3) infusion infections and hematological AEs, indicating that there was no major synergism in the induction of adverse events between the two monoclonals [10].

On the basis of positive results from single-agent trials, ofatumumab was evaluated in *therapy combinations* with various chemotherapies. A randomized Phase II trial (BIFROST) investigated ofatumumab plus fludarabine and cyclophosphamide (O-FC) in 61 untreated CLL patients. Most common AEs were mild/moderate infusion reactions, neutropenia (48 %), infections (38 %), nausea (41 %), thrombocytopenia (26 %), rash (25 %), vomiting (23 %), fever (21 %), cephalea (18 %), and fatigue (18 %) [14].

Similar comparative analysis examined the therapy combinations of ofatumumab, with lenalidomide and with high doses of oral glucocorticoids in resistant-relapsing CLL, with the aim of double targeting resistant leukemic cells and possibly reducing AEs effects by adding a biomedicine in order to lower the dose of the more toxic chemical drugs. Both associations did not show synergistic effects with respect to AEs, which remained in the range of reasonable tolerability. However, a positive synergistic effect was evidenced in vitro between ofatumumab and alemtuzumab, associated in order to benefit of the respective CDC mechanisms of action. This mechanism, together with ADCC, is essential for B cell killing. However, subpopulations of CLL cells could resist to CDC mediated by a single monoclonal antibody. The study was conducted on 21 untreated CLL cases, showing an effective CDC increase in vitro. However, this combination was still unable to kill a residual subpopulation of cells that appear to be intrinsically resistant to activated complement bound to their surface [15]. On this basis, one Phase II trial (NCT01361711) on alemtuzumab-ofatumumab combination is recruiting previously untreated symptomatic CLL patients.

29.4 Off-Label Experience

As previously reported, nine studies submitted with the application for CLL indication tested ofatumumab in other diseases (FL, DLBCL, RA, COPD). At present, this monoclonal is being investigated also in Waldenstrom's Macroglobulinemia (WM), other B cell lymphomas including mantle cell lymphoma (MCL), Hodgkin's disease (HD), malignant melanoma (MM), relapsing-remitting multiple sclerosis (RRMS), rheumatoid arthritis (RA), Crohn's disease (CD), Wegener's Granulomatosis (WG), and other autoimmune disease. In particular, 91 clinical trials are completed, ongoing or recruiting, and include CLL (41), B-NHL (11), other lymphomas (10, FL (9), DLBCL (6), RA (5), MCL (4)), MS (3), WM (2), HD (2), MM (1), GVHD (1), Richter's syndrome (1), and ALL (1).

Overall, AEs profile was similar, with no new signals appearing in both FL and DLBCL cases. As expected, rates of AEs were higher in combined therapies, yet at non-significant levels. Infusion reactions were mostly occurring within the first two days of administration as mild/moderate, and tended to decrease during following treatments. Therefore, no synergistic effects on AEs induction were observed in the examined combinations. Within the limited observation period of these studies, AEs do not seem to increase with time [12, 16–18].

Experience in *non-neoplastic diseases* of ofatumumab is mainly concerned with RA. The basis for these attempts was mainly related to the selective and prolonged B cell depletion induced by this monoclonal, determined via a potent CDC and ADCC activity, due to a unique binding site positioned at close level of the cell membrane.

In the extension study of initial Hx-CD20-403 trial (NCT00291928), 263 RA patients with active disease were treated with two infusions of three different doses (300,700, and 1,000 mg) of ofatumumab after discontinuation of DMARDs, except for the supportive treatment of MTX. Patients were followed up to 24 weeks. In a similar study 130 RA patients with inadequate response to MTX were treated with the intermediate dose (2 infusions of 700 mg) and followed for 24 weeks. Most infusion reactions (70–86 %) occurred during the first administration in spite of premedication, and rapidly decreased (3–20 %) at the second treatment, indicating a early CRS following lysis of normal B lymphocytes, as previously observed on leukemic lymphocytes in CLL. The rate of serious infections in patients treated with ofatumumab was comparable to placebo. Interestingly, no HAHA or other autoantibodies or laboratory test abnormalities occurred, except for the B cell compartment expected modifications [19, 20].

Finally, ofatumumab was planned for use in MS, and two Phase II studies were scheduled. Preliminary data from one of these studies reported the absence of significant safety signals. However, the manufacturer temporary suspended the investigation and announced the intention of considering the subcutaneous administration of ofatumumab in autoimmune diseases, which would need a different procedure for authorization [21, 22].

29.5 Postmarketing Surveillance

Up until first months of 2013, the FAERS database registered 1,056 reports on ofatumumab/Arzerra, including infections (11 %), WBC disorders (10 %), respiratory (5 %), gastrointestinal (3 %), and neurological disorders (3 %) as the most frequent events. Febrile neutropenia (157 reports), pneumonia (60), and neutropenic sepsis (27) were the most relevant infectious disorders. TCP and autoimmune TCP (35 and 2 cases respectively), PNP (14), anaphylactic reactions (10), rash (23), and urticaria (19) were less common. Notably, 16 cases of TLS and 12 cases of PML were registered.

By the end of 2012 EUV database registered 166 reports related to ofatumumab (165 on serious events). General and investigational signs (about 25 %), hematological disorders (11 %), infections (11 %), and cutaneous reactions (6 %) were more frequently reported. Hematological disorders included neutropenia (21 cases), thrombocytopenia (9), and febrile neutropenia (8). Hypersensitivity reaction (3, one drug-related), rash (7), urticaria (8), and one case of anaphylaxis were also reported. Finally, 3 cases of PML, 5 PNP, and one case of TLS, were observed.

29.6 Remarks

The second-generation fully human antibody, ofatumumab, has shown the expected almost total absence of immunogenicity in various clinical applications, together with a manageable safety profile. The most common AEs were infusion reactions and infections, which were primarily grade 1 or 2 events. Experimental evidence in vitro and in clinical trials confirmed the peculiarity of its mechanism of action in destroying B cells with low expression of CD20, such as CLL-cells, without significant damage of the B mediated immune response. In fact, Ig levels showed modest decrease, especially for IgM, infections were infrequent and usually mild, with opportunistic infections virtually absent, and the overall immune response preserved.

Ofatumumab, binding closer to the cell membrane due to the recognition of the inner small loop of CD20 sequence, is able to destroy malignant cells resistant to other monoclonals (rituximab included). This peculiarity, combined with a lower toxicity, has offered the possibility of associated/subsequent therapies showing higher efficiency, in the absence of synergistic effects on AEs induction. Although long-term experience is still lacking, one case of PML has been so far reported, and a few were reported in the postmarketing setting. These cases raised some concerns about the potential application of ofatumumab in CNS autoimmune diseases, such as RRMS, where other monoclonals have shown to induce such devastating complication. Notably, PML has been also reported in CLL not treated with biomedicines.

The CLL patient developing PML in the controlled Study 1 received ofatumumab for about 5 months and developed neurological signs 27 days after the last dose (174 days after the first dose) and died after 63 days after the last dose. However, this case occurred after a number of different aggressive therapies, including another monoclonal antibody (alemtuzumab). It must be noted that other monoclonals potentially inducing PML, either have a BBW on this disease (rituximab) or are distributed under special restricted program (TOUCH), as for natalizumab, or report a warning on PML potential insurgence in the postmarketing observations (alemtuzumab), thus showing a different evaluation of these biomedicines with respect to this important event [1].

Noteworthy, the apparent integrity of the T cell compartment during ofatumumab treatment was also confirmed by the low incidence of viral infections, at least within the time range of present observations (over 24 weeks).

The limited experience in off-label studies indicates that either in neoplastic (lymphomas) or autoimmune diseases (RA, and possibly in MS) the safety profile of ofatumumab remains tolerable and manageable.

A number of third-generation anti-CD20 monoclonals with potential increased binding affinities, such as AME-133v, PRO131921, and GA101, are undergoing clinical evaluation, indicating that this target deserves further attention. The latter experimental molecule seems particularly interesting since it expresses enhanced direct apoptotic death in lymphoma cells in vitro. However, in a recent investigation in vitro comparing cytotoxic activities of rituximab, eculizumab, and GA101, the major killing role was still attributed to CDC for all three monoclonals, while the latter showed a strong activation of NK cells, but only a limited direct cell death of B-CLL cells [23].

A final consideration arises from the recent meta-analysis from the Cochrane collaboration on the impact of rituximab, ofatumumab, and other anti-CD20 monoclonals in the treatment of CLL [24]. The analysis states that CLL patients receiving chemotherapy plus rituximab benefit in terms of overall survival (OS) as well as progressive free survival (PFS), compared to those with chemotherapy alone, and rituximab was recommended in combination with FluC as an option for the first-line treatment, as well as for people with relapsed or refractory CLL. However, the available evidence regarding the other assessed comparisons, including ofatumumab, are not enough to deaw final conclusions, mainly due to the heterogeneity of clinical studies and to the absence of randomized controlled trials, the latter stressing once more their absolute priority in order to determine clinical effects and safety profiles. Of six available trials assessing safety and efficacy with respect to rituximab, only one evaluated different doses of ofatumumab. Expectancies are now focused on three ongoing trials, which may lead to definitive conclusions, comparing ofatumumab with or without additional chemotherapy and no treatment.

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Omalizumab

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Omalizumab (Xolair®, Genentech) is a recombinant humanized IgG1k monoclonal antibody that selectively binds to human immunoglobulin E (IgE). This monoclonal was first registered by TGA in Australia in June 2002 for the treatment of moderate to severe allergic asthma (AA) resistant to inhaled steroids, and with IgE levels corresponding to the recommended dose range. Following a previous rejection in 2001, FDA granted approval in 2003 for adults and adolescents (≥12 years) with moderate to severe persistent asthma, positive to a perennial aeroallergen and whose symptoms are inadequately controlled with inhaled corticosteroids. In October 2005, a marketing authorization was granted by EMEA for the treatment of AA in patients (≥12 years) in whom standard treatment had failed. In December 2005, TGA extended the registration to include management of adult and adolescent patients with moderate to severe AA, who are already being treated with inhaled steroids and have IgE levels corresponding to the recommended dose range. In June 2009, EMEA extended the indication as add-on therapy in young patients (6 to <12 years of age) affected by severe persistent AA with positive skin test or in vitro reactivity to a perennial aeroallergen, frequent daytime symptoms or nighttime awakenings, and multiple documented severe asthma exacerbations despite daily high-dose inhaled corticosteroids, plus a long-acting inhaled beta2-agonist.

Major clinical studies include a pivotal trial (Study 2306) involving 419 patients (12−79 years) with severe allergic asthma and six studies in predominantly severe allergic asthmatics. In particular, four randomized controlled studies (Study 2304, 008, 009 and their extension, Study 011, on over 1,400 patients). conducted in severe persistent AA, and two open-label studies (IA04, and Q2143 or ALTO) on 1,211 patients performed predominantly in patients with severe persistent allergic asthma. Patients with seasonal allergic rhinitis (SAR) and

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perennial allergic rhinitis (PAR) were also included in the mentioned studies, yet only for safety evaluations. All together, the safety profile of omalizumab included about 7,000 patients (over 5,000 on omalizumab) with AA, SAR, PAR, and other indications. Overall more than 57,000 patients were exposed to omalizumab by 2012. At present omalizumab is registered in over 80 countries [1–8].

30.1 Mechanism of Action

The IgE class of antibodies has a pivotal role in Type I hypersensitivity reactions, including allergic asthma. Inhalant allergens are considered as the major antigen class responsible of sensitization and triggering of asthmatic attacks. IgE bind to high affinity receptors (FcεRI) on mast cells and basophils, thus releasing a number of inflammatory mediators, such as histamine, proteolytic enzymes, proteoglycans, and arachidonic acid metabolites. FcεRI consists of two extracellular Ig-like domains. However, this receptor and the low affinity receptor counterpart (FcεRII, or CD23) are also present on other cell types. FcεRII consists of three C-type lectin domains connected to the membrane by a trimeric α-helical stalk (mCD23), which can be cleaved from the cell surface by endogenous proteases in soluble trimeric and monomeric forms (sCD23). The IgE /FcεRII interaction is involved in both IgE regulation and allergen presentation by B cells, and in shuttle (IL-4 dependent) transport mechanisms of food antigens at intestinal level. However, sCD23 are also involved in positive/negative feedback mechanisms for the regulation of IgE synthesis by B cells. So far FcεRI has been found on neutrophils, monocytes, macrophages, dendritic cells, Langerhans cells, eosinophils, platelets and, more recently, on some epithelial intestinal cells, which seem overexpressed in neoplastic (colon cancer) and inflammatory states (Crohn's disease, food allergy). FcεRII is constitutionally expressed on B cells, but can be induced on the surfaces of monocytes, macrophages, eosinophils, platelets, Langerhans cells, and on some T cells and intestinal cells regulated by IL-4. The presence of both high and low affinity receptors on APC cells suggests a crucial homeostatic role at APC level, but also in the promotion of allergic reactions, either via the specific IgE/mCD23 binding, or as allergic reactions to unrelated allergens via the IgE/sCD23 binding and internalization. Interestingly, crystallographic studies pointed out that the binding of IgE to either receptor precludes interaction with the other, thus indicating a mutual exclusion of CD23 and FcεRI binding, which is important for IgE functioning and possibly for the consequences of homeostatic disruption due to IgE therapeutic blockade. Finally, IgE can be bound to Galectin-3 (eBP) protein, which is widely distributed and may play additional regulatory roles in neoplastic and inflammatory diseases.

IgE are considered an important, yet not unique, defense mechanism against parasitic infections, mainly helminthic and protozoal. In fact, they usually induce a strong and specific IgE production in an acute phase, associated to innate immunity. Interestingly, CD23 activation via IgE plays a role in intracellular killing of parasites, such as Toxoplasma [9]. In the chronic phase, in spite of a possible IgE

levels increase, violent manifestations of their actions tend to decrease and appear less specific. It is not clear whether IgE are involved in direct cytotoxic effects, possibly against larval stages of infestants. IgE may be involved in early recognition of exogenous molecules at mucosal level, and even a moderate allergic reaction is considered a physiological defensive attempt to expel foreign antigens by hypersecretions and prompt mechanical actions (sneezing, cough, diarrhea). In spite of the very low serum concentration of IgE, they are prominent at mucosal and epithelial level, and are mostly fixed on their respective receptors situated on highly active effector cells, such as basophils and eosinophils full of preformed bioactive substances. The binding prolongs IgE half-life (months) with respect to the circulating molecules (hours): this may explain their local high efficiency in the presence of very low circulating titers. The role of IgE with respect to neoplasia is unclear. Unconfirmed initial investigations suggested a potential defensive role of IgE against some solid (gastric) tumors and leukemia. Data concerning the occurrence of malignancies during IgE blocking therapy do not support such assumption, but exposures are still too short to allow definitive conclusions.

Omalizumab is a recombinant murine (5 % in Fab) humanized (95 %) IgG1k monoclonal antibody directed to Cε3 constant domain of IgE, containing the binding site for the FcεRI receptor. Therefore, the binding occurs only with soluble free IgE, but not with IgE already fixed to the receptor. In fact, this monoclonal is not indicated for the treatment of acute asthma exacerbations, acute bronchospasm or status asthmaticus. The omalizumab-IgE complex is biologically inert and avoids basophils and mast cells degranulation following IgE binding on their surface receptors, thus inhibiting signals for the release of preformed bioactive molecules (histamine, proteoglycans) and the production of other factors (leukotrienes, IL-4, IL-13). Moreover, FcεRI is downregulated on target cells, including dendritic cells, thus synergizing with the IgE depletion effect of the monoclonal. Circulating levels of IgE are virtually ruled out, and the effect is long lasting (>4 months) after proper omalizumab administration. However, consistent and persistent levels of mAb/IgE complexes remain in circulation, which influence free IgE dosage during treatment and long after (up to 1 year). Omalizumab reduces skin and respiratory reactivity to allergens, both in early and late phase, reduces bronchial mucosal infiltrates of T cells, B cells and has proapoptotic effects on eosinophils [10–13].

30.2 Immunogenicity

As expected, immunogenicity was low (<0.1 %). After a rapid decrease (96 %) of circulating IgE during treatment, there is an apparent increase in IgE levels (up to fivefold over baseline), due to immunocomplex formation with omalizumab, lasting at least for one year, with no rebound effect on free IgE levels [5]. Nonetheless, no significant amounts of anti-IgE HAMA/HAHA antibodies were detected.

30.3 Adverse Events

The overall safety profile of omalizumab has been mostly established on the basis of three studies conducted in 1,412 adolescent and adult patients (718 treated with mAb in study) [1–8]. The prescribing information contains a BBW for *anaphylaxis*, which may occur as an early (first administration) or late (even after one year) serious and life-threatening event. *Malignancies* are the second major concern, followed by *serious systemic eosinophilia* (vasculitis, Churg-Strauss syndrome), and *serum sickness-like syndrome* expressed as pyrexia, arthralgia, and rash. An expected high risk for *parasitic infections* (geohelminthic), and a long-lasting (1 year) circulating presence of *IgE/mAb complexes* are also included in the safety profile of omalizumab. The most common AEs included injection site reaction (45 %), viral infections (23 %), URTI (20 %), sinusitis (16 %), cephalea (15 %), and pharyngitis (11 %), and rash (7 %). Less frequent events (2−8 %) included constitutional signs (pain, fatigue), musculoskeletal disorders (arthralgia, pain), nervous symptoms (dizziness), dermatitis/pruritus, and earache. However, these events were mostly observed at similar rates in treated and control patients. In pediatric patients (<12 years), AEs were evaluated in 926 patients (624 exposed), and serious events, such as anaphylaxis and malignancies were not observed. However, these studies were not planned to address these concerns, and consisted in short-term investigations.

Anaphylaxis has been reported in 0.1−0.5 % of over 3,500 patients, occurring within hours from first doses (70 %) after subcutaneous inoculation. In the post-marketing observation it was estimated as 0.2 % of cases on over 57,000 reports in about 3 years. Patients rechallenged after the event again presented anaphylactic symptoms in 78 % of cases. The FAERS system reported 118 cases of anaphylactic reactions in 15 months, including 33 cases with multisystem allergic reactions, mostly respiratory and skin reactions, and confirmed their primary early appearance within the first two administrations (66 %).

Injection site reactions (45 % vs. 43 % in placebo) were also severe (12 %), and usually lasted about one week, tending to decrease with the following administrations.

Additional signs observed in the postmarketing experience included *serum sickness syndrome, alopecia*, and *severe thrombocytopenia*.

Malignancies, mostly as solid tumors including BC, PC, MM, NMSC and parotid tumors, were observed in 0.5 % of cases in a group of about 4,000 adult and adolescent patients, as compared to 0.18 % of controls. It must be noted that the patients had a less than one year follow-up.

Recent additional and reassuring information came from a pooled analysis on the risk of malignancies related to omalizumab treatment. This study included the outcome of 67 controlled trials (Phase I−IV) enrolling about 11,500 patients, of whom 7,800 patients were exposed to omalizumab. Patients receiving single doses of omalizumab were excluded from analysis, and patients in study received a categorical cumulative dose of 900 mg of omalizumab. The overall incidence rates

were 4.14/1000PY, versus 4.45/1000PY of placebo. The most frequent primary malignancies in the study group were NMSC (45 cases) and MM (52), with a similar time to primary malignancy in both groups, and no evidence of increased incidence related to dose-response or treatment duration [14].

On the basis of an epidemiological study (EXCELS) on long-term safety of omalizumab, conducted in patients with moderate/severe asthma followed-up for 5 years, FDA raised concerns about cardiovascular and cerebrovascular accidents (ATE) that appeared doubled in the treated group (6.3 vs. 3.4 in controls). However, other evaluations indicated that there was no ATE risk association with this monoclonal [7, 15].

Although controlled studies were not programmed for investigating the relevance of *parasitic infections*, some indications were envisaged along, mainly related to particular geographic areas where infestations are more frequent. In fact, in a study conducted in Brazil in patients treated with omalizumab, about 50 % of them experienced at least one helminth infestation, as compared to 41 % of controls, thus indicating a slight risk related to treatment in study, although not reaching statistical significance [16, 17].

30.4 Off-Label Experience

As expected, omalizumab has been used in various conditions where pathogenetic IgE-mediated causes (such as anaphylaxis and food allergy) or IgE-mediated mechanisms (such as chronic urticaria and idiopathic anaphylaxis) are postulated. Off-label tested indications are referable to perennial and seasonal allergic rhinitis, peanut allergy, latex allergy, atopic dermatitis, chronic urticaria, idiopathic anaphylaxis, mastocytosis, eosinophilic gastroenteritis, nasal polyposis, chronic idiopathic urticaria, and to non-prescribed indications for allergic asthma. Moreover, omalizumab has been indicated as add-on therapy to control hypersensitivity reaction during desensitization therapies to allergens and insect venoms. Overall, they consist in small trials and case reports showing alternate beneficial effects, but a substantial absence of serious adverse events.

Studies on *refractory chronic urticaria* in a Phase II prospective dose-ranging trial (MYSTIQUE) included 90 patients (69 treated) treated with a single dose of omalizumab. It must be noted that chronic urticaria is not considered a typical allergic (IgE-dependent) disorder, but an autoimmune disease, during which IgG1 and IgG3 complement binding antibodies directed to IgE and to FCεRI are produced in 35−50 % of cases. AEs were observed in 44 % of patients, and most common events included URTI, nasopharyngitis, cephalea, and dysmenorrhea (all $5 \leq 15$ %). The majority of events were mild/moderate, with low rate of discontinuation (4.4 %) and no SAEs registered. Two patients had hypersensitivity reactions including asthma, yet not other anaphylactic, rash, or injection reactions, nor urticaria. The observed events did not appear as dose-related or time-related, nor revealed new safety signals up to 16 weeks follow-up [18]. In another small trial on 49 patients (27 treated) with refractory chronic urticaria exhibiting

anti-thyroperoxidase IgE, omalizumab was administered once every 2−4 weeks for 24 weeks, in the attempt of reducing potential immuno-complex related mast cell activation and insurgence of allergic skin reactions. The number and intensity of wheals, pruritus, erythema, and angioedema lowered in patients receiving omalizumab, compared with those receiving placebo, including adverse events suspected as drug-related. The most frequent (>5 %) and more represented AEs in the study group were diarrhea (15 % vs. 9 % in controls), and cephalea (37 % vs. 27 %) [19]. Other case reports and some uncontrolled studies, recently reviewed, gave similar responses [20, 21].

Overall, sufficient experience indicates that omalizumab is well tolerated during short-term treatment in chronic urticaria.

Experience in *food allergy* is limited and has been recently reviewed. No safety problems or new signals arose during the analysis [22]. In a study on the association of omalizumab with a cow's milk desensitization protocol with high levels of specific IgE, the monoclonal seemed to accelerate the desensitization process without raising concerns. Interestingly, CD4+T cell response to milk was greatly reduced within a week, milk-specific IgE were reduced and a 15-fold increase in milk-specific IgG4 was detected, although the precise role of omalizumab in the overall "normalization" process was not evidenced [23]. Another interesting association of omalizumab was experienced in various other *desensitization* attempts (pollens, ragweed), where the monoclonal resulted helpful in reducing side effects induced by the desensitization procedure, including anaphylactic reactions [24].

Because of the known omalizumab capacity of reducing the presence of eosinophils in blood and sputum in various conditions, the potential beneficial effects of this monoclonal was studied in *hypereosinophilic syndromes* (HESs), although with some concerns related to the unclear role of omalizumab in the induction of serious systemic hypereosinophilia, including the Churg-Strauss syndrome (CSS). In the first case report of chronic eosinophilic pneumonia (CEP), omalizumab resulted rapidly effective, producing remission lasting at least 15 months, and allowing the reduction or suspension of corticosteroids that had previously produced depression ad amenorrhea. Therefore, steroid-related AEs were effectively reduced, with no new omalizumab-related events reported. Interestingly, laboratory tests indicated a downgrade of CD23 on B lymphocytes. Due to the limited experience and the short-term administration of omalizumab, the mentioned concerns still remain [25].

Another first-case report refers to a *refractory Type 2 diabetes* with elevated levels of specific IgE against insulin of human, porcine, and bovine origin. The patient suffered an anaphylactic shock upon administration of insulin, and therefore subsequently received omalizumab associated with desensitization therapy with beneficial effects and no additional adverse events. Notably, the dosing of the mAb was adjusted on the basis of reappearance of urticaria at insulin injection site, up to 9 weeks intervals, and could be programmed for long-term usage [26].

Overall, omalizumab confirmed the low safety profile depicted in on-label observations, with some additional concerns in anecdotal attempts, mainly involving disorders of the eosinophilic compartment, where basic information is

still lacking and potential serious events were signaled in the postmarketing vigilance. Long-term effects, including malignancies, are still to be evaluated in proper controlled trials.

30.5 Unexpected Adverse Events

Two reports on unexpected events deserve attention for potential future implications, especially in long-term treatments and in possible wider therapeutic indication of omalizumab. One case is about a young adult patient with asthma from childhood, who developed *elevated levels of myeloid cell counts* associated with the administration of this monoclonal. The abnormal myeloid cells were detected after 29 months of omalizumab treatment. Such cells had normal figures before therapy, and returned to normal levels 3 months after discontinuation. Other circulating WBCs were within normal ranges. Notably, the patient also received prolonged corticosteroids therapy and had common variable IgG immunodeficiency treated with subcutaneous immune globulin [27]. The second unexpected event consisted in a *cluster of constitutional signs* registered in a patient with hymenoptera venom allergy and underlying systemic mastocytosis (SM), who was treated with omalizumab as add-on therapy to improve tolerability of a specific venom immunotherapy (VIT). In fact, this patient, in spite of standard prophylaxis, suffered VIT-related SAEs (hypotension, dyspnea, angioedema), before the beginning of omalizumab supportive therapy. While these reactions improved over time, together with pre-existing pruritus and diarrhea (presumably related to SM), new signals appeared, including *sleep disturbance, vertigo, exercise intolerance, diffuse myalgia, joint pain without effusions, and crippling fatigue and feebleness*. Notably, these signs were not detected during previous VIT. After omalizumab discontinuation the patient recovered slowly, and after 6 months all signs have disappeared [28]. Since long-term experience with omalizumab is limited and add-on strategies are increasing, these signals should be pondered.

Finally, a minor albeit intriguing event regards the observation of cases of appendicitis in the pivotal study. Although their number was limited, twice as many exposed patients underwent appendicectomy, and in four of the six cases observed in the study group the appendix was found to be normal.

30.6 Postmarketing Surveillance

By the end of 2012, over 9,300 reports (AEs/R 3.5) included respiratory disorders (12 %), infections (7 %), dermatological (5 %), and allergic reactions (4 %), as the most frequent events in the FAERS database. Anaphylactic reactions were approximately 1.5 % and injection site reactivity 1 %.

In the EUV dataset at about the same date 6,400 reports (SAEs/R 4.0) included respiratory disorders (24 %), constitutional signs (14 %), infections (8 %), and neurological events (7 %) as most frequent events. Malignancies were about 2.4%,

Moreover, 403 cases of anaphylaxis, 53 anaphylactic shock, 32 anaphylactic reactions, and 15 IgE-mediated allergic reactions were registered. Nine cases of SSLS and one CRS were detected.

30.7 Remarks

Omalizumab may induce some consistent and unexpected adverse events, but the general profile is tolerable, at least for the short-term experience so far accumulated. While long-term data are needed for a better evaluation of secondary malignancies potentially inducible by omalizumab, data on anaphylaxis are sufficient to state that this monoclonal can raise concerns for immediate type reactions. At first glance this capacity may appear paradoxical, since the monoclonal inhibits degranulating signals via FCεRI, and indirectly enhances the activity of the counterpart low affinity receptor FCεRII, thus potentially reducing the APC activity in antigen presentation. However, anaphylactoid reactions do not involve this pathway, and the presence of consistent levels of high molecular weight and fully proteic IgE-omalizumab immunocomplexes largely justifies the contemporary possibility of inhibiting Type I hypersensitivity reactions, while allowing anaphylactoid reactions and Type III hypersensitivity reactions to take place. Notably, these complexes may remain into circulation up to one year, i.e., long after anti-IgE effects of omalizumab, and may explain some late AEs occurring with this biomedicine. Another contradiction appears when comparing omalizumab effects on skin tests with the effects on asthmatic symptomatology. In fact, treatment in allergic patients inhibits early intradermal skin tests reactivity (24 %), but is more effective on the late phase (63 %), whereas the suppression of the early phase asthmatic response (80 %) is greater than late phase response (65 %). This may again indicate the presence of multiple and possibly counteracting mechanisms of action, and their different hierarchical role in different districts [24].

The question on malignancies still remains open. The recent finding on the presence of FCεRI in normal and neoplastic intestinal epithelial cells is intriguing [11]. IgE antibodies may participate in tumor immunosurveillance, but the FCεRI pathway induces Ras (the most common oncogene in human cancer) activation, evoking growth cell signals also on mast cells.

Another unbalancing immunological effect of omalizumab deserves attention. The inhibition of IgE binding has been found to be associated with the increase of IgG4 isotype during omalizumab therapy. Many patients (up to 40 %) with IgG4-related diseases, a newly identified set of disorders, have allergic features such as atopy, eczema, asthma, and peripheral blood eosinophilia. The IgG4 production is controlled by the same pathway inducing IgE production (Th2-lymphocytes, IL-4, IL-13). Moreover, IL-10, IL-12, and IL-21 shift the IgE/IgG4 equilibrium in favor of the latter. Therefore, the imbalance consequences should be taken into consideration as potential inducers of IgG4-mediated autoimmune disorders. Noteworthy, IgG4 can bind to other IgG and has also shown a rheumatoid factor

activity, which may explain AEs such as arthralgia or myalgia encountered during treatment [29].

Due to possible proapoptotic effects on eosinophils, omalizumab has been used in off-label conditions, such as hypereosinophilic systemic and local syndromes. Serious concerns may be raised in this respect, not only because CEP and CSS have been reported in omalizumab treatment, but also for potential rebound effects produced by omalizumab or other monoclonals on eosinophils, and on IgE/IgG4 balance and related events [28, 30, 31]. However, these effects were alternatively attributed to tapering or cessation of corticosteroids in patients with severe persistent asthma [25].

Reactivation of primary allergic signs can also be expected after discontinuation of the monoclonal. Although no rebounds over normal levels of IgE and of eosinophils were observed, omalizumab effects—both on IgE reduction and on FCεRI downgrade in basophils and mast cells—are reversible, exposing to returning reactions especially in underlying diseases, such as CSS and SM. In contrast, a pharmacokinetic–pharmacodynamic model, based on omalizumab-IgE binding and feedback control on IgE, indicates that omalizumab reduces the production of IgE, thus suggesting the possibility of avoiding indefinite treatment, or extending dose administration intervals [32]. Due to its low-rate induction of serious AEs, omalizumab can be associated with other therapies, including corticosteroids, without producing synergistic adverse effects, but also contributing to steroids dosage tampering and discontinuation, thus reducing the overall exposure of AEs in these patients.

Finally, until more data from controlled studies are not available, the unexpected reactions encountered in some off-label conditions should be considered as potential risk factors as well.

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Palivizumab

31

Palivizumab (Synagis®, MedImmune, Abbott) is a recombinant humanized IgG1k monoclonal antibody (MEDI-493) directed to specific surface proteins of the respiratory syncytial virus (RSV). FDA approved its marketing in 1998 for the prevention of serious lower respiratory tract disease caused by the virus in pediatric patients at high risk of RSV disease. Palivizumab is the first prophylactic monoclonal licensed for any infectious disease. EMEA approved its use in 1999 for the same indication, and by 2002 over 35 countries had approved the use of palivizumab, including Australia, Japan, and New Zealand. During 2003, the two Agencies extended the indication for disease prevention to children with hemodynamically significant congenital heart disease (CHD), on the basis of additional studies. Within the same year palivizumab was approved by Health Canada.

The pivotal IMpact-RSV (MI-CP018) study, being the only placebo-controlled randomized trial of palivizumab, was the basic reference for approvals. This study evaluated safety and efficacy of palivizumab monthly administration as prophylaxis against serious RSV infection in 1,502 high-risk infants. It must be noted that RSV-infected children with CHD were specifically excluded from this trial since a previous study had showed a higher (threefold) mortality in CHD patients than in non-CHD infected patients. Furthermore, one investigation employing intravenous specific immunoglobulins (RSV-IGIV) in children with CHD revealed an unexpected higher rate of cyanotic episodes in these patients. At a later stage, this adverse event was attributed to the large fluid volume needed for IV administration and not to RSV-IGIV, and thereby the American Academy of Pediatrics eventually recommended the use of palivizumab in children 24 months of age or younger with hemodynamically significant CHD. A subsequent large, multi-center clinical trial (MI-CP048) in CHD patients assessed the safety and efficacy of palivizumab, thus leading to the definitive extension of its indication. The study initially enrolled 248 children, and was subsequently extended to 1,280 children.

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Supportive studies for initial approval included five studies on adult volunteers (Mab9401 a, b, c, MI-CP017, MI-CP007), four studies on prophylaxis (MI-CP 011, 012, 005, 018), and five studies on treatment (MI-CP034, 004, 026, 013, 009) for a total of 1,281 treated subjects and 574 controls. However, 1,009 pediatric subjects received palivizumab in the formulation, dose, and route (IM) of administration under licensure. Healthy adult volunteers (28) of the Mab9401 studies and patients (96) from MICP-005, 009, 013, and 026 received IV injections of an early liquid palivizumab preparation, while only the last group (Study Mab9401c) received IM injections of the same preparation (four subjects). Subsequently, patients of Study MI-CP017 received IV injections of the lyophilized preparation, which was thereon used for the remaining studies as IM treatment (1,120 total subjects). Finally, the five studies enrolling RSV infected patients (treatment) had only IV injections. These studies were not combined with the prophylaxis studies for safety evaluations and were not intended to receive approvals for treatment, nor for the IV route of administration. All these investigations employed the maximal dose of 15 mg/kg, which is the standard therapeutic dose. Subsequently, as suggested by Authorities, 12 healthy adult volunteers in another study (MI-CP035) were administered with 30 mg/Kg, and no increased risk was reported when the product was diluted in sterile water (instead of a dextran solution). Therefore, this dilution was adopted for standard IM injections. Finally, for the EMEA evaluation, it was possible to examine additional data from a second season prophylaxis extension of MI-CP018 study (MI-CP 036) in 88 patients at high risk, including 56 children from the previous study, who had been treated in the first season as well. Overall, the global population included 1,797 children, of whom 1,743 in prophylaxis and 54 in treatment, 62 adult healthy volunteers, and 21 patients with bone marrow or stem cells transplants with RSV infections (MI-CP034). In particular, 1,344 patients were enrolled to receive palivizumab (1,282 children and 62 adults) and 575 patients received placebo [1–8]. Overall, up to 2012 over 400,000 patients have been exposed to palivizumab.

At present palivizumab is approved in more than 60 countries, and over 23 trials are completed or ongoing on various aspects of RSV infection (10), including prematurity states (6), CHD (2), chronic lung disease (2), respiratory infections, chronic lung disease, RSV pulmonary infection in cancer, atopic asthma, and reduction of pain at injection site (one each). Moreover, about five trials compared efficacy and safety of palivizumab to motavizumab (MEDI-524), a recent investigational variant form of palivizumab with potential enhanced activity obtained by in vitro affinity maturation.

31.1 Mechanism of Action

RSV is a single-strand RNA highly contagious seasonal epidemic virus of the family of Paramyxoviridae, infecting up to 70 % of children by 3 years of age, and is the leading cause of serious lower respiratory tract infections as bronchiolitis (50–90 %), pneumonia (5–40 %), and tracheobronchitis (10–30 %) among

hospitalized children. The risk for RSV is increased in prematurity, immunodeficiency, CHD or chronic lung disease. Usually the primary infection occurs within the first two years of life, which sometimes creates an imperfect immune response causing recurrences, and prolonged virus shedding (weeks), mainly in immunocompromised patients (both of pediatric or adult age). In adults, RSV infection is at high risk in 4–10 % of cases, mainly in patients with cancer (80 %), cardiac diseases (60 %), COPD (18 %), and asthma (10 %).

Although the related mortality is less than 1 % in otherwise normal children (3.4 % in CHD and 3.5 % in lung diseased children), RSV is considered the major responsible of respiratory complications up to 10 years after initial infection. The RSV (A and B groups) genomes code for three transmembrane proteins named F, G, and SH. The external portions of the former two structures are immunogenic and induce the synthesis of anti-RSV neutralizing antibodies. The F protein is a disulfide-linked heterodimer with a high antigenic homology between the A and B types, and shows a high stability. Anti-F neutralizing antibodies are active against both groups. Unfortunately, vaccine early attempts performed in 1960 were unsuccessful and enhanced RSV disease.

Palivizumab (MEDI-493) is a recombinant humanized (95 %) IgG1k monoclonal antibody binding with high affinity to an epitope at the A antigenic site of the F glycoprotein. The murine CDR sequences (5 %) derived from Mab1129 were inserted into the human IgG framework. Palivizumab exerts both in vitro neutralizing and syncytial-inhibitory activity on both RSV groups. The neutralizing activity was proved also in vivo in a study on 25 RSV-infected pediatric patients. The overall anti-RSV activity has been estimated in an animal model as 50–100-folds more potent than the previously used anti-RSV polyclonal immunoglobulin (RespiGam) [1–4].

31.2 Immunogenicity

The immunogenicity of palivizumab is low. Studies related to this aspect include MI-CP9401, in which 8/28 normal volunteers had transient and low titers of anti-palivizumab HAHA. In the main Study MI-CP018, no HAHA were detected after one injection on 915 subjects, and after five injections the rate was 0.7 %. Notably, anti-palivizumab antibodies were present also in the placebo group (1.1 %). In another small study (W00-350) only 1/18 had low titers of such antibodies. It is not clear whether they were neutralizing and/or directed to the mouse or human component of palivizumab. In Study MI-CP036, where 56 children received palivizumab for two seasons, 1/56 had transient HAHA titers, which resolved despite the use of palivizumab had continued. More interesting, two children with preexisting low-titer HAHA did not develop antibodies during the second season on palivizumab.

In a subsequent Phase IV Study, MI-CP116 (NCT00233064) on high-risk premature children (379) less than or equal to 24 months of age, immunogenicity of the lyophilized or liquid formulation of palivizumab was evaluated on 379

subjects 4–6 months after the last dose administration. One patient (0.5 %) showed HAHA in the lyophilized group, while none were detected in the liquid formulation group (overall rate 0.3 %). However, these rates may be underestimated due to the known interference of high serum concentrations of the mAb in study in conventional assay systems. Interestingly, palivizumab was immunogenic in Cynomolgus monkeys and led to the generation of anti-palivizumab antibodies in the high dosage group in 1/4 animal, although high levels of free palivizumab were still in circulation.

Anti-RSV antibodies, including anti-palivizumab HAHA, do not negatively influence the RSV infection, and reduce RSV intrapulmonary viral replication. However, the presence of anti-virus antibodies induced by one formalin-inactivated RSV vaccine in a previous experience was correlated with an unexpected severe enhancement of the clinical course of RSV infection. Finally, the presence of HAHA is not correlated to AEs induction, and does not interfere with palivizumab serum concentration [2, 3].

31.3 Adverse Events

The safety profile of palivizumab is mainly based on the 1,502 (1002 exposed) patients of the IMpact-RSV (MI-CP018) Phase III study, and on some of the mentioned supportive studies, in which only 97 pediatric patients of two open-label, non-placebo controlled studies received palivizumab in the formulation, dose, and route of administration under licensure [1]. Overall, at least one AE/subject was observed in 961 treated patients (95.9 %), and in 482 controls (96.4 %). Total AEs were 5,417 in treated subjects, and 2,737 in controls (about 5.6 AEs/patient in both groups). Among these events, the most relevant were *hypersensitivity reactions*, including *anaphylaxis* and *anaphylactic shock*. The most common prevalent AEs in the treated group included URTI (53 % vs. 49 % in controls), otitis media (42 % vs. 40 %), rhinitis (29 % vs. 23 %), rash (26 % vs. 22 %), pain (8.5 % vs. 7 %), pharyngitis (3 % vs. 1 %), and liver functional tests (1.3 % vs. 0.8 %). SAEs events higher than 1 % in the treated group included bronchiolitis (1.5 % vs. 0.8 %), and viral infections (1.5 % vs. 1.2 %), while all life-threatening episodes related to gastroenteritis (0.8 % vs. 0.6 %), pyrexia (0.1 % vs. 0 %), liver abnormal function tests (1 % vs. 0.2 %), respiratory disorders (0.3 % vs. 0.2 %), and URTI (0.1 % vs. 0 %). Among these, severe DRAEs (0.5 %) included pyrexia (0.3 %) and viral infections (0.2 %) in the treated group, while life-threatening DRAEs (0.4 %) included four cases with liver function abnormalities. The rates of DRAEs-related discontinuations were 0.5 % vs. 0.2 % in controls. Additional data emerging from the EMEA evaluations were transient and mild erythema (3.5 % vs. 1.8 % in controls), diarrhea (0.9 % vs. 0.3 %), and a lower discontinuation rate (0.2 %) in the study group.

In *CHD children less than 2 years* of age (MI-CP048) examined by EMEA, the incidence of SAEs was lower in the study group than in controls. No fatalities were attributed to the monoclonal in study, and the safety profile was superimposable to

that of other children treated with palivizumab. AEs were present in 96 % of cases as at least one event/patient, with a mean ratio of 7.03 AEs/patient. The most common events related to the respiratory system (83 %), followed by gastrointestinal disorders (52 %) and cardiovascular (47 %) events. Overall, adverse events mapping was balanced between treatment groups. Events showing a higher frequency in the study group included pyrexia (27 % vs. 24 % of placebo) and infections (6 % vs. 3 %), but their typology was balanced among groups and none was considered related to palivizumab. As for cardiac events, arrhythmia was reported as slightly higher in the study group (3 % vs. 2 %), while other cardiovascular events were either balanced or favored palivizumab. SAEs were similarly balanced (5 % vs. 4 %). Cyanosis and cyanotic events were also balanced between groups. Finally, one study (W00-350) tested multiple doses (up to 7) of palivizumab possibly needed in geographic areas were the seasonal epidemic is prolonged, with no safety profile changes.

Overall, the safety profile resulted predominantly mild/moderate, without unexpected events both as type or incidence, except for the cases with liver function abnormalities. Most signs were typical of prematurity and of bronchopulmonary disorders without active RSV infection, or appeared related to the underlying illness [1–6].

In Western Europe two additional trials were conducted in Belgium/Luxembourg (BEL-99-011) and France (FRAN-05-003), and evaluated in an EMEA assessment report by the end of 2009. The first multicenter trial (Phase III–IV) was conducted in 166 premature children, with or without bronchopulmonary dysplasia (BPD), during the three winter seasons from 1998 to 2001, when palivizumab was not yet officially available. Their follow-up lasted 150 days. Of the total SAEs (23 %), six were hospitalized and one case of dermographism was considered as drug-related. Global hospitalization (17 %) included all three seasons, and was not considered related to palivizumab. One death was attributed to the underlying BPD disease. RSV was searched during hospitalization and found positive in about 2.4 % (negative in 66 %) in the treated group. Overall, safety data of this small group were comparable to those of IMpact-RSV main study, in which RSV positivity was about 5 %. The FRAN-05-003 multicenter study was conducted on a national representative (22 % of patients receiving palivizumab in France) sample of 1,326 premature and 26 CHD children treated for one season (2005–2006) in specialized pediatric centers, with a follow-up to about one year, which confirmed the previous safety profile experienced in IMpact-RSV as evaluated by the EMEA Committee [8].

In Eastern Europe, an open-label trial (NCT01006629) was conducted in Russia on 100 patients at high risk of RSV infection, including CHD and BDP patients. In Russia RSV infection is a prevalent disease, with its highest level in Moscow (42 %), and palivizumab was introduced recently (2010). Most of the encountered AEs (41–44 %) were considered not related to the monoclonal in study, except for three cases of rhinitis, one infection, and one case of atopic dermatitis, which were subsequently excluded from the study. Infections (30 %) were mild/moderate, except for pneumonia and one case of tonsillitis. Severe events (3 %), consisting in

arrhythmia, pneumonia and tonsillitis, were not drug-related. SAEs (10 %) were all considered not related to the study group. Hospitalization during the study was within the expected range (six cases for respiratory, and one for cardiac conditions). Overall, the profile resulted particularly mild and similar to previous larger experiences. It must be noted that RSV antigen was searched only in the seven hospitalized patients and was negative. Moreover, the investigators considered the living conditions of these patients at higher risk of infections, compared to western average standard conditions [9].

Finally, a large systematic literature review and meta-analysis, aimed at evaluating the impact of palivizumab treatment on mortality and morbidity in over 15.000 high-risk children for RSV infections, showed a reduction in all-cause mortality and RSV hospitalizations [10].

31.4 Off-Label Experience

Palivizumab has been used in children not included in the indicated risk groups, in adults, and during RSV active infections. Registries and Study Groups have also been established, such as the Palivizumab Outcomes Registry (POR) in US, the Canadian Registry of Synagis (CARESS), the "Infección Respiratoria Infantil por Virus Respiratorio Sincitial" (IRIS) collecting information on the global population exposed to palivizumab under different conditions. In particular, POR was established in 2000 for prospective collection of data on high-risk US children receiving palivizumab prophylaxis. However, this Registry does not record potential adverse events related to palivizumab use. The CARESS Registry was established with the primary purpose of documenting utilization, compliance, and health outcomes of infants receiving palivizumab prophylaxis in both hospital and community settings. According to an analysis performed on this database, the number of infants receiving palivizumab prophylaxis for non-approved underlying medical conditions has increased from 5.6 % to about 19 % during the last 4 years [11]. Similarly, a nationwide survey conducted in Japan between 2006 and 2008 identified 1,115 RSV hospitalized children treated as off-label patients with palivizumab. Most of them had respiratory disorders (55 %), neuromuscular impairments (16 %) and immunodeficiency (2 %) [12].

The IRIS Registry was established in 1998 to define the national framework for prevention of RSV infections in collaboration with the Spanish Society of Neonatology (SEN). Case reports are also available on a number of rare disorders [13–15].

By utilizing the POR database, safety, and efficacy of palivizumab were evaluated on *cystic fibrosis* (CF) infants and young children. In fact, this cohort of patients is at high risk to develop serious RSV infections, has high hospitalization rates (9–15 %) comparable to BPD patients, but is not included in the official indications for palivizumab. Over 19,000 subjects recorded between 2000 and 2004, none of the 91 CF patients required hospitalization for LRTI as a result of RSV prophylaxis [16].

Additional information came from a recent Cochrane analysis on safety and efficacy of palivizumab prophylaxis in CF. After stringent selection, only one study (186 infants up to 2 years old) comparing five monthly doses of palivizumab (92) to placebo (94) over one season was considered adequate for safety evaluation. At six months follow-up one participant in each group was hospitalized due to RSV infection. No deaths in either group were observed. Any AEs were equally represented (97 % vs. 96 % in placebo), as well as for DRAEs (5.4 % vs. 4.3 %). However, SAEs were slightly higher in the treated group (20.7 %) as compared to placebo (17 %). One case of therapy-related discontinuation for an unspecified SAE was reported. Due to the dimension of the selected study, and the absence of the AEs classification adopted, no definitive conclusions were reached [17]. A similar opinion was expressed by various Authors on CF patients and on *pediatric and adult immunocompromised patients* exposed to palivizumab, including bone marrow and solid-organ transplant recipients, severe combined immunodeficiencies, and patients receiving chemotherapy. However, the Cystic Fibrosis Foundation recommended RSV prophylaxis with palivizumab in children less than 2 years of age [18].

A high risk of complications from RSV infection is observed in patients after allogenic transplantation, pre-engraftment, graft-versus-host disease, high-dose steroids, or neutropenia. The use of palivizumab in *stem cell transplanted patients*, both in children and adults with hematological malignancies or non-tumoral disorders, did not raise particular safety concerns. In some cases palivizumab was administered by IV injections and was associated to ribavirin inhalation. In one early Phase I study, no AEs were attributed to palivizumab, and HAHA were absent [19]. Similarly, in additional experiences with such patients no adverse events were detected, although efficacy of treatment was questioned [20, 21]. In fact, in a study on *leukemic children* treated with palivizumab during an outbreak of RSV infection, no protective effect was observed in most cases, probably because the infants were already in the incubation period of RSV infection. However, infections appeared mild and without drug-related safety concerns [22].

Palivizumab has been used also for *prevention of RSV infections* in lung transplanted children and adults, with various criteria, but no safety concerns were raised [23].

Overall, palivizumab administered in off-label conditions both in pediatric and adult age showed an optimal tolerance, although with occasional cumbersome efficacy.

31.5 Postmarketing Surveillance

The Abbott Postmarketing Safety database (APS) and the REACH program have accumulated a consistent amount of information on palivizumab-exposed subjects. The latter was active from 1998 to 2009 and contained over 20,000 patients safety data, which in 2006 were estimated as 13 % of the total population receiving palivizumab. A particular attention has been given to patients receiving more than

five doses of palivizumab, with the aim of detecting serious events, as also analyzed in the small W00-350 study. Overall, AEs were observed in about 1 % of patients receiving six doses. However, in the APS database 1291 SAEs were registered, of which only 5.5 % occurred over the fifth injection in the same season. Some of the signals encountered were apnea, anaphylaxis, urticaria, thrombocytopenia, and injection site reactions as rare or very rare events, which were subsequently added in the official label [2–4]. Finally, a peculiar concern regarding this monoclonal, as for anti-microbial agents, is the possibility of a drug-induced selection of resistant virus mutants. The existence of such mutants has been observed, but their clinical relevance is still debated [24].

On over 3,600 reports in the FAERS database, respiratory disorders (15.6 %), infections (13.5 %), pyrexia (3.9 %) and GI signs (3.3) were the most frequent reported AEs.

Over about the same number of reports in the EUV database, infections (34.5 %), respiratory disorders (22.9 %), cardiac and GI signs (4.4 % each) were referred as the most frequent events.

31.6 The Motavizumab Experience

Motavizumab (MEDI-524, Numax) is an investigational monoclonal antibody developed by affinity maturation of palivizumab CDRs, with approximately 75-fold greater affinity for the RSV F protein, and 20-fold neutralization activity. In an animal model motavizumab showed a significant reduction of RSV replication and increase of some cytokines (IL-1α, IL-12, TNFα). A number of clinical trials, including a large study enrolling 6,600 infants, showed a similar safety and possibly a higher efficacy profile to palivizumab. In a Phase II study on about 240 high RSV risk infants, motavizumab (M) and palivizumab (P) were administered sequentially (M/P or P/M) within the same season, to compare the effects with motavizumab alone [25]. A detailed analysis of AEs evidenced at least one AE in most patients (89–93 %) with a similar safety profile. However severe (≥grade 3) and serious events were higher (12.8 and 15 % respectively) in M/P group with respect to P/M (4–6 %) and to (5–6 %) motavizumab alone. Most common events included nasopharyngitis (25–31 %), URTI (19–20 %), bronchitis (14–16 %), diarrhea (12–16 %), irritability 12–13 %), teething (11–17 %), rhinitis (9–17 %), conjunctivitis (7–17 %), wheezing (10–18 %), dermatitis diaper (6–12 %), pharyngitis (5–11 %), injection site reactions (4–11 %), rash (4–6 %), and eczema (1–6 %). Overall, this profile was homogeneously distributed among groups. Over 95 % of events were mild. As for DRAEs, three events (visual disturbance, erythema multiforme, and ALT abnormality) were included. Interestingly, each of them received one injection of motavizumab before showing the SAE. Three discontinuations (erythema multiforme, staphylococcal scalded skin syndrome, visual disturbance) were in the M/P group. Over 50 % of all events were infections. Two deaths occurred during the study (pneumonia, sepsis). Both subjects

were in the M/P treatment group and both deaths occurred before receiving palivizumab. One additional death (intestinal obstruction), considered not related to study, occurred after 140 days from one injection of motavizumab. As for immunogenicity, ADAs were detected in 13 subjects of which eight in M/P group, four in the P/M group and one in the motavizumab group. These antibodies were directed to either or both monoclonals and persistent during the study. The subject who developed the DRAE erythema multiforme had anti-motavizumab antibodies. Overall antibody titers ranged from 1:40 to 1:1250 in the M/P treatment group and from 1:10 to 1:250 in the P/M treatment group. Notably, anti-palivizumab antibody titers ranged from 1:10 to 1:20. Cross-antigenicity was low, since only two subjects receiving motavizumab had detectable anti-palivizumab antibodies without receiving palivizumab. The overall profile was considered acceptable by the investigators. The most frequent AEs were infectious in nature, and were events common and expected in this high-risk pediatric population. Although a higher frequency of severe/serious events was indicated in motavizumab recipients, the general profile was considered comparable, and similar to a previous study [26].

In a similar study conducted on CHD patients, comparable results were obtained. In particular, about 90 % of subjects suffered at least one AE and 50 % at least one SAE. Severe events were equally distributed. Most common AEs included pyrexia (M: 30 % vs. P: 29 %), URTI (27 % vs. 28 %), cough (15 % vs12 %), rhinitis (15 % vs. 13 %), and otitis media (12.5 % vs. 11 %;). The overall AE profiles for both treatment groups were similar, with only six events differing by $\geq$2 % between groups: cough (15 % vs. 12 %), rhinitis (15 vs. 13 %), constipation (7 %vs. 5 %), irritability (4 % vs. 6 %), and ALT increased (2 % vs. 4 %).

Skin-related events were observed in detail. Most of them were nonspecific rashes that were mild, transient, and did not result in discontinuation. Motavizumab recipients experienced more hypersensitivity events (1 % vs. 0.2 %), including generalized urticaria (severe and early occurring), and drug-hypersensitivity. SAEs occurred in eight (1.3 %) motavizumab and two (0.3 %) palivizumab recipients. DRAEs were similar between treatment groups (about 8 %). However, a statistically significant higher incidence of drug-related skin events was experienced in motavizumab recipients (2 % vs. 0.3 %). There were no cases of anaphylaxis or evidence of respiratory hypersensitivity in either treatment groups. SAEs related to treatment were infrequent and comparable between treatment groups (about 1 %).

As for immunogenicity, ADAs (1.5 %) were equally distributed, and of the IgG class. No IgE were detected. No association of ADA with AEs was observed. However, three ADA positive patients in the motavizumab group had a skin event of interest, although transient. Notably, no palivizumab patients with ADA experienced such events.

Overall, the safety profile of the most commonly reported AEs was consistent with that of previous studies, except for skin events occurred more frequently (about 3 % higher) in motavizumab recipients compared with palivizumab recipients. In particular, cutaneous hypersensitivity occurred more often among

motavizumab recipients. Such peculiarity was also observed in previous studies. In contrast, no better results could be detected in term of efficacy for the investigational monoclonal in study [27].

While a number of similar studies were ongoing, MedImmune filed the original BLA on January 30, 2008 and received its first complete response by the end of the year. In June 2010, FDA declined (14/17 committee members) approval due to major concerns about safety. In fact, allergic reactions were higher in motavizumab, and particularly concerning was a threefold increase in nonfatal hypersensitivity adverse reactions, including urticaria, while there had been apparent difficulties in showing that the agent was noninferior to palivizumab. After the FDA response, there was a second request of additional data in August 2010. Subsequently, the manufacturer (AstraZeneca, after acquiring MedImmune in 2007) decided to discontinue motavizumab development and withdrew the BLA application.

31.7 Remarks

The evidence supporting the safety of palivizumab is convincing. Previous experiences with two other monoclonals (one IgA applicable on the nasal mucosa and one directed to the same viral F protein) failed to show benefits, but did not raise safety concerns. Similarly, data from postmarketing observations and from off-label experiences do not raise major problems, and indicate the possibility of expanding RSV prophylaxis to other risk groups, although their identification seems to be difficult. Palivizumab has not been associated with an increased risk of localized or systemic adverse events. Behind the typical cohort of AEs related to underlying diseases, palivizumab has not shown specific responsibilities in additional adverse events or in incrementing the incidence of pre-existing reactivities. No significant difference in hematologic, renal, or hepatic abnormalities were observed, even after the fifth dose of conventional treatment, and after repeated prophylactic cycles in following seasons. Severe/serious DRAEs have been constantly rare and manageable. ADA induction has been low and transient, with no signs of progressive increase with dosing and cycles of seasonal therapy. Moreover, their presence was not related to insurgence of AEs. Anaphylaxis and overall hypersensitivity-related events, as early signs or after re-exposures, have been reported in the order of 1:100,000 cases [28].

In contrast, some concern may come from the drug-induced selection of viral mutants resistant to palivizumab, which may open to unexpected modifications of the epidemiological profile, and enhance the hazard of these infections [24]. Therefore, further developments of effective vaccines and of other antiviral therapies are expected, possibly directed to different viral epitopes in order to avoid the spreading of resistant subtypes. Vigilance on this aspect and proper Phase IV studies should be stimulated, including the support to specific Registries on RSV infection.

Finally, the motavizumab experience showed that the consistent increase of affinity did show no better efficacy, while raising AEs rates.

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Panitumumab

32

Panitumumab (Vectibix®, Amgen) is a fully human IgG2k monoclonal antibody specifically directed to the human epithelial growth factor receptor (EGFR) present on normal and neoplastic epithelial cells. In September 2006, FDA granted an accelerated approval as a single agent for the treatment of EGFR-expressing metastatic colorectal cancer (mCRC) with disease progression or following fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy regimens. In July 2009, the indication was restricted to subjects with wild-type KRAS (Kirsten rat sarcoma viral oncogene homolog) tumors. The accelerated approval was based on the pivotal Study 20020408, a Phase III, randomized, controlled trial on 463 mCRC patients (229 exposed) comparing panitumumab monotherapy plus best supportive care (BSC) versus BSC alone. Two Phase III supportive studies provided efficacy and safety data on panitumumab in combination with chemotherapy. In particular, Study 20050181 evaluated the response to panitumumab on patients who had disease progression after FOLFIRI (5-FU, leucovorin, irinotecan) first-line treatment, while Study 20050203 (PRIME) examined patients who had not received previous chemotherapy for mCRC and were treated with panitumumab associated to FOLFOX (5-FU, leucovorin, oxaliplatin) therapy. The former study was also the basis for full approval, while the subsequent studies 20040192, 20050216, and 20060314 on 169 patients showed that benefits of panitumumab were limited to wild-type KRAS tumors and determined the subsequent restriction of indication to this class of patients. Finally, Study 20040249 observed additional safety signals deriving from panitumumab in combination with bevacizumab and oxaliplatin-containing chemotherapy, which contributed to amendments of official labels after June 2008. Overall, 15 studies including four Phase I trials (Studies 200-30138, −30251, −40116, −40192) enrolling 186 subjects, eight Phase II trials (Studies-200-30167, −30250, −25405, −20374, −25408, −30110, −25404, −25409) enrolling 816 patients, and the mentioned

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Phase III (Study 20020408 and extension 20030194) enrolling 638 patients, are the consistent database for this monoclonal. The majority of patients were mCRC (1,012), while the remaining 628 had various types of neoplasms, including NSCLC, RC, PC, pancreatic carcinoma, esophageal carcinoma, CRC and other types of solid tumors. On this basis, in 2007 EMEA granted approval for panitumumab as monotherapy for the treatment of patients with EGFR-expressing metastatic colorectal carcinoma with nonmutated (wild-type) KRAS after failure of fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy regimens. In March 2011, this Agency granted a conditional approval to extend the indication to the use of panitumumab in combination with FOLFOX in first-line treatment and with FOLFIRI in second-line treatment after failure of first-line fluoropyrimidine-based chemotherapy (excluding irinotecan). In 2008, panitumumab was approved by Health Canada as monotherapy for the treatment of patients with EGFR-expressing metastatic colorectal carcinoma with nonmutated (wild-type) KRAS after failure of fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy regimens [1–6].

At present, panitumumab is approved in more than 40 countries as a monotherapy treatment for patients with wild-type KRAS mCRC, when standard monotherapy is no longer effective. In Russia, Japan and Israel, panitumumab is also approved in combination with chemotherapy in patients with wild-type KRAS mCRC. More than 150 trials are completed or ongoing.

32.1 Mechanism of Action

EGFR (cErbB-1, HER 1 in humans) is a transmembrane protein of a subgroup of Type 1 receptor tyrosine kinase (RTK), the ErbB family, which includes EGFR, HER2, HER 3, and HER 4. EGFR is constitutively expressed in many epithelial tissues, including skin and hair follicles as well as in epithelial cancer cells. There are 11 known natural ligands to these receptors, including TGFα, HB-EGF, EGF, epigen, betacellulin, AREG (amphiregulin), and EREG (epiregulin), all interacting with EGFR. However, HB-EFG, betacellulin and EREG also interact with HER 4, but not with the other two ligands of the subgroup. Upon interaction, EGFR forms homo- or heterodimers with other ErbB receptors, a step related to activation of the receptor/ligand complex, via the intracellular tyrosine kinase pathway. The signaling produces DNA synthesis, cell cycle progression, migration, adhesion and proliferation of cells expressing EGFR. Therefore, this pathway is crucial for the homeostasis of epithelia, for the innate immunity and also as a downregulator of myelin regeneration. EGFR is usually overexpressed on neoplastic cells of epithelial origin, and in particular on CRC, lung carcinoma, SCCHN, and on GBM, due to gene mutations/overactivity leading to uncontrolled cell division, angiogenesis, cell migration, and cellular invasion/metastasis.

Panitumumab (ABX-EGF) is the first fully human IgG2k monoclonal antibody specifically directed to the EGFR present on normal and neoplastic epithelial cells. The binding is more pronounced in cells overexpressing EGFR ($\geq$15,000 per cell)

and is inactive in EGFR-negative tumors. The high affinity blockade of EGFR prevents ligand-induced receptor autophosphorylation and receptor-associated kinases activation, thus providing inhibition of cell growth, induction of apoptosis, proinflammatory cytokine (IL-8) and VEGF decreasing, and EGFR internalization. The inhibition of growth and survival is shown also on neoplastic cells expressing EGFR. Moreover, the panitumumab-EGFR complex is rapidly internalized, thus resulting in a downregulation of the receptor. Additional mechanisms of action include the inhibition of angiogenesis, but no immune-mediated cytotoxic actions, since the IgG2 isotype is not able to induce a significant complement activation and ADCC activity. However, certain aspects of the overall capacity of panitumumab to destroy tumor cells are not clear yet, as confirmed by clinical experience and by the comparison of panitumumab with its precursor chimeric cetuximab. Despite they are both directed to the same EGTR receptor, panitumumab revealed a higher receptor affinity and a consequent stronger cytotoxic action on EGFR-positive tumor cells.

On the assumption that the receptor blocking is the only cytotoxic mechanism of panitumumab, its activity should be linearly dose-dependent, reflecting full saturation of EGFR binding sites and their subsequent degradation after internalization. However, PKD of panitumumab showed that its clearance is not linear, thus suggesting that there are other effector mechanisms. Moreover, EGFR-negative tumors, presumably resistant to panitumumab, are sensitive to the action of cetuximab, which acts also via CDC and ADCC. Finally, the levels of expression of EGFR are not correlated to clinical response to panitumumab or cetuximab. Quite recently, an accurate comparative analysis revealed two large, partially overlapping, functional epitopes consisting of 17 critical amino acid positions. Four of them were selectively targeted by cetuximab, and other four were selectively recognized by panitumumab [7].

Since EGFR is consistently expressed at skin level, adverse cutaneous reactions were expected, and found to be dose related and associated with the treatment outcome. However, only 4 % (serious 1 %) of the exposed subjects developed hypersensitivity reactions with panitumumab, with respect to 19 % (serious 3 %) reported for cetuximab. Overall, these differences indicate that either humanization of the former antibody and/or the capacity to express CDC and/or ADCC could be responsible for the differences in clinical response and the induction of major cutaneous AEs, showing that beneficial and adverse consequences could be separated to a certain extent [7–11].

32.2 Immunogenicity

In theory, a fully human monoclonal antibody is still immunogenic, albeit expected at low rate, for at least two reasons: it has nonhuman unique coding sequences in the CDRs, and it is a foreign protein to the patient immune system. In addition, during the cell manufacturing process an extra sequence of light chain (1 %) can be translated as a monomer. Immunogenicity to both proteins was

assessed. According to treatment protocols and laboratory testing, HAHA was detected as <1 to 4.6 % in monotherapy experiences, and <1 to 1 % in associated therapy. Neutralizing antibodies were <1 to 1.6 %. Their presence was not related to clinical performance or to induction of AEs. In a detailed investigation on 1,126 mCRC patients treated with panitumumab in combination with oxiplatin- or irinotecan-based therapy, 1.8 % was found positive to HAHA and 0.2 % of them showed neutralizing antibodies. However, preexisting antibodies were also found in 3.8 % of cases, in part (0.4 %) with neutralizing activity, indicating the existence of previous sensitization and cross-antigenicity. Positivity was higher in the oxaliplatin combination (2.9 and 0.4 % neutralizing) with respect to irinotecan-treated group (0.9 and 0 %). No association with AEs induction was found either in preexisting or in induced HAHA positivity. Similarly, the presence of preexisting antibodies did not affect the post-dose antibody response. Notably, in this study the immunogenic potential of the unique CDR sequences were evaluated and found avoid of agretopes theoretically capable of binding to the eight most common HLA-DRB1 alleles, usually presenting epitopes to TCR-bearing T cells [2, 6, 11, 12].

It must be noted that the chimeric cetuximab showed also some hypersensitivity reactions consistent with preexisting IgE directed to an oligosaccharide, and a cross-reactivity to a sialic acid, both present as residues of manufacturing (see cetuximab, Chap. 15). Both saccharides are absent in the panitumumab processing.

32.3 Adverse Events

The original general safety profile of panitumumab is based on 1,467 patients, 1,293 of them treated as monotherapy and 174 in combination with chemotherapy, from the previously mentioned 15 clinical trials, including mCRC (62 %) and other solid tumors. However, most detailed data refer to the main Study 20020408 (463 mCRC) including 229 patients in panitumumab monotherapy and 234 controls. Updated results up to 2012 were overviewed by EMEA on 2,588 patients in monotherapy and combination treatments, reporting slightly higher frequencies of most common events.

Boxed warnings on *dermatologic toxicity* and *infusion reactions* were included in the official label of panitumumab, since its first release in 2006. They were reported in 90 and 4 % of patients, respectively, and as severe in 16 and 1 % of cases. The overall profile includes also *pulmonary fibrosis/ILD*, *electrolyte depletion, photosensitivity, and increased toxicity/mortality* when combined with chemotherapy.

The *most common* adverse reactions included skin reactions (90 % vs. 9 % in controls), including erythema (65 % vs. 1 %), dermatitis acneiform (57 % vs. 1 %), pruritus (57 % vs. 2 %), hypomagnesemia (38 % vs. 2 %), fatigue (26 % vs. 15 %), abdominal pain (25 % vs. 17 %), paronychia (25 % vs. 0 %), skin exfoliation (25 % vs. 0 %), nausea (23 % vs. 1 %), rash (22 % vs. 1 %) with variable

presentations, diarrhea (21 % vs. 11 %), acne (13 % vs. 0 %), and peripheral edema (12 % vs. 6 %).

The *most serious* adverse reactions were dermatologic toxicity (16 % vs. 0 %) complicated by infectious sequelae and septic death, pulmonary embolism (7 % vs. 4 %, fatal <1 %), dermatitis acneiform (7 % vs. 0 %), abdominal pain (7 % vs. 5 %), infusion reactions (4 % vs. 1 %), hypomagnesemia (4 % vs. 0 %), constipation (3 % vs. 1 %), vomiting (2 % vs. 1 %), nausea (1 % vs. <1 %), and pulmonary fibrosis (<1 % vs. 0 %).

Life threatening and fatal complications included necrotizing fasciitis, abscesses, and sepsis, pulmonary embolism, infusion reactions (postmarketing). Notably, cases of interstitial lung disease (ILD), both fatal and nonfatal, have been reported, mainly from the Japanese exposed population.

Increased mortality and an increased frequency of AEs were observed in studies (PRIME) on panitumumab associated either to FOLFOX or to bevacizumab, and in another oxaliplatin-containing therapy study (20040249), as compared to chemotherapy. In the latter study, there was also an increased incidence of serious/severe events (87 % vs. 72 %), including rash/dermatitis acneiform (26 % vs. 1 %), diarrhea (23 % vs. 12 %), hypokalemia (10 % vs. 4 %), pulmonary embolism (7 % vs. 4 %), stomatitis/mucositis (4 % vs. <1 %), and hypomagnesemia (4 % vs. 0 %).

The safety profile from the EMEA overview, as pooled data from monotherapy and combined therapies, reported *severe skin reactions* in 34 % of patients. Most common events included skin reactions (93 %), diarrhea (50 %), nausea (41 %), vomiting (27 %), constipation (23 %), abdominal pain (23 %), fatigue (37 %), pyrexia (20 %) anorexia (27 %), paronychia (20 %), rash (45 %), dermatitis acneiform (39 %), pruritus (35 %), erythema (30 %), and xeroderma (22 %). When comparing most relevant SAEs differences between monotherapy and associated chemotherapy, all events were 72 % (monotherapy) versus 87 % (combined), severe skin reaction were 16 % versus 26 %, diarrhea occurred in 2 % versus 17 % respectively, with a high incidence of severe diarrhea in the FOLFIRI combined group. In the combination with bevacizumab and triple chemotherapy a greater frequency of pulmonary embolism, infections (mostly cutaneous), diarrhea, electrolyte imbalances, nausea, vomiting, and dehydration was also observed, leading to a worse and shortened survival. Adverse reactions requiring discontinuation of panitumumab were mostly due to infusion reactions, severe skin toxicity, paronychia, and pulmonary fibrosis. Photosensitivity, keratitis and ulcerative keratitis were also reported in studies and in the postmarketing setting. [1–6].

Overall, adverse events of panitumumab in mCRC patients were consistent, but selective and manageable. Skin and infusion reactions were predominant, followed by intestinal disorders. The overall safety profile was similar to other anti-EGFR monoclonals, with a milder profile with respect to the chimeric cetuximab. However, severe/serious events were more frequent in protocols combining panitumumab with chemotherapy and with other monoclonal antibodies.

Since a low efficacy of panitumumab was identified in *KRAS mutated tumors* with respect to wild-type mCRC, differential evaluations of AEs have been attempted. A retrospective analysis in 24 Japanese patients treated with panitumumab as monotherapy in two Phase II studies (20040192, 20050216) examined AEs occurring in wild type (WT) or mutated type (MT) KRAS settings. All patients experienced at least one AE, related or unrelated to panitumumab. Most common events (63, 13 % severe) were constitutional (fatigue, anorexia). Severe (25, 8 % drug-related) and serious (13, 4 % drug-related) but typical skin events (25–60 %) and hypomagnesemia (38 %) were all mild/moderate. Overall, no major differences were reported between AEs expression in KRAS mutated and non-mutated settings [13]. A similar analysis in panitumumab therapy combined with FOLFIRI was performed in a wider cohort of 145/154 KRAS typed patients including 86 subjects with WT KRAS tumors (Study 20060314, or NCT005008404). As expected, most common AEs were dermatologic (98 %). Other frequent events were diarrhea (79 %), stomatitis/oral mucositis (51 %), vascular toxicity (32 %), and hypomagnesaemia (21 %). Severe events included diarrhea (24 %), neutropenia (18 %), acne and rash (10 % each), pulmonary embolism (8 %), and paronychia (6 %). Suspected infusion reaction (13 %) were all mild/moderate. Serious events (55 %) were considered as drug related in 28 % of cases. When considering the KRAS settings, serious diarrhea was predominant in the wild type (15 %WT vs. 8 % in MT), while other signs such as vomiting, neutropenia, fatigue, dehydration and, most importantly, pulmonary embolism were equally distributed (about 2–3 % each). Drug-related discontinuation rates (20 %) were also similarly distributed, but a higher proportion of patients in the KRAS MT (29 %) versus WT (22 %) groups had AEs leading to panitumumab discontinuation. Fatal events were slightly increased in the MT (10 % vs. 8 %) subgroup.

Overall, no significant differences in AEs emerged between the two KRAS settings [14]. Since skin toxicity was a major concern with panitumumab, this aspect was further analyzed. In the same trial (20060314) on 154 patients (86 WT, 59 MT), most of them (98 %) experienced integument-related toxicities, including rash (42 %), xeroderma (40 %), acne (36 %), eye (38 %, mostly conjunctivitis), hair (38 %, mostly alopecia) and nail (32 %, mostly paronychia) toxicities, dermatitis acneiform (21 %), and cheilitis (3 %). Eye toxicities were more frequent in the WT KRAS group compared with the MT group (45 % vs. 29 %), whereas hair (31 % vs. 51 %) and nail (29 % vs. 37 %) toxicities were more present in the MT group. In fact, differences in exposure-adjusted AE rates between the two groups were observed for integument-related toxicities (2938.8 vs. 3284.4 events per 100 P/Y, respectively). Severe cutaneous toxicity rates were 68.8 and 106.5 events per 100 P/Y (WT vs. MT KRAS groups, respectively). Rash (43 % vs. 26 %), xeroderma (45 % vs. 36 %), conjunctivitis (27 % vs. 14 %), skin fissures (23 % vs. 17 %), pruritus (24 % vs. 14 %), skin toxicity (16 % vs. 7 %), and erythema (12 % vs. 7 %) were more common in the WT KRAS group. Alopecia (29 % vs. 44 %) and PPES (14 % vs. 22 %) were more common in the MT group. Among severe/serious only acne (7 % vs. 14 %) and PPES (1 % vs. 3 %), were more frequent in the MT KRAS group [15].

In the 20050184-STEPP (skin toxicity protocol panitumumab) study, cutaneous disorders were evaluated at first as incidence of protocol-specified skin toxicities during the 6-week treatment period, and in a secondary analysis as AEs related to concomitant administration of panitumumab with irinotecan-based chemotherapy in mCRC by KRAS tumor mutation status [16]. A total of 87 patients were screened for KRAS (49 WT, 38 MT), and all had at least one AE. Severe AEs were present in WT (61 %) and MT (37 %) patients. In particular, dermatitis acneiform (16 % WT vs. 11 % MT), fatigue (12 % WT vs. 8 % MT), and diarrhea (24 % WT vs. 21 % MT) were more represented in the WT setting, while nausea (13 % MT vs. 4 % WT), vomiting (13 % MT vs. 4 % WT), paronychia (5 %MT vs. 2 % WT), and dehydration (16 % MT vs. 10 % WT) were more frequent in the MT setting. Serious events were 33 % in WT and 42 % in MT settings, including two neutropenia and two hypomagnesemia in WT KRAS tumor group, and two neutropenia and one paronychia in the MT group. Most severe (grade $\geq$ 3) AEs were reported more frequently in patients with WT KRAS tumors versus patients with MT KRAS tumors. Overall, the profile was within the expected range, and the AEs distribution between the two KRAS settings remains only indicative, due to the sample size of the study. However, data on skin toxicity seem to pinpoint some differences in reactivity according to KRAS status, although they need to be confirmed.

Two meta-analyses on clinical trials involving panitumumab in mCRC WT KRAS status have been recently published. In one recent report, four studies (2 first-line, 2 second-line, and one salvage therapy) on 2,115 patients treated with panitumumab, bevacizumab, and various chemotherapy regimens (FOLFOX, FOLFIRI, and other oxaliplatin-based therapy) or BSC were selected [17]. Severe/serious events related to panitumumab were skin toxicity, diarrhea, hypokalemia, and hypomagnesemia. On the other hand, panitumumab use was not associated with significantly higher incidence of neurologic toxicity, neutropenia, or infusion-related reaction. Reported panitumumab-related death was rare as well as infusion-related reaction or death (<1 %). In the second meta-analysis, 1,270 patients from four studies were treated with cetuximab or panitumumab associated to oxiplatin based. Differently from other similar studies, the effect of chemotherapy with anti-EGFR mAbs to oxaliplatin-based chemotherapy alone in mCRC patients was estimated by excluding the influence of irinotecan-based regimen. No fatal AEs were related to cetuximab or panitumumab. An increased cutaneous toxicity was observed related to anti-EGFR treatment [18].

Overall, the most recent data and meta-analyses confirm the safety profile depicted in pivotal studies, both in term of frequency and typology, possibly indicating some differences of reactivity related to the KRAS status. The general safety profile is more pronounced in combined therapy, but no new signals emerged and their manageability remained acceptable. Although no synergistic effects were seen on AEs induction, some additional drug effects in expanding the AEs typology and major evaluations on efficacy and resistance to panitumumab during treatment remain to be cleared.

Finally, the safety profile of another emerging therapy combination in mCRC, including anti-VEGF and anti-EGFR monoclonals has been recently analyzed. In particular, bevacizumab has been associated to cetuximab or panitumumab on the assumption that the concurrent inhibition of VEGF and EGFR pathways may enhance the respective antitumoral therapeutic effects. The primary concern was about a possible enhancement of the respective adverse events. In particular, relevant AEs such as hypertension, thromboembolic events, proteinuria, bleeding, and gastrointestinal perforation have all been associated with bevacizumab, while dermatologic toxicities and hypomagnesemia are typical effects of cetuximab and panitumumab. A recent overview provided a comparison between severe/serious events occurring in first-line setting of bevacizumab and panitumumab in combination with oxaliplatin- or irinotecan-based chemotherapy, or in second-line setting combination with FOLFIRI, or as monotherapy with best supportive care. In fact, panitumumab profile was similar to cetuximab, albeit milder, and included mainly skin toxicities in first- and second-line settings. However, a higher incidence of severe/serious AEs were observed with the addition of panitumumab, compared with bevacizumab plus oxaliplatin-based chemotherapy alone (36 % vs. 1 %), bevacizumab plus irinotecan-based chemotherapy alone (38 % vs. 0 %; grade 3 only), FOLFIRI (32–37 % vs. 1–2 %), or best supportive care (14 % vs. 0 %). The second concern related to infusion reactions occurring in 15–21 % (2–5 %, $\geq$ grade 3) of patients in various clinical indications. Out of the four studies examined, two reported statistical significant differences among these categories. Finally, ocular complications, including conjunctivitis (4 %), ocular hyperemia (3 %), increased lacrimation (2 %), and eye/eyelid irritation (1 %) represented the third major concerning group of AEs. When comparing panitumumab with BSC, ocular toxicities were observed in 15 and 2 % of patients, respectively ($<$1 % vs. 0 %, $\geq$ grade 3) [19].

32.4 Off-Label Experience

The experience in the treatment of *other solid tumors* with panitumumab is limited. After some encouraging results with cetuximab in SCCNH tumors, panitumumab is being studied in these patients. In two Phase II (PRISM, PARTNER) and one pivotal Phase III (SPECTRUM), the latter enrolling 657 recurrent/metastatic SCCHN patients receiving cisplatin-5-FU chemotherapy with or without panitumumab, the results were not satisfactory in terms of overall survival. However, a more recent subanalysis of these patients showed that an improvement was present only in HPV-negative tumors (78 %), although statistical significance was not reached. Adverse events in the panitumumab arm were as expected. Any SAEs (51–53 % vs. 32–41 %) and SAEs in HPV subgroups (86 % vs. 87 % in HPV + and 77 % in HPV −) were respectively comparable. AEs leading to study or drug discontinuation were also similar in both study and control groups (14 % vs. 12 %)[20].

On the assumption that the concurrent inhibition of VEGF and EGFR pathways may enhance the antitumoral therapeutic effects, a Phase Ib study examined the combination of escalating doses of motesanib (anti-VEGF agent), with panitumumab and gemcitabine/cisplatin in 41 patients with advanced solid tumors, including advanced NSCLC (51 %), and pancreatic (12 %) carcinoma. These patients received a median of six infusions of panitumumab, and were followed for 24 weeks, but subsequently discontinued motesanib (32 %) and panitumumab (27 %) for incoming AEs, or for disease progression. Although the study was mainly directed to explore motesanib safety, selected AEs related to panitumumab were observed in 98 % of patients and included erythema (62 %), rash (54 %), nausea (51 %), fatigue (46 %), pruritus (44 %), hypomagnesemia (41 %), diarrhea (39 %), vomiting (29 %), anorexia (27 %), anemia (17 %), and xeroderma (17 %). Drug-related SAEs (27 %) included DVT (7 %), pulmonary embolism (7 %), and diarrhea (5 %). However, the serious vascular disorders were only observed in patients receiving motesanib. Overall, strictly related panitumumab events were limited to the known cutaneous (rash, dermatitis acneiform, erythema, pruritus) events ranging between 12 and 14 % [21].

In a similar study, 32 patients with solid tumors including CRC (10), ovarian (4), chondrosarcoma (3), NSCLC (2), and other tumors were all treated with everolimus, panitumumab, and bevacizumab (anti-VEGF) drug combinations. Common adverse events were skin rash/pruritus (91 %), mucositis/stomatitis (75 %), hypomagnesemia (72 %), hypocalcemia (56 %) and hypokalemia (50 %). Severe events included hypophosphatemia (19 %), skin rash/pruritus (16 %), hypokalemia (16 %), hypertension (16 %), and mucositis/stomatitis (13 %). Because of the protocol typology, it was not possible to distinguish AEs pertaining to each drug used. However, mucositis/stomatitis appeared as severe (13 %) in patients receiving higher doses of everolimus. Hematologic events included neutropenia, thrombocytopenia, and anemia, each occurring with similar frequency (about 30 %, severe 3–6 %). Bleeding events (34 %) included epistaxis (28 %), hematuria (6 %), and rectal bleeding (3 %). Severe constitutional and laboratory signs were infrequent. Overall, adverse events were all predictable and mostly mild/moderate and manageable [22].

On a similar assumption, the concurrent action of tyrosine kinase inhibitors (erlotinib, sorafenib), one anti EFGR (panitumumab) agent and one new monoclonal (ganitumab) anti IGF-1 (insulin-like growth factor 1) receptor, was recently experienced in various refractory solid tumors including colon (11), ovarian (6), breast (4), and NSCLC (3). The study was mainly directed to assess the potential efficacy of two ganitumab doses associated to four different supportive therapies fractionated in eight small cohorts of patients suffering different tumors. Therefore, AEs were considered mostly with respect to the monoclonal in study, and only two cohorts (10 patients) were associated to panitumumab. Nonetheless, the safety profile potentially related to co-therapies was considered consistent with the respective known profiles. As expected, skin rash was predominant in the panitumumab groups, in which some HAHA positivity (2 %) was also detected. Most importantly, there were no apparent synergistic effects on AEs [23].

Limited experience is present on treatment of biliary tract cancer mainly with cetuximab. A recent open-label Phase II trial evaluated the safety of panitumumab in 45 wild-type KRAS biliary carcinomas receiving at least one cycle of therapy. These patients received also gemcitabine, oxiplatin, and capecitabine in doses adjusted according to evidenced toxicities. Temporary panitumumab discontinuation and subsequent dose reduction were adopted in one case of skin reaction. Total DRAEs included rash 82 % (20 % ≥ grade 3), pain 67 % (4 %), 62 % (4 %) nausea, stomatitis 55 % (4 %), HFS 51 % (4 %), infections 49 % (9 %), diarrhea 40 % (3 %) vomiting, diarrhea 47 % (6 %), and sensory 55 % (7 %) or motor 20 % (2 %) neuropathy. Also in this case the most frequent AE attributed to panitumumab was skin rash, and overall safety profile was considered acceptable [24].

32.5 Postmarketing Surveillance

By the end of 2012, FAERS files contained 3,987 reports on panitumumab/Vectibix including 13,830 events (AEs/R 3.5). Dermatological disorders (8.1 %), infections (7.2 %), electrolyte disorders (4.7 %), GI disorders (4.2 %), WBC unbalance (3.5 %), and GI signs (4.2 %) were most commonly signaled. Most frequent reported signs were diarrhea (605 reports), dermatitis acneiform (595), ILD (310), febrile neutropenia (277), hypomagnesemia (276), and sepsis (108). Other relevant AEs included DIC (29), intestinal perforation (28), PPES (18), and SIRS 17.

Hypersensitivity and cutaneous reactions included rash (193), rash maculo/papular (12), anaphylactic shock (6),), and epidermal necrolysis (6).

Eye disorders included mainly conjunctivitis (25), and keratitis (6).

In the EUV database, at the same endpoint, there were 664 reports including 1,961 AEs (2.9 AEs/P). Cutaneous reactions (25.8 %), gastrointestinal disorders (9,8 %), respiratory disorders (8,4 %), infections (5,9 %), and metabolic disorders (4,3 %) mainly as hypomagnesemia and hypocalcemia, were the most represented.

Most frequent reported signs were rash (87), diarrhea (44), dermatitis acneiform (39), ILD (22), rash pustular (21), hypersensitivity reactions (19, 1 drug-related), neutropenia, (15) and sepsis (10), PPES (7), febrile neutropenia (6), and DIC (1).

Eye disorders were included in 73 reports indicating conjunctivitis (10), corneal ulcer/perforation (3), keratitis (5), and ocular toxicity signs (2).

32.6 Remarks

The safety profile of panitumumab was as expected for an anti-EGFR monoclonal antibody and was considered clinically manageable, as evidenced in particular by the STEPP study, when preemptive skin treatments were adopted. The most commonly reported adverse reactions were integument toxicities and diarrhea, occurring in general with lower severity than in the cetuximab clinical experience.

Immunogenicity and possibly related events were rare. This peculiarity was expected from a fully human monoclonal antibody as panitumumab, although exceptions are known (alemtuzumab). Although targeting the extracellular portion of the same receptor, the panitumumab and cetuximab exerted some differences in terms of efficacy and safety. They showed different affinity, but there was no correlation between this capacity or the level of expression of the target (EGFR) and the clinical response. The recent quasi-difference in recognizing the two epitopes on EGFR by the two monoclonals may explain some of these diversities [7]. The mechanisms of primary and secondary tumor resistance to both antibodies seem to be also different, and mainly related to the KRAS status, since the resistance to one monoclonal can be by-passed by the other, but differences in the generation of AEs in WT and MT variants are not clear yet [11, 25]. Other potential mechanisms of resistance may be involved, such as VEGFR or IGF-1 receptor pathways, which may explain also potential different safety profiles as indicated by some limited observations.

Even if the EGFR presence is necessary for their action and reaction, at least at skin and intestinal level, in some instances cetuximab, but not panitumumab, showed to exert anti-tumoral activity on EGFR-negative targets, indicating that EGFR may not be the dominant oncogenic pathway in all epithelial tumors. Some synergistic effects between panitumumab and chemotherapy were observed in terms of efficacy, but not in enhancing the frequency or severity of drug-related AEs.

Because of their relevance and the possible association with clinical efficacy, cutaneous adverse events have been accurately evaluated. In a recent meta-analysis, global rash (acneiform rashes, acne-like rashes, skin toxicity, skin rashes, and rashes in general) rates induced by EGFR inhibitors were evaluated in comparison with chemotherapy and BSC [26]. These results were presented as attributable to cetuximab and panitumumab, although only 1/13 of the selected trials were related to this monoclonal. Nonetheless, compared with non-EGFR therapy, the monoclonals carry an overall risk of 74 % for rashes of all severities and of 12 % for grades 3 and 4 rashes. It must be noted that usually rash expressions reach their maximal intensity in 3–5 weeks, and then tend to decrease in spite of the continued treatment [9, 10].

More attention should be given to ocular disorders, which have been more frequently observed, and represent the third more frequent AE in this therapy, although they are infrequently reported in postmarketing surveillance databases. Moreover, eye toxicities were more frequent in the WT KRAS patients compared with the MT variants (45 % vs. 29 %). It must be noted that EGFR is expressed in basal epithelial cells of the cornea and conjunctiva, and is present in tears. In recent case reports and retrospective analysis, an interesting aspects was focused. One case of corneal erosion was successfully treated with topical EGF therapy to antagonize the effect of cetuximab. This offers the chance of adopting preemptive therapies, at least at skin and ocular level, thus mitigating the local induction of AEs, and confirms differences between safety profiles of these two classes of inhibitors acting on the same pathway [16, 27, 28].

While most AEs find a reasonable relation to the mechanism of action of panitumumab, hypomagnesemia, and venous thromboembolic events deserve some additional consideration. EGFR is expressed on renal tubules, and glomerular vascular-epithelial structures, being involved in maintaining tubular integrity and regeneration. Therefore, the blockade of EGFR impairs their physiological functions, including Mg readsorption. VTE events may be considered as unexpected in relation to panitumumab mechanism of action. However, EGFR inhibition produces a decrease in VEGF (and other factors, including cytokines), which in turn cause an increase of endothelial damage and apoptosis. VTE and ATE events are frequently encountered in cancer patients. However, VTE, but not ATE, result increased both with cetuximab and panitumumab, as reported by a meta-analysis on over 7,000 patients. In particular, panitumumab showed a VTE incidence of 6.1 % versus 4.8 % in controls. Moreover, the incidence of the two monoclonals was higher (5.9 % vs. 2.6 %) with respect to other TYK inhibitors [29].

Finally, a more comprehensive approach in evaluating the impact of AEs, such as the quality-adjusted time without symptoms of disease or toxicity (Q-TWiST) analysis, should be encouraged. This kind of evaluation allows a better understanding of the overall negative impact of AEs during therapy, instead of considering the specific impact of each adverse event. In a Q-TWiST analysis on panitumumab versus BSC, the association significantly improved quality-adjusted survival, compared with BSC alone [30].

Overall, panitumumab shows an acceptable safety profile in a variety of conditions, with mostly mild/moderate and selected reactions. As for other similar conditions, understanding of its safety profile allows to enhance AEs manageability, including the targeted introduction of preemptive therapy.

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Pertuzumab

33

Pertuzumab (Perjeta®, Genentech) is a recombinant humanized IgG1k monoclonal antibody binding the extracellular dimerization subdomain II of the human epidermal growth factor 2 (HER2). In June 2012, FDA granted full approval for the treatment of HER2-positive metastatic breast cancer (mBC), in association with trastuzumab and docetaxel, in patients not previously treated with anti-HER2 therapy or with chemotherapy. The CHMP Committee from EMEA issued a positive opinion in December 2012 for the use of pertuzumab in the same combination therapy in HER2-positive mBC or in locally recurrent unresectable breast cancer (BC), with the same therapeutic limitations, and final approval was issued in March 2013.

The basis for approval consisted in WO20698/TOC4129g (CLEOPATRA) Phase III Study, performed on 804 (402 exposed) patients with HER2-positive mBC. Supportive studies included 3 Phase II studies (TOC3258g, WO20697–NEOSPHERE-, B017929) enrolling 642 patients, and 8 preliminary dose escalation and combination therapy Phase I-II studies on a number of solid tumors on 453 patients.

The safety profile was based on 804 (397 exposed) of these patients, and on a supportive database of 1,400 patients treated with the drug in study for various malignancies [1–3].

At present, on 39 registered trials 10 are active, 11 completed, and 17 recruiting.

33.1 Mechanism of Action

HER2 (CD340, ErbB-2, Neu, p185) is a cell surface protein encoded by the ERBB2 gene and a member of the erythroblastic leukemia viral oncogene (ErbB) family of tyrosine kinase receptors, which includes EGFR (ErbB-1) and three HER

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types (2,3,4). They consist of four extracellular domains, one transmembrane region, and one intracytoplasmic tyrosine kinase domain. There are 11 known natural ligands to these receptors, including TGFα, HB-EGF, EGF, epigen, betacellulin, AREG (amphiregulin), and EREG (epiregulin), which interact with EGFR. However, no ligands are known to interact with HER2, while HB-EFG, betacellulin, and EREG interact also with HER4, but not with the other two ligands of the subgroup.

Upon interaction, EGFR form homo- or heterodimers with other ErbB receptors: This step is necessary to activate the receptor/ligand complex, via the intracellular tyrosine kinase pathway. In the case of HER2, it has been suggested that the activation follows heterodimerization with EGFR, HER3 or HER4, and the relative receptor ligand(s) binding. For example, the co-expression of HER2 and HER3 leads to high-affinity EREG binding, followed by tyrosine phosphorylation, and a potent mitogenic signal inducing cell proliferation and inhibition of apoptosis via MAPK and PI3K pathways. In particular, the HER2 homodimer formation activates preferentially MAPK, and heterodimers trigger both pathways. Moreover, HER2 dimerization induces the degradation of p27kip1 cell-cycle inhibitor. HER2 is expressed at low level on epithelial cells, including the mammary gland, ovary, lung, liver, kidney, and in CNS. The signaling essentially produces DNA synthesis, cell cycle progression, migration, adhesion, and proliferation of cells expressing EGFR. Therefore, this pathway is crucial for the homeostasis of epithelia, for innate immunity, and also as a downregulator of myelin regeneration. Alternatively, in the presence of HER2 overexpression, the spontaneous formation of homodimers on the neoplastic cell surface triggers transmembrane signaling capable of inducing tyrosine phosphorylation. HER1 and HER2 are overexpressed on neoplastic cells of epithelial origin, and in particular, HER1 is mostly overexpressed on CRC, lung carcinoma, SCCHN and on GBM, and HER2 is expressed on breast and gastric carcinoma, due to gene mutations/overactivity, and leading to uncontrolled cell division, angiogenesis, cell migration, and cellular invasion/metastasis. In particular, HER2 is constitutively overexpressed in 25–30 % of primary breast cancer, in 6–24 % of gastric cancer, in 15–33 % of gastroesophageal junction cancer, in 16–21 % of esophageal cancer, in 7 % of distal gastric cancer, and is usually associated with a poorer prognosis.

Pertuzumab (rhuMAb 2C4) is a recombinant humanized IgG1k monoclonal antibody developed by inserting murine CDRs recognizing the subdomain II of the extracellular portion of HER2, in the same human Fc framework used for trastuzumab. The subdomain II is involved both in HER2 homodimerization and heterodimerization with other HER receptors expressed on the same cell surface. Therefore, the binding of pertuzumab interferes with HER-dependent downstream activation of PI3K and MAPK tyrosine kinase pathways, the latter being particularly activated by the HER2-HER3 heterodimerizaiton. The different binding sites of pertuzumab and trastuzumab on the same extracellular part of HER2 and the enlarged capacity of the former in inhibiting HER dimerization potentiate the overall blocking effects of the two monoclonals, and exert synergistic anti-proliferative activities on human tumor cells in xenograft models. Therefore,

consistent with the mechanism of action, pertuzumab activity is not restricted to tumors with overexpression of HER2. Moreover, both monoclonals activate antibody-dependent cell-mediated cytotoxicity (ADCC). However, the clinical efficacy of pertuzumab in monotherapy is low, but exerts high synergistic effects when combined with trastuzumab. Their complementarity, together with additional combinations of tyrosine kinase inhibitors and chemotherapy, are the present conceptual mainstream for novel therapeutic strategies in the field [1–5]; (see also Chap. 38).

33.2 Immunogenicity

The presence of anti-pertuzumab antibodies was tested in 386 patients treated with both pertuzumab and trastuzumab in the complete regimen arm, and in 372 control patients. However, the cross-reactivity between the two monoclonals could not be avoided. In fact, the presence of anti-pertuzumab antibodies was detected in 2.8 % in the study group and in 6.2 % of trastuzumab-treated controls. No severe hypersensitivity reaction (0.5 %) could be associated to their presence in this study.

33.3 Adverse Events

The present BBW focuses only on *embryo-fetal toxicity*, including olygohydramnios, delayed fetal kidney development, and embryo-fetal death. Additional warnings include *cardiotoxicity* (LVEF decrease), *infusion and hypersensitivity reactions*, and *febrile neutropenia*.

The overall safety profile was based on the CLEOPATRA pivotal study on 808 mBC patients treated with pertuzumab in combination with trastuzumab and docetaxel, as compared to the standard trastuzumab-docetaxel regimen [6]. The most common (>30 %) AEs of pertuzumab experienced in association with trastuzumab and docetaxel include diarrhea, alopecia, neutropenia, nausea, fatigue, rash, and peripheral neuropathy. As expected, the majority of events were comparable among the study groups, except for diarrhea (67 vs. 46 %) mucosal inflammation (28 vs. 20 % in controls), rash (45 vs. 36 %), pruritus (14 vs. 10 %), dry skin (11 vs. 4 %), neutropenia (53 vs. 50 %), febrile neutropenia (14 vs. 8 %), anemia (23 vs. 19 %), cephalea (21 vs. 17 %), URTI (17 vs. 13 %), and paronychia (7 vs. 3.5 %), which showed an increase ≥3 % over the control group. Predominant severe/serious events included neutropenia, *febrile neutropenia*, leukopenia, diarrhea, peripheral neuropathy, anemia, asthenia, and fatigue. The episodes of febrile neutropenia were more frequent during the first cycle of therapy and declined thereafter. Similarly, among events of higher severity showing an increase (≥2 %) over the controls were neutropenia (48.9 vs. 45.8 %), febrile neutropenia (13 vs. 7.3 %), diarrhea (7.9 vs. 5 %), and nail disorders (1.2 vs. 0.3 %). Overall, this preliminary profile indicates a slight general trend of increased toxicity related to the addition of pertuzumab. By contrast,

cardiotoxicity signs were comparable or higher in the control group, as LVEF decrease (4.4 vs. 8.3 % respectively), and CHF (1 vs. 1.8 %). Noteworthy, patients with previous cardiac history were excluded from this study. Overall, the general trend of AEs decreased to <10 % of patients after docetaxel discontinuation, except for diarrhea (19 %), URTI (13 %), rash (12 %), cephalea and fatigue (11 %), while continuing with the pertuzumab-trastuzumab combination.

The most common *neurotoxicity sign* in combination therapy was peripheral neuropathy (12.5-21 %, severe about 2 %), mostly of the sensory type.

Infusion reactions after pertuzumab administration, and before the addition of trastuzumab and docetaxel, were mostly mild/moderate and occurred in 13 % of patients (9.8 % in controls) as fatigue, vomiting, myalgia, and dysgeusia. Severe reactions were <1 %. Hypersensitivity signs, including anaphylaxis (four cases in the study arm and two among controls), appeared in about 11 % of cases (9 % in controls) and were severe/serious in about 2 % in both arms [1–3, 6].

As expected, particular attention was dedicated to cardiotoxic events, because of the presence of two monoclonals targeting HER receptors, and of the previous experience with trastuzumab, which also indicated the possibility of asymptomatic and long lasting cardiac toxicity (see trastuzumab, Chap. 35). Moreover, early studies on cardiotoxicity with pertuzumab indicated an additional toxicity encountered in the dual monoclonal HER2 blocking as expressed by asymptomatic and symptomatic cardiac events [7]. However, most relevant cardiac events were attributed to anthracyclines and other associated chemotherapies, which presumably predispose/synergize with damage produced by the anti-HER2 antibodies. A recent pooled analysis was performed on 598 patients treated with pertuzumab monotherapy (331), either combined with trastuzumab (93), or associated with nonanthracycline cytotoxic therapy (175). Notably, about 6.9 % of patients in pertuzumab monotherapy and 6.5 % of patients in trastuzumab-pertuzumab combination developed asymptomatic cardiac signs, while 0.3 % (1.1 % in controls) showed symptomatic cardiac dysfunction, thus indicating that there was no synergistic effect on cardiac events between the two monoclonals [8].

As for the general safety profile of pertuzumab, additional trials such as NEOSPHERE confirmed in different therapeutic conditions and disease stages the original depicted profile, and indicated the absence of significant synergistic effects on AEs. The study examined efficacy and safety on 417 patients with HER2-positive, locally advanced, inflammatory BC receiving pertuzumab and/or trastuzumab, with or without docetaxel. In the study group (107 patients) the most common AEs included neutropenia (50 %), diarrhea (46 %), alopecia (64 %), nausea (38 %), fatigue, rash and mucosal inflammation (22 % each), myalgia (22 %), asthenia (21 %), and cephalea (11 %). Severe/serious events (10 % as at least one event) included neutropenia (45 %), febrile neutropenia (8 %), diarrhea (6 %), leukopenia (5 %), asthenia (2 %), and hypersensitivity (1 %). However, in this study, it was possible to evaluate in one arm (108 patients) AEs after pertuzumab-trastuzumab, in the absence of docetaxel. In this case, all grade AES included diarrhea (28 %), nausea/cephalea (14 %), fatigue (12 %), rash (11 %), myalgia (9 %), asthenia and mucosal inflammation (3 % each). Notably, severe/

serious events were greatly reduced and limited to 4 % of patients including drug-related hypersensitivity (2 %), and neutropenia (1 %), thus confirming the major responsibility of docetaxel in raising most AEs either in dual or triplet combination with the monoclonals. One of the two fatalities (fulminant hepatitis) occurring during the study in the triplet combination arm was related to treatment. However, about 30 % of patients did not respond to the dual antibody regimen. Cardiotoxicity was represented by CHF in one patient having coronary stents and preexisting cardiovascular history, although the study design excluded subject with impaired cardiac function. Overall, the study confirmed that additional AEs strictly related to HER2 blocking were mostly represented by diarrhea and skin reactions as moderate events [9].

Taken together, the toxicity profile of pertuzumab is low and only partially overlapping that of trastuzumab. For example, asthenia, gastrointestinal, and dermatological reactions are more common with the former monoclonal. Cardiotoxicity remains low (6.9 %), mostly asymptomatic, and did not increase when combined with trastuzumab (6.5 %). However, follow-up observations are still limited and new data from ongoing trials (APHINITY) are expected [10].

33.4 Off-Label Experience

Since in vivo, preclinical studies showed pertuzumab efficacy also on nonmammary tumors, a number of trials are devoted to investigate a number of off-label indications, such as on other solid tumors (3), ovarian cancer (3), neuroendocrine tumors (2), NSCLC (2), and on tumors of stomach, colon, pancreas, and prostate (1 study each).

Preliminary observation on 19 patients with 11 types of solid tumors (gastrointestinal, hepatobiliary, pancreatic, male/female reproductive, and melanoma) treated with pertuzumab and capecitabine, showed a general mild/moderate (72 %) safety profile including anemia (83 %), diarrhea (72 %), asthenia (72 %), nausea (67 %), anorexia (61 %), mucositis (61 %), HFS (44 %), vomiting (39 %), neutropenia (39 %), thrombocytopenia (33 %). Two patients had a severe reaction, but no serious events were registered, except for some infusion reactions, and one case of asymptomatic pulmonary embolism subsequently excluded from the study. Moreover, there was minimal overlapping tocixity with capecitabine [11].

Initial observations on 41 prostate cancer patients treated with pertuzumab as single-agent indicated a relative moderate safety profile, in which gastrointestinal signs were predominant. The most common AEs included diarrhea (61 %) followed by arthralgia/nausea/fatigue (27–34 %), vomiting, constipation and peripheral edema (19.5 % each), pain, myalgia and rash (17 %), mucosal inflammation, dysuria and dyspnea (about 15 % each), URTI, hypoesthesia, cervicalgia, asthenia, and pyrexia (12 % each). Severe AEs were limited to diarrhea (5 %), arthralgia (5 %), fatigue, constipation, vomiting, and peripheral edema (2 % each). Serious events were not observed except for one patient with cervicalgia. Asymptomatic LVEF decrease <50 % occurred in 5 % of cases and other

cardiac dysfunctions occurred in three patients. Overall, pertuzumab resulted well tolerated from these patients, and not significantly associated to cardiac toxicity [12].

Similar results were obtained in another study on 68 prostate cancer patients treated with two different doses of pertuzumab, who experienced diarrhea (37–48 %), and fatigue (9–34 %) as most common AEs, and two cases of serious cardiotoxic events (tachycardia, T-wave inversion) [13].

Pertuzumab associated to gemcitabine was experienced in 130 platinum-resistant in ovarian, fallopian, and primary peritoneal cancer patients (65 treated with the drug in study). All patients experienced at least one AE. The most common all grade events were diarrhea (62.9 %), fatigue (51.4 %), and nausea (42.9 %), which were also higher in the study group compared to controls. Rash (40 vs. 14 %), stomatitis (29 vs. 11 %), lumbalgia (42 vs. 23 %), and cephalea (37 vs. 26 %) were also increased in the study arm. Mild cardiotoxicity was equally represented (12 vs. 17 % in controls) as LVEF decrease. One patient in the study arm suffered CHF [14].

More recent data from a Phase II study were obtained from 149 patients with ovarian cancer treated with pertuzumab and chemotherapy (74 in the study group). AEs were reported in 68–79 % of patients, and were similar in both arms, with a higher incidence in subjects receiving gemcitabine instead of paclitaxel. Similarly, hematotoxicity—in particular, neutropenia—was higher when associated to the former chemotherapy, but not increased with respect to controls. Overall, pertuzumab did not increase the toxicity burden of chemotherapy, including cardiotoxicity, and no new safety signals were produced [15].

Pertuzumab, when administered as single agent showed no efficacy in 43 NSCLC patients. The safety profile was mild and consisted in diarrhea (21 %), nausea, and fatigue (14 % each). Severe/serious event occurred in 9 % of patients, including diarrhea, lung infiltration, hypersensitivity, and ADRS. No cardiotoxicity was reported.

In a more recent Phase I study on 15 NSCLC, a fixed dose of pertuzumab was associated to various doses of erlotinib. All patients had at least 1 AE. Severe events occurred in 30 % of patients including rash (73 %), diarrhea (67 %), pruritus (40 %), asthenia (33 %), cephalea (33 %), anorexia (33 %), vomiting (20 %), and dyspnea (20 %). Six serious events were observed only in the group receiving the higher dose of erlotinib [16].

33.5 Postmarketing Surveillance

Due to the recent marketing authorization of pertuzumab a few cases have been reported to postmarketing surveillance settings so far.

By mid 2013 FAERS registered 234 reports showing GI signs (9 %), infections (6 %), respiratory disorders (4 %), pulmonary vascular (3 %) and cardio-respiratory disorders (2.5 %) among the most frequently reported events.

In the EUV database, 90 reports included GI signs (18 %), constitutional signs (16 %), respiratory (10 %),and hematological disorders (6 %), and infections (5 %) among the most frequent AEs.

33.6 Remarks

On the basis of present data pertuzumab shows a safety profile similar to trastuzumab, both in frequency and typology. The limited early experience as monotherapy has shown a good tolerability, associated with an inconsistent efficacy that remains to be fully explained on mechanistic grounds. The experience so far accumulated with pertuzumab in various therapeutic associations also depicts a tolerable and manageable framework, where usually the AEs of the monoclonal(s) are lower than those induced by the associated chemotherapy. Although in need of long-term confirmations and of better adjusted therapeutic strategies, pertuzumab association with trastuzumab does not seem to exert synergistic effects in terms of safety, nor with associated chemotherapies. Interestingly, in some clinical conditions the spectrum of AEs seems also to be slightly different in typology from that of trastuzumab. In off-label administrations the safety profile does not seem to be significantly modified in frequency, and no new signals have emerged so far.

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Ranibizumab

34

Ranibizumab (Lucentis®, Genentech, Novartis) is a recombinant humanized IgG1k monoclonal Fab fragment binding to human vascular endothelial growth factor-A (VEGF-A). In June 2006, FDA approved ranibizumab for intravitreal (IVI) treatment of neovascular age-related (wet) macular degeneration (AMD). In March 2007, EMEA granted approval for the same indication. In June 2010, on the basis of additional data, FDA extended the indication to macular edema following retinal vein occlusion (RVO), and in October 2012 to the treatment of patients with diabetic macular edema (DME). EMEA extended approval for DME in October 2010 and for RVO in March 2011. Similar steps were taken from Health Canada and from TGA Australia starting from 2007. At present, ranibizumab is approved for RVO in over 70 countries, for DME in over 75 countries and for AMD in over 100 countries.

Three pivotal Phase III studies, FVF2598g (MARINA, 716 patients), FVF2587g (ANCHOR, 423 patients), and FVF3192g (PIER, 184 patients), were the basis for initial efficacy and safety evaluation. They all were prospective, multicenter, randomized double-masked, parallel group studies enrolling 1,323 AMD patients. Supportive studies included two Phase I studies (FVF2425g, FVF1770g); two Phase I-II studies, FVF2428g (FOCUS) and FVF2128g, enrolling 282 AMD patients. Additional data from studies FVF2508g, CRFB1201, and CRFB2201 enrolling 186 patients with AMD and subfoveal choroidal neovascularization (CNV) secondary to AMD were listed in the FDA report, but not in the EMEA application. Overall, the initial safety profile was mostly based on 754 AMD patients treated with ranibizumab and 379 controls of the two studies FVF2587g and FVF2598g [1–7].

Studies on AMD performed after marketing approval include the SUMMIT study—which groups three trials (DENALI, MONT BLANC and EVEREST)

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sharing the same protocols, performed on about 647 patients in North America, Europe and Asia respectively–, EXCITE (353), SUSTAIN (531), PrONTO (40), SAILOR (FVF3689g), enrolling 4,300 patients, and EXTEND I-III (I: on 88 Japanese; II: on 114 Chinese; III: on 95 Asian patients). The large SAILOR study included also the long-term follow-up of previous MARINA and ANCHOR trials, while the EXTEND-I (A1201) trial was the supportive study for ranibizumab approval in Japan, in January 2009. Overall, these trials, along with a number of other studies, helped to better define therapeutic strategies, and confirmed the initial safety profile.

The subsequent indication extension to DME was supported by the two pivotal trials RESOLVE (D2201) enrolling 151 patients, and RESTORE (D2301) enrolling 345 patients. Part of the RESTORE patients (240) continued a long-term extension study with a primary safety endpoint. Additional consistent studies include REVEAL (D2303, with 390 patients), RIDE (FVF4168 g enrolling 382 patients) and RISE (FVF4170 g with 377 patients), which are about to complete their observations.

The extension to RVO treatment with ranibizumab was supported by BRAVO (NCT00486018) and CRUISE (NCToo485836) trials that had respectively recruited subjects with central (CRVO, 392 subjects) and lateral (BRVO, 397 subjects) RVO, with the HORIZON extension study. Overall, these studies enrolled 798 RVO patients (527 treated), of whom 739 reached the first 6-months endpoint. Finally, three relevant trials (CATT -NCT00593450- on 1,208 patients; IVAN -ISRCTN92166560- on 610 patients; LUCAS -NCT01127360- on 420 CNV post AMD patients) are comparing the safety and efficacy of ranibizumab to the off-label bevacizumab intraocular therapy [1–8].

At present, over 300 trials are completed, ongoing or recruiting: this gives an idea of the outmost interest in this new approach to macular/vascular disorders.

34.1 Mechanism of Action

VEGF is a soluble 45-kDa group of cytokines (six homodimeric glycoprotein isoforms) made from a gene splicing family that includes five ligands (VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PGF). The family member recognized by ranibizumab is VEGF-A, the most active variant, which mediates its effects by binding to two tyrosine kinase receptor (TYKR) isoforms, VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1), while VEGF-3 responds to VEGF-C and D ligands. VEGF-A is expressed in four major isoforms (VEGF121, −165, −189, −206) and five minor isoforms (VEGF145, −148, −162, −183, and −165b). Among these, VEGF165b is the only inhibitory factor binding to VEGFR-2. Moreover, VEGF110 is a smaller biologically active ligand derived from the proteolytic cleavage of VEGF121 and VEGF165.

Fibroblasts, neutrophils, endothelial cells, and T cells produce VEGF molecules. Their production is stimulated by hypoxia, nitric oxide, and protein kinase C. VEGFR-1 and VEGFR-2 are expressed in progenitor and mature endothelia,

but also in monocytes, macrophages, neurons, and in renal glomerular, preglomerular and peritubular cells. VEGFR-3 is predominantly expressed in lymphatic endothelium. These receptors are transmembrane Ig-like structures with a predominant extracellular portion (7 domains) and with an intracellular tail that contains one TYK domain. VEGFR-2 is considered the most important angiogenic factor of the family, while VEGFR-1 seems to act as its modulator/competitor. VEGFR-3 is predominantly expressed in lymphatic endothelium and shows lympho-angiogenic properties. VEGFR-2 can be cleaved as a soluble form (sFlt-1), which acts as a physiological competitor of membrane-bound VEGFR-1 and VEGF-2. The natural ligand's synthesis can be stimulated by local hypoxia, which produces a hypoxia inducible transcriptional factor (HIF) capable of enhancing angiogenesis.

Overall, the system generates signals for homeostatic regulation, survival and activation directed to endothelial cells, it regulates angiogenesis and vascular permeability, but also exerts neurotrophic and survival-promoting effects in neural and glial cells, and in the renal epithelial/vascular district. The VEGF network also plays a role in embryonic and postnatal vasculogenesis and angiogenesis, skeletal muscle regeneration, cardiac remodeling, endochondrial bone formation, in the female reproductive cycle and in kidney function. These additional features may help in understanding some AEs related to VEGF-blocking biomedicines, including ranibizumab.

Moreover, VEGF ligands are also produced by various epithelial tumors, thus assuring their proper vascularization and growth.

Finally, VEGF/VEGFR binding at ocular level may induce endothelial cell proliferation and vascular hyperpermeability, which contribute to the development and progression of the neovascular (wet) form of AMD, to the visual impairment caused by DME, or to macular edema secondary to RVO. Despite these processes are considered to be different and multifactorial, hypoxia and a subsequent overexpression of VEGF are present and crucial for AMD. However, increasing evidence indicates that immunologic processes participate to the pathogenesis of AMD through the production of inflammatory cytokines, recruitment of macrophages, complement activation, and microglial activation. In fact, the revisited "immunological privileged site" concept at ocular level is more likely to represent an "endogenous immunological site" protected by the blood retinal barrier (BRB), much similar to the intra-CNS environment protected by the BBB, in which highly specialized immunocompetent cells (microglia, dendritic cells, and even retinal pigmented cells) and perivascular macrophages contribute to internal immune homeostasis. This equilibrium and the BRB integrity are altered by age-related toxic/hypoxic factors in AMD, thus allowing a profound disregulation of internal regulatory processes and the entrance of exogenous immune cells. DME arises from breakdown of the BRB, also mediated by VEGF, thus producing edema and accumulation of macromolecules in the retina. Notably, in these cases there is a dramatic increase of VEGF in the retina, where it is hardly detectable under normal conditions.

RVO is the second most common retinal vascular disorder after diabetic retinopathy, and may cause the central retinal vein (CRVO) or a lateral branch (BRVO) occlusion subsequent to multifactorial etiopathogenetic events, mainly including compression at the arteriovenous crossing, parietal vascular disorders, and hemocoagulative disorders.

Ranibizumab is a recombinant humanized IgG1k monoclonal antibody fragment (Fab) binding with high affinity to all VEGF-A isoforms, including the small biologically active isoform VEGF110, thus preventing the binding of any VEGF-A to its natural receptors. In particular, murine anti-VEGF-A complementarity-determining regions (CDRs) were inserted into a consensus human IgG1 framework with proper amino-acid substitutions to increase the binding affinity to VEGF-A. Due to the absence of the Fc portion, ranibizumab could not exert CDC or ADCC properties.

Ranibizumab reaches maximal intraocular concentration in one day and is eliminated in about 9 days, as estimated on serum concentrations. Most PKD data refer to AMD patients, but no relevant differences have been reported in other groups. Ranibizumab penetrates through all layers of the retina in order to reach the target tissue. It is quickly removed from the system and is characterized by a low level of immunogenicity. At present, three other anti-VEGF agents are used for ocular diseases: (i) Pegaptanib (Macugen), which is a pegylated oligonucleotide, adopting a three-dimensional conformation that enables the binding to extracellular VEGF165; (ii) Bevacizumab (Avastin), a humanized monoclonal IgG1 antibody directed to VEGF-A, licensed as an IV anti-tumoral agent, but extensively used as intraocular off-label therapy in AMD, DME and RVO; (iii) Aflibercept (Eylea), approved in November 2012, which is a fusion protein binding to VEGF-A.

In fact, bevacizumab is the full antibody used to derive ranibizumab, as its Fab fragment, subsequently modified to bind with a higher affinity to the same VEGF-A epitope [7, 9–13]. Quite recently, aflibercept (Eylea), a fusion protein binding to VEGF, has been approved for the treatment of AMD and CRVO (see Chap. 10, 42).

In order to better understand the mechanism of intervention of anti-VEGF agents and the framework of their safety profiles, it must be stressed that the VEGF/VEGFR system is not the only angiogenic/neoangiogenic regulator at systemic and ocular level. For example, other cytokines such as IL-2, IL-6, IL-8 and TNF-a, have been related to the activity of CNV. Second, hypoxia and other toxic/degenerative mechanisms are not the only VEGF inducers at ocular level, since EGF, TGF, PDGF, IL-1a, IL-6, and insulin-like growth factor 1, all involved in inflammatory processes, are able to enhance VEGF mRNA synthesis [14].

34.2 Immunogenicity

As expected, immunogenicity was detectable, yet low (1–8 %). However, pre-existing anti-ranibizumab antibodies (nonspecified) were present up to 5 %. As a Fab fragment, ranibizumab is not expected to cross the placenta. However, this

monoclonal was detected at fetal level in one case with high maternal anti-ranibizumab antibodies. It was assumed that such ADA, containing a fully active Fc portion, may have acted as carrier of ranibizumab for the placental transfer. No clear relation between hypersensitivity and the presence of ADA could be documented, and therefore their induction was considered a potential risk [6, 7].

34.3 Adverse Events

The initial safety profile of ranibizumab was mostly based on 754 AMD patients treated with ranibizumab, and 379 controls of ANCHOR and MARINA trials. Another 2-year similar trial (PIER), currently ongoing, has enrolled patients with or without CNV. The last label update (Feb 2013) refers to 1,323 AMD patients (879 treated) due to the fact that PIER patients who had completed, and data from the first two years observation on AMD and DME could be added. Because of the extensions to DME and RVO disorders, additional safety studies have been also included. In particular, 759 DME patients (502 of them treated), and 789 RVO patients (528 treated) from the mentioned pivotal trials and extensions were observed for safety evaluations. Most treated patients received local intravitreal injections of ranibizumab, or sham inoculations. In some studies, photodynamic therapy (PDT), such as in ANCHOR and FOCUS studies, or laser therapy, such as in RESTORE and REVEAL studies, were compared. Therefore, the emerging safety profile describes the *local* encountered AEs and the *systemic* occurring events, while individual safety profiles are depicted for each ocular disease officially admitted for this therapy.

In the general ocular safety profile, *endophthalmitis*, *retinal detachment* and *increased ocular pressure* (IOP) or in a minor number of cases persistent or delayed *ocular hypertension* (OHT) are mentioned, *Arterial thromboembolic events* (ATE) were the most relevant systemic events common to all cohorts of patients, while *fatalities in DME patients* showed a potential relation with IVI administration of ranibizumab [1–7].

34.3.1 Ocular Adverse Events

Three serious events are strictly related to the intravitreal injection of ranibizumab: *endophthalmitis, rhegmatogenous retinal detachment,* and *iatrogenic traumatic cataract*. However, their overall frequency is estimated as <1 % of cases. The most common ocular events can be grouped as *general discomfort* signs (pain, lacrimation, dry eye, pruritus, and foreign body sensation), *palpebral* (blepharitis), *conjunctival* (hyperemia, hemorrage, and injection site hemorrage), *anterior eye* (cataract, posterior capsule opacification), *vitreal* (increased pressure, floaters, detachment, inflammation, posterior capsule opacification, and blurred vision), and *retinal* (detachment, degeneration, minor disorders) reactions. They are all occurring in AMD, DME and RVO, yet at different frequencies.

Overall, the most frequent (47–74 %) events include eye pain (17–35 %), floaters (7–27 %), IOP (7–24 %), vitreous detachment (4–21 %), and general irritative signs (7–15 %), which are distributed among the three diseases in treatment. The remaining signs are usually observed in <10 % of cases, or as particularly occurring in some of the diseases in study, as described below. As a general trend, most ocular AEs occur with high frequency (5–74 %) in AMD compared to DME (1–47 %) for the same observation time (2 years), except for cataract (12 % in AMD, 28 % in DME), which possibly reflects the respective underlying diseases since the DME control group showed a high rate (32 %) compared to DME treated patients. In contrast, RVO patients showed a higher frequency of new maculopathy (11 % in RVO, 5 % in DME, and 9 % in AMD patients). By comparing frequencies of AEs ≥5 % among the three treated groups and with the respective controls, AMD registered higher scores for conjunctival hemorrage (74 AMD, 47 DME 48 % RVO), floaters (27 vs. 10 vs. 7 %), IOP (24 vs. 18 vs. 7 %), intraocular inflammation (18 vs. 4 vs. 1 %), foreign body sensation (16 vs. 10 %, 7 %), xerophthalmia (12 vs. 5 vs. 3 %), and injection site hemorrage (5 vs. 1 vs. 0 %). All the remaining less frequent reactions were more frequent in AMD.

34.3.2 Systemic Adverse Events

The overall systemic safety profile was more homogeneous. Most common disorders involving all treated pathologies include *URTI* (5–16 %, mainly nasopharyngitis), and *constitutional signs* (3–12 %), while a number of additional signs show a more marked difference among them or are present as <5 %. Overall, RVO patients report both ocular and systemic AEs with less frequency than AMD and DME patients. However, the reported data refer to a shorter period of observation for the former (6 months) with respect to the two other groups (1–2 years). In DME a slightly higher frequency of *renal failure* (7 % in DME, 1 % in AMD, 0 % in RVO), *anemia* (11 vs. 8 vs. 1 %), *edema peripheral* (6 vs. 3 vs. 0 %), and *neuropathy* (5 vs. 1 vs. 0 %) occurred, mainly reflecting the underlying disease as for the mentioned cataract complication. *ATE*s are the most relevant, albeit rare, systemic adverse event. In AMD-treated patients the risk of ATE is 1.9 versus 1.1 % in controls during the first year of ranibizumab administration, while in the second year it was slightly increased (2.6 %), but remained within the controls' values (2.9 %). Similarly, the *stroke* rates were 2.7 % in the treated groups and 1.1 % in the control arms. In DME the rates of ATEs and stroke were higher (5.6 % and 1.2 %, respectively) than in controls; however rates in controls also resulted elevated (5.2 and 1.6 %). After 3 years of treatment ATE reached 10.8 % and stroke rate was 4.8 % with higher doses (0.5 mg) of ranibizumab, but no controls were present at that time. In RVO patients ATE and stroke were low (0.8 and 0.2 %, respectively) and similar to controls (0.4–0.8 %), but the observational period was shorter (6 months) [1–7].

While the general safety profile of ranibizumab in AMD, DME and RVO has been widely confirmed in subsequent trials, some *peculiar aspects of single diseases* and other remain to be investigated, mainly about potential long-term therapy, administration strategies, and associations with supportive therapies.

In a wide German overview (WAVE), 3,470 *AMD patients* received an initial 3-month upload of ranibizumab followed by on demand (or pro re nata—PRN) injections for 1 year. The overall safety profile was low and showed adverse events in 6.5 % of patients (2.2 AEs/P), that were serious in 3.9 % of cases (2.3 SAEs/P). Most events related to ocular reactions (59 %) and included drug-related events as 1.5 % (0.8 % serious). A reduced drug-related visual activity was observed in 0.8 % (0.6 % serious) of cases, and metamorphopsia in 0.4 % (0.3 % serious) of patients. It must be noted that overall ocular AEs occurred in only 3.8 % of the patients, mostly as transient and as consequence of the procedure. Moreover, drug-related extra-ocular events were particularly low, either as general disorders (<0.2 %), or as cerebrovascular serious events (stroke 0.4 %). Overall cerebral and cardiac vascular accidents were far lower than previously reported in SAILOR and other similar trials [15].

In other recent studies on ranibizumab safety and efficacy, based on currently available data on AMD from clinical trials, the genesis of some AEs was better clarified. In particular, most ocular events were related to the IVI procedure and were mostly transient, such as irritation, conjunctival hemorrage, intraocular inflammation, and increased ocular pressure. In contrast, SAEs potentially related to intravitreal ranibizumab treatment included endophthalmitis, uveitis, vitreous hemorrhage, rhegmatogenous retinal detachment, retinal tear, and lens damage. A retrospective analysis of 14,320 IVIs of ranibizumab revealed an incidence of endophthalmitis of 0.02 %, which is slightly lower than the rate encountered in pivotal trials (0.05 %), while retinal tear ranged 0.6–2.2 %. Differences derived from regimen administration of ranibizumab were inconsistent, except for cataract encountered at higher doses, suggesting a potential accelerating effect on its progression.

As for systemic AEs, the main concerns are related to vascular thromboembolic events, which seem to increase with ranibizumab IVI treatment. In a retrospective analysis of most relevant trials, *ATE* occurred in 2.5 % of treated patients and in 0.7 % of controls. Interestingly, myocardial infarction and stroke rates, which had been reported as slightly increased in AMD patients at ANCHOR and MARINA first year endpoints, were not confirmed at the end of the second year. Moreover, the increase in stroke events observed in patients treated with the higher dose of ranibizumab at SAIL 6-month endpoint, was confirmed at one year although not reaching statistical significance. In particular, no association with myocardial infarction was observed. Nonocular hemorrhagic events including ecchymosis, gastrointestinal hemorrhages, hematoma, vaginal hemorrhages, and subdural hematomas were about twofold higher than control levels (7.8 vs. 4.2 %).

Overall, when ranibizumab is administered at a reduced frequency, ocular AEs seem to be reduced, especially serious events, yet longer observation data are still needed and expected from final endpoints of SECURE, EXCITE and SUSTAIN

trials. Interim data from the SECURE trial (derived from EXCITE and SUSTAIN studies) on 234 patients treated with the high dose (0.5 mg) ranibizumab showed that the most frequent ocular AEs were retinal hemorrhage (12.8 %; one event related to study drug), cataract (11.5 %; one event related to treatment procedure), and IOP (6.4 %; one event related to study drug). Main extraocular AEs were hypertension and nasopharyngitis (9.0 % each). ATEs were reported in 5.6 % of patients. Longer term data provided by over 4-year treatment (HORIZON) have already shown that AEs do not increase with time and no new signals emerge. However, all AEs were 79–82 % in treated groups as compared to 49 % in controls. SAEs occurred in 4–8 % of cases, versus 0 % in controls. Cataract events were 12 versus 6 % in controls, and intraocular inflammations were 2–3 versus 0 %. Cataract was partly reported as serious, due to the fact that hospitalization for surgery occurred in 2.6 % of cases; none was suspected to be related to study drug or procedure. Moreover, the most common AE were AMD worsening/progression (32–37 vs. 8 % in controls) and retinal hemorrage occurring in 28–35 versus 9.5 %. Nonocular AEs ranged 79–81 versus 71 %, and SAEs were 28–32 versus 29 % in controls. ATE occurred in 5–9 versus 5 % of controls. Overall, the safety profile was lower than in previous studies. Notably, the reduced incidence of intraocular inflammation was due to a new liquid formulation of ranibizumab for monotherapy. In the HORIZON trial, being the long-term safety of multiple PRN intravitreal injections the primary objective, the remarkable incidence of AMD worsening/progression (considered an AE) emerged as unexpected. This occurrence was interpreted as a consequence of delayed injections and of a less-frequent follow-up, while the low incidence encountered in controls was attributed to a possibly nonrepresentative population of patients, given that the group included patients who may have not needed treatment (selection bias). Although in terms of efficacy monthly IVI scored better, the safety profile was more favorable in PRN regimen. However, this procedure was also considered as an additional selection bias since patients with better outcomes received fewer injections or were not followed as frequently as those with worse outcomes. All together, these data remain cumbersome [16–18].

A recent in-depth analysis on cerebrovascular accidents (CVA) encountered in AMD patients treated with ranibizumab in large trials (MARINA, ANCHOR, FOCUS, PIER, SAILOR) has shown that CVA rates from pooled 2-year observations were <3 %. However, treated patients had a potential increased risk compared to control groups. The risk was also dose-related (0.5 mg in high dose vs. 0.3 in low dose regimens). Although data pooling allows evaluations on larger sample of patients, confounding factors were also increased, and CVA cases were still too few to reach statistical significance [19].

A detailed literature review (532 citations) on *DME disease*, examined by the AAO, compared long-term (over 2 years) effects of ranibizumab, pegaptanib, and off-label bevacizumab, confirming that *DME* patients receiving IVI anti-VEGF injections were at no greater ocular risks than the other subgroups of patients; but longer term follow-up were needed. In fact, DME patients are usually younger than AMD patients and therefore they may become at greater risk of cataract

progression and/or IOP at a later time and after more injections. Additional data reported from other trials (CATT, RISE, and RIDE) and from the literature confirmed that there seem to be no greater systemic risks in DME patients receiving intravitreal anti-VEGF injections [20].

An indirect attempt to compare ranibizumab and bevacizumab in DME showed that no consistent increase in adverse events was observed in either treatment group. In particular, the DRCRN 2010 trial, the one with the longest present follow-up, reported more cardiovascular events in controls than in the ranibizumab group (11.5 vs. 5.1 %). However, in a 2-year retrospective study, hypertension was slightly more prevalent in the study groups than in controls, in contrast with previous data from DRCN2007 trial. Similarly, endophthalmitis was slightly prevalent in some intervention arms, except for the DRCRN 2010 trial, where this event was more common in the control arm [21]. Although indirect comparisons need to be interpreted with caution, no particular differences in the respective overall safety profiles emerged. However, in previous studies on over 7,000 IVIs, bevacizumab showed to induce more AEs, such as corneal abrasion (0.15 %), mild ocular discomfort (0.14 %), inflammation or uveitis (0.14 %), and hypertension (0.21 %) [22].

In a recent review and meta-analysis on four trials enrolling 1,313 DME patients followed for 12 months, the incidence of AEs showed no statistical difference between ranibizumab administration as monotherapy, its combination with laser therapy, and placebo [23].

As for the safety profile of ranibizumab in *RVO patients*, a different evaluation of risk/benefit is demanded, since vision may improve spontaneously within 3–6 months in about 50 % of cases. Moreover, it is difficult to distinguish improvements induced by the therapy from spontaneous recovery after 6 months, mainly in the absence of untreated controls, which is often the case of this kind of long-term studies. It is also difficult to assess the real outcome in RVO subtypes, such as recurrent forms or progression to retinal ischemia, which may be in more need of such therapy. Overall, drug-related AEs were similar to those observed in other clinical situations, and mainly consisted in ocular hemorrhage, pain, and IOP, while systemic AEs, when present, usually remain within the range of controls. However, among the systemic AEs, hemorrage, cardiovascular ischemic and functional disorders seem to increase after the second year treatment. No major differences were seen between CRVO and BRVO, both in efficacy and safety. No new safety events were identified in these patients [7, 24, 25].

Finally, a series of most recent studies on AMD patients have been aimed at *comparing safety and efficacy of ranibizumab to other anti VEGF agents*, including bevacizumab, aflibercept, and pegaptanib. It must be noted that in the majority of these studies no head-to-head observation was performed, except for the CATT trial, and most of them were not powered to identify differences in drug-related adverse events. In the 2-year results report on CATT trial enrolling 1,107 AMD patients (571 with ranibizumab), SAEs were higher in the bevacizumab arm (40 %) than in ranibizumab arm (32 %). In particular, VTE were increased (1.7 vs. 0.5 %), while ATE events were equally distributed (4.7 vs. 5 %). Similarly,

endophthalmitis (1.2 vs. 0.7 %), and GI disorders (4.8 vs. 1.8 %), such as hemorrhage, hernia, nausea, and vomiting, were significantly higher in the bevacizumab arm [26].

A stringent comparative analysis on SAEs in a large ranibizumab (891) and bevacizumab (693) cohort of patients, showed that subjects receiving bevacizumab were likely to develop severe intraocular inflammation following each injection 12 times more than those who received ranibizumab (OR:11.71; 95 % CI 1.5–93). A trend toward an increased risk for ATE in patients receiving bevacizumab was also noted, although the confidence interval was wide (OR = 4.26; 95 % CI 0.44–41). In particular, among a total of 1,584 administered injections, nine cases of ocular inflammations (in 5 AMD, 3 DME, 1 BRVO, 1 myopic) occurred in bevacizumab arm (693 injections in 173 patients), while one case (AMD) occurred after ranibizumab (891 injections in 351 patients). No other adverse ocular events, such as retinal detachment, infectious endophthalmitis, or vitreous hemorrhage, were noted within one month from injection in either group. The Authors noted also in discussion that among CATT's data in appendix uveitis, scleritis, or anterior chamber inflammation had occurred in six patients receiving bevacizumab, while only one pseudoendophthalmitis had been reported for ranibizumab. Moreover, the ATE rates in this trial were lower than in their experience, and in contrast to ATE incidence in on-label applications of bevacizumab. [27]. The higher trend of AEs induction in the bevacizumab group was confirmed by: (i) the cumulative analysis of at least one systemic SAE/P up to the second year endpoint; (ii) SAEs occurring within the second year of treatment (24.4 vs. 18 %); (iii) the increase observed in all MedDRA classes, except for neoplasms. Notably, patients treated on PRN regimen showed more AEs than patients on monthly regimen, either as two-year trend or as events within the second year of treatment (22 vs. 18, 5 %). However, endophthalmitis occurred more frequently in patients treated with monthly injections (91 %), being also prevalent in the bevacizumab groups (1.2 vs. 0.7 % in ranibizumab arms) [26].

In a separate study on 186 AMD eyes in patients who had completed the study (60 treated with ranibizumab, 85 with bevacizumab), significant ocular AEs were low and consisted in one vitreous hemorrage (with bevacizumab) and two retinal pigment epithelium rips (with ranibizumab) [28]. Four systemic cardiovascular AEs (two strokes, one fatal, myocardial infarction, angina pectoris) occurred in the bevacizumab group, while no systemic events occurred in the ranibizumab group. However, 1-year findings of the IVAN trial on 610 AMD treated patients (314 ranibizumab) showed that SAEs were prevalent in bevacizumab arm (12.5 vs. 9.6 %), but ATE or cardiac failures were more present in the ranibizumab one (2.9 vs. 0.7 %) [29]. In a meta-analysis on ranibizumab and bevacizumab in direct comparison on 1,333 patients (enrolled in 3 trials), and in indirect comparison on 4,050 patients (5 trials) the 2-year results showed that absolute rates of serious ocular AE were low (2.1 %), but the risk of ocular and of multiple systemic AE with bevacizumab was significantly raised (RR 3.1; 95 % CI 1.1–8.9). In contrast, a significant increase in nonocular hemorrhage with ranibizumab was observed (RR 1.7; 95 % CI 1.1–2.7). Overall, a higher concern for bevacizumab than for

ranibizumab was confirmed, but a precise safety profile of bevacizumab could not be depicted due to the poor quality of AE monitoring and reporting in the relative trials [30]. Finally, an interesting retrospective review examined the incidence of delayed OHT elevations after intravitreal injection of anti-VEGF agents in 302 treated eyes, compared to 226 controls. Patients included AMD with or without glaucoma. In the former, the incidence of OHT was 3.1 % (eye/year) versus 5.7 % in respective controls, while the rate was 0.5 % in the latter group (1 % in controls) [31]. These data are reassuring and exclude a significant risk of OHT after repeated injections during a four-years treatment, even in the presence of glaucomatous states.

34.4 Off-Label Experience

As previously mentioned, most of the over 300 trials on ranibizumab potential applications are ongoing. As expected, most of them concern eye studies (289), and in particular AMD (224), but also include a number of intra ocular off-label indications, such as uveal diseases (67), choroid disorders (63), visual disorders (39), diabetic ocular disorders (28), metastatic neoplasia (56), neurovascular disorders (91) melanoma (6), neuroectodermal tumors (12), sensation disorders (20), skin disorders (10), teleangectasia (9), ocular hypertension (8), cystoid macular edema (6), ocular ischemic disorders (6), bone diseases (5), anemia (4), von Hippel-Lindau syndrome (2), pterygium (1), corneal vascularization (1), Eales' disease (1), etc. Among these, particular attention is given to the treatment of *uveitis*, where other biomedicines such as infliximab, adalimumab, rituximab and etanercept are being experienced. The rate of AEs to ranibizumab is expected to be low compared to the other full monoclonals in study.

In a recent survey, safety problems related to some of these agents were compared to results included in the CATT experience with ranibizumab and bevacizumab. Despite the expectations of lower SAEs rate with these monoclonals in off-label treatment of uveitis, some concerns were raised because of the different inflammatory pathways underlying AMD and uveitis. Another concern relates to the doubtful capacity of a local and transient immunomodulation to prevent recurrence, due to potential autoimmune T cell targeting processes occurring in uveitis [32].

Although ranibizumab monotherapy has shown little effect on *choroidal vascular abnormalities*, a recent association with photodynamic therapy (PDT) experienced also in the EVEREST trial, produced some results and, interestingly, in some cases limited AEs related to PDT by allowing a reduction of its targeting area [33]. However, in another experience the benefit decreased during the second year because of *recurrence* (70 %) *of polypoidal lesions* and insurgence of massive *subretinal hemorrage* (21 % of recurrent patients) [34]. In a 2-year experience on 12 patients (13 eyes) with *retinal angiomatous proliferations* (a recent AMD variant), PDT was associated with bevacizumab and afterwards with ranibizumab without any AEs insurgence [35]. In a similar experience 19 patients (20 eyes)

with *polypoid choroidal vasculopathy* (including an AMD variant) were treated with ranibizumab, and most AEs were mild, with no significant or serious AEs reported. The most visually significant ocular AEs included cataract progression (3), mild vitreous hemorrhage (2), and macular hole (1). No systemic drug-related adverse events were observed [36]. In *myopic choroidal neovascularization*, ranibizumab (27 eyes) showed efficacy at an inferior number of intravitreal injections compared to bevacizumab (28 eyes), thus exposing to a potential lower number of related AEs [37].

Finally, a single case of 5-year long treatment with ranibizumab in a secondary choroidal neovascularization was beneficial and did not produce significant AEs [38]. All together, these experiences are still limited and only indicative in terms of efficacy and safety. However, the safety profile does not seem to be changed and no new signals have been so far perceived.

34.5 Postmarketing Surveillance

Most common events in the FAERS database on over 10,500 reports are vision abnormalities (11 %), ocular hemorrage (9 %), ocular structural changes (9 %), fatalities (5 %) cardiovascular disorders (5 %), and infections 4 %. Nonocular thromboembolic event were uncommon (0.9 %).

In the EV database on over 6,317 reports (99 % serious) the most common events were eye disorders (39 %), general system disorders (12 %), infections (8 %), and cardiac disorders (5 %). In particular, relevant eye disorders included vision abnormalities (10 %), hemorrage and pain (2.3 %), cataract (1.5 %) and retinal tear (0.6 %). The latter is also mentioned as a postmarketing report in the official label. AMD and CNV persistence/recurrence were reported as in 1.5 % of reports. ATEs were rare (0.01 %).

34.6 Remarks

The safety profile of ranibizumab is reassuring for all on-label indications, at least up to 3-4 years of treatment. The utilization of a Fab fragment instead of the whole antibody offers the advantage of a better penetration at retinal level and the absence of ADC and ADCC activation, while its shorter half-life (about 9 days vs. 20 days of the full monoclonal analogue) does not seem to raise efficacy or safety concerns at ocular level. However, the smaller size of this molecule may increase its potential systemic diffusion, which may be relevant for nonocular AEs. In fact, both monoclonals reach circulation, and systemic AEs were detectable during treatment with both biomedicines, with some distinctive features.

As for ranibizumab, AEs frequency was different in AMD, DME, and RVO. Interestingly, plasma concentrations of ranibizumab varied among them and elevations in plasma ranibizumab in DME patients were more transient and declined

faster than in subjects with AMD, thus indicating a different systemic exposure to the drug in these patients [4]. Overall, these data support the possibility of generating extra-ocular AEs during ranibizumab IVI, although in most of the direct clinical observations the event did not reach statistical significance, or could be excluded in large cohorts of patients [39].

VEGFRs are present on various cell types, including neural and renal cells. VEGF/VEGFR homeostasis is crucial also for pregnancy, lactation, as observed in experimental models, and fetal neoangiogenesis. It must be noted that ranibizumab inhibits VEGF-induced human umbilical vascular endothelial cell proliferation and reaches consistent serum levels.

In a recent case report, the presence of ranibizumab and bevacizumab has been measured in serum and in the breast milk after intravitreal injections. Both monoclonals were detected in the serum, with concentrations that interfered with the circulating VEGF-A after one IVI. In one week bevacizumab lowered circulating VEGF-A to an undetectable level, which started to increase within 3 weeks. Ranibizumab was detected after 4 days, and caused a VEGF-A decrease of about 10 %, which started to recover after 6 days from IVI. In the breast milk bevacizumab caused a VEGF-A decrease of about 35 % after 2 weeks, followed by a slow recovery, while ranibizumab did not cause significant modifications. The case report requires wider investigations, but is consistent with some reported differences in nonocular AEs to these monoclonals, and more importantly suggest a potential risk of AEs in mother and infant, due to the reduction of VEGF-A in pregnant patients induced by IVI therapy [40].

Another potential concern relates to the possibility of causing retinal nerve damage, both for OHT and as a direct effect of ranibizumab on neural fibers, given that VEGFRs are present and active also in neuronal components. A recent investigation on 49 AMD treated patients showed a significant reduction (5.2 %) of retinal nerve fiber thickness in the study group, after 12-month follow-up, with no modifications in controls [41]. However, another study has given more reassuring results showing that repeated IVI of ranibizumab did not have adverse effects on any retinal layers, nor on the retinal ganglion cell function [42].

A significant improvement in the safety profile has been provided by the PRN administration of ranibizumab that reduces the risk of IVI-related AEs and in some instances seems as effective as monthly injection. PRN administration in DME patients seems to have a more positive outcome, possibly due to the fact that such disease requires a lower level of sustained therapy compared to AMD [43]. Therefore, the improvement of sustained intraocular delivery of biomedicines, with reduction of injection-related AEs and costs abatement, represents a crucial point for the future development of IVI therapies [20]. Overdosage of ranibizumab seems also rather safe. In the SAVE trial (87 AMD) and in a pilot study on nine patients with persistent/recurrent AMD treated with 2.0 mg of ranibizumab, no AEs were reported [44]. In the pilot study, one case of tachyphylaxis was observed, thus indicating that such event may occur also with high dose of ranibizumab.

In fact, tachyphylaxis is a relevant phenomenon in terms of risk/benefit evaluation, which may develop after bevacizumab or ranibizumab. This rapidly decreasing therapeutic effect seems to develop more frequently within the eye microenvironment, and at rates of about 2 % of cases with bevacizumab. Surprisingly, in one reported case of cystoid macular edema, the progressive reduction of response to bevacizumab did not affect the subsequent administration of ranibizumab, which showed to be fully effective [45]. Since these two molecules share the same paratope and have a quasi-identical Fab, it may be speculated that in this case thachyphylaxis could be related to the Fc fragment presence and function. Noteworthy, macrophages are rich of Fc receptors, produce VEGF, and are stimulated by inflammatory signals and hypoxia [46]. Their presence in retinal perivascular areas and the possibility of activation via the Fc portion of bevacizumab suggest a potential contrast with its therapeutic action, which is absent in ranibizumab and may explain its capacity of overcoming thachyphylaxis.

Additional differences in the overall safety profiles of these two monoclonals are stressed by a number of dissimilar outcomes concerning their affinity, Fc-related immunologic reactivity, dimensions and glycosylation, and even the presence of distinct contaminants [27]. Therefore, the possibility of comparing ranibizumab and bevacizumab has offered a unique chance to evaluate their respective potential adverse consequences. However, as it happens for other drugs, the off-label use of bevacizumab in a number of different clinical conditions still raises more concerns than benefits, especially when experienced on occasional basis, in small groups and out of well-organized trials. In some occasions, the striking difference in costs of treatment has been taken as a supportive motivation for off-label use and for overcoming safety problems, thus unbalancing the risk/benefit evaluation at clinical level [47]. Costs may be another aspect of disease, yet should be treated with different prescriptions.

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Rituximab

35

Rituximab (Rituxan®, MabThera®, Genentech/Roche) is a chimeric murine/human monoclonal IgG1k antibody directed against the CD20 antigen located at the surface of normal and malignant *B* lymphocytes.

In November 1997, FDA granted its approval and in June 1998 EMEA licensed the product for the treatment of relapsed refractory low-grade or follicular CD20 positive B cell non-Hodgkin's lymphoma (NHL). In February 2002, FDA authorized the use of rituximab as a component of the ibritumomab (Zevalin®) therapeutic regimen (see ibritumomab, Chap. 23). During 2006, the same Agency extended the use of rituximab in combination with methotrexate (MTX) as first therapy, to reduce signs and symptoms of moderately to severely active rheumatoid arthritis (RA) in adult patients who have had an inadequate response to one or more TNF antagonist therapies. The extension also included the use of this biomedicine in combination with CVP (cyclophosphamide, vincristine, and prednisolone) chemotherapy as first-line treatment of follicular CD20 positive B cell NHL. In 2008, the indications were further extended to include first-line treatment of B cell NHL in combination with CHOP (i.e., CVP+ adriamicine) or other anthracycline-based chemotherapy regimens. In February 2010, the extension to first-line therapy for CD20-positive chronic lymphocytic leukemia (CLL), in combination with fludarabine and cyclophosphamide (FC), was approved. During 2011 two new extensions were approved, concerning the use of rituximab as single-agent maintenance therapy in patients with previously untreated follicular, CD20-positive, B cell NHL who achieve a response to rituximab in combination with chemotherapy, and the use of rituximab in combination with glucocorticoids for the treatment of patients with Wegener's Granulomatosis (WG) and Microscopic Polyangiitis (MPA).

Between 2004 and 2010 EMEA granted similar extensions. In particular, the Agency approved the following rituximab uses: (i) combined with CVP

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chemotherapy, as first-line treatment, in patients with follicular lymphoma (2004); (ii) the use in RA patients resistant/intolerant to DMARDs and TNF therapy, in combination with MTX (2006); (iii) as first-line treatment of patients with CLL, in combination with chemotherapy (2008); (iv) for the treatment of advanced (stages III–IV) follicular lymphoma, in combination with any dedicated chemotherapy (2008); (v) for the treatment of patients with previously untreated and relapsed/refractory CLL (2009); and (vi) for the treatment of follicular lymphoma patients responding to induction therapy (2010).

However, the requests for extension to MTX-naive patients (as first-line treatment) and to MTX-IR patients (as second-line treatment) were not accepted. At present, rituximab has been approved in over 100 countries for most or all of the mentioned indications.

Pivotal trials for initial approval of NHL treatment were the Phase III controlled study 102-105 on 203 NHL patients, and supportive Phase I–II study 102–02, for a total of 240 enrolled patients. Six additional studies were presented: two completed Phase I–II studies, three Phase II ongoing studies, and one other study that was planned yet not implemented. Overall, 322 patients were evaluated for safety, and 306 for efficacy. Subsequent extension was supported by the several additional studies listed below:

NHL: (i) Pivotal Study EORTC20981 on 465 (334 exposed) patients, along with studies GLSG-FMC, SAKK35/98, and LYM-5 for rituximab maintenance therapy on about 500 patients; (ii) Study U0824 and ECOG1496, that enrolled 384 previously untreated patients; (iii) Main studies GLSH'00, OSHO-39, FL2000 and supportive studies from various Authors, for the extension in combination with any chemotherapy indicated for B cell follicular lymphoma on 987 (563 exposed) patients; and (iv) Study MO18264 (PRIMA) for maintenance therapy in 321 (162 exposed) patients responding to induction therapy.

DLBCL: Main studies SO15165, U071.5 s, and efficacy data from Study LNH98-5 for treatment in combination with CHOP on 486 patients.

CLL: Pivotal Study BO17072 on 546 patients, and supportive studies from various investigators for a total of 1564 (880 exposed) patients.

RA: (i) Pivotal WA17042 (REFLEX) on 520 (308 exposed) patients, and supportive studies WA16291 and WA17043 for a total of 585 patients; (ii) An extension of Study WA16291 continued in Study WA16855; (iii) IMAGE (WA17047) on 755 (513 exposed) patients, SERENE (WA17045) on 520 (209 exposed) patients, MIRROR (WA17044) on 377 exposed patients, and REFLEX extension study (WA17042D) on 468 patients. Studies, that supported the evaluation of rituximab when combined with various MTX regimen; and (iv) SUNRISE (U3384 g) and DANGER studies, that were included but not evaluated.

WG/MPA: The most recent approved indications are for WG, MPA, and ANCA-associated vasculopathy (AAV) group, and are based on data from the RAVE (NCT00104299) trial on 197 AAV patients.

Overall, a total of 3,587 NHL patients (739 treated, 1,427 for maintenance studies) were evaluated for NHL. CLL treatment was experienced on 1,564 patients (880 treated). RA studies were conducted in 2,385 patients (1,439 exposed), and

WG/MPA were analyzed on 197 patients [1–8]. At present, over 1,250 trials on various rituximab applications are completed, ongoing or recruiting.

35.1 Mechanism of Action

CD20 (Bp35) is a tetraspanning transmembrane human B lymphocyte-restricted differentiation antigen, located at the surface of normal and primate B lymphocytes and on human malignant B cells. During B cell maturation, this nonglycosylated hydrophobic phosphoprotein is first expressed on pre-B cells, but is lost during the final stage of maturation to plasma cells. In particular, CD20 is expressed in a subpopulation (about 30 %) of precursor B cells, in mature B lymphocytes, and in follicular dendritic reticulum cells. CD20 is also expressed by low-grade B cell NHL, precursor B cell neoplasms, precursor B-Lymphoblastic leukemia/lymphoma, (B-LBL), HCL, B-CLL (weak), and by B-PLL. It is also present in lymphoplasmacytic cells in Waldenström's macroglobulinemia, but is absent in myeloma and B cell ALL.

CD20 has a role in B cell activation/proliferation through the Src family tyrosine kinases, and enables optimal B cell immune response against T cell-independent antigens. Moreover, CD20 is also expressed in a minor population of T lymphocytes (2 %), pertaining to the memory cytotoxic compartment that tends to increase with age. It is also presumed that CD20 may act as a store operated calcium ion channel. However, no natural ligands are known, and no soluble CD20 forms have been detected.

Rituximab (formerly C2B8) is a glycosylated chimeric IgG1k murine/human monoclonal antibody directed against the human CD20 antigen. The antibody has murine light- and heavy-chain variable regions and human constant region sequences. Rituximab was developed from the fully murine parent ibritumomab, which recognizes the same epitope. The basic effector mechanisms of this antibody are related to the Fc portion, and include complement-dependent cytotoxicity (CDC) resulting from C1q binding, and antibody-dependent cellular cytotoxicity (ADCC), mediated by one or more of the Fcγ receptors at the surface of granulocytes, macrophages, and NK cells. However, rituximab is also able to induce cell death via apoptosis, yet it is not clear which of these potential aggressive mechanisms are prevalent/effective in vivo. Administration of rituximab induces a rapid and massive destruction of circulating and tissue-based normal and malignant B cells. In some studies, depletion was evaluated by assessing the presence of residual B cells by another marker (CD19) and resulted being over 95 %. However, rituximab has no effect on hemopoietic progenitor cells and T cells. In hematologic malignancies, B cell depletion in circulation and in tissue localizations occurs within 3 weeks, and recovery begins in 6 months, reaching normal values within one year after treatment. In RA patients, there is an almost total depletion of circulating B cells within 2 weeks of treatment, lasting about 6 months and reaching normal levels within one year in most patients. A delayed recovery occurs, over 3 years after treatment, in a subgroup of subjects (4 %).

Consequently, there is a reduction of both IgM and IgG serum levels, reaching concentrations below normal limits in 15–20 % of cases. A reduction of IgA was also observed in RA patients, being below normal levels in a minority of cases (<1 %). B cell depletion was also rapidly detectable in WG and MPA, and lasted up to one year [7, 8].

Other potential and less known depletion mechanisms are induced by rituximab. For example, rituximab is effective in restoring platelet levels in immune-mediated thrombocytopenia (ITCP), but CDC and ADCC depleting action on B cells seems not sufficient to explain its action, since levels of antiplatelet autoantibodies remain virtually unchanged in these patients, while the platelet count increases rapidly. Among the proposed additional involved mechanisms, there is a saturating binding effect of rituximab-coated B cells to macrophages via Fc receptors, which may compete with platelet phagocytosis actively occurring in ITCP [9].

In a recent overview on anti-CD20 mAbs, five mechanisms of action were recognized, and the therapeutic mAbs were grouped according to their capacity to induce the reorganization of CD20 molecules into lipid rafts upon binding [10]. In particular, Type I antibodies induce the formation of lipid rafts and efficiently activate the classical pathway of the complement system, while Type II antibodies do not translocate CD20 into lipid drafts, yet induce cell death upon direct binding, and poorly activate complement. Both types are capable of inducing ADCC in the presence of effector cells. Rituximab is a Type I mAb and expresses the cytolytic action mainly by ADCC via FcγR-expressing monocyte/macrophages, while CDC may play an additional role. In contrast, Type II mAbs, such as tositumomab, mostly act by direct apoptosis. These cytolytic functions may be differently expressed in various situations, being CDC more effective in circulation, where complement factors are highly represented, than on a solid tumor mass. Moreover, their efficacy varies in dependence of the tumor burden. In this case, multiple events are expected to be necessary in the presence of significant tumor masses. Further mechanisms are involved during therapy, such as the opsonization of mAb-covered targets mostly occurring as late effect. CD20 is not shed from the cell surface and is not endocytosed during the antibody binding [8]. However, Type I mAbs can be internalized when bound to some FcγR, more than Type II mAbs. As for rituximab, the internalization requires the cross-link between the Fc tail to FcγRIIb, an ITIM-containing inhibitory receptor, and the Fab portion to CD20 on the same target cell. This mechanism is crucial in reducing therapeutic efficiency; it lowers the available amount of mAb at the target cell surface, necessary for effector cells recruiting, and drags the rituximab-CD20 complex into lysosomes for degradation. Interestingly, the expression of FcγRIIb on different targets correlates with resistance to rituximab while the presence of FcγRIIIa, the ITAM-containing stimulatory receptor, is related to mAb efficacy [11].

However, a genetic polymorphism of this receptor produces differences in the binding of IgG1, and subsequent activation of ADCC. In particular, homozygous valine in the position 158 exerts higher ADCC capacity with respect to heterozygous or homozygous phenylalanine alternatives in the same position. About 49

different FcγRIIIa phenotypes have been identified in NHL patients showing differences in therapy response. Similarly, a correlation between B cell depletion induced by rituximab and receptor phenotype has been shown in SLE patients. Another mechanism potentially capable of influencing the therapeutic response is related to the rituximab-induced downregulation of IL-10 that enhances apoptosis and synergize with chemotherapeutic and steroid agents on the target [12]. Finally, rituximab seems to increase the number of Treg lymphocytes and prevents the activity of specific autoreactive T cells. These two additional mechanisms support in principle the administration of rituximab in autoimmune diseases. Overall, rituximab shows multiple and complex functions, which may explain the different target sensitivities and safety profiles under various clinical conditions. These mechanisms are shared with other anti-CD20 mAbs, but their efficiency is expressed with a different hierarchy among them. In fact, clinical experience indicates that cases of noncross-reactive resistance and synergistic effects between anti-CD20 mAbs and chemotherapy occur, and are possibly related to mechanistic diversities (see also Chap. 23, 29, 37).

35.2 Immunogenicity

As expected, anti-rituximab antibody response was detected after treatment, with different frequencies. In particular, HACA were present in 23 % of WG/MPA, followed by 11 % of RA and 1.1 % of NHL-treated patients. In the RA group, most HACA-positive cases had an objective clinical response, and about 1 % of HACA-positive RA patients had associated serious infusion reactions. Overall, among 3,095 RA patients treated with rituximab 13 % had HACA, (4 % of them had previous anti-rituximab antibodies detected at baseline), which may interfere in efficacy and safety profiles of this subgroup of patients. Interestingly, in some off-label trials enrolling RRMS patients HACA were observed in 25 % of rituximab recipients.

Because of the lowering of Ig levels, which is not profound but can be prolonged during rituximab treatments, live viral vaccines were contraindicated and nonlive vaccines may produce a reduced response. In one study on NHL patients, the primary response to a conventional antigen (KLH) was remarkably reduced (4 % vs. 69 % in healthy subjects), as well as the immune recall to tetanus toxoid (18 % vs. 81 % in healthy controls). Similar results were obtained in CLL patients, but not in RA patients where the primary response was reduced of about 50 %, and secondary response was comparable to controls [6–8].

35.3 Adverse Events

According to the 2012 update of the official label, safety data on rituximab are based on 2,783 (2,427 exposed) patients with malignancies, including 1180 NHL, 927 DLBCL, and 676 CLL. The safety profile for RA is based on 2,587 (540

exposed) patients, and for WG/MPA disorders on 197 subjects. The 2012 EMEA EPAR update reports evaluations on 3,189 exposed patients, including 1,193 untreated NHL, 166 in monotherapy, 881 in combination with CHOP, 322 with CVP, and 44 with FCM chemotherapy. Responders from these groups were put on maintenance therapy as 513 FL (505 controls). Relapsed/refractory FL consisted in 465 patients, of whom 234 in combination with CHOP (231 controls). Of 334 responders, 167 were put on maintenance therapy (167 controls). Of 399 previously untreated DLBCL, 202 were treated with rituximab in combination with CHOP chemotherapy (197 controls). The CLL cohort consisted in 817 previously untreated CLL and 552 relapsed/refractory CLL treated in association with FC chemotherapy. In particular, 403 of the untreated group (407 controls) and 276 of the relapsing/refractory patients (276 controls) received the combined therapy. The RA safety profile was based on 3,100 patients from clinical trials receiving at least one cycle of rituximab in combination with MTX, and followed from 6 months up to over 5 years. On this basis, and considering postmarketing data so far accumulated, a general safety profile of rituximab has been depicted, reporting most relevant and occurring AEs. Moreover, some peculiarities encountered in each treated disease have also been reported.

The updated official label includes BBW for *infusion reactions*, *tumor lysis syndrome* (TLS), severe *mucocutaneous reactions*, and *progressive multifocal leukoencephalopathy* (PML). They all can be fatal. The initial 1997 label contained only a warning for infusion reactions, while the 2004 update included a warning box for the first three SAEs. PML was included in 2007 on the basis of postmarketing reports.

Infusion reactions were predominantly seen as *cytokine release syndrome* (CRS) in >50 % of patients. Additional complications, such as hypotension and bronchospasm, associated in about 10 % of cases, can be serious. The reported incidence at first infusion was up to 77 % for patients with malignancies and 32 % for RA patients, and decreased to 9–11 % after the second infusion. In the smaller group of WG/MPA patients treated with rituximab (99), infusion reactions were reported as 12 % at first treatment, with a similar decreasing trend after the following administrations. Typical manifestations included urticaria, cardiovascular and respiratory hypersensitivity signs, ARDS, and anaphylactoid events. *TLS* is mainly expressed as renal failure and is observed in malignancies with a high number of circulating malignant cells or high tumor burden. *Mucocutaneous reactions* usually appear within the first 13 weeks of treatment as paraneoplastic pemphigus, Stevens-Johnson syndrome, lichenoid dermatitis, vesiculobullous dermatitis, and toxic epidermal necrolysis. *PML* after rituximab has been reported in 114 cases in one major database (WHO Drug Monitoring AE databank) and is fatal [13].

The most relevant reactions in the general profile of rituximab include *infections*, *cytopenias*, and *hypogammaglobulinemia*. However, due to the very different treatment indications for rituximab and the consequent encountered AEs, a general safety profile will be provided for malignant and nonmalignant diseases,

while additional relevant AEs peculiarities will be further described for each treated pathology.

The *safety profile in NHL and CLL* is based on patients treated with rituximab as monotherapy, or in combination with various chemotherapies. Under these circumstances, the most common drug-related AEs were infusion reactions occurring in the majority of patients, followed by infections (mostly bacterial and viral) and by cardiovascular events (mostly arrhythmias). Additional rare events include HBV hepatitis reactivation and PML. In particular, during monotherapy treatment of B cell malignancies, the overall incidence of infections was 13–17 % (serious 4 %) and occurred as bacterial (19 %), viral (10 %), and fungal (1 %), mainly as URTI and UTI. Localized Candida and Herpes zoster infections were also reported at higher incidence in the study groups. Cytopenias at induction and maintenance regimen were usually mild and transient. However, severe neutropenia (4–10 %), anemia (1 %), and thrombocytopenia (2 %) were observed as increased on control levels (<1–4 %). IgM and IgG levels decreased in about 14 % of patients. Ig levels were reduced also in control patients receiving chemotherapy alone, but they recovered up to normal levels. In long-term observations (2 year), IgG levels remained reduced in 60 % of patients in the study group, and in 36 % in controls [7].

When combined with chemotherapy, constitutional signs were present in 86 % of patients with malignancies (57 % severe/serious), hematological signs occurred in 67 % (48 %), followed by dermatological reactions as 44 % (2 %), respiratory signs as 38 % (4 %), metabolic/nutritional abnormalities as 38 % (3 %), gastrointestinal signs as 37 % (2 %), nervous system disorders in 32 % of cases (1 %), musculoskeletal signs as 26 % (3 %), and cardiovascular disorders as 25 % (3 %). In particular, severe leukopenia ranged 88–97 % in NHL when rituximab was associated with CHOP chemotherapy, and was present in 30 % of CLL patients treated in association with FC chemotherapy, with frequencies in controls of 79–88 % and of 19 %, respectively. Cardiovascular disorders during monotherapy were about 19 %, being arterial pressure unbalance the most frequent reported event. Serious cardiac events (ischemia/infarction, atrial fibrillation, LVF) were 3 % in the study group and 1 % in controls. Noteworthy, only functional cardiac disorders increased (7 %) in groups with associated CHOP chemotherapy. Cardiac disorders in CLL patients treated in combination with FC chemotherapy were low and equally distributed between study groups and controls. Cerebrovascular accidents, respiratory serious events (ILD), and gastrointestinal disorders were also observed at lower frequencies, mostly in patients treated with rituximab associated to chemotherapy.

Additional differences in the safety profiles, either in typology or frequency, were observed in patients with B cell malignancies. In *untreated NHL* patients, peripheral sensory neuropathy occurred in 30 % of treated patients (18 % in controls), rash/pruritus occurred in 17 % (5 %). Pulmonary and hepatotoxicity (17–18 % vs. 7–10 %), and neutropenia (8 % vs. 3 %) were also reported. Infections reached 37 % (22 % in controls) in some studies and were serious, together with neutropenia, in 4 % of treated patients (1 % in controls). In *DLBCL*,

rituximab was associated with CHOP chemotherapy, with 80–100 % of patients experiencing at least one AE. The events were severe/serious and drug-related in 20–40 % of cases. Infusion reactions were usually mild and observed in about 30 % of patients (severe in <10 %), mostly at first administration. Pyrexia (56 % vs. 46 % in controls), lung disorders (31 % vs. 24 %), cardiac disorders (29 % vs. 21 %), and chills (13 % vs. 4 %) were more frequently reported as severe/serious events. Other SAEs included thrombocytopenia (9 % vs. 7 %), cardiac toxicity (4.5 % vs. 1 %), and lung disorders (6 % vs. 3 %). Moreover, infections (about 45 %), neutropenia (>80 %), and anemia (15–20 %) were also among the registered events in both study groups and controls. Drug-related infections were about 19 %. Moderate hypogammaglobulinemia was present in 15–30 % of cases. In *CLL*, all AEs were 99 % versus 96 % in controls. Severe reactions (80 % vs. 74 %) and serious events (50 % vs. 48 %) were similarly distributed. Infusion reactions were frequent (59 and 7 % severe/serious). Neutropenia (49 % vs. 44 %), febrile neutropenia (15 % vs. 12 %), thrombocytopenia (11 % vs. 9 %), hypotension (2 % vs. 0 %), and hepatitis B reactivation (2 % vs. <1 %) characterize the safety profile. Prolonged neutropenia was observed in 25 % of cases. Overall, the general SOC safety profile in the study group was slightly higher than in controls, but no new/unexpected signals were detected. When the biomedicine was used in combination with FC chemotherapy, all AEs were observed in 83 % of cases in the study group and in 71 % in controls receiving only the FC chemotherapy. However, serious cardiac events (4 % vs. 3–4 %), cerebrovascular accidents, neurologic events (3–4 % vs. 3–4 %), leukopenia (30 % vs. 19 %), and pancytopenia (3 % vs. 1 %) were consistently lower in CLL than in NHL. Nonetheless, CRS, TLS, and some PML cases were observed in CLL treated patients and reported in the postmarketing experience.

Overall, in studies where rituximab was associated with various chemotherapies, the incidence of AEs in most SOC categories was higher than in patients receiving mAb monotherapy or chemotherapy alone. However, no synergistic drug effects on AEs induction could be detected. In contrast, the incidence of HACA in combined therapy was lower than in rituximab induction monotherapy. It was also low compared to treated patients with nonmalignant disorders.

During treatment of *RA* and *WG/MPA*, infections occurred in 39 and 62 % respectively, which also reflect the rates of infections in control groups (34 and 47 % respectively). They were mainly featured as URTI (7 %) and UTI (<5 %). Serious infections occurred in about 2 % (1 % in controls) and 11 % (10 %) in the two groups of patients, and involved LRTI including pneumonia, cellulitis, and UTI at ≤0.5 % rates. Overall, the most common AEs included constitutional signs (2–8 % in RA, 1–18 % in WG/MPA), dermatological reactions (2–5 and 10 % respectively), gastrointestinal signs (2–3 and 17 %), hypertension (8 and 12 %), and arthralgia (6 and 13 %). Moreover, in the WG/MPA group leukopenia/anemia (10–16 %), dyspnea (10 %), and epistaxis (11 %) were also reported.

Additional AEs peculiarities in RA-treated patients include vascular (7 % vs. 4 %), respiratory (5 % vs. 1 %), and dermatologic events (5 % vs. 2 %), while musculoskeletal complaints were prevalent in control groups. Infusion reactions

occurred in 36 % (serious <0.5 %) of patients under treatment, mostly (26 %) during the first administration. Most common SAEs after rituximab were infections, some of them fatal, with statistical significance when compared to controls (7–10 % vs. 3 %). Cardiac events were 10 % in the study group and 3 % in controls. However, serious cardiac events were more equally distributed (1.7 % vs. 1.3 % in controls), although they resulted fatal in one study as 0.4 % on 769 treated subjects (0 % in controls). Infections were low (1 % vs. <1 %) but serious. Hypophosphatemia (12 % vs. 10 %) and hyperuricemia (1.5 % vs. 0.4 %) were also observed as new cases, infusion-related and transient. In an enlarged cohort of RA-treated patients, hypophosphatemia was observed in 21 % of cases. AEs tended to be more frequent and severe in patients treated with higher doses of rituximab.

Taken together, the overall risk of serious AEs is more elevated in RA patients, and is more frequently associated to allergic reactions and to an increased incidence of HACA, as revealed by four extension studies (IMAGE, SERENE, SUNRISE, and MIRROR) where they were detected in 2–12 % of cases. PML cases occurred in rituximab-treated patients as well as in untreated RA, SLE, and vasculitis. HBV reactivation and PML cases were also reported. Moderate hypogammaglobulinemia was also detected, but was not associated with increase of infections or serious infections.

Finally, the safety profile in WG/MPA was further depicted in a relatively small cohort of treated patients (99) and was characterized by the presence of CRS during infusion, and by a consistent number of infections of any type (62 % vs. 47 % in controls), including URTI, UTI, and Herpes zoster infections. However, serious infection frequency was similar to controls (11 % vs. 10 %), being pneumonia the most common serious event. Overall, the general profile, except for CRS, TLS, and PML cases, may be considered acceptable. No opportunistic infections or cases of latent/overt tuberculosis were observed. No risk of increased malignancies has been raised so far. Subsequent courses of rituximab therapy do not seem to increase rates and types of AEs, up to four cycles [1–8].

Rituximab is also used in *Waldenstrom's disease*, which is classified as a lymphoplasmacytic lymphoma by WHO, and is mainly associated with chemotherapy. However, as a single agent rituximab is also used in patients with *IgM-related peripheral neuropathy*, with no concomitant evidence of symptomatic lymphoma. A peculiar drug-related AE is referred as *hyperviscosity syndrome*, caused by a transient increase of serum IgM levels, which usually almost returns to baseline level within 4 months. The syndrome is commonly expressed by local hemorrhage (epistaxis, gingival and retinal episodes), dizziness, and fatigue. The general safety profile in chemotherapy-associated treatment of Waldenstrom's disease is within the range of other lymphomas and includes hematologic (neutropenia, thrombocytopenia, and anemia) and nonhematologic signs (infusion reactions, constitutional signs, arrhythmias, etc.). In a recent study on 43 patients treated with multiple cycles of rituximab, fludrocortisone and cyclophosphamide, infusion reactions (49 %) were mostly mild and associated to first administration. One of them was severe and associated with rash. Major events included anemia

(30 %), neutropenia (12 %), thrombocytopenia (3 %), nausea/vomiting (21 %), arrhythmia (5 %), dyspnea (2 %), biliary/liver dysfunction (2 %), and polyuria (2 %). Hyperviscosity syndrome was absent. However, there was at least one episode of severe neutropenia in about 88 % of patients during the study, and it was long lasting in 44 % of them. Two cases of severe thrombocytopenia and one of serious hemolytic anemia were also observed. A total of nine infections (six severe) including pneumonia (5) and sepsis (1), UTI (2) and Herpes zoster infection (1) were observed during treatment. Three additional cases of pneumonia were observed as tardive and two of them were associated to late neutropenia. One patient required hospitalization for pemphigus vulgaris. None of the patients developed high-grade NHL [1–8, 14].

35.4 Off-Label Experience

Rituximab has been used in nephrotic syndrome, ITCP, immune-mediated glomerular diseases, refractory granulomatous ocular diseases, multiple sclerosis, peripheral nervous system autoimmune disorders, SLE, neuropsychiatric lupus, kidney transplant rejection, autoimmune hepatitis, primary biliary cirrhosis, mucous membrane pemphigoid systemic sclerosis, cryoglobulin vasculitis, pemphigus vulgaris, and chronic neutropenic leukemia. Rituximab has been also used for purging stem-cell transplants and for posttransplant treatment of residual disease. At present, over 1,250 trials are completed, ongoing or enrolling patients, and most of them investigate on-label indications (970). However, part of them also include or are expressly dedicated to off-label pathologies, such as Burkitt's Lymphoma (86), plasma cell neoplasms/Waldenström (84), ALL (64), HD (54), precancerous conditions (30), AML (18), autoimmune diseases (164), arthritis/joint disorders (77), blood protein disorders (70), urologic diseases and kidney transplant rejection (37), virus reactivations (36), thrombocytopenias and thrombotic microangiopathies (32), purpura (28), skin disorders (28), GVHD (22), HIV infections (22), nephritis/lupus nephritis/glomerulonephritis (18), SLE (14), multiple sclerosis (10), and vasculitis (14). Among all of these, nonmalignant pathologies offer the possibility of long-term evaluations of rituximab regimen for NHL or RA on different underlying diseases. Most of all, recent experiences on autoimmune and dermatologic disorders seem more promising and potentially expanding, such as in AIHA, ITCP, thrombotic thrombocytopenic purpura, SLE, refractory dermatomyositis, and cryoglobulinemias.

The removal of autoreactive B cell clones is the basic assumption of these off-label treatments, which should inhibit/eradicate autoimmune aggressions. A secondary effect has been attributed to the decrease of the B-APC function, which reduces potential T cell activation in autoimmune disorders.

Rituximab is increasingly used in refractory *pemphigus vulgaris*. In a wide overview of the past 12 years publications, 272 patients in 42 studies received rituximab either with the NHL protocol (180) or with RA protocol (92). Interestingly, the former was less effective and produced less serious infections, but

higher mortality, while the RA protocol was more effective, produced more infections, but with lower mortality rates. None produced sustained clinical remission. The overall major concern was the high infection rates, some of which were fatal. SAEs (16.6 %) associated to the NHL protocol in 48 patients included two cases of pneumonia (P. Carinii, one fatal), and single cases of septic shock (fatal), multi-bacterial sepsis, bacterial pneumonia, DVT/pulmonary embolism, hip arthritis (P. Aeruginosa), and gastritis/retinitis (CMV). Two late-onset neutropenias, one severe and one associated to bacterial pneumonia, were observed after 19–27 weeks from treatment. Seven case series on 88 subjects reported SAEs in 2.3 % of patients, including one septicemia (fatal) and one pyelonephritis.

SAEs associated to the RA protocol were reported in 20 % of patients and included three cases of sepsis, one fatal (S. Aureus), and one associated with spinal hemorrhage and paraplegia, three cases of pneumonia, six UTI, and two herpes infections (one ocular).

Additional SAEs (5.8 %) were observed in 51 patients enrolled in six case series including one gastric perforation (fatal), one cardiac complication, and late-onset neutropenic sepsis. Overall, the incidence of serious infections was about 4 % in the NHL protocol and 15 % in RA protocol, while mortality rates were 2.2 and 1.1 %, respectively [15]. The same Authors also reviewed the literature on *mucous membrane pemphigoid* (MMP). Serious adverse events included 10 % infections in 28 patients treated with one or two cycles of the NHL protocol associated with immunosuppressive and anti-inflammatory agents, and included one pyelonephritis, and one new-onset pulmonary TB (fatal), both associated with hypogammaglobulinemia. Although some benefits were reported in both reviews, serious infections and related mortality remained the major concerns, mainly because of their low occurrence in untreated patients [16].

Rituximab in ITCP treatment as second-line therapy has given more encouraging results, also due to a longer experience of more than 5 years.

In a systematic review on 313 ITCP adult patients treated with rituximab, AEs were observed in about 97 % of patients, and were mild/moderate in about 22 % of cases, mostly as infusion reactions (60 %). However, the rate of infusion reactions was variable across all studies. Serious and life-threatening events were reported in about 4 % of cases. Mortality was reported in 2.9 % of treated patients as caused by respiratory insufficiency, pneumonia, cerebral hemorrhage, hemorrhagic complications, infections, pulmonary embolism, and hepatic failure. This rate was in the range of similar studies. However, only two of nine reported deaths were referred to drug in study by the Authors [17]. In the first systematic review on *pediatric ITCP*, 352 patients were examined for efficacy in 18 studies. In 304 patients the diagnosis was primary ITCP, while 48 patients were diagnosed with secondary ITCP associated to other diseases, including Evans' syndrome, SLE, and autoimmune lymphoproliferative syndrome. Patients received a variety of IV doses (1–6) of rituximab. Safety data were considered in 23 studies reporting adverse events. In particular, 91/208 (44 %) patients reported 108 AEs as mild/moderate (84 %). The most common events included allergic reactions with

pruritus, urticaria, chills, and pyrexia. Serum sickness-like syndrome was observed in seven patients (6.5 %), and was severe in three cases. Infusion reactions were present in two cases, causing therapy discontinuation. Transient neutropenia was observed in three cases. Infections related to treatment in study were reported in 3.7 % of cases, and included pneumonia, one life-threatening enteroviral meningoencephalitis, and two cases of varicella. Finally, one patient developed cephalea with brain MRI abnormality, and one developed common variable immunodeficiency with prolonged hypogammaglobulinemia and increased susceptibility to infections.

Overall, a good response to therapy, both in primary and secondary ITCP, was accompanied by a limited number of mild/moderate AEs and a very limited number of serious infections. No death was reported [18].

Similar results have been reported in another recent review, which also provided some indications for reducing the impact of most common drug-related AEs. Among these are premedication and infusion slowing to minimize serum sickness signs, which occur more frequently in children, and prevaccinations against all encapsulated bacteria to reduce the risk of serious infections [19].

Recent *updates on SLE* have been provided by a number of open studies, along with secondary analysis of EXPLORER and LUNAR (lupus nephritis) trials and previous pivotal trials. All of these failed in terms of efficacy, except for a subgroup of African-American patients, who are known to develop more frequently lupus nephritis. This result stresses the opportunity to direct this therapy to specific and selected cohorts of patients, who could thus benefit from the reduction of AEs caused by alternative treatments such as corticosteroids or estrogens. Tolerability and patient dropout rates were similar to placebo.

Similar results were obtained in *Sjögren's syndrome* (SS), where some benefits were observed on peripheral neuropathy, vasculitis, and cryoglobulinemia, but worsening and serious effects were observed on CNS manifestations of SS, such as MS-like signs, transverse myelitis, anxiety, depression and cognitive dysfunction, all with MRI abnormalities. Interestingly, some SS patients treated with rituximab revealed a higher clonal expansion and mutational rate in IgA and IgG-expressing cells in the parotid tissue, despite the almost complete peripheral B cell depletion [20]. In contrast, a recent review and case report on *refractory neuropsychiatric SLE* (NPSLE), enrolling 36 patients treated with rituximab, showed a partial therapeutic response (in 85 % of cases, but with 45 % recurrence), yet along with a number of expected and moderate AEs. Infections were the most common registered event (29 %) and included pneumonia (four cases), Herpes zoster (two cases), UTI (two cases), infected decubitus ulceration (one case), chickenpox (one case), and enteritis infection (one case). No cases of severe infusion reactions or hematologic abnormalities were reported. Only one patient died due to SLE progression (fatal pancarditis) in the study group. The apparent discrepancy with results of EXPLORER and other studies were attributed to the exclusion of severe and refractory patients, including NPSLE [21, 22].

Other uncontrolled clinical studies on SLE have shown contrasting results. Nonetheless, no new signals were reported, and a consistent sparing effect on steroid-related AEs was confirmed. Once again discrepancies were attributed to patients selection, heterogeneity of SLE disease, and clinical assessment.

Experience on MS is expanding, on the assumption that B cell depletion reduces autoreactive B cell clones and the production of T cell mediated proinflammatory cytokine response. A number of open-label and controlled trials are completed or ongoing on relapsing remitting multiple sclerosis (RRMS) and on primary progressive disease (PPMS) with uncertain results. So far, the side effect profile in studies using rituximab in monotherapy is similar to that previously reported in RA and other autoimmune disorders. Infusion reactions were the most common ($\geq$10 %) drug-related AEs, and were mild/moderate in more than 90 % of cases (7 %, severe). Other severe events were similar in the study groups and in controls (about 14 %), as well as withdrawal rates (about 5 %). Similarly, overall infections were comparable in placebo and study groups (about 70 %), although nasopharyngitis, URTI, UTI, and sinusitis were prevalent in rituximab recipients (5–6 % over controls). No opportunistic infection was reported. However, long-term data are still lacking, and more prolonged observations in PPMS studies (about 2 years) indicate that serious infections are increased in rituximab older recipients (4.5 % vs. <1.0 %). Notably, in these patients HACA to rituximab were observed in about 25 % of RRMS recipients [23].

A number of *glomerular diseases*, including on-label AAV and various off-label disorders such as lupus nephritis, mixed cryoglobulinemia-associated glomerulonephritis, idiopathic membranous glomerulopathy, and focal glomerulosclerosis are treated with rituximab with variable success, on the assumption that autoantibodies play a crucial role in their development. Overall, the safety profile follows the mentioned AAV experience. However, the major advantage in the whole group of disorders is mAb low toxicity compared to conventional therapies, rather than its higher efficacy. In fact, conventional immunosuppressive regimens produce cumulative toxicity and heavily compromise the quality of life of these patients, also exposing to infertility and to a major risk of malignancies in the long term.

In a recent retrospective survey on 74 *pediatric* patients with various forms of *nephrotic syndrome* resistant to conventional therapy, who were treated with rituximab in Japan, the best achievement was the steroid sparing in terms of serious adverse effects. Among these, short stature, obesity, hypertension, cataract, glaucoma, spinal fracture, glucose intolerance, and psychological disturbance are steroid-related AEs, while hypertrichosis, nephropathy, gingival hypertrophy, and drug-resistance are related to CsA treatment. During treatment with rituximab it was possible to discontinue steroids in 77 % of cases, and CsA in 60 % of the recipients. About 50 % of patients recovered from these drug-related events, including CsA resistance. In contrast, rituximab-related AEs were mostly mild infusion reactions expressed as constitutional signs, pyrexia, rash, cardiac frequency disorders, and blood pressure unbalance. Severe events were limited to one infection and two cases of late granulocytopenia recovered without sequelae.

However, many patients relapsed, thus requiring repeated rituximab treatments and re-introduction of some immunosuppressive agents for maintenance [24].

When administered in eight patients with early diffuse *systemic sclerosis* (dSS) who were followed for 2 years, rituximab safety profile registered five SAEs, including one sepsis (fatal), a secondary infection after coronary bypass grafting (fatal), noninfectious pyrexia, secondary infection on a digital ulcer, and one episode of hyperventilation, which were all considered probably nondrug related. No neutropenia or hypogammaglobulinemia were observed. Minor AEs occurred in four patients (3.5 AEs/P) and included URTI (3), one skin infection, GI signs (4), cardiovascular manifestations (hypertension, thrombosis), one COPD exacerbation, depression (1), and one tendinitis. Interestingly, the treatment seemed to stabilize dermatological and internal organ progression of the disease [25].

In a recent review on *cryoglobulinemia vasculitis* (CryoVas) results seem more encouraging. However, about 50 % of treated patients developed AEs, and in a subset of the 23 patients (aged, Type II CryoVas), severe infections were observed in 26 % (about 3 fold rates of RA patients). Severe infections were also observed in a larger subsequent series of patients (242) treated with a different regimen, particularly when high doses of corticosteroids were associated, whereas death rates did not differ between the two therapeutic regimens [26].

A particular concern with some mAbs, as well as with chemotherapy and immunosuppressive regimens, is the *reactivation of viruses*, such as HBV and to a lesser extent HCV reactivation. In particular, HBV reactivation has been reported with considerable variation (1–23 %), and is a potentially fatal complication after rituximab-containing chemotherapy. HCV infection seems to be a risk factor mainly in DLBCL-treated patients, which can lead to therapy discontinuation and lymphoma progression [27].

Rituximab has been also used to treat *granulomatous ocular episcleritis* and *iritis associated to AAV or sarcoidosis*. In a retrospective case report analysis, nine patients (six after AAV, four after sarcoidosis) were treated with rituximab systemic therapy for 12–41 months. Two patients had recurrent herpetic mouth and skin infections. Two cases of neutropenia were also observed and the severe one caused discontinuation of rituximab for one year, with relapse of eye disease causing reintroduction of monotherapy and subsequent improvement [28].

Recently, rituximab has been experienced in *chronic fatigue syndrome* with some benefit, and four trials have been registered (one completed and three ongoing). Data form the completed study on 30 patients (15 treated) reported infusion reactions as mild (13 %), without serious AEs or major toxicity. Insomnia and psoriasis exacerbation were also observed (two cases each) [29]. As for the latter disease, a patent for the use of anti-CD20 mAbs has been registered in Norway (IPC8 Class: AA61K39395FI (USPC Class: 4241731).

Finally, serious and fatal viral infections and reactivations, including HBV, HCV, CMV, herpetic viruses, and JCV (responsible for PML fatalities) have been observed in off-label administrations. Moreover, progression of Kaposi's sarcoma has also been observed during off-label treatment with rituximab, mostly in HIV patients [7].

35.5 Postmarketing Surveillance

In the 2012 label, a number of postmarketing reports were considered as relevant on the basis of their severity, frequency of reporting, and causality with rituximab administration. They included hematologic (bone marrow hypoplasia, prolonged/late pancytopenia and neutropenia, and hypogammaglobulinemia), cardiac (fatal), infective (severe/serious, HIV-associated, PML), dermatologic (mucocutaneous reactions), intestinal (obstruction and perforation), pulmonary (fatal ILD and bronchiolitis obliterans), neurologic (RPLS), and malignant (Kaposi sarcoma progression) events. Moreover, a number of immune/autoimmune events were observed, which enlarge the spectrum of possible drug-related AEs at ocular (neuritis, uveitis) and systemic level (vasculitis, LLS, polyarticular arthritis, and serum sickness).

Initial observations on about 13,000 rituximab-treated patients with B cell malignancies reported 39 fatalities and 66 serious infusion reactions. Serious dermatological events included toxic necrolysis, paraneoplastic pemphigus, lichenoid dermatitis, and lichen planus.

In the FAERS database over 16,700 reports (3.5 AE/R) included infections (6.6 %), WBC disorders (5.7 %), respiratory (3.7 %), and GI signs (2.8 %) as the most signaled events.

In the EV database, 16,766 (16,633 serious) reports included 36,744 AEs (2.19 AEs/P). Most common AEs were infections (13 % of reports), respiratory signs (11 %), hematological events (10 %), nervous disorders (7 %), GI disorders (6 %), and infusion reactions (4 %). TLS (145 reports) and CRS (44 reports) were also registered. Infections included pneumonia (3 %), sepsis (2 %), neutropenic sepsis (0.2 %), HBV (3 %) and HCV (0.3 %) infections/reactivations, herpes zoster infections (1 %), CMV infections (0.8 %), UTI (0.6 %), and URTI (0.2 %). Most frequent cytopenias were neutropenia (6 %), febrile neutropenia (2 %), thrombocytopenia (3 %), and pancytopenia (2 %). Bone marrow failure was reported in 137 cases. Noteworthy, 423 cases of PML, and 37 cases of JCV infections, and 33 cases of leukoencephalopathy were also reported. Fatalities were about 6 %.

35.6 Remarks

Since initial observations in experimental animals (monkeys), the overall safety profile of rituximab appeared limited to the B cell line and reassuring. However, one apparent concern was about the long-term depleting effect on these cells, since B cell recovery was not complete after 90 days postdose [1]. After about 15 years of clinical experience, rituximab has confirmed the expectancies and remains a manageable biomedicine, mainly when compared to alternative therapies for the same indicated diseases. The lack of serious long-term toxicity and of myelo-suppressive effects, although massively depleting the B cell compartment without

interfering with the stem cell compartment, remains the most valuable characteristic of this mAb. However, anemia and thrombocytopenia are among the encountered drug-related AEs.

Rituximab exerts its action through a number of effector mechanisms that still need to be fully elucidated, which support the selective and prolonged activity on the B cell compartment. However, recent experience is showing that genetic polymorphism of Fc receptors may explain differences in efficacy and safety, and possibly enlighten the progressive resistance to therapy observed with this drug, mainly when used as monotherapy [10]. The intervention of different mechanisms of action may also be involved in diversity of expressions at local level, both in efficacy and safety, as well as in early versus late interventions. For example, CDC is more likely to cause early events in circulation, while internalization of rituximab-CD20 complexes may led to resistance episodes and more persistent late events. On the other hand, they also explain some enhancing and synergic effects of rituximab, when associated with chemotherapy and/or other monoclonals, through mechanisms of sensitization of the targets.

The major concern of rituximab is about infections, often serious and sometimes fatal, which occur in most therapy regimen and in almost all treated diseases, while the risk for developing an immune or allergenic response is very low. The tumor burden has also an important impact on efficacy and expression of typical AEs, such as TLS and CRS, which are usually moderated by proper initial treatment regimens.

Particular concerns rise from off-label experiences, not only due to lack of controlled trials, extreme heterogeneity of uncontrolled studies, and observational data bias, but also because serious infections and related fatalities remain frequent also in cohorts of patients with low levels of diseases-related mortality. This approach exposes patients to an additional unjustified risk, when considering the overall low efficacy profiles so far experienced.

In some studies on ITCP, drug-related mortality rates were higher than in NHL cohorts, and the real efficacy of rituximab treatment was hard to estimate, due to the known spontaneous recovery of some ITCP in the absence of controlled trials [17]. In other clinical conditions overlapping multiple cytotoxic and immunosuppressive therapies do not allow to discriminate among AE causalities. In other published experiences, NHL or RA protocols were adopted for the treatment of other diseases without an apparent scientific rationale, and in the presence of a number of serious and typical rituximab-related AEs, such as severe and fatal infections [15, 16]. Therefore the off-label treatment with rituximab should be more carefully considered.

In both on- and off-label experience, the rate of Ig decrease is not severe and tends to recover, together with the number of B cells. This may appear in contrast with the rate and severity of encountered infections, thus suggesting the existence of other immunological impairments. In fact, postrituximab B cell late reconstitution appeared to be preferential and earlier for naive B cells, rather than for memory B cells, although transient decreases of CD4+ and CD8+T cells were also

observed, suggesting the possibility of a different remodulation of the immune lymphocyte compartment after rituximab therapy [23].

A different approach to reduce mAbs-related AEs incidence without hampering efficacy may come from ongoing studies on lower and fractionated doses. In fact, a higher dose of rituximab seems to reduce cell killing with respect to intermediate doses, due to hypothetical mechanisms of effector exhaustion, including complement consumption at high B cell burdens.

Both in NHL and SLE experience an underlying heterogeneity of the disease affects efficacy, but it is not yet clear if it influences also the AEs response. Studies in this direction are lacking and may help in selecting best curable subpopulations of individuals.

Amid the discouraging experience on SLE patients, rituximab seems to be more effective in refractory diseases than in nascent lupus nephritis, suggesting the possibility of selecting populations with a better risk/benefic outcome for this disease, such as Afro-Americans [20].

Overall, the risk of infections and of serious/fatal infectious complications seem elevated in many autoimmune diseases, including RA and pemphigus, which may be influenced by a variety of heavy immunosuppressive therapies. Whether rituximab confers a uniquely elevated risk remains unclear. Nonetheless, in most instances the toxic effects of chemotherapy and immunosuppressive therapy are more elevated than rituximab ones, and their association considerably contributes to lower the overall rates of AEs.

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Tocilizumab

36

Tocilizumab (Actemra®, RoActemra®, Genentech/Roche) is a humanized IgG1k monoclonal antibody against interleukin-6 receptor (IL-6R), thus inhibiting the biological activity of IL-6, and thereby reducing the production of acute phase molecules active in inflammatory and autoimmune processes. Tocilizumab was approved in Japan in April 2005 for the treatment of multicentric Castleman's disease, and in April 2008 for the treatment of adult rheumatoid arthritis (RA), systemic juvenile idiopathic arthritis (SJIA), and polyarticular juvenile idiopathic arthritis (PJIA). EMEA approved tocilizumab by the end of 2008 for the second-line treatment of moderate to severe active RA in combination with methotrexate (MTX), or as monotherapy when MTX treatment is inappropriate or not tolerated. In December 2009, FDA granted approval as second-line treatment in RA patients after inadequate response to TNF antagonists, as monotherapy or in combination with DMARDs, and in April 2011 for the treatment of active SJIA in patients 2 years of age and older [1–8]. Initial approval in Japan for Castleman's disease was essentially based on Nishimoto studies followed by one (ML19367) post-marketing study [9]. Subsequent extensions to RA were based on MRA213JP (SATORI), MRA012JP (SAMURAI), and MRA010JP (STREAM) trials and on MRA215JP study, an extension of the SATORI trial. Extensions on SJIA and PJIA patients include the Japanese studies MRA011JP (Phase II) and MRA316JP (Phase III), one long-term study (MRA324JP) and one extension study (MRA317JP) enrolling patients from the two previous MRA011JP and MRA316JP trials, one postmarketing study (ML21940) for SJIA, and two Japanese postmarketing studies for PJIA (ML21939, ML21943). An additional study (LRO320) was conducted on Caucasian SJIA patients to test on other populations the Japanese experience. The multicenter WA18221 (TENDER) study followed, giving additional 1 year interim data, and is currently in the 5 years extension phase [5, 10–12]. Finally, one ongoing study (WA19977) is dedicated to PJIA long-term follow-up.

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Subsequent approvals in other countries for RA patients were based on five additional Phase III trials, two long-term extension studies, and four supportive studies, including two of the previous studies performed in Japan. In particular, OPTION (WA17822, enrolling 623 RA patients, 419 exposed to tocilizumab), LITHE (WA17823, 1,196 patients, 802 exposed), and TOWARD (WA18063, 1,220 patients, 805 exposed) trials evaluated safety and response to tocilizumab in RA patients who had failed MTX or DMARDs therapy, while study RADIATE (WA18062, 498 patients, 338 exposed) enrolled patients who had failed anti-TNFα therapy, and AMBITION (WA17824, 572 patients, 288 exposed) compared tocilizumab to MTX in a non-inferiority study.

Finally, studies WA18695 (GROWTH95, with 537 exposed) and WA18696 (GROWTH96, 1,902 patients, all exposed) were respectively extensions of WA17822 and of the remaining four studies mentioned above. They were conducted to provide further long-term safety and efficacy evaluations.

Because of their relative homogeneity, data from the major studies could be pooled and evaluated in meta-analyses [1–12].

Overall, more than 4,200 RA patients were evaluated for FDA and EMEA approvals. Supportive data were also obtained from Roche and Chugai databases on 3,800 patients. About 50 % of them were followed for 12 months and 39 % up to 18 months after treatment with the higher dose of tocilizumab (8 mg/kg). The population of SJIA patients, up to 2010, included 285 subjects (225 exposed). At present, over 95 countries have approved tocilizumab for RA, and over 165 trials are completed, ongoing or recruiting, including long-term extensions of previous pivotal trials.

Quite recently (2013), both FDA and EMEA approved the indication for PJIA on the basis of study WA19977 consisting in three parts, including 251 patients for the first two controlled studies followed by an open-label ongoing investigation. Supportive studies were MRA318JP and its long-term extension MRA319JP on the same 19 patients, and the postmarketing study ML21939 , all on Japanese patients.

36.1 Mechanism of Action

IL-6 (formerly known as IFNβ2) is a glycoproteic cytokine with a four-helix structure inducing cell differentiation and modulating antigen-specific immune responses and inflammatory reactions. It is one of the major physiological mediators of acute phase reactions and shows a wide range of biologic activities in immune regulation, hematopoiesis, inflammation, and oncogenesis. IL-6 is produced by a variety of cell types including lymphocytes, monocytes, fibroblasts, adipocytes, endothelial cells, and synovial cells in response to endogenous (TNF, IL-1, IFN, etc.) and exogenous viral and bacterial stimuli. In particular, IL-6 stimulates hemopoietic progenitor cell proliferation and differentiation, induces B cell differentiation, promotes differentiation of CD4 T cell into the Th17 subset, particularly active in autoimmune processes, and of CD8 T cells into cytotoxic

effectors. IL-6 stimulates fibroblasts to produce RANKL and VEGF, which promote osteoclastogenesis and angiogenesis, respectively. Moreover, IL-6 induces the production of collagen in fibroblasts, and stimulates hepatocytes to produce a number of acute phase proteins (CRP, amyloid A, haptoglobin, α1-antichymotrypsin, fibrinogen), reduces the intestinal uptake of Fe and increases uptake of Zn ions in hepatocytes, thereby causing hypoferremia and hypozinkemia usually occurring during inflammation. Upon stimulation of Toll-like receptors on monocytes and macrophages by pathogenic bacterial lipoproteic and lipopolysaccharidic motifs, IL-6 also triggers innate immune response, by promoting leukocyte cell recruitment at inflammatory sites. IL-6 is also produced by synovial and endothelial cells in joints affected by inflammatory processes. Finally, IL-6 acts also on neuronal cells and regulates glial cells activation, thus showing to exert pleiotropic functions in various systems as a differentiating and activator factor.

IL-6R is naturally occurring as soluble (sIL-6R) or membrane-bound (mIL-6R) receptor. The former is a proteolytic cleavage product of the latter, which consists of two glycoproteic chains. The short unit acts as the IL-6-binding receptor and generates also sIL-6R. The long unit (gp130) transduces the IL-6 signal via an intracellular domain.

After IL-6 binding, the IL-6/mIL-6R complex induces the gp130 homodimerization (tetrameric or hexameric), which in turn activates the intracellular JAK tyrosine kinases pathway. However, sIL-6R can also bind IL-6, and the soluble complex can trigger gp130 even at cell surfaces lacking endogenous mIL-6R. In fact, the gp130 chain is also expressed on a wide number of cells which can receive IL-6 signals via the sIL-6R, and is shared by other receptors, such as IL-11, IL-27, leukemia inhibitory factor (LIF), oncostatin M (OM), ciliary neurotrophic factor (CNTF), and other structures which show overlapping functions with IL-6R. However, IL-27 may also act as inhibitor if IL-6 and IL-11 functions.

The pivotal role of IL-6 in inflammation and autoimmunity has been proved in different experimental and clinical situations. Il-6 levels are elevated in RA, Castleman' disease, in JIA, SJIA, Crohn's disease, polymyalgia rheumatica, Takayasu arteritis, polymyositis, SLE, progressive systemic sclerosis, neuromyelitis optica, and MS. Notably, in the latter two diseases IL-6 levels are elevated in the CNS. Furthermore, in MS the IL-6-dependent autoreactive Th17 T cell subset and B cells are considered the crucial cerebral damage effectors.

At articular level, IL-6 can stimulate pannus formation through VEGF-induced angiogenesis and increase bone resorption as a result of osteoclastogenesis. These are usually accompanied by a marked increase of acute phase proteins, including serum amyloid A (SAA), related to IL-6 dysregulation. Finally, IL-6 is overexpressed in many malignancies, including lymphoproliferative disorders and epithelial cancers, possibly after disruption of downregulating agents, such as p53 and Rb gene inhibitory products. Elevated levels of IL-6 in these patients have been correlated with poor prognosis. Because of the angiogenic function via VEGF upregulation, IL-6 may also have a role in metastatic diffusion.

Tocilizumab (RO4877533), formerly myeloma receptor antibody (MRA), or rhPM-1, is a recombinant humanized IgG1k monoclonal antibody binding with similar affinity to both sIL-6R and mIL-6R receptors. Tocilizumab was obtained by engrafting CDR murine regions of an anti-IL-6R monoclonal antibody (PM1) into a human IgG1 framework. The binding site of tocilizumab is located within the IL-6 binding site, and thereby the monoclonal blocks the IL-6 biological activity on both receptors. In particular, tocilizumab interferes with the dimerization of gp130 component of IL-6R, thus blocking the classical and the trans-signaling pathways inducible by IL-6 via mIL-6R and sIL-6R, respectively. Notably, the administration of proper doses of tocilizumab increases the level of circulating IL-6, with early peak levels and a relative dose–response effect. These levels tend to decrease with time, possibly as a consequence of the anti-inflammatory effect of the monoclonal. However, a marked and dose-dependent increase of sIL-6R was also observed in healthy and RA subjects up to 7-fold higher than the baseline after a single administration, with peak levels after 3–4 weeks and returning to baseline in about 8 weeks. Both elevations are considered a consequence of accumulation of IL-6 and sIL-6R, due to the lower clearance of tocilizumab/IL-6R complexes with respect to the free soluble molecules. No significant CDC or ADCC activities induced by tocilizumab have been detected in vitro. However, tocilizumab binds to FcγI and FcRn [1, 4, 13–15].

36.2 Immunogenicity

Over a large cohort (2,876) of tested RA patients, anti-drug antibodies (ADA) were detected in 1.6 % of cases (1.1 % neutralizing). Hypersensitivity events were associated to a minority of ADA-positive subjects (0.2 %). About 6 % of AEs were considered of immunogenic origin. The presence of ANA seroconversion, either negative or positive, did not correlate with tocilizumab treatment. In the small cohort of SJIA treated patients, ADAs were present in 1.8 % of cases. However, these values were considered as possibly underestimated, due to the higher serum concentrations of tocilizumab observed in pediatric patients [5, 6]. Discontinuation rates for anaphylaxis and other hypersensitivity reactions were 0.1–0.2 % for RA, and 0.9 % for SJIA [3, 5, 6]. In a subset analysis of pivotal clinical trials on 4,199 RA patients (LITHE, RADIATE, OPTION, TOWARD, AMBITION, and GROWTH95/96), ADA were detected with three different methods and confirmed in 2.3 % of cases (2,816 patients), while their rate was 1.3 % (on 2,315 patients) in the extension studies after 24 weeks of treatment. A potential immunogenic event was associated in 7/14 cases to ADA-positivity. The neutralizing capacity of these antibodies was not tested in this study. Interestingly, 8/14 patients experiencing anaphylactic reactions had ADA of the IgE isotype, while ADA-positive patients at baseline (1.7 %) were IgM directed to the human Fc portion of tocilizumab. It was concluded that hypersensitivity-like reactions, including some infusion reactions in ADA-negative patients were induced by antibody-independent mechanisms. [16].

36.3 Adverse Events

Overall, the safety analysis performed by EMEA in RA patients was based on the five pivotal studies WA17822, WA17823, WA17824, WA18062, and WA18063 for a total of 4,109 enrolled patients, while the long-term safety information derived from 2,439 patients subsequently followed in the extension studies of WA17822 and WA18696 trials, for a duration of 5 years. Safety in SJIA patients was based on WA18221 part I and part II extensions (112 patients observed over 1 year, 75 treated) and on the supportive studies MRA011JP and MRA324JP, for a total of 149 treated patients observed for 2 years (median), and up to 3.5 years. FDA safety analysis was based on the same studies and on other additional information from various sources. In particular, ML21136, MA 21573, and WA19923 provided information on RA; ML 21940 on SJIA; trials ML21939, ML21943, and postmarketing studies conducted in Germany (ML21469) and in China (ML21753) on PJIA; six studies and reports (121 patients), along with one postmarketing study (ML19367) conducted in Japan on Castleman's disease. Additional information was available also from applicants' databases (Roche/Chugai) on Crohn's disease (24 patients), multiple myeloma (37), and SLE (14). The major selected safety population consisted in 3,778 patients with RA followed for at least 6 months; 56 % of them were followed for one year, and 6 % for 2 years. In the last 2012 update of the official labels (EMEA, FDA) the safety population included over 4,009 RA patients (74 % treated for 2 years, 55 % treated for 3 years), and 112 SJIA patients followed for 12 weeks and still ongoing [7, 8].

The BBW in the updated version of the official label reports *serious infections,* including TB, bacterial, invasive fungal, viral, and other opportunistic infections. Special additional warnings include *viral reactivations*, *gastrointestinal perforations*, primarily as complication of diverticulitis, *hypersensitivity reactions, hepatic impairment, active hepatitis and enzymatic abnormalities, severe neutropenia and thrombocytopenia, hyper/dyslipidemia, demyelinating nervous disorders, cardiovascular disorders,* and *malignancies.* Within this general safety profile, there are some RA and SJIA safety peculiarities. In fact, these diseases share most types of AEs, but show some differences in their frequency and in the induction of *macrophage-associated syndrome* (MAS), which seems typical of SJIA patients. Additional details are given also on profiles occurring in Castleman's disease and in PSJIA diseases approved in some countries for this biomedicine, as well as on other off-label occasional features described in the following dedicated section.

The most *common AEs in RA* were *infections*, which included nasopharyngitis/URTI (5–8 %), and less frequently cellulitis, pneumonia, and herpetic infections. Serious infections of all origin, including opportunistic infections, occurred at rates of 5 % P/Y. In the all-exposure initial population (4,009 patients) they included pneumonia, cellulitis, abscesses, gastroenteritis, and sepsis. Opportunistic infections included TB (9 cases), Herpes exacerbation (10), atypical mycobacterial

infections (2), 2 cases of pneumonia (P. Jiroveci, Cryptococcus), and one fungal sinusitis [4]. Other common events included cephalea, hypertension, ALT increase, hypersensitivity (rash, pruritus, urticaria), gastrointestinal (pain, oral ulcers, gastritis), hepatic enzymes abnormalities (ALT and bilirubin increase), leukopenia (neutropenia), respiratory signs (cough, dyspnea), peripheral edema, and conjunctivitis.

Infusion reactions were reported in about 7 % of cases (5 % in controls), mostly showing as hypertensive crisis during infusion, with cephalea and rash/urticaria following within 24 h. Anaphylactic reactions were observed in about 0.2 % of cases. Unlike other similar treatments, infusion reactions appeared after the first infusion (usually after 2–5 administrations) and were not dose-dependent. Fatal events were registered in the postmarketing experience.

Gastrointestinal perforations were mainly reported as complications of diverticulitis at an overall rate of <0.3 % P/Y and were not dissimilar in the long-term experience. In the all-exposure initial population (4,009 patients) 41 cases of gastrointestinal perforations were detected, with rates above the background RA population and the incidence for TNF inhibitors, but below the frequency after corticosteroids treatment. *Hematological abnormalities* were mainly represented by neutropenia (3.4 vs. <1 % in controls) and thrombocytopenia (1.7 vs. <1 %), while cytopenias/pancytopenias were observed in the postmarketing setting.

Other laboratory abnormalities were limited to transaminase ALT/AST increase (2/6 % respectively) and dyslipidemia, which occurred more frequently as total cholesterol (24 %) and LDL increased levels (15 %).

It must be noted that a *cardiovascular risk* is evidenced in the EMEA official product information and has been related to the existence of risk factors in RA patients, including hypertension and hyperlipidemia also induced by tocilizumab [7]. However, IL-6 overexpression is associated to coronary artery disease, CHF, and other cardiac risk factors such as obesity and diabetes, all considered disease states with low-grade inflammation. Overall, the incidence from seven pivotal trials on 4,009 patients was 0.25 % P/Y for myocardial infarction and 0.19 % P/Y for stroke, remained stable during follow-up, and therefore was not higher than expected rate in the RA population.

The *safety profile in SJIA* was similar to that of RA patients, except for the serious/life threatening MAS, and for a tendency to express some AEs at a higher frequency. In fact, infections were more frequent, and serious infections rates were about 11 % P/Y, but remained stable after one year of treatment. Infusion reactions occurred during administration (4 %) or within 24 h (16 % vs. 5.4 in controls) and showed additional gastrointestinal signs (pain, diarrhea) and arthralgia, associated with rash/urticaria and cephalea events also observed in RA adult patients. Neutropenia was also more frequent (7 vs. 3.4 % in RA and 0 % in SJIA controls) and tended to increase with time (15 %). Thrombocytopenia was slightly increased (3 %), but remained stable. Transaminases tended also to be more elevated (5–12 % ALT, 3–4 % AST vs. 0 % in controls) with time, while lipids tended to be less altered than in adult RA (1.5 vs. 24 % in RA, vs. 0 % in SJIA controls for total cholesterol). The real peculiarity in SJIA patients is the

development of MAS, which occurs in about 7–8 % of cases, and is induced by a massive activation of macrophages and of T lymphocytes leading to a profound inflammatory reaction with persistent pyrexia, lymphadenopathy, and hepatosplenomegaly (see Chap. 3). However, the incidence of MAS in WA18221 and in Chugai database on SJIA-treated patients ranged from 2.7 to 3.8 %, thus being lower than respective background rate of untreated SJIA patients, thus indicating a potential beneficial effect of anti IL-6 therapy. The syndrome is also associated with liver dysfunction, cytopenia, and serious coagulopathy with elevated levels of fibrinogen and thrombocytopenia. However, MAS occurs in SJIA patients during other conventional anti-inflammatory therapies, and also during treatments with etanercept and anakinra. During pivotal trials in Japan, no MAS cases were observed after tocilizumab, and only two cases were reported in the national postmarketing setting. However, it is believed that diagnosis is difficult in tocilizumab-treated patients, due to a possible masking effect of the treatment on the clinical features. In fact, basic signs of MAS such as pyrexia and serious sickness, are mainly related to IL-6 activity in these patients, which can be greatly mitigated by therapy, while drug-related laboratory abnormalities are not modified. In contrast, constitutional signs are unchanged during conventional therapy, and corticosteroids are effective in controlling the syndrome [1–14, 17].

Necrotizing fasciitis is a serious event with a considerably high mortality (70–80 %) when not promptly diagnosed, and even after due surgery remains high (20–30 %). It has been reported in the postmarketing experience after adalimumab, efalizumab, infliximab, panitumumab, and tocilizumab administration. Two recent cases of necrotizing fasciitis during tocilizumab treatment in adult RA have confirmed this possibility and raised concerns for potential missing/delaying diagnosis and treatment, since serious signs of infection, including pyrexia and sickness, were absent at initial presentation due to the suppression of acute phase reactants induced by tocilizumab. Therefore, tocilizumab may expose to a peculiar and *increased risk of infections in the absence of typical clinical signs* [18, 19].

During clinical development *demyelinating disorders* were observed in RA patients. The real impact of this event is unknown, but is expected to be <1 %. In the overall exposed original population, eight cases of demyelinating disorders were registered, including one cranial and 2 poly-neuro/radiculopathies, one optic neuritis, one MS (probably antecedent to treatment), one leukoencephalopathy, and 2 other MRI white matter lesions (one chronic ischemia). However, on subsequent data review they were not considered as new cases, and only three of them were accepted as true demyelinating disorders. Two of the suggestive demyelinating events were observed in Study WA17823 and in WA18696 (extension of WA18062), and one case of repeatedly JCV-negative leukoencephalopathy was reported in the long-term MRA215JP study [1, 2, 4, 20]. In the SJIA population one suspected case was also reported showing a tic disorder and mild MRI signs.

In a systematic literature review and meta-analysis of the major six controlled trials (OPTION, TOWARD, RADIATE, CHARISMA, SATORI, and AMBITION) on RA patients, including three studies with tocilizumab administered as monotherapy, *safety pooled data* revealed a small but significant increased risk

(OR 1.53) of AEs in the 8 mg/kg plus MTX combination groups, mainly for infections (OR 1.30), but not for total SAEs and for serious infections. Moreover, no increased incidence was observed for TB reactivation, hepatitis, and malignancies. Significantly increased categories of infections included URTI, skin/soft tissue and GI disorders, while all other typologies were not different among study groups and controls. Overall, 3,012/3,501 patients completed the 24 weeks follow-up [21].

In a recent postmarketing analysis, the safety profile of tocilizumab was assessed in 3,881 RA Japanese patients treated *in clinical care* with monthly injections for 28 weeks. This cohort differs from the groups enrolled in clinical trials since they are not selected for risk factors. Total AEs were observed in 52.3 % of patients (1.8 AEs/P), and SAEs were reported in 22 % of cases (1.4 SAEs/P). Tocilizumab-related events were attributed to 84 % of patients reporting at least one AE, and SAEs were registered in 20 % of them (1.3 SAEs/P). The most clinically relevant events were infections, which resulted higher (9 % P/Y) than in previous clinical trials and included pneumonia in 44 patients (1.1 AEs/P), and sepsis/septic shock in five patients. Four new cases of TB (0.1 %) were observed and ILD signs occurred in 23 patients. Gastrointestinal perforations were observed in six patients (1.2 AEs/P). Infusion-related anaphylactic episodes were observed in seven patients.

Overall, *serious infections* and *ILD disorders* resulted increased with respect to clinical trials' history, while the remaining safety profile was substantially confirmed, including the standardized mortality ratio (1.66) [22]. Similar and complementary data were reported in another investigation on 1,681 clinical care RA patients with inadequate response to DMARDs and/or TNFα inhibitors. In this cohort, AEs were present in over 77 % of patients (3.5 AEs/P) and infections occurred in 35 % (2 % serious). Infusion reactions were about 17 %. SAEs occurred in about 8 % of cases and were considered as tocilizumab-related in 44 % of cases. Overall, serious infections were less frequent than in the previous study, but slightly higher in patients showing inadequate response to TNFα inhibitors [23].

Clinical practice did not evidence new signals compared to those reported in selected cohorts of RA patients enrolled in the mentioned trials. Moreover, the general profile emerging from both selected and unselected patients were similar to that experienced in tocilizumab monotherapy as shown by the AMBITION study.

A peculiar aspect among drug-related AEs is represented by induced *malignancies*, which are difficult to assess in the short range, and on limited populations of patients, mainly when the underlying disease also expresses increased rates of neoplastic events. In a recent meta-analysis on the risk of malignancies on over 29,400 RA patients treated with biomedicines, such risk seems to be excluded, since a statistical significant increase in the overall tumor rates was not observed, nor in specific cancer types (see adalimumab, Chap. 3). The 211 encountered malignancies included 118 solid tumors, 48 skin cancers, 14 lymphomas, and five cases of leukemia/myeloma. In the tocilizumab-treated cohort, 18 neoplasms were

registered in the 3,271 patients included in the safety analysis. The risk ratio was higher (2.22) for solid tumors, while skin tumors and lymphoma were below (0.22 and 0.05 respectively) the Peto OR index [24]. However, these data were limited to 52 weeks follow-up for tocilizumab patients.

Finally, a recent experience from a new trial on 556 RA patients (ACT-RAY) has provided new data to the safety profile of *tocilizumab monotherapy*, in addition to the previous data that had mostly derived from the AMBITION study, also allowing comparison of such data to the addition of MTX profile. In particular, over 72 % of patients in monotherapy had at least one AE (70 % in combined therapy). SAEs were also similar between groups (5.8 and 6.1 % respectively), as well as for serious infections (2.2 %), which represented the most common serious event. ALT/AST levels were higher in combined therapy (7.8/1.9 % as 3 fold upper normal levels at baseline) compared to monotherapy (1.2/0.4 %). Similarly, AEs-related discontinuation (3.6 vs. 2.5 %) and mortality rates (0.7 %) were also comparable in the two study groups [25]. Overall, the safety profile of tocilizumab monotherapy was confirmed as a mild/moderate, and MTX association induced only minor modifications in its features.

36.4 Off-Label Experience

The off-label use of tocilizumab is rather limited. Among 165 ongoing trials, more than 150 concern RA (147), SJIA (7), and Castleman disease (2). Occasional studies are dedicated to polymyalgia rheumatica (4), giant cell arteritis (3), uveitis (3), GVHD (2), and skin disorders including Behçet's and systemic sclerosis. Case reports have been also published on Hemophilia A, GVHD, and TRAPS. Vasculitis, psoriasic arthropathy, seronegative arthropathy, temporal arteritis, ankylosing spondylitis, polyarthritis, and pancreatic carcinoma are also included in postmarketing records and case reports.

Although approved in Japan and India, the treatment of *Castleman disease* and of *PJIA* is not included in the UA and EU indications for tocilizumab. Castleman's disease is a localized or multicentric lymphoproliferative disorder characterized by lymphonodal follicular hyperplasia, with capillary and endothelial proliferation, and elevated levels of IL-6 [26]. The multicentric form tends to be refractory to conventional therapy and therefore has a poorer prognosis. Infections, renal failure, and malignancies (including Kaposi's sarcoma) are common serious complications, and the suspected etiological agent is the Kaposi's sarcoma-associated herpesvirus (KSHV or HHV-8), mainly in HIV infected subjects [27]. Notably, these viruses share an IL-6 homologue (vIL-6), which binds to IL-6R, yet with low affinity, and can activate the JAK kinase pathway [26]. The initial experience of Nishimoto, with the initial formulation of tocilizumab (rhPM-1) administered in seven patients with Castleman's disease, reported only a transient and mild-granulocytopenia in two patients, which recovered spontaneously within two days. No decrease in T cell function was observed as measured by PPD skin test and

allogenic MLC test [28]. In the subsequent experience on the same group of 28 patients, a more detailed description of AEs was reported. The Chugai formulation of the tocilizumab (MRA) was used, and one patient was withdrawn after 40 weeks of treatment (continuing up to 55 weeks for compassionate use) because of aggravation and development of acute leukemia. All but one patient reported adverse events, which were mild/moderate and included common cold (57 %), pharyngitis (18 %), pruritus/rash (18–21 %), eczema (18 %), moderate pyrexia/malaise (18–21 %), diarrhea (18 %), UTI, chest pain, and aphthous stomatitis (14 % each). Infusion reactions (21 in 14 patients) were also mild and transient. SAEs were observed in 2 patients as cellulitis. Transient, mild/moderate leukocytopenia (43 %) and liver enzymes abnormalities (32 %) were also detected. Overall, the typical safety profile subsequently experienced in larger scale on other diseases was anticipated, although with particularly mild and transient features [29].

Data on PJIA are limited and often collected together with SJIA and Still's diseases events. A postmarketing study (ML21940) on about 231 PJIA patients and data from case reports and postmarketing reporting include 125 subjects suffering at least one SAE (1.8 AE/P). Among them there were 18 reports in PJIA inside Japan (1.5 AE/P) and no fatal events. All together there were 65 serious infections including two cases of pneumonia in PJIA, and one unspecified serious event in a compassionate program [4].

A recent off-label experience in *spondyloarthritis* and peripheral spondyloarthritis has been conducted in France on 21 patients with inconsistent results. However, no serious AEs occurred and active phase reactants were reduced by therapy. The registered events included one abscess, oral ulcers (2), nasopharyngitis, Q fever, peripheral vasospasm, liver enzyme and lipids elevation, and one unspecified allergic reaction. Notably, one case of anterior uveitis, two cases of reactivation uveitis, and one case of psoriasis reactivation were observed. A few previous reports on the same diseases showed comparable results, thus indicating that the reduction of IL-6 and other active phase parameters induced by tocilizumab did not parallel with a significant clinical improvement in this disease [30].

In another off-label experience in pediatric refractory *Takayasu arteritis*, which is particularly serious in children, tocilizumab administered every 2 weeks up to 4–5 years was effective and no relevant adverse events were registered, including infusion reactions or serious infections. Notably, IL-6 and other acute phase reaction laboratory parameters returned within normal levels, thus enabling discontinuation of corticosteroids [31].

36.5 Postmarketing Surveillance

In the FAERS database, over 6,000 records refer to tocilizumab-related AEs. The most common events include infections (11 %), respiratory disorders (3 %), dermatologic reactions (2.8 %), hepatic (2 %) and CNS disorders (1.8 %).

In the EU database, 3,289 reports (3,226 serious) were registered from November 2005 to December 2012, including 7,231 AEs (2.2 AEs/P). The most frequent events were infections (21 %) followed by gastrointestinal signs (9.5 %), dermatologic (7.3 %), nervous and respiratory disorders (6.5 % each). Among infections, the most frequent and serious were pneumonia (11 %), sepsis/septic shock (6/2.8 %), cellulitis (5.3 %), diverticulitis (4.2 %), Herpes zoster infections (3.9 %), UTI (2.3 %), and abscess (1.7 %). Neutropenia (4.8 %), thrombocytopenia (3.5 %), and anemia (2 %) were also observed. Infusion reactions (3 %), and hypersensitivity reactions (rash, urticaria, pruritus, etc.) were reported in about 4–5 % of cases. Two systemic syndromes, reported as "systemic inflammatory syndrome" and "acute phase reaction", were presumably referable to MAS. Notably, 20 cases of TB, 36 cases of disseminated intravascular coagulation, and 11 cases of necrotizing fasciitis were also registered. Six cases of encephalopathy, 5 demyelinating disorders, and five leukoencephalopathies were also reported.

36.6 Remarks

The safety profile of tocilizumab is in the framework of events related to its specific mechanism of action. The major concerns are related to serious infections and malignancies due to the immunosuppressive activity. However, by considering the pivotal role in inflammation of IL-6 and the pleiotropic effects exploited on different systems, the frequency and typology of AEs so far experienced seems more limited and moderate than expected. However, some discrepancies between the biological consequences of IL-6 blocking and safety have also emerged. For example, abnormalities in the hepatic enzymes due to direct interference of tocilizumab with IL-6R expressed on hepatocytes do not seem to induce much damage at hepatic level, and remain widely reversible even after prolonged treatment. Similarly, the frequently occurring drug-related hyperlipidemia is not strictly associated with a significant increased risk of cardiovascular events. Although long-term observations are still limited, malignancies do not show a trend to increase with time, a characteristic shared also by all major encountered AEs to this biomedicine. However, a dose–response trend is suggestive, as better evidenced by tocilizumab treatments in monotherapy, but the AEs increment rate remains often within the range of underlying diseases background, as shown for cardiovascular and cerebrovascular events, and malignancies in the RA population, or when compared to conventional therapies. Even serious infections, which are elevated after tocilizumab, tend to become equivalent to controls with longer duration of exposure, or to high dosages of conventional treatments, and their typology is similar to that occurring during DMARDs treatments. Infusion reactions are present in the expected range of frequency and typology. Interestingly, they do occur mainly after 2–5 infusions, in contrast with similar biomedicines which reactions tend to appear at first administrations and to decrease afterwards. No significant CDC- or ADCC-related AEs have been identified. However, MAS

episodes are present among SJIA patients, although at lower levels than in the SJIA background population, indicating a potential beneficial or masking effect on some MAS clinical expressions. In fact, a concerning capacity of the treatment to cover clinical signs of disease, with potentially life-threatening consequences, has emerged (MAS, necrotizing fascitis, and other silent local infections) [17, 18, 32].

Opportunistic infections, new/reactivated TB, and virus reactivations are rare, indicating that the overall immune defense is still reasonably operative. The immunogenicity, as expected, remains low and does not seem to interfere with the presence/induction of autoimmune reactions, such as ANA. However, demyelinating disorders and leucoencephalopathies are being reported in the postmarketing setting in addition to initial suspected episodes, suggesting a closer vigilance in the field. Hematologic abnormalities do not raise particular concern, since neutropenia is usually moderate and reversible and not associated to infection. Similarly thrombocytopenia and serious hemorrhagic disorders are limited. Nonetheless, AEs remain the most frequent reason of tocilizumab discontinuation [33], although pooled data and indirect comparisons in the absence of head-to-head studies indicate that there is no higher risk with respect to controls and to other similar treatments [34].

Overall, due to the limited time of observation and treatment with tocilizumab a high vigilance is still demanded mainly for serious infections, silenced infections, which can become life threatening if not promptly cured, demyelinating disorders, and malignancies. Particular vigilance on RA treatment is demanded, especially on the insurgence of malignancies in the exposed RA population [24] since observations so far conducted are incomplete, and because of the frequent inadequacy of a number of studies in this field [35].

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Tositumomab

37

Tositumomab (Bexxar®, Corixa, GSK) is a murine IgGλ2a monoclonal antibody directed to the CD20 antigen expressed by normal and primate B lymphocytes, and on human malignant B cells. In the therapeutic regimen it is usually associated with tositumomab-Iodine^{131} to exert anti-neoplastic radioimmunotherapeutic effects. In May 1994, FDA granted to this therapeutic association the orphan designation for the treatment of B cell nonHodgkin's lymphoma (NHL), including rituximab-refractory cases. In February 2003, EMEA granted the orphan designation to both products for the treatment of follicular lymphoma. In June 2003, FDA, following an initial request submitted in 1999 and subsequent revisions of 2000 and 2001, granted full approval for the treatment of rituximab-refractory, low grade, nontransformed NHL. In December 2004, this Agency extended the indication to all previously treated CD20$^+$ relapsed/refractory, low grade, follicular or transformed (NHL), granting an accelerated approval. Approvals from Canada and Japan followed in November 2008 for the same setting.

Initial approval (BLA125011) for the therapeutic regimen was based on two studies conducted in 100 patients with low-grade, transformed low-grade, or follicular large-cell lymphoma, namely CP-97-012 (40 patients) and RIT-II-004 (60), and on three supportive studies, RIT-I-000 (59 patients), RIT-II-001 (47), and RIT-II-002 (78). Data provided for the expansion submission (BLA125011/S024) were based on the same studies of the original application, except for updated safety data on delayed AEs from RIT-II-004.

Overall, safety evaluations were performed on 230 NHL patients in five clinical trials, and safety data from the expanded access program including 765 patients for delayed AEs observations on myelodysplastic syndrome (MDS), and for HAMA and TSH testing.

The postmarketing experience up to March 2004 was based on Study RIT-II-004 enrolling 60 patients, and on the ongoing SB39229/028 (formerly CCBX001-

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049) Phase III study (rituximab vs. tositumomab regimen) on relapsed NHL cases planned to enroll about 500 patients in US and EU centers [1–4]. However, the latter was early stopped due to lack of feasibility, and its patients (15) have been transferred for long-term evaluation to BEX104528, The manufacturer proposed as alternative the SWOG study S0016 on 554 (265 exposed) patients. Study 393229/029 comparing ibritumumab-tiuxetan to tositumumab-iodine131 was not feasible as well. Additional relevant trials include Study 104517 (NCT00996996) on 77 untreated advanced NHL, and Study 104514 (NCT01663714) on 30 untreated low-grade NHL patients, which have been recently completed. As for Iodine^{131} tositumomab associations with other nonmAb therapies, Study LYM 2005-01 (NCT00479167) evaluated toxicity and efficacy combination with bortezomib in 20 refractory/relapsed NHL, while Study CP-98-025 (NCT00933335) evaluated the combination of fludarabine followed by tositumomab regimen in 38 cases of untreated follicular lymphoma, followed for 10 years in Study 104528. Five studies from the SWOG group, either completed (S9911, S0433, S0016) enrolling 714 patients or ongoing (S0801) have focused on safety and efficacy of various chemotherapy regimen followed by rituximab and/or tositumomab administration.

Finally, 10 additional active or recruiting studies, including one long term completed Study FHCRC-1734.00 (NCT 00098566) on 36 patients, are evaluating the tositumomab regimen with or without chemotherapy, followed by autologous stem cell transplant in relapsed/refractory NHL patients.

37.1 Mechanism of Action

CD20 (Bp35, B1 antigen) is a tetraspanning transmembrane human B lymphocyte-restricted differentiation antigen, located at the surface of normal and primate B lymphocytes and of human malignant B cells. During B cell maturation, this nonglycosylated hydrophobic phosphoprotein is first expressed on pre-*B* cell, but is lost during the final stage of B cell maturation to plasma cells. In particular, CD20 is expressed on a subpopulation (about 30 %) of precursor B cells, on mature B lymphocytes, and on follicular dendritic reticulum cells. CD20 is also expressed on low-grade NHL B cells, precursor B-cell neoplasms, precursor B-lymphoblastic leukemia/lymphoma, (B-LBL), HCL, B-CLL (weak), and B-PLL. It is also present on lymphoplasmacytic cells in Waldenstrom's macroglobulinemia, but is absent on myeloma and on B-ALL cells.

CD20 has a role in B-cell activation/proliferation through the Src family tyrosine kinases, and enables optimal B cell immune response against T cell-independent antigens. Moreover, CD20 is also expressed on a minor population of T lymphocytes (2 %) pertaining to the memory cytotoxic compartment, which tend to increase with age. It is also presumed that CD20, regulated by the insulin-like growth factor (IGF-1), may act as a store operated calcium ion channel. However, no natural ligands are known, and no soluble CD20 forms have been so far detected.

Tositumomab is a murine IgG2aλ monoclonal antibody directed to CD20 antigen expressed by normal human and primate B lymphocytes, and on human malignant B cells. The therapeutic regimen includes also the same antibody covalently linked to Iodine^{131}, a beta/gamma emitter with a physical half-life of 8 days, for a single course treatment. The pretreatment with unlabeled tositumomab increases the cytotoxic effects of the radioisotope-labeled antibody. Therefore, the antibody effector mechanisms, such as induction of apoptosis, complement-dependent cytotoxicity (CDC), and antibody-dependent cell cytotoxicity (ADCC), are associated with the radiation cell toxicity expressed by the linked isotope on the same target (radioimmunotherapy). Murine IgG2a can bind all the three types of Fcγ receptors in humans, thus potentially binding to macrophages, granulocytes, and NK cells. This binding capacity goes beyond the mentioned anti-tumor cytotoxic action, affecting also the physiology of the immune system.

The high-energy beta particles emitted by I-131 are cytotoxic up to a distance of approximately 1–2 mm, thus permitting eradication of antigen-negative tumor cells by crossfire from neighboring antibody-coated cells. The treatment course induces a profound and persistent depletion of CD20^{+} cells, with lowest levels (about 0 %) at 7 weeks and initial recovery after 12 weeks, which still remains below the normal limits in 15–30 % of cases after 6 months from treatment. Nonetheless, as observed also for other anti-CD20 mAbs, there is no consistent reduction of Ig levels of the three major classes.

In a recent overview on anti-CD20 mAbs, five mechanisms of action were recognized, and the therapeutic mAbs were grouped according to their capacity to induce the reorganization of CD20 molecules into lipid rafts upon binding. In particular, Type I antibodies induce the formation of lipid rafts and efficiently activate the classical pathway of the complement system, while Type II antibodies do not translocate CD20 into lipid drafts, but induce cell death upon direct binding, and poorly activate the complement. Both types are capable of inducing ADCC in the presence of effector cells. Rituximab is a Type I mAb and expresses the cytolytic action mainly by ADCC via FcγR-expressing monocyte/macrophages, while CDC may play an additional role. In contrast, Type II mAbs like tositumomab mostly act by inducing direct apoptosis. These cytolytic functions may be differently expressed in various situations, being CDC more effective in circulation, where complement factors are highly represented, than on a solid tumor mass. Moreover, their efficacy varies in dependence of the tumor burden. In this case, multiple events are expected to be necessary in the presence of high tumor masses.

Further mechanisms, such as opsonization of mAb-covered targets, are involved, possibly as long-term effects during therapy. Upon CD20 binding the complex is not shed or internalized. However, tositumomab can be internalized when bound to some FcγR, although to a lesser extent than Type II mAbs. The internalization requires the crosslink between the Fc tail and FcγRIIb, an ITIM-containing inhibitory receptor, and between the Fab portion and CD20 on the same target cell. This mechanism is crucial in reducing therapeutic efficiency, since it

reduces the amount of mAb available at the target cell surface to recruit effector cells, and drags the mAb-CD20 complex into lysosomes for degradation.

Interestingly, the expression of FcγRIIb on different targets correlates with resistance, while the presence of FcγRIIIa, the ITAM-containing stimulatory receptor, is related to mAb efficacy. These mechanisms are common to other anti-CD20 mAbs, but their efficiency is expressed with a different hierarchy among them. In fact, the clinical experience indicates that cases of non cross-reactive resistance and synergistic effects between anti-CD20 mAbs and chemotherapy occur, possibly due to the mentioned mechanistic diversities. The presence of radioisotopes linked to tositumomab (or to ibritumomab) allows the overcome of residual resistances and enhances the cytotoxic action of these monoclonals.

Therefore, the double stage therapy seems the most appropriate for destroying circulating $CD20^+$ B cells, allowing to direct the radio-immuno conjugate more specifically to the bulk of lymphoma B cells and to antigen-negative tumor cells through the crossfire from neighboring $Iodine^{131}$antibody-coated cells. The crossfire seems also to circumvent the residual disease and to prevent from its diffusion. The tositumomab-$Iodine^{131}$ regimen is similar to ibritumomab-tiuxetan-^{90}Yttrium regimen, the latter being the murine parent of the chimeric rituximab linked to a different emitting isotope [1–7; see also rituximab, ibritumomab, and ofatumumab, Chap. 23, 29, 35].

37.2 Immunogenicity

The incidence of HAMA tested on over 980 patients was about 11 % among subjects who were negative at baseline, with a delayed trend to increase with time (167 days median time for HAMA positivity), and was up to 20 % after 18 months. HAMA positivity at baseline was about 1 %. HAMA conversion was also low, occurring in < 10 % of cases over a period of 15 months. However, in a smaller study the incidence of seroconversion was calculated as 70 % within one month. [2–4]. In another study on 30 patients where tositumomab regimen was associated with previous chemotherapy, no patients developed HAMA, and 24 % of them had elevated TSH [8].

37.3 Adverse Events

The safety profile is mainly based on the experience of five pivotal clinical trials conducted on 230 NHL patients treated with one course of tositumomab regimen and followed for one year. Additional data on SAEs, HAMA, and TSH testing were obtained from 765 patients enrolled in one expanded access program. Long-term ongoing studies (SB393229/028, studies 104514, 104517, and CP-98-025) have provided interim data, and lifetime or 10 years follow-up on their patients will provide further information.

The BBW of tositumomab regimen includes *severe prolonged cytopenias, radiation exposure and allergic/anaphylactic reactions*. Additional warnings refer to *infusion reactions* and delayed consequences such as *hypothyroidism*, and *malignancies.*

Severe and prolonged cytopenias are reported as neutropenia (63 %), thrombocytopenia (53 %), and anemia (29 %), which usually peak after 4–7 weeks and last about 1 month. Secondary infections were reported in 34 % of cases, while serious infections, including pneumonia, bacteriemias, sepsis, bronchitis, and skin infections, were reported in 9 % of cases.

Allergic reactions (6 %) such as facial edema, injection site reaction, anaphylactic reaction, angioedema, laryngismus, bronchospasm, and serum sickness were observed. Infusion reactions were observed in about 30 % of cases, mostly occurring within 48 h.

Radiation consequences of the therapeutic regimen concern mostly testes and ovaries—although maximal estimated absorbed radiation was referred to thyroid, due to its preferential capture of iodine isotope—followed by kidney, intestine, and heart wall. The risk of infertility was calculated to persist for at least 12 months. A secondary concern relates to the lowering of testosterone levels after radiation therapy, affecting both fertility and quality of life. Recently, it has been shown that the absorbed dose at testicular level after radioimmunotherapy is variable, but is associated to a significant reduction of serum testosterone and to an increase in FSH, together with an increasing trend of LH, in patients receiving highest testicular exposures. Notably, 42 % of the examined patients absorbed a radiation dose at upper limit for the treatment of testicular leukemic relapse, a much higher dose compared to that usually absorbed after thyroid treatment with radioactive iodine [8].

In patients with normal hormone level at baseline (88 %), after the therapy the incidence of *hypothyroidism*, in those with elevated TSH or in need of replacement therapy, was 18 % with a median time of 18 months and a cumulative incidence of 19 % after 5 years. The onset of hypothyroidism signs could be delayed up to 90 months from treatment. Adverse reactions at gastrointestinal level (38 %) included some early reactions, such as nausea (36, 3 % severe), emesis (15, 1 % severe), and abdominal pain (15, 3 % severe) occurred mainly within days of infusion, whereas diarrhea (12 %) was generally reported days to weeks after infusion, possibly associated to localization of the radiolabeled antibody in the bowel, which increases the level of local radiation damage. An additional risk of external radiation, potentially involving also other subjects, is related to the gamma-emitting radiation of the same isotope.

The grade of induced hypothyroidism is usually moderate and was reported in 7 % of cases, tending to increase with time (19 % at 5 years follow-up). Finally, the risk of miscarriage and of embryo-fetal toxicity due to $Iodine^{131}$ transplacental passage was cautioned, in the absence of specific studies in pregnant animals or humans.

The possibility of observing localized radiation consequences may derive from the an imbalanced biodistribution of radioimunotherapeutics. In a postmarketing retrospective study (BEX114606) on 2,649 patients exposed to dosimetric regimen of tositumomab-iodine131, 0.2 % showed an altered biodistribution.

Malignancies occurred in the overall safety population of 995 patients as myelodysplastic syndrome (MDS)/secondary leukemia (3–10 %) after a median time of 31-39 months, with a cumulative index of 6–15 %. Solid neoplasms occurred in 5 % of cases and included NMSC (26), CRC (7), HNC (6), BC (5), lung cancer (4), bladder cancer (4), melanoma (3), gastric cancer (2), prostate (1), renal (1), Burkitt's (1), and other tumors (4).

Overall, constitutional signs of all grades were present in 81 % of patients (severe/serious 12 %), followed by hematological (65 %, mostly, severe), digestive (56 %, severe 9 %), and respiratory (44, 8 % severe) system signs. Dermatological disorders (44, 5 % severe) included signs of hypersensitivity and NMSC. Signs of cardiovascular toxicity (26, 3 % severe) were mostly limited to hypotension/vasodilatation. Musculo-skeletal and nervous disorders (23–26, 3 % severe) were mainly represented by pain and sleep disturbances. Urinary disorders (14, 3 % severe) were mainly related to UTI. In synthesis, the most common registered events were neutropenia, thrombocytopenia, and anemia followed by pneumonia, pleural effusions, and dehydration. Delayed AEs were hypothyroidism, MDS, and signs of increased immunogenicity (HAMA) [3, 4].

A relevant aspect on AEs in combined therapies regards the possibility of their potential synergistic effect in generating additional events. In a study, where tositumomab regimen was associated with previous chemotherapy (CVP), it was possible to evaluate AEs originated by each treatment among nonhematological events. For example, alopecia (70 %), asthenia, constipation, dyspnea, stomatitis, and pain in the extremities (all in the range of 20–27 %) were related to CVP treatment, with no substantial increment after tositumomab treatment.

In contrast, somnolence (43 %) was only related to radioimmunotherapy, while in other studies an additional increase (> 10 %) of fatigue and chills were observed after tositumomab treatment. Overall, the safety profile of tositumomab therapy was more tolerable than CVP [8].

Similarly, a large study (SWOG S0016) on 430 untreated NHL cases in CHOP therapy combined with tositumomab regimen (263 eligible patients per group to be followed for 5 years) revealed that CHOP had the worse safety profile, except for severe neutropenia (51 % in combined vs. 48 % CHOP alone), thrombocytopenia (18 vs. 2 %), thyroid dysfunction (7 vs. 3 %), MDS/AML (3 vs. 1 %), and presence of HAMA (17 vs. 2 %). None reached the level of statistical significance, thus reassuring on the potential additive effect of radioimmunotherapy on chemotherapy, including the major hematological risks. These patients were followed for 2–3 years after therapy [9].

Finally, in a study on 23 patients with low-grade bulky NHL disease, fractionated external radiotherapy was administered just before tositumomab regimen. In this case, hematologic toxicity (15 %) was not worsened by additional radiation

and no patients were hospitalized or developed serious additional AEs complications [10]. This therapeutic combination seems therefore more safe and potentially capable of reducing the tumor burden before immunotherapy without additional or synergistic AEs. New data are expected from two trials, IRB7883 and LYMNHL0046, the former just completed and the latter ongoing.

Finally, a recent update on long-term safety and efficacy of 12 recurrent indolent and 4 transformed, relapsed/refractory lymphomas treated with radioimmunotherapy alone showed that 50 % of patients with the indolent form were in complete remission after 10 years, with an unchanged safety profile. All had transient bone marrow depression and mostly mild nonhematologic AEs, including pneumonia, thyroiditis, and elevated TSH (1 each). Two cases of febrile cytopenia were also observed shortly after therapy. However, one case of MDS observed after 6 years developed in a fatal leukemia.

Taken together, the safety profile of tositumomab regimen resulted as expected in relation to the targeted antigen and the additional radiation toxicity, and it seems stable with time, up to 10 years for the recurrent indolent NHL disease. The serious concern remains for MDS/leukemia, which can develop as late as 6 years after treatment [7, 11, 12].

37.4 Off-Label Experience

The off-label experience of tositumomab is limited. Among 51 trials, completed or ongoing most relate to lymphoproliferative disorders, including NHL (47), mantle cell lymphoma (8), CLL (5), Burkitt (5), Waldenstrom (5), Hodgkin (1), and multiple myeloma (1). Occasional reports indicate the use of tositumomab as an immunosuppressant therapy (FAERS). Most data are still unavailable, and no new signals from interim results have so far appeared.

37.5 Postmarketing Surveillance

Hypersensitivity reactions including anaphylaxis and axonal neuropathy leading to quadriparesis were observed during post-approval use of tositumomab regimen and were enclosed in official update label. During 2003 and 2004, in between approval and subsequent extension of therapeutic indications, five AEs were reported in 67 patients followed for about 1 year. Two events were serious (one anaphylaxis, one nausea/vomiting), and three were moderate (leg pain, cephalea, pyrexia, and muscular pain). Two cases of fatal anaphylaxis were reported after observational cut off in the RIT-II-004 Study on 60 treated patients. No additional cases were reported in the SWOG study S0016.

Up to December 2012, the FAERS database registered 247 ^{131}I-tositumomab tositumomab reports and 178 as Bexxar. The most common serious events relate to MDS (6.3 %), dyspnea (2.8 %), AML (2.7 %), asthenia (1.6 %), pancytopenia, and pneumonia (1.5 % each). Infections were described in 49 reports and were

mainly bacterial, including four cases of sepsis. Seven cases of anaphylactic reactions and three hypersensitivity reactions were detected.

37.6 Remarks

Overall, the safety profile of tositumomab standard regimen resulted as expected in relation to the targeted antigen and the additional radiation toxicity, and seems stable with time. Both hematological and nonhematological events seem manageable and of limited severity. However, hematological toxicity was more prolonged than that observed after a single cycle of chemotherapy. Interestingly, the incidence of infections associated to neutropenia was relatively low, possibly due to the low toxicity of tositumomab regimen for mucosal epithelia, as compared to traditional chemotherapy.

Immunogenicity against the murine monoclonal and related serious events, including anaphylaxis are of concern and can be fatal, although the risk is limited by the single course indication of treatment for this regimen, and HAMA conversion was also low, occurring in < 10 % of cases over a period of 15 months. Hypothyroidism is a delayed event, with a cumulative incidence of 19 % at 5 years follow-up, that poses concerns and need long lasting follow-up.

The most serious long-term concern remains for MDS/leukemia, which can develop as late as 6 years after treatment. However, the overall background data estimate MDS occurrence at about 10 % of cases, secondary to NHL or AML, and long-term data are still limited for drawing final conclusions. Murine IgG2a can bind all three types of Fcγ receptors in humans and thus can potentially bind to macrophages, granulocytes, and NK cells. Moreover, CD20 is also expressed on a minor population of T lymphocytes (2 %) pertaining to the memory cytotoxic compartment. These two peculiarities may enhance impact with the immune system.

Resistance to anti-CD20 mAb, including tositumomab, has been observed in on and off-label experience (CLL), and is mainly related to Fcγ polymorphism as well as to the different capacity of organizing lipid rafts at the surface of targeted B cells. Therefore, an accurate selection of patients is demanded to avoid the exposure to adverse events of patients refractory to these biomedicines [13, 14].

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Trastuzumab

38

Trastuzumab (Herceptin®, Genentech, Roche) is a recombinant humanized IgG1k monoclonal antibody binding with high affinity to the extracellular domain of the human epidermal growth factor receptor 2 (HER2).

In September 1998, FDA granted first approval as second-line treatment after chemotherapy, or as first-line therapy in combination with paclitaxel, of metastatic breast cancer (mBC) overexpressing HER2. In November 2006, the indication was extended to include adjuvant treatment for node-positive breast cancer in combination with doxorubicin, cyclophosphamide, and paclitaxel. In January 2008, the indication for trastuzumab as single agent was revised and extended to the adjuvant treatment of node-negative or node-positive breast cancer following multimodality anthracycline-based therapy. On May 2008, the indications included trastuzumab treatment in combination with doxorubicin, cyclophosphamide, and docetaxel, or in combination with docetaxel and carboplatin for node-positive or high-risk node-negative breast cancer. Finally, in October 2010, the extension for treatment of patients with HER2 overexpressing metastatic gastric or gastroesophageal junction adenocarcinoma, who had not received prior treatment for metastatic disease, was also approved.

Initial approval from EMEA was granted in August 2000 for the first two indications, and subsequent extensions followed in 2006 for adjuvant therapy of early breast cancer (eBC) after surgery and completion of chemotherapy, for treatment of hormone receptor positive breast cancer in combination with an aromatase inhibitor in 2007, for the treatment of metastatic HER2-positive advances gastric (mGC) or gastroesophageal junction adenocarcinoma in 2010, for adoption of chemotherapy with docetaxel and carboplatin in combination with trastuzumab, and in combination with neoadjuvant chemotherapy followed by

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adjuvant therapy with trastuzumab for locally advanced (including inflammatory) breast cancer or tumors >2 cm diameter, during 2011.

At present, trastuzumab is approved worldwide, including Japan, Australia, New Zealand, Israel, and India (Trastuzumab, Herclon).

Basic experience for first approvals consisted of 10 studies on mBC, including three Phase I (H0407, H0452, H0453) trials enrolling 48 patients, six Phase II (H0551, H0552, H0649, H0650, H0659, H0693) enrolling 689 patients, and one Phase III (H0648) study enrolling 469 patients. Additional information was collected from Study BO16216 (TANDEM) on 207 patients (103 treated), planned to support an extension to the treatment of HER2-positive, hormone receptor positive mBC in combination with an aromatase inhibitor (anastrozole). Extensions to eBC were mostly based on the HERA trial, enrolling 3,386 patients, and on two supporting studies (NSABP B-31, NCCT N9831). Additional information were provided by Study BCIRG006 to support the extension to adjuvant concurrent administration of trastuzumab with chemotherapy in over 3,351 patients, to be followed up to 5 years for cardiotoxicity events. Extensions for mGC were mainly based on the ToGA trial, enrolling 584 patients [1–9].

Finally, in June 2012 FDA approved a new combined strategy for mBC, including the newly licensed pertuzumab—a monoclonal antibody blocking HER2/HER3 dimerization and active in ADCC—into the standard combination of trastuzumab with docetaxel.

At present, over 540 trials on trastuzumab are completed, active, or recruiting, including 19 studies as monotherapy and the majority in combination therapies, 119 studies planning the use of the monoclonal before (119) and/or after (116) surgery. Safety evaluations are planned to be provided by 358/540 trials.

38.1 Mechanisms of Action

HER2 (CD340, ErbB-2, Neu, p185) is a cell surface protein encoded by the ERBB2 gene, and is a member of the erythroblastic leukemia viral oncogene (ErbB) family of tyrosine kinase receptors, which includes EGFR (ErbB-1) and three HER types (2, 3, 4). They consist of four extracellular domains, one transmembrane region, and one intracytoplasmic tyrosine kinase domain. There are 11 known natural ligands to these receptors, including TGFα, HB-EGF, EGF, epigen, betacellulin, AREG (amphiregulin), and EREG (epiregulin), all interacting with EGFR. However, no ligands are known to interact with HER2, while HB-EFG, betacellulin, and EREG also interact with HER4, yet not with the other two ligands of the subgroup.

Upon interaction, EGFR form homo- or heterodimers with other ErbB receptors, a step necessary for activation of the receptor/ligand complex, via the intracellular tyrosine kinase pathway. In the case of HER2, it has been suggested that the activation follows heterodimerization with EGFR, HER-3, or HER-4, and the relative receptor ligand(s) binding. For example, the co-expression of HER-2

and HER-3 leads to the high-affinity EREG binding, followed by tyrosine phosphorylation and a potent mitogenic signal inducing cell proliferation and inhibition of apoptosis via MAPK and PI3K pathways. In particular, the HER2 homodimer formation activates preferentially MAPK, and heterodimers trigger both pathways. Moreover, HER2 dimerization induces the degradation of p27kip1 cell cycle inhibitor. HER2 is expressed at low level on epithelial cells including the mammary gland, ovary, lung, liver and kidney, and in the CNS.

The signaling essentially produces DNA synthesis, cell cycle progression, migration, adhesion, and proliferation of cells expressing EGFR. Therefore, this pathway is crucial for the homeostasis of epithelia, for innate immunity and also as a downregulator of myelin regeneration. Alternatively, in the presence of HER2 overexpression, the spontaneous formation of homodimers on the neoplastic cell surface triggers transmembrane signaling capable of inducing tyrosine phosphorylation. HER1 and HER2 are overexpressed on neoplastic cells of epithelial origin, and in particular HER1 is mostly overexpressed on CRC, lung carcinoma, SCCHN and on GBM, and HER2 is expressed on breast and gastric carcinoma, due to gene mutations/overactivity, and leading to uncontrolled cell division, angiogenesis, cell migration, and cellular invasion/metastasis. In particular, HER2 is constitutively overexpressed in 25–30 % of primary breast cancer, in 6–24 % of gastric cancer, in 15–33 % of gastroesophageal junction cancer, in 16–21 % of esophageal cancer, in 7 % of distal gastric cancer, and is usually associated with a poorer prognosis.

Trastuzumab (anti-p185, rhuMab HER2) is a recombinant humanized IgG1k monoclonal antibody binding with high affinity to the extracellular domain of HER2, and was developed by inserting the CDR of the 4D5 murine mAb into a human IgG1 framework. Trastuzumab has an increased affinity and an improved antibody-dependent cytotoxicity (ADCC) compared to 4D5 antibody. In particular, this monoclonal seems to attract immune cells expressing FcR on their surface, such as macrophages and NK cells, within the treated tumor mass. ADCC subsequently leads to the activation and expansion of tumor-specific cytotoxic T lymphocytes. ADCC is considered the most powerful mechanism of immune tumor cells destruction mediated by this mAb.

Trastuzumab binds to the C-terminal of HER2 extracellular domain, thus blocking intracellular phosphorylation, and suppresses the growth of HER2-positive human tumor cells. The receptor-antibody complex is subsequently endocytosed. In addition to ADCC, multiple mechanisms are involved in the antitumoral activity, such as downregulation of HER2, inhibition/disruption of receptor dimerization, inhibition of cell cycle, and enhancement of apoptosis. In particular, the HER2 downregulation reduces the activation of MAPK and PI3K pathways, leading to the inhibition of cell cycle, while the interaction of trastuzumab with HER2 blocks the cleavage of the extracellular domain, which precludes both the formation of the active signaling p95-HER2 protein and HER2-HER3 dimerization. The action on the underlying cell cycle is stimulated by p27kip1 and by the inhibition of cdk2 regulators. Similarly, apoptosis is enhanced via the inhibition of the anti-apoptotic Bcl-2, leaving the pro-apoptotic BAX

protein to prevail. These pathways are affecting the mitochondrial outer membrane permeabilization (MOMP), which is essential for cell functioning, especially at cardiac level.

Trastuzumab downregulates also neuregulin-1 (NRG-1), a protein acting on EGFR, which is essential for normal myelination in the nervous system, and for activation of cardiomyocytes.

Finally, a crucial role of trastuzumab on tumor cell growth and progression consists in the inhibition of powerful angiogenetic stimulation induced by the HER2-dependent kinase pathways.

However, clinical experience with trastuzumab has shown a rapid increase of drug resistance induced by different and alternative mechanisms. Some patients show primary resistance, but more frequently they acquire resistance during therapy. In fact, less than 35 % of HER2-positive breast tumors initially respond to trastuzumab, and over 70 % of the responders become resistant within a year. Higher levels of resistance have been observed in advanced gastric cancer. The presence of overexpressed membrane mucins such as Muc4 on particularly aggressive breast tumors, or after acquired resistance, reduces the binding of trastuzumab to HER2, while the knockdown of Muc4 removes this effect in vitro. It has been postulated that Muc4 masks the binding epitope on HER2 recognized by the monoclonal.

Alternative mechanisms of resistance are related to the binding of the autocrine motility factor (AMF) cytokine, overproduced by cancer patients, or to the spontaneous expression of constitutively active p95HER2 protein on some resistant tumors, which is not blocked by trastuzumab.

Moreover, various isoforms and truncated forms of HER2 have been recently identified, which become crucial for the individuation of resistance. Cross-talk signals to HER2 may bypass the action of trastuzumab blocking, thus inducing resistance. Among them, the IGF-1R and other receptors may also interact with HER2 and be overexpressed on tumor cells, thus vanishing/reducing the effect of trastuzumab. Hyperactivated downstream signaling, such as constitutive activation of PI3K, can override the action of trastuzumab and drive progression of disease.

Finally, impaired activation of ADCC has been associated with trastuzumab resistance in relation to the FcR presence and typology. These mechanisms of resistance have been recently grouped according to their dependence either from HER2 steric changes, or from alternative activating cross-signals (IGF-1R) bypassing trastuzumab blocking. Additional mechanisms of resistance are due to mutation-derived constitutive activation of downstream intracellular signals, such as the PI3K pathway (25 %), or to loss of PTEN (a downregulator phosphatase and tensin homologue) observed in about 36 % of primary trastuzumab-resistant breast tumors and in 4 % of gastric cancers.

One recent approach to overcome resistance to trastuzumab, which reaches 100 % in some therapeutic regimens, is the dual blocking of HER2 by the combined action of trastuzumab and one TKI, such as lapatinib. The complex interactions among the different mechanisms of action of anti-HER2 drugs and of counteracting mechanisms of resistance are depicting a more complex safety

profile and a consequent more personalized approach of intervention on these tumors.

At present, it is still difficult to evaluate the role of activating and suppressive mechanisms on the safety profile emerging from combined anti-HER2 therapy, or even from supportive therapies employed during treatment. For example, the recombinant human erythropoietin used to mitigate anemia and fatigue antagonizes trastuzumab effects by triggering PI3K and Src kinase pathways, leading to PTEN suppression and tumor resistance. Recently, the latter pathway has appeared to be crucial for both primary and acquired resistance and may become a possible future unique target for resistant tumors (see pertuzumab, Chap. 33).

A different approach consists in coupling a cytostatic agent to mAb (trastuzumab-emtansine), quite recently approved (Kadcyla®, Genentec) by FDA in February 2013 in mBC with the aim of destroying residual and resistant cancer cells. In this case, the complex can bear up to eight molecules of emtansine (a derivative of maytansine, or DM1), which, upon internalization, are released from lysosomes, thus preventing microtubule polymerization and interfering also with the TK pathways. The stable complex, on the line of radiolabeled mAbs (see Chaps. 23 and 37), is expected to reduce adverse events of the coupled cytotoxic agent by directing emtansine to specific targets [10–16].

38.2 Immunogenicity

Immunogenicity was tested in 903 mBC patients and found (HAHA) in only one subject, not associated with allergic events. The incidence of these antibodies in eBC and mGC is not known [7]. However, a higher level of anti-drug antibodies (11.5 %) was observed after SC administration of a new formulation of trastuzumab [26].

38.3 Adverse Events

In *adjuvant BC,* the safety profile of trastuzumab was based on HERA, NSABP B-31, and NCCT N9831 studies, for a population of 6,663 subjects (3,355 exposed to trastuzumab). AEs were documented on 6,592 subjects (3,313 exposed).

In *mBC*, it was based on initial HO649 (213 patients), HO648 (464 patients) for a total of 586 patients treated with trastuzumab (352 in monotherapy). Part of treated patients (90) from Study H0648 entered the long-term extension study HO659.

In *mGC*, the safety profile was based on ToGA Study on 584 patients (294 exposed patients).

The initial 1998 BBW included cardiomyopathy, while infusion-related reactions were listed among the observed adverse reactions. The last updated (2010) BBW included *cardiomyopathy*, *infusion reactions, pulmonary toxicity,* and

embryo-fetal toxicity, which were added on the basis of additional studies and of postmarketing observations.

The primary concern in the general safety profile is about serious and fatal *cardiomyopathy* (4- to 6-fold higher) associated with LVEF decrease, CHF, arrhythmias, and hypertension. These events were rather unexpected and were observed after trastuzumab monotherapy or in association with chemotherapy in women, and are related to the mentioned MOMP mechanism altered by the mAb. The incidence of CHF, in various therapeutic conditions, ranged 2 % (0.3 % in controls). In adjuvant BC, overall cardiac failure occurred in 0.6 % of patients (HERA), and LVEF decrease was about 4 % in combined therapy versus 0.9 % in chemotherapy-treated controls (supportive studies). In mBC, the incidence of heart failure/cardiomyopathy was over 2 % in patients receiving trastuzumab in combined therapy versus 0 % in controls, being the highest in association with anthracyclines, mainly when given after chemotherapy (4 %). No significant cardiotoxicity related to trastuzumab was found in mGC clinical trials, including heart failure, except for an asymptomatic decrease of LVEF (4.6 % vs. 1.1 % in controls).

It must be noted that initial trials were not designed to evaluate cardiac dysfunctions, and tended to exclude subjects at risk. Most patients were treated for about one year, and follow-up lasted about 2 years in some groups.

Infusion reactions were reported in about 40 % of patients in clinical trials, mainly at the first infusion, and were mild. However, permanent discontinuation occurred in <1 % of cases. Serious signs of infusion toxicity were reported in 1.4 % in monotherapy and in 9 % of patients when in combination with chemotherapy.

Signs of *pulmonary toxicity* (ARDS, edema, respiratory insufficiency, infiltrates and interstitial pneumonitis, and pulmonary fibrosis) may occur as sequelae of infusion reactions. Severe/serious pulmonary toxicity was 3.4 % (1 % in controls) in some eBC studies, and pulmonary infiltrates/pneumonitis 0.7 % (0.3 % in controls).

Embryo-fetal toxicity was reported in the 2010 label as postmarketing reports of oligohydramnios, pulmonary hypoplasia, skeletal abnormalities, and death.

Most common *non-cardiac AEs* in the general safety profile include about all the SOC categories, with differences in frequency among the various disease groups, yet without peculiarities in typology [4]. Among these, *neutropenia and febrile neutropenia* induced by chemotherapy were exacerbated by the addition of trastuzumab in combined therapy.

Overall, safety evaluation on eBC, mostly based on HERA trial and on the three mentioned supportive studies, showed a total frequency of all-grade AEs ranging from 2 to 10 %, and events were higher (>1–7 %) compared to controls. They included constitutional signs, arthralgia/myalgia, infections, anemia, rash/desquamation, nail changes, and insomnia, which were at least 2 % higher than in controls. Severe diarrhea was also reported (1.6 % vs. 0 %). Infections were mainly represented as URTI and UTI (about 3 % vs. 1 %), while the incidence of septic deaths was similar to controls.

In *mBC* studies, mainly represented by the initial pivotal trials, cardiac AEs were 5–7 % in monotherapy and rose to 10–28 % in combined therapy. Allergic reactions were 3 % in monotherapy and 4–8 % in combined therapy. Constitutional signs ranged from 10 % to 47 % in monotherapy, and from 12 % to 62 % in combined therapy. Gastrointestinal signs were 8–33 % in monotherapy and 14–76 % in combined therapy. Cytopenia was 3–4 % in monotherapy and 14–52 % in combined therapy. URTI were found in 9–14 % of cases, and UTI in 5 %, with a respective raise to 20–42 % and 13–18 % over time. Both in some eBC and mBC studies, thromboembolic events were also registered at higher frequency than controls (2–3 % vs. 0–2 %).

In *mGC*, serious neutropenia (34 % vs. 29 % in controls), febrile neutropenia (5 % vs. 3 %), thrombocytopenia (5 % vs. 3 %), anemia (16 %), and diarrhea (9 % vs. 4 %) were the most relevant signs. Constitutional and gastrointestinal signs were frequent, but severe events were limited (2–4 %), except for anorexia (20 %), nausea (14 %), and fatigue (8 %). Moreover, mild/moderate renal impairment (54 %), and renal failure (2.7 % in the study group vs. 1.7 % in controls) were also reported. No serious infections were detected.

Taken together, the most serious and/or common adverse reactions reported in trastuzumab usage are cardiotoxicity, infusion-related reactions, hematotoxicity (in particular neutropenia), and pulmonary adverse events [1–9].

Due to the great variability among the various studies, and to different associated therapies on different underlying diseases, these profiles are only indicative in terms of frequency, but define a rather homogeneous typology framework.

Recently, a number of studies have focused on the most important risk—*cardiotoxicity*—in long-term controlled studies and in clinical care patients receiving trastuzumab associated with chemotherapy including anthracyclines. In a 7-year follow-up of the mentioned NSABP B-31 Study on 1,830 patients receiving trastuzumab plus chemotherapy as adjuvant therapy, 4 % had at least one cardiac event (1.3 % in controls). Interestingly, only two patients experienced the event after 2 years from the beginning of trastuzumab treatment, and most patients recovered (LVEF normalization) after stopping trastuzumab [17]. A retrospective cohort study focused on trastuzumab risk of cardiotoxicity when associated with different chemotherapeutic regimens, including anthracyclines, in clinical practice. Among 12,500 women with incident invasive breast cancer, 0.8 % received trastuzumab in monotherapy, or associated with anthracycline (3.5 %) or other chemotherapies (19.5 %), while 29.6 % were treated with anthracyclines alone. The risk of heart failure/cardiomyopathy was high in the anthracycline-trastuzumab regimen (HR: 7.19), followed by trastuzumab alone (HR: 4.12), compared to chemotherapy (HR: 1.49), and to anthracycline alone (HR: 1.40).

Data from another large group of unselected BC patients confirmed previous experiences in clinical trials for aged women, although at a higher level, and revealed an unexpected higher risk in younger subjects who were preferentially receiving the combined association with anthracycline, although with some selection bias related to observations in community settings [18].

However, in a similar but smaller experience, 83 patients with stage II–III BC were treated in clinical practice with trastuzumab administered concurrently with chemotherapy (including anthracycline), followed by surgery, and observed for 50 months. In this case, the safety cardiac profile appeared less aggressive. The most frequent severe events related to hematotoxicity (neutropenia 8 %, febrile neutropenia 5 %). No patient developed HF, and a transient mild/moderate LVEF decrease was observed in 11 % of cases. One patient had atrial fibrillation. The Authors, consistently with previous data from three Phase II trials, attributed the most favorable cardiac tolerability to the concurrent use of trastuzumab and chemotherapy, and to a better selection/monitoring of patients [19].

More recent data on primary systemic trastuzumab therapy associated with chemotherapy (HER2NAT study) on 38 patients with locally advanced primary BC reported severe neutropenia (60 %) and leukopenia (37 %), with a lower rate of febrile neutropenia (5 %). Serious allergic reactions (about 8 %) included infusion reactions related to trastuzumab (2.6 %), and other events related to docetaxel (5.3 %). Interestingly, no CHF or LVEF decrease <50 % were observed. The Authors attributed the consistent decrease in cardiotoxicity to the introduction of epirubicin, rather than of doxorubicin, and to the concurrent administration of trastuzumab, allowing the use of low cumulative doses and less cardiotoxic anthracyclines, thus supporting the hypothesis that moderate reversible cardiac dysfunction determined by trastuzumab do not synergize and may be distinct from cardiotoxicity associated with anthracyclines [20].

An alternative interpretation attributed the variability in cardiotoxicity rates to the renal function, in a study on 499 patients with eBC undergoing combined therapies. In fact, the presence of even a mild renal impairment in these patients increases the risk of cardiac damage, which progresses in parallel with renal dysfunction. The Authors implied that the use of trastuzumab in addition to anthracyclines increases the risk of toxicity on myocytes, due to HER2/neuregulin impairment mediated by both drugs [21].

The second most relevant risk, *fetal toxicity*, is an increasing concern because of the extension of trastuzumab therapy to younger women. A recent review and meta-analysis evaluated all available studies by examining the safety of trastuzumab administered during pregnancy, and selected 17 investigations including 18 pregnancies and 19 newborns (15 alive). These patients suffered eBC or mBC (56 %), and received trastuzumab (mean total dose 2.85 mg) for about 15 weeks, as monotherapy or in various combinations. Occurrence of oligohydramnios/anhydramnios was the most frequent AE (61 %). The majority of patients (73 %) were exposed to trastuzumab during the II/III trimester. Ten neonates (53 %) were born and remained healthy up to the end of the 9 months follow-up, while two newborns had prematurity-related disorders.

The remaining relevant AEs include ARDS (two cases), lung disease and renal failure, severe pulmonary hypoplasia, sepsis, persistent infections with respiratory failure, and necrotizing enterocolitis (one each). Interestingly, children exposed to trastuzumab only during the first trimester were healthy. No fetal cardiotoxicity or congenital malformations were observed, and no data were reported on AEs

occurring in the treated mothers. These data are in line with previous observations and indicate that trastuzumab is slowly transferred across the placenta via FcR active transport. The Authors implied that the amniotic disorder might be related to the HER impairment, which interferes with fetal kidney development and function, including the production of amniotic fluid [22].

In the *dual blocking therapy* approach, in which trastuzumab is associated with one TKI, sunitinib was added to the standard combination of trastuzumab/docetaxel as first-line therapy of unresectable and locally recurring mBC in 26 patients. Most patients (88 %) reported at least one severe event. The most common serious non-hematologic events were fatigue/asthenia (28 %), diarrhea (16 %), stomatitis (8 %), dyspnea (8 %), and vomiting (8 %). One fatal ARDS and one intestinal perforation also occurred. Cardiotoxic events were observed in 3 patients: one had mild LVEF decrease, and 2 had severe supraventricular arrhythmias. Neutropenia was reported in all patients, and was severe in 79 % of cases, followed by all grade lymphopenia (79 %) and thrombocytopenia (64 %). Febrile neutropenia was present in 20 % of patients. Transaminase elevations were observed in 4 cases. Due to protocol settings, the investigators could attribute ARDS, fatigue/asthenia, and one transaminase elevation leading to discontinuation to trastuzumab-related events, while the intestinal perforation was related to sunitinib.

Overall, no new or unexpected events were registered. However, the low efficacy profile experienced in this study and in a following Phase III (SUN) trial discouraged such approach [23]. In a similar study lapatinib was associated with trastuzumab and chemotherapy with better results. A recent meta-analysis on selected studies showed no statistical differences among the safety profiles of different combinations, except for severe diarrhea, discontinuation rates, and dermatologic events ($p < 0.001$), occurring more frequently in the lapatinib arms [24]. Interestingly, the dual blocking approach did not result in a higher risk of cardiotoxicity, despite anthracyclines were present in some of the evaluated studies. Overall, trastuzumab resulted more effective and safe compared to lapatinib.

A very recent experience (EORTC 16023) has tested the association of trastuzumab with paclitaxel and lonafarnib, an inhibitor of RAS proteins interfering with intracellular transduction of MAPK, in 23 BC patients. The addition of the RAS inhibitor revealed new AEs compared to the known safety profile of previous cycles of the trastuzumab-paclitaxel setting. In particular, new gastrointestinal signs (diarrhea, nausea/vomiting, dyspepsia, abdominal pain), allergic reactions, and myelosuppressive toxicities caused interruption and modifications in the lonafarnib administration setting. However, the latter did not increase cardiotoxic events [25].

Another interesting study compared the standard intravenous (IV) trastuzumab treatment versus the *subcutaneous administration* (SC) of a new formulation of the same product (HannaH study). An equivalent proportion of patients (52 %) developed at least one severe adverse event, and the overall pattern was comparable between the study groups. However, the incidence of serious AEs was higher

in the SC group (18.1 % vs. 7.7 %), mainly because of infections (8.1 % vs. 4.4 %). Interestingly, such increase was not accompanied by an imbalance in hematological laboratory parameters, and no infections were observed at the SC trastuzumab injection site. Over four fatalities occurred during the neoadjuvant phase of the study, three subjects (1 %) enrolled in the SC group died for septic shock in febrile neutropenia and thrombocytopenia, myocardial infarction, and hypertension/diabetes, and two of them were judged as treatment-related events, but not related to trastuzumab per se. Notably, non-neutralizing anti-trastuzumab antibodies were higher in the SC group (11.5 % vs. 3.4 %), which also showed antibodies directed to rHuPH-20, a recombinant human hyaluronidase excipient used to favor trastuzumab delivery. The cardiac safety profile was as expected and comparable in the two study groups, but no severe CHF were observed [26].

Finally, the just approved trastuzumab-emtansine (T-DMI) conjugate-Kadcyla—was evaluated in Phase III trials (MARIANNE, EMILIA) for HER2-positive BC, and the association of the highly cytotoxic microtubule dimerization inhibitor (DM1) seems to overcome tumor resistance to the monoclonal, as well as to other TKIs. Initial data from Phase I–II studies have depicted a preliminary safety profile of T-DM1 as monotherapy in over 300 patients. The most common AEs include fatigue (37–65 %), anemia (10–29 %), nausea (25–51 %), thrombocytopenia (33–54 %), and hypokalemia (4–24 %). Among these, severe/serious events were <5 %, except for hypokalemia in one study (8 %), and for early thrombocytopenia (7–8 %) that was rarely associated to bleeding. Cardiotoxicity signs were observed in about 8 % of cases and were lower, compared to standard trastuzumab-docetaxel regimen (16.4 %). However, a potential minimal QT prolongation effect was observed and is currently under close monitoring [27]. The product is marketed from about three months, and more data are needed before a better safety assessment can be depicted.

38.4 Off-Label Experience

Among a remarkable amount of launched trials on trastuzumab (542), the majority related to BC studies (469). Off-label evaluations include sarcoma (7), lung tumors including NSCLC, (16), pancreatic cancer (7), bladder/UT carcinoma (7), ovarian cancer (7), prostate cancer (6), endometrial adenocarcinoma (4), and oral carcinoma (2). Most of these ongoing studies are based on the HER2 positivity of part of these tumors, as detected in vitro or in xenograft models of trastuzumab binding, sensitization and cytotoxicity. However, insufficient clinical data on safety have been so far reported.

Recently, in a Phase I study, 21 patients with solid tumors including breast (7), colon (6), esophageal (4), gastric (2), pancreas (1), and thyroid (1) cancer, were treated with trastuzumab in combination with paclitaxel and IL-12. Grade 1 fever/chills associated with IL-12 were common, and 2 patients had dose-limiting fatigue [28]. Initial studies on pancreatic cancer showed HER2 positivity in about

16 % of the 155 patients treated with trastuzumab and gemcitabine. AEs in the combined regimen were comparable to those of the gemcitabine monotherapy, being severe neutropenia (29 %) and thrombocytopenia (15 %) the most common events. One patient suffered LVEF decrease [29]. However, in a recent Phase II trial on 17 patients (8 % of the screened population) overexpressing HER2 in metastatic pancreatic cancer, only 4 % of them showed a related gene amplification. These patients were treated with trastuzumab and capecitabine. Severe AEs reported after 12 weeks included neutropenia, diarrhea, nausea, and HFS (7 % each). No cardiac toxicity, anemia, or thrombocytopenia was observed [30]. Case reports have also been published on HER2 positive salivary ductal carcinoma [31], and on renal collecting duct carcinoma [32].

Overall, both safety and efficacy profiles on off-label tumor treatment are still lacking of solid data.

38.5 Postmarketing Surveillance

In the FAERS setting including more than 7,500 records (3.3 AEs/R), the most frequently reported AEs categories included infections (5 %), gastrointestinal signs (4.2 %), respiratory disorders (3.7 %), WBC abnormalities 3.5 %, and dermatologic events (2.9 %). Most common events included diarrhea (3 %), dyspnea (2 %), neutropenia/febrile neutropenia (1.5 %), pyrexia (1.4 %), and nausea/vomiting (2 %).

In the EUV setting, 7,116 reports (7,002 for serious events) registered 15,904 events (2.3 AEs/R). The most frequently reported AEs categories included cardiac events (11 %), respiratory (10 %) and gastrointestinal (6.8 %) disorders, dermatological reactions (5.9 %), and nervous disorders. Cardiotoxic events were 6 % of all cardiac events. Noteworthy, renal impairments (27 cases) and renal failures (55) were rather frequent. Infections were present in 4.8 % of reports and included as most relevant events 56 cases of pneumonia, 37 sepsis, 15 neutropenic sepsis, 20 cellulitis, 20 UTI, 17 URTI, and 15 cases of herpes zoster infections. Anaphylactic/anaphylactoid reactions were present in 95 reports, and cytokine release syndrome in 3 cases. Gastrointestinal most common signs were diarrhea and nausea/vomiting (about 16 % of GI events), followed by abdominal pain (6 %). Moreover, 39 cases of stomatitis, and 29 cases of pancreatitis were also reported. The most common respiratory sign was dyspnea (24 %). The most relevant respiratory reports included 181 cases of ILD, and 49 cases of lung infiltrations. Finally, among 673 hematological events the most common and relevant included neutropenia (25 %), febrile neutropenia (11 %), thrombocytopenia (13 %), and anemia (13 %). Twenty-two cases of bone marrow failure were also registered.

Overall, the safety typology of trastuzumab was comparable among clinical trials, clinical practice, and postmarketing reporting, with a rather consistent variability in frequency mainly related to combined therapies and underlying treated diseases.

38.6 Remarks

Although the incidence of side effects is considered acceptable and expected, due to trastuzumab target specificity, major concerns are about cardiotoxicity, severe diarrhea, and to a lesser extent neutropenia and related infections. The overall incidence of cardiac dysfunction related to trastuzumab monotherapy in patients with eBC/mBC ranges from 3 to 7 % and increases to 11–18 % when combined with chemotherapy. Severe diarrhea, sometimes life threatening, was observed as 2–9 % in monotherapy and increased to 16 % in combination therapy. Infections were usually manageable and included mainly URTI (9–14 % monotherapy; 20–40 % in combined therapy), and UTI (5 % and 13–18 % respectively), and could be serious in 5–8 % of cases.

In particular, cardiotoxicity has been related to the specific HER2 blocking mechanism of action of trastuzumab, and to renal impairment as an enhancer factor [20]. Fetal toxicity was also related to anti-HER2 activity due to fetal renal damage and the consequent reduction of the amniotic fluid. Interestingly, no cases of fetal cardiotoxicity have been so far reported [22]. As expected, AEs appeared underestimated in controlled studies, compared to observations in clinical practice, due to the selective criteria usually adopted when enrolling patients in clinical trials [18].

Because of insufficient efficacy and of primary/secondary resistance to trastuzumab of various types of tumors, combined therapeutic strategies have been introduced, including different chemotherapy associations and dual blockade of HER2. Both the type of associated chemotherapy and the timing of trastuzumab administration influence the induction of AEs, being anthracyclines responsible of the most cardiotoxic events in the combined therapy. However, additional cardiotoxicity experienced in multi-combination treatments may not be solely an additive effect of toxic events, but may derive from different mechanisms of action, as suggested for trastuzumab and anthracyclines [20].

One recent approach to overcome resistance to trastuzumab, which reaches 100 % in some therapeutic regimens, is the dual blocking of HER2 by the combined action of trastuzumab and of TKIs, such as lapatinib and sunitinib. The latter combination did not show improvements in efficacy, while the combination with lapatinib, although more effective, raised some safety concerns, mainly the increased cardiotoxicity and the elevated discontinuation rates (30 %) [24, 25, 33].

In other recent experiences these concerns were confirmed, and additional discontinuations due to severe diarrhea (6–11 %) were reported, although in a better risk/benefit balance compared to standard trastuzumab-chemotherapy associations [34, 35].

Unfortunately, the SC administration of trastuzumab did not show a better safety profile compared to the IV administration, although it remains the more acceptable regimen. However, a twofold increase in serious AEs, the presence of anti-trastuzumab antibodies, and allergic reactions observed in the former group demand further attention. Notably, the serum concentration of trastuzumab after

SC administration was non-innferior to IV administration, and could be 30 % higher than after IV injection [26].

While most efforts of anti-BC cancer are focused on the multiple blocking of TK pathways due to strong biologic and clinical rationale, less attention is devoted to the role of ADCC and other immune interventions, such as T cell activation/cytotoxicity, both in efficacy and in their potential AEs induction. Immune events, in fact, are crucial for trastuzumab ultimate efficacy and for prognosis in BC, since they may be involved also in long-term tumor control via the activation of specific cytotoxic T lymphocytes, Treg downmodulation, and the arming of macrophages and NK cells infiltrating the tumoral mass. The immune-mediated events seem less prone to induce cardiotoxicity and fetal toxicity, compared to HER2 blocking activity of trastuzumab. Therefore, the potentiation of the immune arm may improve efficacy and produce less toxicity than an overwhelming block of TK pathways and multi-HER inhibition [15]. However, the recent introduction of new anti HER2/HER3 monoclonal formulations (pertuzumab) and of new TKIs synergistic combinations (neratinib, afatinib, erlotinib) continue to follow the multi-HER blocking strategy, while the experience with trifunctional monoclonals (ertumaxomab) privileging the anti HER2/CD3 immune targeting of breast cancer cells (NCT00452140) was discontinued [36, 37].

Long-term studies are still limited and mainly restricted to cardiotoxicity monitoring [17]. Therefore, additional unexpected events may still occur. For example, patients treated with trastuzumab for 1 year show a higher frequency of brain metastases than those treated with chemotherapy alone, although it is not clear whether this finding is drug-related or is part of the natural course of the disease. Similarly, the observed absolute increase of serious/severe infections and of vascular disorders associated with long-term treatment with trastuzumab, as well as the reported (BCIRG006 study) persistence of subclinical toxic effect for several years, have raised concerns about the treatment of early small BC because of the increased risk/benefit unbalance [38].

Taken together, the safety profile of trastuzumab is tolerable and manageable, having a limited spectrum of SAEs to monitor, but it demand further attention in the evaluation of risk/benefits and in the AEs preventability setting events [39].

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Ustekinumab

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Ustekinumab (Stelara®, Janssen-Cilag, Centocor Ortho) is a fully humanized IgG1k monoclonal antibody binding with high affinity to the p40 subunit shared by interleukin-12 (IL-12) and IL-23 heterodimeric cytokines, thus preventing their binding to the IL-12Rβ1 receptor expressed on the surface of immune cells and therefore neutralizing their biological activity. In December 2008, Health Canada authorized ustekinumab for the treatment of chronic moderate to severe plaque psoriasis (Ps) in adult patients suited for phototherapy or systemic therapy. EMEA granted first approval of ustekinumab in January 2009 for the treatment of adult patients with moderate to severe chronic plaque psoriasis, who are intolerant or have a contraindication, or failed to respond to systemic therapies including cyclosporine, methotrexate, and PUVA. Following the initial submission in November 2007, approval from FDA was granted in September 2009 for adult ($\geq$ 18 years) patients with Ps, who are candidates to phototherapy or systemic therapy. In December 2012, the sponsor requested both Agencies to extend the treatment to patients with active psoriasic arthritis (PsA). The request was accepted in September 2013 by both Agencies.

Pivotal data for original approval derived from two ongoing Phase III trials, C0743T08 (PHOENIX 1) and C0743T09 (PHOENIX 2) including 1,965 Ps patients exposed to ustekinumab. They followed three previous studies (C0379T01, C0379T02, C0379T04) in 295 Ps patients examining various IV (18 patients) or SC (in 227 patients) doses. Additional studies included two investigations, C0379T03 and C397T06, evaluating SC single or multiple doses of ustekinumab in 137 MS patients; Study C0379T07 evaluated single IV or multiple SC doses of ustekinumab in 120 CD patients, and another Study (C0743T11) on 31 healthy subjects injected with a single SC dose. The subsequent approval from FDA was based on PHOENIX-2 trial on 1,212 Ps exposed patients, one additional Phase III trial, C0743T12 (ACCEPT) on 556 Ps patients, and one Phase II trial

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(C0743T10) on 133 PsA patients. Data from PHOENIX-1 study, initially submitted in November 2007, were also considered, although data were not updated for the final evaluation (not included in Complete Response submission), except for deaths and a summary on serious adverse events. Both PHOENIX 1 (766 enrolled patients) and PHOENIX 2 (1,230) studies are planned to continue treatment and follow-up for a total of 5 years [1–4]. The extension for the treatment of PsA was based on two main ongoing studies (PSUMMIT I, II).

39.1 Mechanism of Action

IL-12 is a tetra-alpha helicoidal heterodimer composed by p35 and p40 subunits, while IL-23 has a similar structure composed by p19 and p40 subunits. The p40 subunit is also homologous to IL-6 receptor, and to ciliary neurotrophic factor (CNTF) receptor, while p35 is homologous to IL-6 and G-CSF. Monomers and homodimers can also be found, albeit with insignificant biological activity. Both cytokines bind to IL-12Rβ1 receptor expressed by activated NK cell, CD4+, and CD8+ T lymphocytes. IL-12 and IL-23 are produced by dendritic cells and macrophages, in response to microbial stimulation, and are essential for immune host defense and tumor surveillance. Moreover, some human B-lymphoblastoid cell lines produce IL-12, while IL-23 is produced also by CNS resident cells (macrophages, microglia). IL-12 induces differentiation of native CD4+ T cells into Th1 mature lymphocytes, and activates NK cells. Both cell subsets produce IFNγ, which plays an important pathogenetic role in the formation of psoriasic plaques. IL-23 stimulates another CD4+ T cells subset (T17) to produce IL-17, which upregulates TNFα, and has also a role in autoimmune inflammation by synergizing with IFNγ to increase the production of proinflammatory cytokines by keratinocytes. More recently, the role of these cytokines has been found crucial also for gut inflammatory and autoimmune diseases, such as Crohn's disease (CD), ulcerative colitis (UC), and other forms of inflammatory bowel diseases (IBD). They are also implied in the pathogenesis of experimental allergic encephalomyelitis (EAE), and possibly in human multiple sclerosis (MS).

Ustekinumab (CNTO 1275) is a fully humanized IgG1k monoclonal antibody binding with high affinity to the first domain of the shared p40 subunit between IL-12 and IL-23 heterodimeric cytokines. The binding site located on the second and third domain of p40 is different from the site involving p35 and p19 chains. However, this monoclonal cannot bind to the cytokines already bound to their receptors, and is unlikely to mediate ADCC or CDC immune effector functions. Ustekinumab was developed in transgenic mice, in which the immunoglobulin genes were replaced with human antibody transgenes. In particular, the IL-12Rβ1 chain is shared by both receptors and binds the p40 subunit of the cytokines, while the IL-12R β2 chain of the IL-12R binds to the p35 subunit of IL-12, and the IL23R chain binds to the p19 subunit of IL-23, thus assuring the specificity of activation via the two distinct receptors. Ustekinumab prevents the binding of IL-

12 and IL-23 to the heterodimeric IL-12R receptors expressed on the surface of immune cells, and therefore neutralizes their biological activity. It must be noted that IL-23 was discovered after the development of ustekinumab. Both IL-12 and IL-23 are overexpressed in a number of autoimmune diseases, such as Ps, CD, and MS. Ustekinumab decreases mRNA expression of IL-12 p40, IL-23 pl9, and IFNγ in the skin. It also inhibits IL-12- and IL-23-induced IFNγ, IL-17A, TNFα, IL-2, and IL-10 secretion [5–9].

Overall, ustekinumab expresses a general immunosuppressive activity, which is therapeutically useful in these diseases, but increases the risk of infections and malignancies, as experienced during clinical studies. Subjects genetically deficient in IL-12/IL-23 are particularly vulnerable to infections (Mycobacteria, Salmonella, and to BCG), which can be fatal. The inhibition of IL-12/IL-23 in rodents inccreases the rate of malignancies. Moreover, IFNγ is known to have some anticancer activity. However, a recent analysis on the influence of ustekinumab on T cell differentiation, cytokine production, and on the T cell receptor repertoire diversity conducted in healthy and psoriasis subjects, showed no significant suppression of naive T-cell differentiation and of cytokine production from memory CD4 T cells. The number of Treg cells was not altered by the administration of ustekinumab, nor was the TCR diversity repertoire in Ps patients. Therefore, according to these studies, ustekinumab seems to improve Ps manifestations without a significant immunosuppressive effect on the production of Th1 and T17 cells and on other crucial functional parameters of the immune system [10].

39.2 Immunogenicity

The rates of antibodies to ustekinumab across all-phase trials ranged from 3.8 to 5.4 %. However, the frequency of inconclusive testing was high (about 80 %), possibly due to interference of circulating ustekinumab. Approximately 1 % of positive subjects had an associated injection site reaction, and no association was found with serious immunologic events including anaphylaxis or delayed hypersensitivity reactions. Immunogenicity rates remained low (5 %) through the 5 years follow-up, with antibodies to ustekinumab detected in 5.2 % of 746 tested patients. Titers remained primarily low (1:40 in 67 % of cases), but most antibodies were neutralizing (64 %), and in some cases associated with a reduced efficacy of treatment. No additional patients developed antibodies after 3 years follow-up [4, 11–13].

39.3 Adverse Events

Safety evaluations from Health Canada and EMEA for Ps were based on PHOENIX 1, PHOENIX 2, and ACCEPT Phase III trials. Ustekinumab was usually administered in two SC doses (40 and 80 mg) in 2,266 patients, including

1,970 exposed for 6 months, 1,285 exposed (five injections) for at least one year and 373 exposed for at least 18 months (seven injections). According to EMEA reviewers, the safety population from the PHOENIX studies was too small and therefore data from ACCEPT (ustekinumab compared directly to etanercept without a placebo group) were added in order to better define the drug safety profile. Additional data from previous studies C0379T01, C0379T02, and C0379T04 were also comparatively considered.

Safety data evaluated by FDA in the final Ps approval were mainly based on PHOENIX 2 patients including up to 100 weeks updates and ACCEPT patients through week 24. Data from PHOENIX 1 trial, previously submitted to the Agency (in November 2007) were also considered, yet summarized in a report concerning only serious events and deaths. Similarly, investigations on PsA patients from Study C0743T10 were included only for updated serious events. Overall, the safety typology and frequency were based on 1,212 patients from the pivotal trial exposed for one additional year, and on 556 exposed patients from Study C0743T12 for additional 6 months of exposure. However, data from all studies could not be pooled because of differences in study designs.

Finally, in the updated prescribing information at May/June 2012, the safety profile reflects exposure on 3,117 Ps patients, including 2,414 exposed for 6 months, 1,852 exposed for at least 1 year, 1,650 exposed for 2 years, 1,129 exposed for 3 years, and 619 exposed at least for 4 years. The global population of controlled and uncontrolled Ps patients was 6,791 patients/years. However, tabulated data usually refer to initial settings during the placebo-controlled period.

The most recent PsA approval was based on the PSUMMIT trials on 927 patients. All together, Ps and PsA profiles considered a total of 4,031 patients including 3,106 subjects exposed to ustekinumab for 6 months, 1,482 exposed for 4 years, and 838 exposed for over 5 years.

The most relevant adverse events related to the administration of ustekinumab include *infections*, *malignancies, hypersensitivity reactions including anaphylaxis* and *reversible posterior leukoencephalopathy syndrome (RPLS)*. In controlled studies (12–20 weeks) *infections* were reported in 27 % of treated patients (24 % in controls). However, serious infections (0.3–0.4 %) were equally distributed. In the global exposed population, 70 % of subjects reported infections, being serious in 2 %. The rate of infections in Ps controlled studies was 1.39 P/Y (1.21 in controls) and serious events were 0.01 P/Y (0.02 in controls). Interim data on PsA reported infections as nasopharyngitis and URTI at about 4–7 % up to 24 weeks observation. Most common infections were mild/moderate *nasopharyngitis* (8 %) and *URTI* (3–7 %). One case of suspected opportunistic infection (H. zoster) was also observed. At later observations (crossover phase of the two PHOENIX trials), infections ranged 30–40 % with the same typology (nasopharyngitis, 9–10 %; URTI, 7–11 %) and dental infections especially in PsA patients. Overall, these values did not significantly increase during follow-up, and duration of infections was not prolonged in the study groups with respect to controls [1–3, 14, 15]. However, some differences occurred during the placebo-controlled period between

the Phase II and the Phase III studies, particularly with regard to SAEs, which occurred in 3.6 % of treated subjects and in 1.5 % of placebo subjects in Phase II, whereas SAEs occurred at similar rates in the different treatment groups in both Phase III trials [2]. In a recent long-term analysis on 1,247 Ps patients from the same trials treated for up to 3 years, infections remained stable and serious infections were in the range of controls, with a tendency to decrease in the group treated with the lower dose of ustekinumab, toward frequencies comparable to the general Ps population. Most serious events (0.5 % of 3,117 patients treated up to 3 years) were of bacterial and viral origin, and included cellulitis, osteomyelitis, pneumonia, sepsis, diverticulitis, and UTI [11, 16]. A longer 5 years follow-up on 753 patients of PHOENIX 1 confirmed the stable trend of infections, and a slight dose effect. Thirty-two serious infections were reported in 30 patients. No cases of anaphylaxis or serum-sickness syndrome, nor TB or other opportunistic infections were reported. Overall, no indications of cumulative toxicity emerged and a tendency to decrease of AEs causing discontinuation was also observed [17]. Uncommon infections included cellulitis, Herpes zoster and viral URTI.

The *reactivation of latent TB infections* (LTBI) is considered a major risk during immunosuppressive therapy. A recent investigation on the efficacy and safety of TB-specific prophylaxis identified 167 cases (5.3 %) of LTBI among 3,177 Ps patients enrolled in the major trials and treated with ustekinumab. Although the preventive treatment raised a number of expected isoniazid-related AEs, no cases of active TB were reported in these patients, and one case of asymptomatic pulmonary TB was observed among subjects not receiving chemoprophylaxis [18].

The second major concern refers to *malignancies,* reported in 1.3 % of the general population of Ps patients exposed to ustekinumab, because of the potential immunosuppressive activity of this monoclonal antibody. Their incidence, excluding NMSC was 0.16 per 100 P/Y (0.35 in controls). NMSC incidence was 0.65 per 100 P/Y (0.7 in controls), which represented the most frequently observed tumors, followed by prostate cancer, CRC, BC, and melanoma in situ. However, NMSC incidences were within values of the general population (0.95; 95 % confidence interval: 0.70, 1.22). The ratio basal vs. squamous carcinoma was 4:1. However, the respective rates were considered equivalent to values observed in the general population. In the 3 years follow-up of the mentioned Phase II and Phase III trials, 1.9 % of 3,225 patients reported a malignancy, mostly as NMSC (56 %), suggesting a slightly higher, but stable incidence over time without dose response effects. In particular, the overall rate of NMSC was higher in the study groups in early observations (within 20 weeks) with respect to placebo (1.13,100P/Y). However, over 4 years of follow-up the rate in study groups decreased (0.61,100P/Y) with respect to initial controls, thus excluding cumulative effects. Cutaneous tumor histotypes included BCC (25), squamous carcinoma (6) and basosquamous skin cancer (3), and melanoma (3). Noncutaneous tumors included prostate cancer (9), BC (3), CRC (2), RCC (2), HNC (2), mycosis fungoides (1), and HL (1), which were considered within background levels [16].

In the 5 years follow-up, over 753 Ps treated patients, malignancies occurred in 3.8 % and included NMSC (14 patients), mostly as BCC, and other malignancies in 15 patients. Overall, the profile confirmed a rather stable trend for all types of tumors over 5 years in this selected population of Ps patients [17]. Recently, two cases of *multifocal cutaneous squamous cell carcinoma* were observed in Ps patients in clinical practice, soon after administration of ustekinumab. These patients were also treated with PUVA and other therapies, including etanercept. However, the timing and their reversibility after discontinuation of ustekinumab were indicative for a drug-related serious event. The Authors reported also five more cases provided by the Australian TGA national Agency, as possibly related to ustekinumab therapy [19].

The risk of *RPLS* has also been related to the immunosuppressive activity of ustekinumab. However, one case was reported among the PHOENIX 2 Ps patients and remains the only reported event on 3,758 exposed subjects of the general exposed population. The patient received twelve doses of ustekinumab in 2 years. No other reports were registered up to 5 years follow-up. In the postmarketing settings 5 cases in FAERS and 3 cases in EUV have been registered [4].

The general safety profile observed in Phase II and Phase III trials and in the ACCEPT Study up to week 76, in addition to the previously mentioned major events, included low rates of cephalea (6–15 %), fatigue (3–5 %), pruritus (2–6 %), lumbalgia (2–7 %), arthralgia (3–5 %), and injection site reactions (3–4 %). Serious events included gastrointestinal disorders (0.3–0.6 %), renal and urinary disorders (0.1–0.3 %), cardiac disorders (0.1–0.5 %), psychiatric and nervous disorders (0.1–0.5 %), vascular (0.1–0.5 %), musculoskeletal (0–0.1 %), and dermatological disorders (0–0.1 %). However, similar rates were reported in controls. Allergic reactions, such as asthma, atopic dermatitis and seasonal allergy were observed in 0.1–0.5 % of cases. Rash and urticaria were observed each in <1 % of Ps ans PsA patients. Serious hypersensitivity reactions including anaphylaxis were reported in the postmarketing settings. Psoriasic exacerbation and PsA were also observed (0.3–1.3 %), but at lower rates than in controls. Discontinuation rates were about 1 % and were lower with respect to controls. In the 3 years follow-up, both profiles and frequencies remained stable, or showed a slight decrease with time. The major events emerging in the long period were *cardiovascular disorders* (0.3–0.6 % P/Y), as myocardial infarction, death, and stroke.

As previously mentioned, the ACCEPT trial was planned to evaluate two doses of ustekinumab compared to one dose of etanercept and did not include controls. The overall AEs profile of ustekinumab was similar, albeit slightly higher than pivotal trials, and slightly lower than the etanercept profile. However, the latter produced a much higher rate of injection site reactions (about 25 vs. 4 %) [11, 12, 16, 17, 20].

The cardiovascular risk can be underestimated in clinical trials, and is difficult to detect as a rare event, even with meta-analytic evaluations. In a large meta-analysis in 10,183 Ps patients treated with various biomedicines, including ustekinumab (1,771 treated; 820 controls), no significant differences in the rate of major adverse cardiovascular events (MACEs) were observed [21]. However, in a

more recent meta-analytic re-evaluation MACEs (myocardial infarction, cerebrovascular accident and cardiovascular death) related to anti-IL-12/23 therapy (ustekinumab or briakinumab) were found significantly increased in the pooled treated population with respect to placebo (OR: 4.23; p 0.04), indicating the existence of a potential drug class effect. However, the specific MACEs rate for ustekinumab was 0.28 % (5 cases) on 1,771 selected patients, and the calculated risk difference was not statistically significant (OR: 3.96; p 0.19) [22].

As for potential *ethnical differences* in Ps patients treated with ustekinumab, it is known that the rate of psoriasis is lower in Asians with respect to Caucasians, shows a higher frequency in males, and seems to have a lower response to some TNF inhibitors. In a recent controlled Phase III trial (PEARL), 121 of 159 enrolled Taiwanese/Chinese and Korean Ps patients, equally distributed between the two ethnicities, entered the study. Notably, the first cause of exclusion was latent TB (15 cases), followed by laboratory (12 cases), and 14 % of patients (all Taiwanese) had also PsA. Ustekinumab was administered up to week 16 in 61 SC patients (60 controls), who were then followed up to week 36. At week 12, the safety profile was generally mild and similar in study groups and controls (66 vs. 70 % respectively). Infections were higher among patients receiving ustekinumab (33 vs. 23 %), and URTI (11.7 vs. 11.5 %) represented the most common encountered event. However, pruritus was much higher in placebo (27 vs. 8 % in study group), and serious events (3.3 %) were only observed among controls (anal abscess, Ps worsening) as well as abnormal hepatic functions (3 %). During follow-up, the total number of patients (59 treated, 55 controls) suffering at least one infection was similar in all groups (32–33 %). However, there was a slight tendency to increase of some infections, such as nasopharyngitis, as compared to controls (13.5 vs. 5.5 %). Notably, hepatic functional abnormalities increased in all groups (8.5 vs. 7.3 % in controls). Moreover, the majority of hepatic dysfunctions were observed in patients receiving concomitant prophylaxis with isoniazid. Seven serious events were registered, one in the treated group (facial fracture and Schönlein-Henoch purpura) and six among cross-over patients, including one case of TB reactivation (not receiving prophylaxis), two appendicitis, muscle injury, and one benign parathyroid tumor. No other opportunistic infections were observed.

Anti-ustekinumab antibodies were found in 5 patients (4.4 %). These patients did not show an increased association with injection reactions, but had a lower response to therapy. Overall, the safety profile was considered similar to the Caucasian experience, except for hepatic events, which were mostly attributed to isoniazid prophylaxis [23].

The first Phase II-III study of ustekinumab on 152 Japanese Ps patients treated for 12 weeks and followed for 72 weeks reported safety data in line with previous reports. The most common event was nasopharyngitis (16 %). Serious events were observed in 5 % of patients treated with the higher dose, and were lower than controls (6 %). Infections (20–24 %) included one serious case of pneumonia with peripheral and pulmonary eosinophilia. The proportion of patients with at least one AE was increased at week 72 (96–99 %), with no additional AEs typologies.

Infections were observed in about 70 % of cases with two serious cases (cellulitis, pharyngitis). No cases of TB or opportunistic infections were observed. One case of cerebral hemorrhage and two cases of malignancies (prostate, and cervical cancer) were also reported [24].

A recent retrospective study was recently performed in UK and Ireland on 129 Ps patients treated with ustekinumab for a short-term in clinical practice. A total of 10 AEs and 5 SAEs (3 depression; 1 hospitalized URTI; 1 bladder cancer) causing treatment discontinuation, were observed. Interestingly, in this short-term study certain patients with comorbid disease such as MS (3), ischemic heart disease (2), HCV hepatitis (2), and cardiomyopathy (1) did not show SAEs [25].

Overall, no major differences emerged among the examined ethnical groups, except for hepatic dysfunction and possibly an increase in infection rates in Asians.

Reactivation of latent viruses is an expected AE during immunosuppressive therapy. However, a recent retrospective study showed that the first four Ps patients with concurrent HCV (3 cases) or HBV (1) hepatitis treated with ustekinumab did not show increases in the viral load or hepatic enzymes abnormalities, during a follow-up of 25 (7–47) months. The remaining subjects were treated with anti-TNF therapy, with similar results [26]. It must be noted that IL-12 is supposed to exert a crucial protective role in HVC and HBV infections, and was also used in HCV hepatitis therapy with poor results, while anti-TNF drugs are contraindicated.

Most of the experience on Ps in clinical trials derives from patients with moderate to severe cases of psoriasis vulgaris, while limited observations relate to highly *severe forms of psoriasis*, such as erythrodermic psoriasis. In fact, these cases and other forms of the disease (Ps guttata, pustular) were excluded from pivotal trials.

In a recent retrospective study, 28 erythtrodermic patients, most of them resistant to conventional therapy, were identified and treated with different biomedicines. Among them, there were three cases treated with ustekinumab. AEs were present in 43 % of the cohort and were mostly classified as severe, being infections (64 %), and cutaneous infections (78 %), the predominant observed typology. In particular, the three patients receiving ustekinumab showed one forunculosis, and two severe widespread cutaneous staphylococcal colonization (one fatal). The overall profile appeared similar, yet of higher severity when compared to pivotal trials' experience [27]. Two previous cases of erythrodermia in psoriasic patients, who developed also PsA and anti-nuclear antibodies (ANA, dsDNA) during anti-TNF therapy, were successfully treated with ustekinumab, in the absence of adverse events, with beneficial effects on Ps, PsA, and with unmodified levels of anti-nuclear antibodies [28]. In another small case series on eight Chinese patients with erythrodermic syndrome treated with ustekinumab, no data on safety were reported [29].

Finally, *unusual AEs after ustekinumab* were also reported, such as an *eczematous eruption*s during treatment of one case of cutaneous plaque psoriasis/palmoplantar pustular psoriasis [30], and a *linear IgA bullous dermatosis* (LABD)

in a case of plaque psoriasis, which appeared after two doses of ustekinumab and lasted over one year [31].

As for PsA treatment recently approved in adult patients, the overall AEs profile was consistent with that of Ps, with an higher incidence of arthralgia, nausea (3 % each vs. 1 % in controls), and dental infections (1 vs. 06 %). Latest data from PSUMMIT 2 showed AEs in about 63 % of cases, being infections the most common event, and SAEs were comparable to controls. No opportunistic infections, TB, MACEs or deaths occurred. One SCC and one additional serious infection in ILD were detected through week 24 [32, 33].

39.4 Off-Label Experience

At present, the majority of 39 active trials on ustekinumab are dedicated to Ps (26), 6 to PsA, 4 to CD, 1 to MS (completed). In addition, studies on sarcoidosis, primary biliary cirrhosis, hidradenitis suppurativa, and uveitis are active (one each). Among the principal trials on PS, one study (CADMUS, NCT01090427) was planned to evaluate the safety and efficacy of ustekinumab in young (<18 years) Ps patients.

A number of studies and case reports have been published on CD, UC, pityriasis rubra pilaris, pyoderma gangrenosum, and hidradenitis suppurativa. In the post-marketing settings cases of atopic dermatitis, acute febrile neutropenic dermatosis, sarcoidosis, dermatitis exfoliative, and hidradenitis are also recorded.

The completed Phase II trial on *relapsing-remitting MS* (NCT00207727) was conducted in North America, Europe, and Australia on 249 patients treated with different doses (27–180 mg) of ustekinumab at various time intervals (6–8 SC injections every 4–8 w). The administration showed no therapeutic benefit and the study was stopped at week 37. However, 85 % of treated patients had at least one AE and the most common events were infections (URTI, nasopharyngitis), reported in about 50 % of patients, injection site reactions (32 %), and constitutional signs. Serious events were 2 %, and the overall profile did not reveal new signals [34].

The experience of *gut inflammatory diseases* with ustekinumab, such as CD, UC, and other IBD was stimulated by recent findings on the role of IL-23 and Th17 cells in these diseases, and in particular on their powerful proinflammatory activity [7]. Experience of ustekinumab in CD treatment has been evaluated in two initial Phase II studies (C0379T03 and T06). In a more recent report on 526 patients, ustekinumab was injected intravenously (1–3 mg/kg), with a maintenance SC dose (90 mg) administered up to week 16, and was assessed at week 22. During the induction phase, the proportion of AEs, infections included, was similar in study groups and controls. Six serious events (5 in the study groups) included Clostridium infection, Staphylococcal infection, UTI, viral gastroenteritis, anal abscess, and vaginal abscess (one each). One anal abscess was observed in the placebo group. Infusion reactions were not serious and were equally distributed

(4.3–4.5 %). Similarly, during the maintenance phase, rates and severity of AEs were equally distributed among all groups. No TB, serious opportunistic infections or cardiovascular events were observed. One BCC was reported in one study group. The presence of anti-ustekinumab antibodies was 0.7 % on 427 tested patients. Overall, the safety profile did not show new/unexpected AEs [35].

Experiences in the treatment of *pyoderma gangrenosum* (PG) and of peristomal PG, are based on the frequent association with CD and on recent findings on the overexpression of IL-23, both at transcriptional and protein level in PG lesions. In two separate case reports, the treatment with ustekinumab was well tolerated with no adverse effects [36, 37].

Experience for the treatment of *hidradenitis suppurativa (HS)* with TNF inhibitors, and more recently with ustekinumab, is based on a possible multiple pro-inflammatory pathogenesis and on a frequent association with CD. In this case, a concomitant beneficial effect on the associated hidradenitis was observed after treatment with anti-TNF agents. In the first three published cases, no adverse events, infections, or reopening of HS lesions were observed within the study period, and no safety data were reported for the other two [38].

One case of *pityriasis rubra pilaris* treated with ustekinumab has been published, even as first-line treatment, apparently without safety events, and has been criticized because of inconsistent scientific support for such off-label approach [39].

Finally, a peculiar aspect of off-label use of a drug is related to nonstandard regimens administration, and/or interruption followed by retreatment. A recent study examined the possible consequences of such medical decisions with some biomedicines, including ustekinumab. A number of AEs were reported, including serious infections and malignancies during dose escalations and interruptions. Anti-ustekinumab antibodies appeared increased in dose intensification regimens in about 13 % of cases. However, due to the small number of participants, the results were difficult to interpret [40].

39.5 Postmarketing Surveillance

In the FAERS database, 3,778 reports were included for ustekinumab by the end of 2012. The most common categories include infections and skin reactions (8 % each), neurologic (4 %), and respiratory disorders (2.6 %). Most common reported events include psoriasis exacerbation (3 %), cephalea (2 %), myocardial infarction (2 %), and pneumonia (1.5 %). Notably, five cases of RPLS were reported.

The EUV records include 1394 events. The most common AEs were infections (12 %), dermatological disorders (11 %), nervous disorders (9 %), malignancies (6 %), muscular (6 %), and cardiac disorders (5 %). In particular 64 cases of myocardial infarction, 10 cases of cardiac failure, and 6 CHF were reported. Eighteen cases of hypersensitivity, 3 cases of RPLS, 2 cases of anaphylactic shock, and 2 cases of serum-sickness syndrome were also reported. Most common

infections included pneumonia (28), sepsis (18), cellulitis (17), Staphylococcal infections (17), TB (11), and Herpes zoster infections (19). Reports on malignancies (183) included prostate cancer (15), malignant melanoma (12), BC (12), lymphoma and multiple myeloma (5 cases each), HL (6) and NHL (5). Cerebrovascular accidents (26) and depression (38) were the most common serious CNS reported events.

39.6 Remarks

Ustekinumab is considered an immunosuppressive agent. In fact, the safety profile indicates infections and malignancies among the major concerns. However, the overall spectrum is usually mild. Infections are limited mainly to the upper respiratory tract and are serious in a limited number of cases. Reactivation of TB is among them, but specific prophylactic measures seem effective. Other opportunistic infections have been occasionally reported [4, 17]. Recently, a limited experience in Ps patients with concurrent HCV and HBV hepatitis did not show signs of viral load increase, or hepatic enzymes additional abnormalities [26]. Among malignancies, the rate of NMSC is increased, but stable, during follow-up, while the incidence of other solid tumors seem to remain within the expected rates of underlying diseases populations. However, some concerns have been raised by two reported cases of multifocal cutaneous squamous cell carcinomas observed in clinical practice, showing a strict association with ustekinumab treatment [19], and by a discontinuation-related clinical reversibility. No lymphoproliferative disorders were reported at the end of the 5 years follow-up. Immunogenicity and hypersensitivity reactions, including injection site reactions, were limited, usually mild, and did not increase with time. The risk of RPLS seems so far very low, being related to the one and only case reported during the initial studies, and 8 cases reported in the postmarketing settings.

The overall profile indicates a moderate and selective immunosuppressive potential of ustekinumab, with respect to other agents of the same class. This has been also evidenced in recent studies, where no significant suppression of naive T cell differentiation and cytokine production, or in the number of Treg cells and in the TCR diversity could be found [10]. Nonetheless, additional effects related to the mechanism of action of ustekinumab cannot be excluded, and further vigilance for long-term and rare events is necessary. Therefore, a drug class safety assessment—an increasing approach in the safety evaluation of biomedicines—is debatable [41]. For example, according to some recent meta-analyses MACEs may appear as underestimated, and in need of further accurate surveillance [21, 22].

Recent evidences suggest that cardiovascular morbidity is increased in PsA. It must be noted that most of the early and long-term experience on Ps are based on the common type of disease (psoriasis vulgaris), while the observation on PsA and most severe forms of Ps, where AEs of higher severity have been reported, is very limited. In fact, these cases—together with other risk factors such as latent TB,

serious infections, malignancies and major cardiovascular events—were excluded from pivotal trials, and occasional experience is coming only form the clinical practice. Recently a number of RPLS cases were signaled to postmarketing surveillance major settings. In the recent experience in PSUMMIT 1 study, 8 major cardiac events (myocardial infarction (2), stroke) were observed, thus posing again the possible existance or a relation between cardiac disorders and IL-12/IL-23 blockade [32, 33].

So far, the off-label experiences did not show additional new signals. Early-stage human clinical trials have demonstrated some therapeutic potential of ustekinumab in CD, but not on MS, while AEs did not raise new concern [13, 34, 35].

However, a major concern comes from other pathologies treated with a merely "extrema ratio" approach in administrating ustekinumab, as well as other biomedicines, after or in concurrence with other unsuccessful treatments in isolated and uncontrolled cases. Although the safety typology remained within the known profile, AEs frequency and severity resulted increased and unbalanced with respect to efficacy, the latter being sometimes remarkable but rarely long lasting.

Another major limiting factor in depicting the safety profile of such diseases and in planning proper controlled studies is related to their rarity. More controlled studies are needed along with the establishment of specialized Registries providing wider data collection (including rare clinical forms such as PSOLAR, NORDIC, and BADBIR).

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Part III
Fusion Proteins

Fusion Proteins

40

A fusion protein (FP) is a chimeric product of a fusion gene, i.e. a hybrid gene derived from the joining of two different genes originally coding for separate proteins. Translation of a fusion gene results in a single polypeptide, which may carry functional properties of both original proteins. Fusion genes may naturally occur in the body as the result of DNA transfer by translocation, interstitial deletion or chromosomal inversion; often they are oncogenes found in hematological malignancies, prostate cancers and sarcomas. Fusion genes and proteins can also be made in the laboratory by recombinant DNA technologies.

As for the structuring of FPs biomedicines, they are usually composed of an *epitope* linked to a larger proteic *carrier* consisting in an immunoglobulin (Ig) fragment; they are in fact *antibody fusion proteins*. With this respect, chimeric and humanized mAbs can be considered fusion proteins as well.

The epitope is usually an extracellular domain of a cell-surface receptor, or a soluble form of it, linked to the Fc portion of a human IgG acting as carrier.

The carrier can be rendered functional by selecting proper IgG isotypes actively binding to the Fc receptors widely distributed on leukocytes and dendritic cells, thus activating their specific functions. The carrier acts also as a stabilizer by binding to the so called neonatal Fc receptor (FcRn) that protects it from degradation. The receptor has also the capacity of prolonging the construct's half-life, which still remains short compared to native antibodies or full-size mAbs (1–2 weeks vs. 3–4 weeks). Because of the increased size of the whole molecule, its renal clearance is also lowered, although the major elimination of all proteic substances is provided by the reticulo-histiocytic system.

The binding site of FcRn is situated at CH2-CH3 interface and its binding affinity can be improved by inducing mutations in the Fc sequence, a crucial aspect for FPs that usually exert a lower affinity for the target with respect to mAbs.

The Ig structure is particularly favorable for the proteic fusion due to its arrangement in domains, preserving the tridimensional conformation necessary to express its native function, even after separation in single domains. In particular, the function is retained as long as the heavy (V_H) and light (V_L) variable regions remain intact. Since isolated V_H-V_L heterodimers are rather instable, usually a

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flexible peptide linker is used for their covalent connection. The second advantage offered by Ig molecules relates to the biological functions of the Fc fragment.

The four human IgG isotypes bind Fcγ receptors (FcγRI, FcγRIIa, FcγRIIIa), the inhibitory FcγRIIb, and the first component of complement (C1q) with different affinities (Kd 10^{-6}–10^{-9}), yielding very different effector functions.

In particular, IgG1 and IgG3 express higher affinity for these receptors and are strong complement activators as well, being IgG3 the most potent isotype with this respect. IgG2 has very low affinity for Fc receptors on phagocytic cells and intermediate capacity as complement activators. The IgG4 isotype shows intermediate affinity for FcRs, and virtually no binding for C1q. Therefore, when properly assembled, the Fc carrier not only prolongs the half-life of the whole fused protein, but is able to exert different degrees of complement-dependent cell lysis (CDC), and antibody-like dependent cell cytotoxicity (ADCC), just as the native Ig molecules.

Should these properties be unfavorable for the intended therapeutic use, the Fc portion can be inactivated, or the FP can be provided by an "inert" carrier, thus acting only through the selected fused epitope.

The third advantage of Fc as carrier is represented by its small size compared to mAbs (approximately 25 kDa vs. 150 kDa, respectively), allowing FPs to better and faster penetrate tissues. However, a potential drawback of genetic fusion technology is the potential misfolding of the epitope after fusion with the carrier, thus producing loss of efficacy, unexpected activity, or adverse events.

The effector functions of the Fc portion can be altered either as component of a mAb or of a FP structure. For example, two regions of the CH2 domain are critical for both FcγRs and C1q bindings, and have unique sequences in IgG2 and IgG4 isotypes. Substitutions in positions 233–236 for IgG1 and IgG2, or in positions 327,330, and 331 for IgG4 greatly reduce ADCC and CDC functions. Alanine substitutions at various Fc positions reduce complement activation, but increase both functions at position 333. Therefore, in the manufacturing of mAbs and FPs it is possible to induce "maturation" affinity and function modulation by specific mutations, according to the scope of the designed biomedicine, being IgG1 and IgG2a the preferred backbones for enhancing Fc functions, and IgG4 the inactive counterpart.

Following these procedures FPs of various combinations were initially constructed with insulin-like growth factor, plasminogen activator, Factor VIII or Factor IX-Fc, TNFα-antitransferrin receptor, TNFβ-antiganglioside GD2, angiopoietin-Fc, and IL-2-antiT cells to generate, among others, directional activation of cytotoxic effectors. Some of them have reached advanced clinical testing.

As for the more recently approved therapeutic FPs, their principal mechanism of action is related to the competitive inhibition of specifically targeted receptors for the binding of their respective natural ligands (abatacept, aflibercept, belatacept, etanercept, rilonacept). They may alternatively act as agonists to modulate the immune response (alefacept, romiplostim). Some of the approved FPs act as "traps" or "decoy receptors" for soluble mediators of inflammation, such as aflibercept for VEGF-a isoforms. A number of new agents with similar properties,

directed to capture other angiogenic cell growth factors, are also under investigation.

Fusion proteins do not show direct toxicity, but they may induce a number of indirect, mostly cell-mediated AEs. Interestingly, when directed to the same target, FPs and mAbs do not produce identical clinical effects (see for example etanercept vs. infliximab Chap. 24, 45), and their capacities to induce AEs are nor completely overlapping. This also indicates the possibility of sequential treatment, and of splitting efficacy from unwanted adverse reactions in future drug developments. On this respect, not only the proteic structuring may be critical, but also their glycosylation. In fact, their overall content can influence the immunogenicity of FPs and of mAbs, and therefore affect their safety as well [1–5].

At present, seven FPs are licensed for human therapy, their area of intervention being cancer, autoimmune diseases, coagulative disorders, auto-inflammatory diseases, AMD, and kidney rejection. They are all based on IgG1 Fc fragment fusion. The present work provides data on the following seven approved biomedicines: Abatacept, Aflibercept, Alefacept, Belatacept, Etanercept, Rilonacept, and Romiplostim. Table 40.1 reports some information on their targets, official clinical indications, and approval dates. Recently, the manufacturer discontinued

Table 40.1 Fusion proteins in human therapy

INN	Trade name Company	Target Type	Indications FDA and/or EMEA	Approval[a] FDA/EMEA
Abatacept	Orencia BMS	CD80, CD86 CTLA4-Fc (IgG1)	RA, JIA	2005/2007
Aflibercept	Eylea/Zaltrap Regeneron Sanofi-Aventis	VEGF VEGFR1,2-Fc (IgG1)	AMD,CRVO/CRC	2011-12/ 2012-13
Alefacept[b]	Amevive Astellas	CD2 LFA-3-Fc (IgG1)	Ps	2003/NA 2003
Belatacept	Nulojix BMS	CD80, CD86 CTLA4-Fc (IgG1)	Renal graft rejection	2011
Etanercept	Enbrel Immunex, Amgen, Pfizer	sTNFα, sTNFβ TNFR2-Fc (IgG1)	RA, JRA, JCA, JIA, ERA Ps, PsA; pediatric PsA[c]	1998/2004
Rilonacept[d]	Arcalyst Regeneron	IL-1α, IL-1β IL-1RI/IL-1RAcP-Fc (IgG1)	CAPS	OD 2008/OD 2007
Romiplostim	Nplate Amgen	TPOR TPO/TPO-Fc (IgG1)	ITCP	2008/2009

For targets and therapeutic indications acronyms see text and list
[a]Initial approval date. Some of the reported indications approved at a later time, [b]discontinued in 2011, [c]indication withdrawn in US and Canada, [d]discontinued in 2012
OD: Orphan drug; NA: not approved

alefacept production in U.S., and the distribution was supported up to March 2012. However, the FDA licensure is still active (while EMEA did not approve the product) and a new analogue molecule is being produced in China (Jan Xi Fu, Shanghai Zhanjiang Biotechnol).

Finally, a different type of fusion protein, denileukin-diftitox, consisting of a whole recombinant human IL-2 fused to diphtheria toxin is described in Chap. 50 among the interleukin analogues.

The amount of ongoing studies on new FPs is impressive. By the end of 2012, therapeutic fusion proteins were under investigation in 448 clinical registered trials (www.clinicaltrials.gov), and about 100 of them were at Phase III level. Among these, 21 trials included 13 new fusion proteins under investigation in coagulative disorders (8), cancer (8), rheumatic disorders (2), infections (2), and AMD (1).

All together monoclonal antibodies, which can be considered as particular types of fusion proteins, and fusion proteins represent the 43 % (2008 data) of all therapeutic approved proteins, while FPs are the 20 % of all antibody-based biomedicines.

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Abatacept

41

Abatacept (Orencia®, Brystol-Meyers Squibb) is a soluble fusion protein binding to CD80 and CD86, thus blocking their interaction with CD28 receptor and the consequent costimulatory signal to activate T lymphocytes.

FDA approval was granted in December 2005 for the IV treatment of adult patients with moderately to severely active rheumatoid arthritis (RA), who have had an inadequate response to one or more DMARDs, other than TNF antagonists. In April 2008, the indication was extended to the IV treatment of moderate to severe polyarticular juvenile idiopathic arthritis (JIA), in patients 6 years of age and older. In July 2011, the SC use of abatacept was approved only in RA patients after the first IV infusion, or as starting administration in patients who were unable to receive the IV treatment. The administration of abatacept is indicated as monotherapy in RA and in JIA, or concomitantly with DMARDs other than TNF antagonists in the former disease, or concomitantly with methotrexate in JIA patients. Health Canada approved abatacept in May 2007 for the IV treatment of RA patients with the similar indications, and the Australian TGA approved the product by September of the same year for the same indications.

In May 2007, EMEA granted approval for the treatment of moderately to severely active RA in adult patients, who have had an insufficient response or intolerance to other DMARDs, including at least one TNF inhibitor. In December 2009, CHMP approved the extension to JIA in combination with methotrexate in pediatric patients (6 years and older) who had insufficient response to other DMARDs, including at least one TNF inhibitor. In May 2010, the treatment indication for RA patients was modified to include adult patients, who had inadequately responded to previous therapy with one or more DMARDs, including MTX or a TNFα inhibitor. In October 2012, the European Agency also approved

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SC administration of abatacept in RA adult patients. Abatacept monotherapy was not approved by EMEA.

Pivotal trials for initial approvals of RA treatment included three Phase III studies enrolling a total of 2,484 patients. In particular, in Study IM101102 (AIM) 433 patients (219 controls) were treated with a fixed IV dose (10 mg/kg) of abatacept; Study IM101029 (ATTAIN) treated 258 patients (133 controls), and Study IM101031 (ASSURE) treated 959 patients (482 controls) with the same dose. The latter study was designed for the evaluation of abatacept safety in clinical practice patients.

Previous studies included one Phase IIa (IM103002) on 122 patients, with 90 patients receiving different doses of abatacept (0.5−10 mg/kg), and 2 Phase IIb (IM101100, IM101101) for a total of 305 patients treated with 2 or 10 mg/kg and 155 controls. All patients, except those in Study IM103002, had a background therapy with MTX (IM101100, IM101102), etanercept (IM101101), or anakinra (IM101029), associated with one dose-adjusted DMARD. In particular, the efficacy evaluation was primary focused on studies IM101100, IM101102, and IM101029, with supporting information from IM101103 and IM103002. Data collected in Study IM101101 (combination of abatacept and etanercept) did not reach statistical significance.

As above mentioned, EMEA approved the extension in 2010 to RA patients inadequately responding to previous therapies. This extension was based on the mentioned trials; on additional accumulated data on 4,632 patients from the pivotal Phase II-III studies, subsequently passed to long-term studies up to 8 years observation; on one additional study (IM101023) conducted in 483 with early severe RA; and on data from the postmarketing experience (about 32,000 P/Y).

The approved extension for JIA treatment was based on a three-part study (IM101033) enrolling 190 pediatric patients (6–17 years old) with inadequate response to previous DMARD, and in particular on 153 subjects that completed the study (5 years, long term, open-label extension arm, part C).

Additional relevant studies on RA include ARRIVE (IM101064) conducted on 1,046 RA patients after anti-TNF therapy, AGREE (IM101023, NCT00122382) enrolling 509 naive patients, and ADJUST (IM101046, NCT00124449) on 56 patients at risk of RA.

The approval extension for the SC use in RA patients was based on the ACQUIRE (IM101174) Phase III study on 1,457 patients, on ALLOW Study (IM101167, NCT00533897) on 270 RA patients evaluating safety and immunogenicity upon withdrawal and reintroduction of abatacept, and on ATTUNE (IM101185, NCT 00663702) enrolling 123 RA patients to evaluate the effect of switching from long-term IV to SC administrations. Finally, a head-to-head noninferiority study (IM101235, NCT00929864) on 649 naive RA patients compared abatacept with adalimumab SC regimen [1–5].

At present, 108 trials evaluating the safety and efficacy of abatacept are completed, ongoing, or recruiting.

41.1 Mechanism of Action

CD80 (B7-1) and CD86 (B7-2) are the ligands of CD28 and of CD152 (CTLA-4), all members of the same immunoglobulin superfamily. The ligands are expressed on the surface of antigen-presenting cells (APC), while CD28 and CD152 (CTLA-4) are T-cell surface proteins. Upon B7/CD28 binding, a costimulatory signal necessary for T cell activation is generated, in concurrence with the pivotal binding of the T-cell receptor (TCR) to the antigen-MHC complex situated on APCs (dendritic, monocyte/macrophage, and B cells). In contrast, the B7/CTLA-4 binding, or the CTLA-4 crosslinking generate negative signals, which terminate T cell activation [6]. In fact, CD28 is constitutively expressed on naive T cells, while the CTLA-4 expression on lymphocytes follows their activation (24–48 h), and strongly competes with CD28 because of a higher affinity (about 20-fold) for B7 ligands. Interestingly, memory T cells (especially CD8+ memory cells) are not entirely dependent on CD28 costimulatory signals for activation. In fact, other costimulatory signals are generated by the CD40/CD40L binding between T cells and APC cells, and by 4-1BB (CD137)/4-BBL interactions. Notably, CD137 is expressed on T cells (mainly CD8+), dendritic cells, NK cells, granulocytes, and endothelia at sites of inflammation. Interestingly, CTLA-4 is expressed by fetal cells and by placental fibroblasts, indicating a potential role in the induction of maternal-fetal tolerance during pregnancy. The prolonged activation of T cells and the consequent overproduction of proinflammatory cytokines are considered crucial for the pathogenesis of RA and other autoimmune disorders. In addition to T and B cells, monocytes migrate to the synovial membrane, differentiate in macrophages and osteoclasts, and induce the proliferation of fibroblasts and condrocytes, which participate to the local destructive/proliferative inflammatory processes typical of this disease [7].

Abatacept (CTLA-4Ig, BMS188667) is a soluble recombinant human fusion protein binding with high affinity to CD80 and CD86, thus blocking their interaction with CD28 and the consequent costimulatory signals to activate T lymphocytes. The blocking of CD28 predominantly prevents naive T-cell activation, since memory T cells are not entirely dependent on CD28 costimulatory signal for activation. The binding to CD80/86 is reversible. Abatacept was developed by combining the extracellular domain of CTLA-4 with a fragment of the Fc domain of human IgG1 [6]. This fragment was genetically modified to greatly reduce the binding capacity to FcRIII and FcRI receptors, thus inhibiting the expression of Fc-mediated immune activities, such as CDC and ADCC. Abatacept reduces T-cell proliferation, survival, and cytokine production (TNFα, IFNγ, IL-2, IL-4) as well as the serum concentration of acute-phase proteins. In addition to the principal action on the B7/CD28 costimulatory pathway, abatacept has been found to exert inhibitory effects on intracellular signaling mediated by the same pathway in some APC cells. In fact, intracellular signaling events can be activated in B cells after B7/CD28 binding, which increase immunoglobulin production, while exerting inhibiting effects on T cells proliferation via p38MAPK and NF-kB pathways. More recently, abatacept administration in RA patients has produced an

early increase in circulating CD14+ monocytes and a reduction at their surface of some adhesion molecules (CD15, VCAM-1, E-selectin) essential for transmigration of these cells trough vascular endothelia [8].

Abatacept is the parental antibody of belatacept, approved in 2011 for the prevention of kidney transplant rejection; they only differ in two aminoacids, but the former has a higher avidity for the same targets. (See belatacept Chap. 44).

41.2 Immunogenicity

Antibodies to abatacept, or to the CTLA4 portion, were estimated in the range of 2 % in 1,993 adult RA patients following repeated IV injections for up to 2 years, and in 4.8 % of 3,985 patients, treated for up to 8 years. Subjects who discontinued therapy were more frequently positive than subjects who did not (7.4 vs. 2.6 %). [2, 4].

A considerable variation in positivity was observed among different studies, with values shifting from 2 to 20 % [3]. Variability was also related to the type of antibody assay used in the study.

A lower positivity was found with abatacept SC administration, with respect to IV injections (1.1 vs. 2.3 %). However, SC administration in adult RA patients gave 0 % positivity, and about 9.6 % after reintroduction, as detected by an ELISA assay. In another similar study, positivity was 9.3 % after discontinuation, and 12.7 % after reintroduction according to an ECL higher sensitivity assay.

By contrast, in ATTUNE study an overall positivity of 6.6 % was found with the ELISA testing, 8.2 % had anti-abatacept antibodies, and 0.8 % showed anti-CTLA4 terminal antibodies, but no antibodies were detected with the ECL assay [9].

Noteworthy, the concomitant use of MTX did not have a significant impact on immunogenicity as usually experienced with other biomedicines.

Anti-abatacept antibodies were searched in 128 Japanese RA patients and resulted absent in one study. In another smaller investigation, anti-abatacept antibodies were also absent, but 33 % of them were positive as anti-CTLA4 terminal portion, and were in part neutralizing [10, 11].

In JIA, 13-17.5 % of tested patients had anti-abatacept or anti-CTLA4 antibodies, which appeared stable in the post-treatment observation. However, after reintroduction the positivity increased up to 40 % in another study arm. Overall, the immunogenicity of abatacept in JIA patients was higher than in adult RA patients. However, no apparent correlation of antibody development to clinical response or adverse events was found in both RA and JIA studies. Moreover, when compared to other similar biologic agents, such as adalimumab, etanercept, and infliximab, the immunogenicity of SC abatacept resulted at the lower end of values [12].

As already known, testing for antibodies to the whole molecule are usually confounded by preexisting anti-Ig antibodies, particularly in RA patients with RF. Conversely, the assay to detect antibodies directed to CTLA-4 is more sensitive and clear. These antibodies, when neutralizing, may have an adverse impact on

efficacy and on the immune system of the patient, potentially leading to uncontrolled immune responses, including autoimmune reactions [1].

The antibody response to inactivated bacterial vaccines has been reduced in some experiences, but is considered effective and their use is advisable. Live vaccines are contraindicated.

41.3 Adverse Events

Safety basic evaluations from FDA were conducted on 2,944 patients from the five core RA studies (IM100100, IM100101, IM100102, IM100029, and IM100031), including 1,955 treated patients (total exposure 1,687 P/Y in controlled studies) and 989 controls. Treatments continued for 6 months (258 treated patients) to one year (1697) in patients on concomitant DMARD therapy. Study IM103002, and an additional experience with a similar molecular entity (BMS-224818), evaluated abatacept in monotherapy for a total of 182 treated patients and 32 controls. EMEA initial safety evaluations were based on the same studies on 2,778 subjects exposed to abatacept for at least one year (83 %), and on 1,378 patients (50 %) exposed for at least 2 years. Safety evaluations on JIA were based on a three-part study (IM101033) on 190 pediatric patients (6 to 17 years old) with inadequate response to previous DMARDs treatments, and in particular on 153 subjects that completed the study (5 years, long term, open-label extension arm, part C). In the last updated product information, safety evaluations referred to 1,955 RA IV treated patients (989 controls) and 1,457 RA patients, from a non-inferiority study comparing SC and IV administrations of abatacept.

The overall general safety profile includes *infections*, *malignancies*, *infusion reactions,* and *hypersensitivity reactions*.

Infections occurred in 54 % of treated patients (48 % in controls) during the placebo-controlled phase, and were mainly represented by URTI, nasopharyngitis, sinusitis, UTI, influenza, and bronchitis, ranging from 5 to 13 %. Serious infections were observed in 3.1 % of treated cases (1.9 % in controls). There was a relative increase of Herpes simplex infections (2 vs. 1 % in controls) in the five main controlled studies.

Malignancies, evaluated in 1,955 patients exposed for one year, were similar to controls (1.3 vs. 1.1 % respectively). However, hematological malignancies (0.13/100 PY), in particular lymphomas (0.06,100 P/Y), were more frequent in the treated groups and higher (over threefold) than expected in the general population. However, these rates are consistent with the expected rates in the RA population.

Infusion and hypersensitivity reactions were mild/moderate (68/28 %), and caused discontinuation of treatment in <1 % of cases. Rash was observed in 4 % of treated cases versus 3 % in controls. Two cases of anaphylaxis were observed among the 2,688 treated patients. Among minor adverse events (3–18 %) resulting at higher frequency in the treated groups, the most common disorder was cephalea (18 vs. 13 % in controls).

Some clinical situations were identified at increased risk for AEs, such as chronic obstructive pulmonary disease (COPD) or the concomitant use of TNF antagonists during treatment with abatacept. In the first case, AEs frequency increased up to 97 % in one study group (88 % in controls), while the concomitant abatacept/anti-TNF therapeutic regimen resulted ineffective and increased the rate of infections (63 vs. 43 % in TNF monotherapy) and serious infections (4.4 vs. 0.8 % respectively) [1–5].

Transaminase elevations (0.1–1 %) were usually mild and reversible, and were mainly observed in associated therapy with MTX.

Overall, this general safety framework was derived from a majority of RA patients in short-term observations, and is subject to variations in relation to previous and concomitant therapies, to their typology (conventional or biologic), to duration of treatment, age, and underlying disease (JIA). For example, in Study IM101031 the number of SAEs in abatacept plus biologic DMARD-treated subjects was almost twofold higher than in placebo, while no difference was observed in the combined therapy with nonbiologic DMARD. However, serious infections were high in both combined therapies compared to placebo, but the association with other biologicals resulted at higher risk [1].

The first study on *abatacept as monotherapy* was the dose-response Study IM103002, with 122 patients receiving three different doses of abatacept, 90 patients administered with the analog BMS-224818, and 32 patients receiving placebo infusions. Safety evaluation showed that treated patients had a greater incidence of AEs, yet without dose-response effects also on SAEs, infections, malignancies, and death [1].

In the ARRIVE trial, a group of 43 patients received abatacept as monotherapy. AEs and SAEs were, respectively, reported in about 84 and 9 % of cases, including one serious infection (Salmonella gastroenteritis) [10]. Interestingly, the safety profile of abatacept in this study was not modified in patients directly switching from anti-TNF therapy to abatacept.

Recently, a Phase I study was conducted on 19 Japanese RA patients, one of them receiving a single IV dose, and 18 receiving multiple doses of abatacept up to 18 weeks. After the first dose, about 90 % of them developed mild/moderate AEs. The most common events included hypertension (42 %), nasopharyngitis (21 %), tachycardia and paresthesia (10 % each). One case of psoriasis exacerbation was observed. After multiple doses, 100 % of patients developed AEs as mild/moderate with a similar profile, and an additional stomatitis also occurred (17 %). One observed SAE (subcutaneous hematoma) was not related to treatment [11]. A Phase II study followed, conducted on 194 Japanese patients (129 treated, 66 controls) with active RA and an inadequate response to MTX, who remained in combined therapy during the study for 24 weeks. AEs were reported in 72−73 % of treated patients (62 % in controls), mostly as mild/moderate. Infections ranged from 33 to 42 % in the treated groups (24 % in controls), being nasopharyngitis the most representative event (21−27 % vs. 12 % in controls) [13].

Experience on early MTX-naive RA with poor prognosis was based on 509 patients (256 treated with abatacept plus MTX for up to one year).

At completion, the frequency of AEs (85 vs. 83 % in controls), SAEs (7.8 vs. 7.9 %) and discontinuations (3 vs. 4 %) were similar in all groups. The most common events (>10 %) included nausea, URTI, and cephalea. Infusion reactions were more frequent in patients receiving abatacept (6 vs. 2 %). Infections (52 vs. 55 %) and serious infections (2 %) included pneumonia, gastroenteritis, cellulitis, pseudomonal lung infection, postoperative wound infection in the study group receiving combination therapy, and pneumonia (3), gastroenteritis (1), breast cellulitis (1), and also one staphylococcal infection in controls receiving MTX. No opportunistic, TB cases or discontinuations occurred due to infections. Various types of autoimmune events (2 %) were also observed in both groups and included Sjögren's syndrome, sicca syndrome, SLE, psoriasis, and atrophic gastritis occurring in one patient in each group; one erythema nodosum was observed in the study group. Overall, the safety profile was similar to previous studies in abatacept-treated RA patients with longer disease duration [14]. Similar data were reported in a clinical and imaging study (ADJUST) in 56 patients at risk or with very early RA, also followed for one year. AEs were frequent in both groups (64 vs. 71 % in placebo) and showed the same safety profile. SAEs were also equally distributed (about 4 %), and one malignancy (BCC) was observed in an abatacept-treated patient [15]. These two studies are relevant since they seem to indicate that an early intervention on T-cell activation with abatacept can modify the progression of the incoming disease.

Long-term analysis of safety treatment with IV abatacept in 317 RA patients (223 treated) from the ATTAIN study was recently reported [16]. Overall, safety over the 5-year cumulative study period remained consistent with the previous 6-months results. In particular, the incidence of malignancies (2.1 events per 100 P/Y) and infections (107.7 events per 100 P/Y) did not increase over time. Serious infections remained in the lower range of previous similar reports. However, the Authors cautioned against the extrapolation of these data to clinical practice, since patients in study were highly selected, as it happens in the majority of clinical trials.

Recently, an overall analysis of clinical trials data, representing 10,366 P/Y exposures to abatacept, concerning RA patients from early disease to established cases with inadequate response to previous biologic and nonbiologic treatment, confirmed that AEs and most SAEs in abatacept and placebo-treated patients were comparable. Moreover, the long-term safety profile was consistent with previous data and remained stable up to 7 years. Pooled analysis on the most concerning events, such as infections and malignancies, were conducted on 4,150 patients exposed to abatacept. Serious infections were infrequent and remained stable with time, but showed higher rates in treated patients than in controls (3.5 vs. 2.4 events 100 P/Y). The most common hospitalized infections were pneumonia, bronchitis, cellulitis, and UTI. However, only few opportunistic infections were observed,

including TB (0.06 events 100 P/Y), aspergillosis (0.02), blastomycosis (0.01), and systemic candida (0.01). Similarly, the integrated incidence of malignancies (about 0.6 cases 100 P/Y) excluding NMSC was comparable between treated and control subjects, and remained stable over time [17]. The overall risk was high compared to nonbiologic DMRAD, but did not reach statistical significance. Therefore, the risk of NMSC is increased with this treatment, but no specific rates were reported in the study, while the risk for lung cancer and lymphoma remained within the rates of the RA background population, which is higher than the general population.

Among events of special interest, autoimmune disorders were detected in early placebo-controlled observation (1.4 vs. 0.8 %), but remained stable over time (1.4 vs. 1.6,100 P/Y). Psoriasis was the most frequently reported autoimmune event in study. Importantly, no new safety signals emerged from the long-term observation.

A number of studies have been dedicated to the evaluation of efficacy and *safety of abatacept SC administrations*, compared with IV administrations, or in head-to-head comparison with other SC administrable biomedicines. In the ATTUNE study, 123 RA patients enrolled in previous two trials (AIM and ATTAIN) switched from monthly IV to weekly SC regimen, and were followed for 12 months. Cumulative AEs were reported in about 76 % of cases, including one SAE (RA worsening) within 3 months observation, and twelve additional serious events within the year. Injection SC site reactions (about 2 %) were mild. The infections (45 %) were as expected, and included one serious event (pneumonia). Two malignancies (BC, uterine cancer) and two autoimmune disorders (sarcoidosis, erythema nodosum) were also observed [9].

In the ALLOW study, 167 RA patients receiving a leading IV dose of abatacept, followed by an SC regimen, were observed up to 24 weeks with the aim of assessing the effects of a temporary interruption of the SC protocol. Interestingly, safety was comparable regardless of withdrawal, with no unexpected events upon reintroduction. AEs were observed in 49 % of patients, and were mild/moderate. Six SAEs were observed including pulmonary embolism, cellulitis, fatigue, and one URTI. Infections were observed as vaginal, UTI, URTI, influenza, and in 2 cases were serious (cellulitis, gastroenteritis salmonella). Two patients experienced a SC site reaction, yet no reactions were observed upon treatment reintroduction. Five patients experienced transitory hypertension within 24 h from the SC injection. One patient experienced a slight worsening in efficacy, which improved following abatacept reintroduction [12].

Finally, in a head-to-head ongoing study (IM 101235, AMPLE) the SC abatacept regimen was compared with that of adalimumab in combination with MTX in 646 RA patients (318 and 328 patients, respectively), followed for one year. Overall, most AEs (88 vs. 86 % in adalimumab) and SAEs were balanced between the two groups (10, 9 %). However, discontinuations were higher in the abatacept group (6 vs. 3.5 %), while infection rates (63 vs. 61 %), mostly nasopharyngitis, URTI, and serious infections (2.2 vs. 2.7 %) were comparable. They included pneumonia

(2), UTI (2), gastroenteritis (1), and Helicobacter gastritis (1) in the abatacept group, while pneumonia (3), bacterial arthritis (3), and meningitis, abscess, bursitis, diverticulitis (one each) were observed in the adalimumab group. Malignancies (1.6 vs. 1.2 %) included cutaneous SCC (2), lung SCC, DLBCL, prostate cancer (one each) in the abatacept group, while cutaneous BCC (2), SCLC (1), and transitional cell carcinoma (1) were observed in the adalimumab group. Autoimmune disorders were more represented in the abatacept group, but none was considered serious. However, adalimumab-treated patients developed seropositivity for ANA/dsDNA autoantibodies. Similarly, injection site reactions were more frequent and severe after adalimumab administrations (9 vs. 4 %). Interestingly, this study indicates that the two biomedicines, acting through different mechanisms of action lead to comparable efficacy and safety profiles [18].

Recently, a first retrospective observation on 8 *RA patients with chronic B hepatitis* treated with abatacept showed that all patients (4) without antiviral prophylaxis developed HBV reactivation, whereas none in antiviral prophylaxis had reactivation. There were no other adverse effects noted in the study [19]. An additional case report on one RA patient with occult HBV infection confirmed the possibility of HBV reactivation during abatacept treatment, and the development of a severe hepatitis with lobular necrosis and portal inflammatory infiltrates [20].

The safety profile in *pediatric JIA* patients is based on 190 patients in the IM101033 trial, and in particular on 153 patients completing the third phase of the study. In the lead-in phase, 70 % of subject showed adverse events and 6 SAEs, included articular exacerbation, ALL, and varicella. In the long-term extension, 91.5 % developed at least one AE, with a higher frequency in the abatacept group than in controls (62 vs. 55 %). The most common category remained infections (78 %), being URTI the most frequent (16 %). SAEs were reported in about 20 % of subjects, 6 % were considered related to the treatment in study, and 6.5 % related to serious infections, which included dengue fever, erysipelas, gastroenteritis, Herpes zoster, bacterial meningitis, and pyelonephritis. Moreover, one case of uveitis and one MS developed in the study group. Discontinuation rates ranged around 4 % of cases. Noteworthy, no opportunistic infections and malignancies were observed. Infusion reactions were not increased (4 %) with respect to adult experience in RA patients, but showed 2 cases of serious hypersensitivity signs. Anti-nuclear antibodies developed in about 11 % of subjects. Overall, safety observations up to 3 years of the 5 years long-term study appeared stable and similar to that of adults with RA [5, 21, 22] with a possible increase in frequency and typology of some autoimmune disorders, such as vasculitis, psoriasis, Type 1 diabetes, vitiligo exacerbation, multiple sclerosis, and uveitis.

In a small case study (7 patients), on uveitis in JIA treated with abatacept, no new ocular complications or worsening were observed. One patient developed oral mycosis and arthritis flare, leading to discontinuation [23].

The overall safety profile of abatacept in official indication appears acceptable and stable over time [24].

41.4 Off-Label Experience

Among 109 trials evaluating safety and efficacy of abatacept, about 40 % are exploring new potential applications of this agent in various directions. In particular, they include studies on SLE (5), Type 1 diabetes (5), lupus nephritis (4), psoriasis (4), vasculitis (Wegener granulomatosis, Behçet's disease, Takayasu's arteritis, and ANCA associated vasculitis), MS (2), GVHD (2), IBD (2), spondyloarthropathies (2), uveitis (2), psoriasic arthritis (1), polymyositis (1), and systemic sclerosis (1). In the postmarketing settings, there are also AEs reports on AS, CD, UC, SLE, vasculitis, Wegener granulomatosis, glomerulonephritis, and arthritis/polyarthritis.

An exploratory Phase II study (IM101042, NCT00119678) evaluated the safety of abatacept in 175 *SLE patients* (118 treated) with active polyarthritis, discoid lesions, or pleuritis and/or pericarditis followed for one year. Most patients (over 90 %) in both groups had any AE. However, SAEs were more frequent in the study group compared to placebo (20 vs. 7 %), as for discontinuation rates (8 vs. 5 %) and SAEs-related discontinuations (6 vs. 2 %). In particular, treatment-related SAEs were higher in the study group (6 vs. 3 %). The most frequent events included URTI (215 vs. 15 %), cephalea (21 vs. 17 %), diarrhea (12 vs. 7 %), and UTI (11 vs. 8.5 %). Serious infections were infrequent and more leveled (3 cases vs. 1 case in placebo), with only one (gastroenteritis) causing discontinuation of abatacept. One malignancy (BCC) and one accidental death (gunshot) were reported in the study group. Other signs, such as musculoskeletal pain, glomerulonephritis, and some laboratory abnormalities were more difficult to relate to the treatment in use or to the underlying disease, and were also confounders for efficacy evaluation. However, a higher incidence of SAEs in the abatacept group clearly emerged from this study [24].

Abatacept has been evaluated in various controlled studies on *CD and UC patients*, either as induction or maintenance therapy. A recent review on four main trials reported unsatisfactory results in terms of efficacy and a number of AEs.

In CD studies, AEs (70−75 %) and SAEs (11−20 %) were equally distributed among treated groups and placebo, being higher in the placebo group during the maintenance period (20 %). Infections in both treated and placebo groups (36 vs. 39 % respectively) were higher during maintenance than in the induction phase (15−17 % vs. 16 % in placebo). By contrast, serious infections were reported in 3−7 % of abatacept-treated patients, and in 2.3 % in the placebo group during the induction phase. However, during maintenance they lowered to placebo levels (2 %). Malignancies were reported in one (low-dose abatacept) treated group (two SCC, BC). No opportunistic infections were reported.

In UC, safety data were also comparable in the induction phase (56−66 % vs. 61 % in placebo) and during maintenance (54−60 % vs. 54 %). However, an increase in SAEs occurred with abatacept in the latter phase, primarily as serious infections (7.7 vs. 3.0 %), including three opportunistic infections in the study groups [25].

Abatacept has been experienced also in *spondyloarthropathies* with unsatisfactory results. In an open-label Phase II study (NCT00558506) follow-up of 24 weeks on 30 AS patients (15 after inadequate response, 15 naive to TNF inhibitors), the treatment in study was well tolerated with no new safety signals either in frequency and severity, with respect to initial observations. No serious infections, opportunistic infections or malignancies were reported. However, some serious events caused discontinuation of treatment [26]. In a similar small case series study, 5 AS and 2 undifferentiated spondyloarthropathy patients refractory to TNF inhibitors were treated with abatacept for 6 months, with no clinical benefit. No adverse events were recorded. Interestingly, one AS patient showing an associate uveitis did not show flares during the study period [27].

For what concerns psoriasic arthritis, in a Phase II study on 170 patients (128 treated with abatacept for 6 months) AEs were reported in about 70 % of cases in all groups, including placebo. However, SAEs were reported in six patients treated with higher doses of abatacept (2 BCC, osteomyelitis, gastroenteritis, cholecystitis, dizziness), and one in the placebo group. Infusion reactions occurred only in the study groups (5 %) and were mild/moderate [28].

Finally, in one case report on an axial spondyloarthropatic woman with a history of psoriasis and uveitis, an exacerbation of psoriasis with nummular plaques and a diffuse erythematosus involvement was observed after abatacept treatment, and resolved after discontinuation [29].

In a recent study, from the EUSTAR group on *systemic sclerosis* (SS) associated with refractory polyarthritis (5 patients) or with refractory myopathy (7 patients) treated with abatacept, no significant AEs were reported, with partial beneficial effects on joint parameters, but not on myopathy [30].

In a controlled ongoing trial (NCT00505375) on 112 patients (6−45 years) with recent-onset *Type 1 diabetes*, 27 infusion of abatacept were administered for over 2 years. The treatment was well tolerated. Overall AEs rates, including laboratory abnormalities, were low and equally distributed between treated and placebo recipients. Infusion reactions (2 %) were mild and occurred more frequently with abatacept than with placebo (22 vs. 17 %). Infections were frequent but equally distributed (42 vs. 43 % in controls). AEs more frequently observed (>5 %) in the study group included constitutional signs (19 vs. 6 %), and cutaneous reactions (17 vs. 11 %) [31].

Finally, exposure to *abatacept during pregnancy* has been reported in 8 RA patients in association with MTX or leflunomide. Spontaneous abortions occurred in three of them during the first trimester, while one delivered a healthy baby. In another case of accidental exposure to one dose of abatacept, during the first trimester of pregnancy, no harm occurred to the fetus and the mother [32, 33].

Overall, the safety profile emerged in these studies was generally similar to that of abatacept studies on RA, with no new emerging safety signals, although with a more unbalanced risk/benefit ratio, due to a substantial loss of efficacy in most off-label attempts.

The overall experience in off-label treatments did not show new emerging safety signs, but the severity profile tended to be higher and unbalanced, mainly in autoimmune disorders, due to insufficient benefits obtained in various treatment regimens.

41.5 Postmarketing Surveillance

In the FAERS database, over 4,400 reports registered by October 2012 included 9,280 AEs (2.2 AEs/R). Most common categories included infections (7.5 %), gastrointestinal signs (4.5 %), cutaneous reactions (4.5 %), respiratory (4.4 %), and neurological disorders (3.4 %). The most relevant infections included pneumonia (1.5 %), nasopharyngitis (0.5 %), UTI (0.5 % each), sepsis (0.4 %), and septic shock (0.2 %).

In the EV database, 2,305 reports were submitted by the end of 2012 on AEs observed during abatacept therapy including 4,627 reactions (2.0 AEs/R). The most common categories included infections (15.4 %), respiratory disorders (8.0 %), nervous disorders (7.3 %), GI and muscular disorders (7.2 % each), malignancies 6.9 %, dermatological reactions (5.8 %), and cardiac disorders (4.1 %). Most common infections included pneumonia (129 reports), UTI (42), sepsis (33), herpes zoster infections (31), bronchitis (23), cellulitis ((15), and diverticulitis (12). Malignancies included BC (27 reports), malignant pulmonary tumors (23), lymphoma (16), malignant melanoma (11), BCC (11), and CC (10). Among relevant nervous disorders there were cerebrovascular disorders (23), optic neuritis (5), peripheral polyneuropathy (5), demyelinating disorders (3), and one case of reversible posterior leukoencephalopathy syndrome (RPLS).

41.6 Remarks

The overall safety profile of abatacept is considered tolerable and rather stable. AEs and SAEs mostly relate to infections and to some malignancies. Infections were usually frequent, but manageable and infrequently serious. Infusion reactions were also mild/moderate, and usually not related to the presence of anti-abatacept antibodies. The rates of serious hypersensitivity reactions causing discontinuation were rare (0.4 vs. 0.2 % in controls). However, 2 cases of anaphylaxis were reported in studies on 2,688 RA patients, 3 cases of anaphylactoid reaction were recorded in the FAERS database, 39 anaphylactic reactions (1.7 % of reports), and 9 cases of anaphylactic shock were reported in the EU database. Although infections remain the main identified safety risk of abatacept, the spectrum was usually limited to URTI and UTI with a manageable asset, and was stable over time. However, pneumonia, sepsis, and septic shock were also experienced during trials and were reported in postmarketing settings. The rates of serious infections

were higher than in controls (3 vs. 2 %), but were lower when compared with anti-TNF therapy. Malignancies were slightly increased, mainly as NMSC, compared to controls (IR 0.69 vs. 0.59). Frequencies of other solid tumors remained within the range of the general population, and lymphomas were in the range of the RA populations. However, 5 cases of lung cancer were identified during clinical trials in the study groups versus none in placebo, with a slight trend to increase (SIR 1.51) over time. Cardiovascular events were exceedingly rare in controlled and open-label studies, even after prolonged exposure. The overall profile did not significantly change over time, up to 8 years of observation. The safety profile of abatacept as monotherapy was not substantially modified in typology, but resulted predominantly increased in associated therapies, mainly with other biologic DMARDs [32]. Overall, AEs and SAEs resulted more increased in JIA than in adult RA patients, and showed more autoimmune disorders.

Experience in a number of off-label treatments did not show new emerging safety signs, but the severity profile tended to be higher and unbalanced, due to insufficient benefits obtained in various treatment regimens [32]. The experience in one study on Type 1 diabetes showed a particularly mild safety profile, and an initial benefit of abatacept indicated a possible role of T cell activation at early phases of the disease, that subsided over time. An additional limitation to the clinical applicability of this regimen derives from the contraindication for live vaccines during abatacept administration and up to 3 months from treatment, due to a predominantly young age of the potential target population.

A critical area to be monitored in longer observations may be related to the insurgence of autoimmune disorders, such as vasculitis, psoriasis, Sjögren's syndrome, Type 1 diabetes, vitiligo exacerbation, multiple sclerosis, and uveitis, mainly observed in JIA patients that may be manifested after several years. Autoimmune risk rates in abatacept-treated patients on 12,132 P/Y pooled data and exposure up to 5 years, were low. The major risk (IR 0.57) was found for psoriasis, followed by Sjögren's syndrome (0.19) and vasculitis (0.18). These rates remained stable over time.

However, RA disease can be associated with other autoimmune diseases per se.

These events may also appear paradoxical considering the mechanism of action of abatacept. However, not all T-activating pathways are inhibited by this biomedicine, which may interfere over time with the general homeostasis of the T cell compartment. Alternatively, the immunogenicity of the whole antibody or part of it (CTLA4 terminal) may be involved in the triggering of autoimmune events over time. For this reason, more accurate and continuative monitoring of abatacept antibodies in long-term studies has been demanded [3].

Finally, occasional potential alert signs should be followed in relation to possible long-term neurological complications. A few cases of demyelinating disorders, including MS, and one case of RPLS were reported in the studies and in the postmarketing setting. Moreover, two cases of PML occurred after belatacept treatment, a molecule much similar to abatacept with higher affinity for the same target [34].

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Aflibercept

42

Aflibercept is a fusion protein consisting of the Fc portion of human IgG1 and the extracellular domains of vascular endothelial growth factor receptors (VEGFR-2 and VEGFR-1), which bind to circulating VEGF, thus acting as a decoy receptor.

In November 2011, FDA approved the first commercial version of aflibercept (Eylea®, Regeneron) for the treatment of neovascular (wet) age-related macular degeneration (AMD). In February 2012 TGA (Australia) granted its apporval. In September 2012, the indication was extended to the treatment of macular edema following central retinal vein occlusion (CRVO). In November 2012, EMEA granted approval for the treatment of AMD, and in September 2013 for CRVO.

In August 2012, FDA approved a new commercial version of aflibercept (Zaltrap®, Sanofi-Aventis) for the treatment of metastatic colorectal cancer (mCRC) that is resistant to or has progressed following an oxiplatin-containing regimen, in combination with irinotecan, 5-FU, and leucovorin (FOLFIRI). The product is closely related to Eylea, yet differing in strength, formulation and purity, and has received a different commercial name (Zaltrap, or Ziv-aflibercept) in order to minimize medication errors. The European CHMP recommended approval of Zaltrap in combination with 5-FU, irinotecan and folinic acid (FOLFIRI) for the treatment of metastatic colorectal cancer (mCRC), when resistant or progressing after oxiplatin-containing regimens. The official approval was released in February 2013.

Pivotal trials for FDA approval for aflibercept (Eylea®) intravitreal injection (IVI) were based on two noninferiority Phase III studies in comparison with ranibizumab, VIEW 1(VGFT-OD-0605, NCT005095) on 1,217 AMD patients, and VIEW 2 (Study 311523, NCT00637377) on 1,240 AMD patients. Study VGFT-OD-0702 was a supportive long-term trial, and was a rollover of a previous Phase I–II study enrolling 157 patients. Overall, patients receiving up to 13 IVI administrations had been followed-up for one year at the time of approval, and then for another year. Data from VIEW 1 and VIEW 2 could be pooled together

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for efficacy and safety analysis. Additional studies included two Phase I trials (VGFT-OD-0603, VGFT-OD-0502) on 71 AMD patients, one Phase II study (VGFT-OD-0508, CLEAR-IT AMD-2) on 167 AMD patients, and one Phase I–II study (Study VGFT-OD0910, an extension of VIEW 1) on 140 patients. Overall, safety evaluations considered 2,984 AMD patients from the seven mentioned studies, but had the main support on 2,578 patients.

FDA approval for CRVO was based on two main studies, VGFT-OD-0819 (COPERNICUS) enrolling 273 (187 exposed) patients, and Study 14130 (GALILEO) on 171 exposed patients receiving one monthly IVI injection up to week 24, for a total of 218 patients exposed to aflibercept.

EMEA approval for aflibercept was based on the two VIEW 1 and VIEW 2 pooled data on 2,412 AMD treated patients, and on COPERNICUS and GALILEO studies for a total of 366 (210 exposed) patients.

Two studies, VGFT-OD-0706 (DA VINCI) and VGFT-OD-0307, conducted on diabetic macular edema (DME) patients were also enclosed in the initial application, yet the sponsor did not submit a request for this indication.

Initial approval for aflibercept (Zaltrap®, Sanofi-Aventis) in combination with FOLFIRI was based on one Phase III study, EFC10262 (VELOUR) on 1,061 mCRC patients (531 exposed in FDA application; 611 in EMEA). Additional datasets came from two other Phase III studies, VITAL (EFC10261) and VANILLA (EFC10547), conducted in 452 and 270 exposed patients, respectively. Integrated data on toxicity came from previous 10 Phase I-II studies (TED6115 and 6116, single agent; ARD6122, 6722 and 6125 on ovarian cancer; ARD6123 on NSCLC; TCD6117, 6118, 6119, 6120 and 6121 in combination therapy on solid tumors) on 404 patients, both as monotherapy and in combination regimens. Initially, aflibercept was tested in a Phase I study as SC regimen (TED6113/6114) in patients with advanced cancer. Since the dose required a large volume, the following studies focused on IV administrations. In particular, various IV regimens were experienced in three Phase I studies in combination with irinotecan (TCD6118), with FOLFOX4 (TCD6117), with gentamicine/erlotinib (TCD 6121), with docetaxel/cisplatin/5-FU (TCD6119), and with docetaxel/cisplatin//pemetrexed (TCD6120). Additional Phase II studies evaluated aflibercept monotherapy in advanced ovarian cancer and symptomatic malignant ascites (EFC6125, ARD6122, ARD6772) and in NSCLC (ARD6123). Overall, safety evaluations considered 1,253 exposed patients from pivotal Phase III studies, and 2,073 patients from additional studies (Phase I–II) for a total of 3,326 exposed subjects. Finally, the recent Study EFC6546 (VENICE) experiencing aflibercept in metastatic androgen-independent prostate cancer in combination with docetaxel/prednisone was discontinued because of efficacy failure [1–9].

At present, 89 trials on aflibercept have been launched, 38 with the ocular IVI formulation (Eylea), and 51 with the IV anti-tumoral formulation (Zaltrap).

42.1 Mechanism of Action

VEGF is a soluble 45-kDa group of cytokines (six homodimeric glycoprotein isoforms) made from a gene splicing family that includes five ligands (VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PGF). The family member recognized by ranibizumab is VEGF-A, the most active variant, which mediates its effects by binding to two tyrosine kinase receptor isoforms VEGFR-1 (Flt-1), and VEGFR-2 (Flk-1), while VEGF-3 responds to VEGF-C and D ligands. VEGF-A is expressed in four major isoforms (VEGF121, 165, 189, 206) and five minor isoforms (VEGF145, 148, 162, 183, and 165b). Among these, VEGF165b is the only inhibitory factor binding to VEGFR-2. Moreover, VEGF110 is a smaller biologically active ligand derived from the proteolytic cleavage of VEGF121 and VEGF165.

Fibroblasts, neutrophils, endothelial cells, and T cells produce VEGF molecules. Their production is stimulated by hypoxia, nitric oxide, and protein kinase C. Local hypoxia produces the Hypoxia Inducible transcriptional Factor (HIF) capable of enhancing angiogenesis. VEGFR-1 and VEGFR-2 are expressed on progenitor and mature endothelia, but also on monocytes, macrophages, neurons, and renal glomerular, preglomerular, and peritubular cells. VEGFR-3 is predominantly expressed on lymphatic endothelium. These receptors are transmembrane Ig-like structures with a predominant extracellular portion (7 domains), and the intracellular tail containing one TYK domain. VEGFR-2 is considered the most important angiogenic factor of the family, while VEGFR-1 seems to act as its modulator/competitor. Moreover, VEGFR-2 signaling generates nitric oxide and prostaglandin 12, which induce vasodilatation. VEGFR-3 is predominantly expressed on lymphatic endothelium and shows lympho-angiogenic properties. VEGFR-2 can be cleaved as a soluble form (sFlt-1), which acts as a physiological competitor of membrane-bound VEGFR-1 and VEGF-2.

Overall, the system generates signals for homeostatic regulation, survival and activation directed to endothelial cells, regulates angiogenesis and vascular permeability, but also exerts neurotrophic and survival-promoting effects on neural and glial cells, and on the renal epithelial/vascular district. The VEGF network also plays a part in embryonic and postnatal vasculogenesis and angiogenesis, in skeletal muscle regeneration and cardiac remodeling, in endochondral bone formation, in the female reproductive cycle, and in kidney function. These additional features are relevant for the pathogenesis of some AEs related to VEGF-blocking biomedicines, including aflibercept.

VEGF/VEGFR binding at ocular level may induce endothelial cell proliferation and vascular hyperpermeability, which contribute to the development and progression of the neovascular (wet) form of AMD, to the visual impairment caused by DME (diabetic macular edema), or to macular edema secondary to RVO (retinal vein occlusion). Despite these processes are considered to be different and multifactorial, hypoxia and the subsequent overexpression of VEGF are considered crucial for AMD. However, increasing evidence indicates that immunologic

processes participate to the pathogenesis of AMD through the production of inflammatory cytokines, recruitment of macrophages, complement activation, and microglial activation. In particular, proangiogenic and angiogenic cytokines, such as VEGF, HGF, FGF, TNFα, PDGF, and PEDF are all active at ocular level in the induction of choroidal neovascularization. In fact, the revisited "immunological privileged site" concept at ocular level is more likely to represent an "endogenous immunological site" protected by the blood retinal barrier (BRB), much similar to the intra-CNS environment protected by the BBB, where highly specialized immunocompetent cells (microglia, dendritic cells, and even retinal pigmented cells) and perivascular macrophages contribute to the internal immune homeostasis. This equilibrium and the BRB integrity are altered by age-related toxic/hypoxic factors in AMD, thus allowing a profound disregulation of internal regulatory processes and the entrance of exogenous immune cells. DME also arises from breakdown of the blood retinal barrier (BRB), which is also mediated by VEGF and other proinflammatory cytokines, thus producing neovascularization, edema, and accumulation of macromolecules in the retina. Notably, in these cases there is a dramatic increase of VEGF in the retina, where it is hardly detectable under normal conditions. RVO is the second most common retinal vascular disorder after diabetic retinopathy, and may cause the central retinal vein (CRVO) or a lateral branch (BRVO) occlusion subsequent to multifactorial etiopathogenetic events, mainly including compression at the arteriovenous crossing, parietal vascular disorders, and hemocoagulative disorders. Cellular and circulating levels of VEGF are increased also in patients with solid tumors and hematological malignancies. In fact, VEGF ligands are produced also by various epithelial tumors, thus ensuring their proper vascularization and growth.

Aflibercept (BAY86-5321, AVE0005) is a recombinant IgG1 glycoprotein consisting of the Fc portion of human IgG1 fused to a hybrid dimer consisting of the VEGFR-2 domain 3 and the VEGFR-1 domain 2. It contains disulfide bonds matching the patterns of both VEGFRs and the IgG Fc portion, and has approximately 15 % glycosylation, without extraneous linker sequences between any of the peptide domains. The complex is also called "VEGF-Trap", due to the high affinity binding and inhibiting action on circulating VEGF-A and on other related proangiogenic VEGFR ligands, such as VEGF-B and placental growth factors (PGF-1, PGF-2). Therefore, VEGF-Trap exerts a wide antiangiogenic effect. In fact, aflibercept acts as a soluble decoy receptor and binds with high affinity VEGF-A121 and VEGF-A165 isoforms, even exceeding the affinity of native receptors and the affinity for PGF and VEGF-B molecules.

Despite VEGFR-1 has high affinity for the natural ligands compared to VEGFR-2 (about 500-fold), it has low pharmacokinetic features and shows a higher nonspecific toxicity. These different properties brought to the development of the hybrid dimeric fusion protein aflibercept. Moreover, the third domain of VEGFR-2 and the second domain of VEGFR-1 were included in the fusion, because of their respective essential role in binding the natural ligands.

Blockade of VEGF induces angiogenesis inhibition, as demonstrated in vitro and in vivo. In addition, aflibercept induces vasoconstriction and vascular rarefaction leading to tumor vasculature regression, and causes hypertension due to blockade of nitric oxide and prostaglandin 12 production. It has been calculated that about 20 % of intravitreal-injected aflibercept reaches the systemic circulation.

In animal models, aflibercept inhibits vascular endothelial proliferation in colon tumors xenografts. However, the tumoral neovasculature appears to be more heterogeneous and complex than expected. This may explain, at least in part, the heterogeneity of response of human tumors to anti-VEGF therapy and the consequent acquired resistance to treatment. In fact, innate resistance of some tumors and adaptive resistance after an initial response to therapy have been attributed to the upregulation of VEGF receptors and/or to a loss of VEGF dependency of some types of tumoral vessels, resulting in unresponsiveness to antiangiogenic therapy. Aflibercept targets the same VEGF family members of bevacizumab and ranibizumab, yet it shows a higher binding activity (140-fold over ranibizumab, and 500-fold over bevacizumab) and seems to have a longer binding duration after administration (2.5 months). In contrast to the mentioned monoclonals combining in multimeric complexes, aflibercept forms stable monomeric inert complexes with VEGF. Moreover, while bevacizumab and ranibizumab inhibit some VEGF-A isomers and pegaptanib (a pegylated modified oligonucleotide) inhibits the VEGF165 isomer, aflibercept can inhibit all VEGF-A isoforms, plus PGF [10–14].

42.2 Immunogenicity

The incidence of antiaflibercept (Eylea) antibodies ranged between 1 and 3 % in both AMD (1 year observation) and CRVO (6 months observation) studies [5].

Antiaflibercept (Zaltrap) antibodies occurred in 3.1 % after IV administrations (1.7 % in controls), and were neutralizing in about 35 % of some tested cases [6, 7]. In the subsequent EMEA assessment report, the overall incidence of such antibodies after Zaltrap administrations in 1,671 treated patients was 3.8 %, with 1.3 % showing neutralizing activity. However, in the pivotal study on mCRC, patients positivity was found higher in placebo group, including neutralizing antibodies. No impact on drug PK profile was observed. No data are available on the presence of aflibercept in breast milk [8].

42.3 Adverse Events

The safety profile of aflibercept (Eylea) was originally based on the seven mentioned studies enrolling 2,984 patients administered with IVI injections. Among these, VIEW-1 and VIEW-2 noninferiority Phase III trials in comparison with

ranibizumab together with VGFT-OD-0702 (Phase III extension) and VGFT-OD_0910 (Phase III extension) were pivotal studies.

The CRVO safety profile was based on VGFT-OD-0819 (COPERNICUS) and on Study 14130 (GALILEO) enrolling 366 patients (218 treated, 142 sham controls). The EMEA CHPM evaluated a total of 3,237 IVI treated subjects, including 2,647 AMD patients.

The safety profile of aflibercept (Zaltrap) in mCRC was based on EFC10262 (VELOUR) trial enrolling 1,061 patients (531 exposed), in combination with FOLFIRI regimen. Two other Phase III studies, VITAL (EFC10261) and VANILLA (EFC10547)—with 452 and 270 exposed patients, respectively—provided additional datasets. Integrated data on toxicity came from previous Phase I–II studies on 404 patients, both as monotherapy and in combination regimens. Overall, safety evaluations considered 1,253 exposed mCRC patients from pivotal Phase III studies, and 2,073 patients from additional studies (Phase I–II) for a total of 3,326 exposed subjects [1–9].

42.3.1 Aflibercept (Eylea)

The updated profile in the last prescribing information reports a total of 2,024 patients exposed to aflibercept. The AMD safety population of 1,824 AMD exposed patients, including 1,223 patients enrolled in VIEW-1 and VIEW-2 studies received the standard dose (2 mg) of aflibercept for up to 96 weeks, and 218 patients with macular edema following CRVO, treated with the same dose in COPERNICUS and GALILEO studies.

The *intraocular* main adverse events include *endophthalmitis*, *retinal detachment, increased intraocular pressure* (IOP), and arterial *thromboembolic events* (ATE).

Some of the most common and relevant events are IOP (5 and 8 % in AMD and CRVO, respectively), cataract (7 and <1 %), vitreous detachment (6 and 3 %), and detachment of retinal pigment epithelium (3 and 0 %). The observed minor events are local hemorrhagic events, corneal erosions, vitreous detachment, floaters, pain, foreign body sensation, lacrimation, and vision blurred all ranging between 3 and 6 %, except for conjunctival hemorrhage (25 and 12 %). Serious local AEs occurred in <1 % and included retinal tear, retinal detachment, endophthalmitis, hypersensitivity reactions in both AMD and CRVO patients, the latter showing also edema (eyelid, corneal) and cataract.

Systemic effects of IVI therapy mainly include *nonocular hemorrhages and ATE*. The incidence of ATE in AMD pivotal studies was 1.8 % of cases during the first year of observation, and reached 3.3 % after 96 weeks. In the analysis of EMEA, AEs were evaluated on three groups of patients; the major one (2,647 patients) included the two VIEW studies where patients were examined on a monthly basis up to one year. A second group of patients (230) included those studied in Phase I–II trials with various doses. Patients of the third group—some of

them (2,235) from the two VIEW studies, others enrolled in the ongoing extension Study VGFT-OD-910—remained under long-term observation (up to 2 years).

Overall, *safety pooled data* from AMD main studies showed rates of ocular and nonocular treatment–emergent adverse events (TEAEs), equally distributed among aflibercept IVI receivers and comparator groups equally treated with ranibizumab (active control). Nonocular TEAEs were similar among all treated groups and included nasopharyngitis (7–8 %), hypertension (5–7 %), cephalea (4 %), bronchitis (4–5 %), and UTI (4–6 %). ATEs were also similar in aflibercept and ranibizumab groups, and reached 8.3 % in the long-term studies. However, ATEs of interest were higher in aflibercept-treated subjects (3.2 %) when compared to ranibizumab (1.8 %). In long-term observations all grade AEs, AEs of interest, TEAEs and any injection related AEs were similarly distributed. However, more discontinuations were observed in the aflibercept patients' pool (4–6.5 %) compared to ranibizumab (3.5 %). Severe AEs ranged around 20 %. Severe TEAEs were slightly higher in aflibercept (3 %) with respect to ranibizumab (2 %) patients. One case of severe uveitis and one retinal vascular disorder were recorded in Phase I–II studies.

In the GALILEO Study on CRVO patients (104 treated, 68 controls) followed for 6 months, ocular AEs included eye pain (11.5 vs. 4.4 % in sham controls), increased IOP (9.6 vs. 5.9 %) and conjunctival hemorrhage (8.7 vs. 4.4 %) as the most common events. No cases of endophthalmitis, rhegmatogenous detachments, or systemic events, such as ATE, were observed. One additional case of mild uveitis, which resolved without change of therapy, was observed in the GALILEO study. Nonocular severe TEAEs (6–10 %) were also equally distributed among treated groups, and included myocardial infarction (0.5 %), CHF (1 % in Phase I–II studies), and TIA (aflibercept-treated patient) [15]. The COPERNICUS experience on 189 CRVO patients followed for 6 months showed a similar profile, with most frequent AEs including conjunctival hemorrhage (17.6 %), eye pain (2.7 %), and maculopathy in the study group. SAEs (3.5 %) were reported in four patients (retinal artery occlusion, endophthalmitis, corneal abrasion). However, all-grade ocular AEs occurred in similar proportions in treated patients and sham controls (about 68 %). Ocular SAEs were more frequent in the controls (13.5 %), although showing a different typology. Nonocular AEs were usually mild and equally distributed, except for hypertension (8.8 %) and URTI (5.3 %) that were most commonly reported in the study group. Abnormalities in laboratory values, which were balanced among the groups, were clinically nonsignificant [16].

Long-term observations in AMD studies reported serious ocular TEAEs as 3.8 %, mostly as procedure-related events or AMD-related disorders (visual acuity reduction, retinal hemorrhage, and cataract) ranging from 0.5 to 0.8 %. Five cases of TEAE-endophthalmitis (2 after aflibercept, 3 after ranibizumab) were observed in the two pivotal trials. After one year observation, data confirmed the 6-month profile, both in typology and frequency. Ocular SAEs were still balanced (2.7 vs. 3.3 % in controls). In particular, three patients in the aflibercept group and two patients in the control group experienced new ocular SAE between 6-month and 21-year endpoint. No new cases of endophthalmitis, corneal abrasion, or retinal

artery occlusion occurred. Similarly, the incidence of nonocular TEAEs was equally distributed.

Nonocular severe TEAEs during 2-year observations were more frequent in the aflibercept group only in Study VIEW 1, including cerebrovascular events (0.8 vs. 0 % in ranibizumab), and TIAs (2 vs. 0.3 %). Laboratory abnormalities were low and balanced among all groups [1–9, 17].

Finally, a recent experience assessed the *effect of high-dose* (4 mg) and low dose (0.15 mg) IVI aflibercept in 28 AMD patients. Previous data from one Phase I study showed that the 4 mg dose was well tolerated. The study confirmed the safety of such single dose, with no evidence of inflammation or other serious events. The most common reaction was conjunctival hemorrhage (71.4 %), both with high and low dose of aflibercept. However, a higher incidence of refraction disorders (35.7 vs. 28.6 %) and reduced visual acuity (21.4 vs. 7.1 %) was observed after the high dose IVI administration, although results in terms of efficacy privileged the higher dose [18].

The incidence of anti-aflibercept (Eylea) antibodies ranged between 1 and 3 % in both AMD (1 year observation) and CRVO (6 months observation) studies [5].

42.3.2 Aflibercept (Zaltrap; Ziv-Aflibercept)

The updated safety profile reported in the last prescribing information was mainly evaluated on 1,216 mCRC patients (VELOUR study) treated with IV aflibercept dose (4 mg/kg in 611 patients, 605 placebo) in combination with the FOLFIRI regimen, for a total of 9 cycles in 18 weeks, enrolled in controlled studies and in part on a wider population predominantly consisting in mCRC patients. Additional datasets were obtained from the VITAL (905 patients, 452 exposed) and VANILLA (541 patients, 270 exposed), and from Phase I-II studies on 404 patients treated with aflibercept as monotherapy or in combined regimens. Total patients for safety evaluations were 2,073 treated and 1,354 placebo in the EMEA assessment report.

The BBW included *severe hemorrhage*, some of them fatal, *gastrointestinal perforation*, and *compromised wound healing*. Additional severe AEs included *fistula formation*, *hypertension (hypertensive crisis, encephalopathy)*, *ATE*, *nephrotic syndrome/thrombotic microangiopathy* (TMA), *neutropenia and related complications, diarrhea and dehydration,* and *reversible posterior leukoencephalopathy syndrome* (RPLS).

All grade bleeding/hemorrhage were observed in 38 % of cases in the study group, with respect to 19 % reported in control patients receiving only FOLFIRI. Severe/serious events including GI hemorrhage, hematuria, and postprocedural hemorrhage occurred in about 3 % of patients versus 1 % in controls.

GI perforation was estimated in 0.8 % of treated mCRC (as for pancreatic and lung cancer patients) versus 0.3 % of controls of the same population. Compromised wound healing was in the range of 0.3 %, and it was not observed in

controls. Fistula formation (anal, enterovesical, enterocutaneous, colovaginal, and intestinal) was reported in 1.5 % of mCRC patients, and in 0.3 % of controls. Severe hypertension occurred in 19 % of patients in study and in 1.5 % of controls, mainly during the first two cycles of combined treatment. ATEs included TIA, cerebrovascular accidents and angina pectoris, with an incidence of 2.6 % in mCRC patients (1.7 % in controls). Proteinuria occurred in 64 % of treated patients and in 41 % of controls, and was severe in 8 % of cases (1 % in controls). Nephrotic syndrome was observed only within the treated group (0.5 %). Other AEs of relevance were severe neutropenia (37 vs. 30 %), febrile neutropenia (4 vs. 2 %), and neutropenic infections/sepsis (1.5 vs. 1.2 %). The incidence of severe diarrhea was 19 % in the study group and 8 % in controls, while dehydration was 4 and 1 %, respectively. Palmoplantar erythrodysesthesia syndrome was reported in 11 % of patients (3 % severe) in the study group and in 4 % (0.5 % severe) of patients in the placebo/FOLFIRI group. Finally, RPLS was reported in 0.5 % of a total population of 3,795 patients receiving aflibercept as monotherapy or in combination chemotherapy.

All together, the most common all grade AEs (>20 %, with at least 2 % increase over controls) included leukopenia, diarrhea, neutropenia, AST increased, stomatitis, fatigue, thrombocytopenia, ALT increased, hypertension, weight and appetite decrease, epistaxis, abdominal pain, dysphonia, serum creatinine increased, and cephalea. The most severe/serious events (SAEs) occurring as >5 and >2 % over controls were neutropenia, diarrhea, hypertension, leukopenia, stomatitis, fatigue, proteinuria, and asthenia. Discontinuation rates were ≥1 %.

Anti-aflibercept (Zaltrap) antibodies occurred in 3.8 % after IV administrations (1.7 % in controls), and were neutralizing in about 35 % of some tested cases [6, 7].

The completion of a pharmacokinetic Phase II study on 74 aflibercept-treated patients and a recent detailed update of the VELOUR trial on 1,226 patients followed for 36 months, and confirmed the reported safety profile [19, 20].

Finally, in the first Phase I dose-escalation study on mCRC in Japanese patients, the safety profile showed no new signals. SAEs were observed only in two patients. All AEs resolved, except for anemia. No anti-aflibercept antibodies were detected, nor major allergic reactions were observed [21].

Overall, the multi-VEGF-trap aflibercept showed an acceptable safety and tolerability profile in all-phase clinical trials. No unexpected AEs were reported. The observed AEs were typical of anti-VEGF therapy, and the association with chemotherapy did not develop synergistic effects on the whole treatment-related toxicity [8, 22].

42.4 Off-Label Experience

At present, 89 trials on aflibercept have been launched, 38 with the ocular IVI formulation (Eylea), and 51 with the IV formulation (Zaltrap). As for the former, six studies are dedicated to DME, representing the most common off-label use of

the IVI aflibercept therapy. The latter has been experienced in various advanced adult and pediatric solid tumors either as monotherapy or, more frequently, in combination with chemotherapy. In adults, aflibercept (Zaltrap) has been evaluated in NSCLC, mesothelioma, glioblastoma, ovarian cancer, NSCLC, sarcoma/angiosarcoma, breast cancer, endometrial carcinoma, thyroid cancer, pancreatic, and prostate cancer. Aflibercept is also experienced in studies on B cell lymphoma, multiple myeloma and myelodysplastic syndrome. In pediatric patients, embryonal tumors (hepatoblastoma, neuroblastoma), ependymoma, pylocytic astrocytomas, Ewing sarcoma, rhabdomyosarcoma, synovial sarcoma, and hepatocellular carcinoma have been investigated in preliminary Phase I dose escalation study, in order to establish optimal doses and maximal levels of toxicity [23].

42.4.1 Aflibercept (Eylea)

The initial DME safety IVI profile mainly derived from the completed Study VGFT-OD-0706 (DA VINCI) on 219 patients, one preliminary Study VGFT-OD-0512 on 5 patients treated with one dose of 4 mg aflibercept, and on VGFT-OD-307 Study on 24 patients treated with 0.3 mg/kg IV injections. At present, there are three active studies (VGFT-OD-1009, NCT01331681 –VIVID/DME-, and NCT01512966 –VIVID/JAPAN) on 53, 404, and 73 DME patients, respectively. One additional study (NCT01627249 or Protocol T) is enrolling 660 DME patients to compare efficacy and safety of IVI aflibercept, bevacizumab, and ranibizumab.

Results at 6-month observation of 221 DME patient treated with two IVI doses of aflibercept were similar to previous experience in AMD. Most common ocular AEs included conjunctival hemorrhage (18.9 %), increased IOP (9.7 %), eye pain (8.6 %), hyperemia, and floaters (5–6 %). SAEs included two cases of endophthalmitis, one uveitis, retinal tear, and corneal abrasion (1 case each).

Nonocular SAEs included hypertension (9.7 %), three cases of ATE, one myocardial infarction and one cerebrovascular accident in the study group, while none of them was reported in the laser treated controls. Three cases of death occurred in the study group (1 renal failure, 2 multiorgan failure), and none among controls. Results of one year outcomes of the same trial on 175 treated patients showed an aflibercept safety profile similar to the previous one, with conjunctival hemorrhage, eye pain, increased IOP, ocular hyperemia, cataracts and floaters, as the most common events. Nonocular SAEs were present in about 26 % of cases, and included CHF (3.7 %), cellulitis (3.7 %), hypertension, cerebrovascular accident, and anemia (1.7 % each). Interestingly, all SAEs, but one cerebrovascular accident, were absent in the control group (laser photocoagulation), and most of the systemic AEs were attributed to the underlying disease [24, 25].

A recent Cochrane review and metaanalysis on anti-VEGF therapy in DME compared different anti-VEGF treatments at about 1-year follow-up. Overall, ocular and systemic AEs were considered rare. There was no significant difference in ATE and mortality, compared with sham controls. However, it was underlined

that available studies were not suited for the investigation of AEs in a sensitive population, such as people with diabetic microangiopathy, thus indicating the need of additional long-term studies to assess ocular and systemic AEs [26].

42.4.2 Aflibercept (Zaltrap; Ziv-Aflibercept)

Aflibercept in combination with docetaxel has been experienced in *metastatic NSCLC* after platinum failure in a Phase III study (VITAL) on 913 patients (456 in combination therapy; 457 in placebo/docetaxel) followed-up for a median time of 23 months. Overall, the co-administration regimen increased the rate of severe events (71.5 vs. 49.7 % in placebo). The most common and/or relevant events were stomatitis (41.6 vs. 15.2 %), fatigue (31.6 vs. 15.2 %), hemorrhage (27.9 vs. 13 %), hypertension (21 vs. 5.1 %), epistaxis (20.4 vs. 6.2 %), neutropenia (34 vs. 29.4 %), dysphonia (18.4 vs. 3.5 %), and cephalea (13.1 vs. 5.5 %). Severe/serious events included neutropenia (28 vs. 21.1 %), fatigue (11.1 vs. 4.2 %), stomatitis (8.8 vs. 0.7 %), proteinuria (7.6 vs. 0.9 %), febrile neutropenia (6.6 vs. 4.2 %), diarrhea (4.2 vs. 2.4 %), and GI perforation/fistula (0.9–1.3 vs. 0.2 %). At cutaneous level the palmoplantar erythrodysesthesia syndrome appeared increased in combination therapy (6.2 vs. 1.3 %). Fatal events excluding those caused by disease progression were more frequent in the study group (7.1 vs. 4 %), and were mostly related to neutropenic complications and pulmonary embolism.

Overall, the safety profile was considered tolerable, although showing an increase of AEs related to the anti-VEGF therapy toxicity in the combined regimen. However, some SAEs, such as ATE, were less frequent than expected. The therapeutic association had a safety profile comparable to other anti-VEGF/chemotherapy regimens [27].

Early experiences in 58 cases of *glioma/glioblastoma* showed SAEs as hypertension (10 %), lymphopenia (7 %), fatigue (5 %), CNS ischemia (3 %), and GI hemorrhage (2 %) [28]. In 28 of these patients circulating cytokines and angiogenic factors were measured, with the aim of correlating their presence with AEs and identifying potential markers of toxicity. It was found that IL-13, IL-6, and IL-10 levels were repeatedly correlated with general toxicity. Baseline elevations of e-selection, RANTES, and MIP1b anticipated the development of inflammatory-mediated toxicities. Moreover, it was suggested that IL-13 and MCP3 early changes could individuate patients at risk of endothelial damage, such as hypertension, proteinuria and bleeding, while increases of IL-6, IL-10, and IL-1b correlated with fatigue. It is believed that these data can be extrapolated to other targeted tumors and to different antiangiogenic agents [29].

Final results of a Phase II study on 55 patients (29 receiving aflibercept as monotherapy) with *malignant ascites in advanced ovarian cancer* showed a more complex safety profile. The trial included a double blind period (from 2 to 6 months) followed by an open-label study. All patients experienced at least one TEAE, which was serious in 73–90 % of cases (56–72 % in controls) and related

to death in 10–13 % of treated patients. The most frequent events were constitutional disorders, such as fatigue (47 %, serious 13 %), nausea (30 and 7 %), anorexia (23 and 7 %), peripheral edema ((33 and 7 %), respiratory disorders including dyspnea (43 and 20 %), cough (23 and 3 %), and diarrhea (43 and 7 %). Other relevant TEAEs included hypertension (17 and 7 %), GI perforation (10 %), VTE (7 %), CHF (3 %), and hemorrhage (10 %), and one case of suspected pulmonary embolism, all observed in the aflibercept-treated group, except for one GI perforation. As for laboratory abnormalities, a consistent increase of hepatic enzymes (ALT, AST, ALP, and hyperbilirubinemia), and hematological disorders (lymphopenia, anemia) were more frequent in the study group. Severe proteinuria occurred only in five treated patients, and lowered during follow-up [30].

These results indicated a safety profile composed of signs related to the underlying or progressive intraperitoneal disease and serious signs more strictly related to treatment, such as GI perforation, hypertension, VTE, proteinuria, and respiratory disorders, leading to a significant overall risk. Notably, in one previous Phase I-II study evaluating the association of aflibercept to docetaxel therapy in 9 patients with ovarian, peritoneal and fallopian tube cancers, and in one Phase II study on 16 patients with advanced ovarian cancer treated with aflibercept as monotherapy, safety results were contrasting. The first study assessed that the combination therapy with docetaxel was feasible and did not generate unexpected or severe events (no intestinal perforations, VTE, or RPLS) after 22 infusions. However, the second study experienced a higher rate of drug-related SAEs among patients receiving aflibercept long-term monotherapy (up to 394 days), such as hypertension, or immediately after therapy initiation (6 days), such as intestinal perforations. Moreover, five cases of severe intestinal obstruction were also observed [31, 32].

A number of studies examined the association of aflibercept with different chemotherapy regimens in various *advanced solid tumors*. In the first dose-escalation study, aflibercept was associated with docetaxel/cisplatin or pemetrexed/cisplatin in the treatment of 30 patients (mostly with breast cancer, ovarian, colon/rectum carcinomas, and sarcoma), 16 of them enrolled in the dose escalation part, and the other 14 in the expansion phase. The maximal administered dose of aflibercept was 6 mg/kg. All but one patient had a history of prior chemotherapies, and all discontinued the study either for diseases progression or AEs (11 patients). The most common all grade events related to aflibercept included epistaxis (83.3 %), proteinuria (53.3 %), and hypertension (50 %). One case of GI perforation, one pulmonary embolism and one DVT were also observed. Other common events were related to constitutional signs (fatigue 100 %, nausea 73 %, dysphonia 70 %, and cephalea 57 %), GI signs (diarrhea/vomiting 60 %), stomatitis (77 %), and alopecia (40 %), and were confounded among disease signs and combined chemotherapy signs. The main hematological AEs included lymphopenia and anemia (90 % each). All grade neutropenia (70–100 %), and severe neutropenia (23.5–100 %) were present in all aflibercept-treated patients, but severity was not dose-related. No deaths were considered treatment-related.

Overall, the highest dose (6 mg/kg) was found effective in combination with chemotherapy, but the type, frequency, and severity of some AEs were considered not easily manageable in the experienced conditions [33].

Another Phase I dose-escalation study examined the association with pemetrexed/cisplatin, and the same range of aflibercept doses were experienced in 18 patients with various advanced solid tumors (mostly mesothelioma and NSCLC). All patients showed at least one AEs, including fatigue (89 %) and nausea (83 %) as the most common all grade events, followed by GI signs (56–83 %). The most common all grade events related to aflibercept included hypertension (56 %), thromboembolic events (17 %), proteinuria (6 %), and mild dysphonia (39 %). Severe fatigue was observed in three patients treated with the high dose of aflibercept. No dose response effects were related to hypertension. Two patients had pulmonary embolism, one associated with DVT. Laboratory abnormalities included severe neutropenia (33 %), but no febrile neutropenia occurred. Overall, the safety profile was similar to the previous Phase I studies and to more general experiences with aflibercept in combined therapies, including confounding signs from therapeutic agents and the different underlying diseases. A higher frequency of thromboembolic events was attributed to the cisplatin component of the therapeutic regimen, known to induce activation of platelets and endothelia, thus exacerbating the anti-VEGF effects of coagulability and endothelial integrity. Six patients with neurocognitive disturbances suggesting the insurgence of RPLS were studied in detail. Eventually, such diagnosis was not confirmed [34].

Results from an expansion cohort of 27 patients with advanced solid tumors, enrolled in a Phase I study of aflibercept in combination with irinotecan-LV5FU2, reported the known safety profile of anti-VEGF combined therapy. All patients had at least one AE and most of them (89 %) had a severe AE. The most frequent aflibercept-related AEs were proteinuria (88 %), hypertension (67 %, severe 30 %), dysphonia (85 %), bleeding (74 %), fistula formation (4 %), and severe proteinuria (8 %). No serious renal events were registered. No anti-VEGF antibodies were detected. All patients discontinued study treatment, either for disease progression (74 %) or for AEs (19 %). Overall, the safety profile was compatible with the adopted combination therapy and no new signals emerged [35].

Finally, one Phase I dose-escalation study on 21 *pediatric patients* with refractory solid tumors assessed both maximum tolerated dose (MTD) and dose-limiting toxicities (DLT) of aflibercept. The most common non-DLTs were hypertension (9 patients) and fatigue (7 patients). However, one of six patients receiving 2 mg/kg dose developed intratumoral hemorrhage, and two patients receiving 3 mg/kg dose had tumor pain or tumor necrosis. These effects were considered a strong argument in favor of the biological activity of aflibercept, although no objective clinical responses were observed during this study [36]. The study seems to indicate a higher toxicity on pediatric age for aflibercept, as compared to other anti-VEGF agents showing a similar class-toxicity between adults and younger patients. However, the main limiting factor was intratumoral hemorrhage attributed to the tumor histology/vasculature or to the higher

aflibercept activity, possibly related to a major contribution of VEGFs to pediatric tumor growth [37].

Overall, the VEGF multiblockade of aflibercept shows a potent antiangiogenic effect, with a safety profile in the range of previous anti-VEGF agents for frequency, typology, and manageability [23]. However, its role in childhood cancers raises more concern and needs further studies to better customize doses and therapy regimens.

42.5 Postmarketing Surveillance

In the FAERS setting 274 reports were registered as aflibercept, mostly related to Zaltrap treatment of oncologic patients, and 131 reports related to the aflibercept (Eylea) IVI formulation. No data were recorded as Zaltrap or ziv-aflibercept administrations.

In the EUV database, 66 reports as aflibercept (Eylea) included 127 SAEs (1.92 AE/R). The most frequent and relevant reported events included endophthalmitis (14) and pseudoendophthalmitis (8), uveitis (5), ocular/retinal hemorrhage (3), retinal tear (3), and retinal detachment (2). All other ocular and nonocular AEs were reported as single events.

By the end of April 2013, the EUV database had 85 reports registered as Zaltrap, mostly as general disorders (31, mostly as disease progression), gastrointestinal disorders (24, including 1 perforation), and nervous disorders (18, including 4 cerebral hemorrhage and 1 TIA).

42.6 Remarks

The assessment of aflibercept safety encompasses an exposure to IVI aflibercept (Eylea) of over 3,000 AMD patients, 400 CRVO patients, and about 250 DME off-label treated patients. Part of these cohorts has been followed up to 2 years. The overall safety profile has repeatedly proven to be well tolerated, both for ocular and nonocular drug-related events, and rather stable over time. The AEs typology did not significantly change in CRVO and DME patients, where some SAEs occurred even at a reduced rate, allowing to identify a series of events clearly related to the mechanism of action of aflibercept, and in general to anti-VGEF/VGFR therapy, as local and systemic reactions. However, there was no clear aflibercept dose-relation with respect to AEs insurgence and typology. In addition, a number of frequent events were more related to intervention (IVI procedure) or to underlying disease, than to the drug in study.

Interestingly, aflibercept seems to allow more prolonged intervals between IVI administrations, thus reducing at least some of the procedural AEs. In fact, aflibercept seems to have a longer duration of clinical action (possibly up to 2.5 months) with respect to other anti-VEGF agents [38]. Immunologic reaction

were negligible, both as local or systemic events. However, concerns remain for permanent therapy, especially for AMD, since beneficial effects of anti-VEGF agents tend to disappear shortly after therapy discontinuation [39], and possible intervention of tachyphylaxis [40].

Experience with aflibercept (Zaltrap) encompasses about 3,000 treated patients in mCRC and over 700 off-label treated patients with a variety of solid tumors. The safety profile after IV administration appears to be at higher risk, but with the same typology of systemic AEs related to anti-VEGF therapy, associated with underlying disease signs and more pronounced AEs related to associated chemotherapy regimen. However, the addition of aflibercept to some chemotherapy regimens did not seem to aggravate the safety profile of these patients, nor the mortality rates.

The overall safety situations encountered in off-label treatments did not show new signals, but were concerning for a more unbalanced risk/benefit ratio and, in the case of pediatric patients, for a possible increased risk of anti-VEGF-related AEs on the vasculature of pediatric tumors.

Taken together, while experience in ocular angiogenic diseases seems favorable and relatively safe, the impact of aflibercept in oncology needs more convincing long-term data in terms of efficiency, while the safety profile remains within an acceptable framework, considering the effects of alternative therapy regimens. Most, if not all, AEs induced by aflibercept can be referred to the principal mechanism of action of this agent. However, it is not clear yet if the multiblockade of VEGFs produces more benefit and/or induces a higher frequency of AEs in the long term, with respect to similar biomedicines blocking only one or some of these factors [41]. For example, proteinuria seems to be more frequent after aflibercept therapy compared to similar agents, and some signs of toxicity were increased in combined therapy, thus indicating a possible synergistic effect on the safety profile, such as for hematological cisplatin toxicity. However, in some AMD studies aflibercept has proven to be effective in patients who poorly respond to other VEGF blockers, although it does not seem to generate different/additional AEs. Nonetheless, the multiblocking action of aflibercept does not seem to overcome tumor resistance to blockers of few VEGF isomers. Finally, further studies on the complex heterogeneity of tumor vasculature and its differential resistance to aflibercept are needed, in order to assess their role in the AEs induction, and for a better selection of patients.

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Alefacept

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Alefacept (Amevive®, Astellas) is a recombinant dimeric fusion protein combining the Fc fragment of human IgG1 with the CD2-binding portion of the human leukocyte antigen-3 (LFA-3). The binding interferes with T lymphocytes activation by blocking CD2/LFA-3 interaction. In 2003, FDA granted approval for the treatment of moderate to severe chronic plaque psoriasis (Ps) in adult patients who are candidates for systemic therapy or phototherapy. Approvals followed in Canada, Switzerland, Australia, and Israel. EMEA rejected approval for safety issues. On December 15, 2011, the manufacturer announced discontinuation of production, distribution, and sales. The decision was officially taken for business needs and any specific safety concern was excluded. The supportive programs were provided up to March 2012, and patients were invited to apply for alternate therapy. Alefacept was officially discontinued in US since September 2008 for the lower dose formulation (7.5 mg vial) and in September 2012 for the higher dose (15 mg vial), but has still a prescription market status (www.accessdata.fda.gov). The last prescribing information was updated in May 2012 to add postmarketing reports of malignancies and infections. No BBW was included, and major reported serious AEs are *lymphopenia, malignancies, serious infections*, and *hypersensitivity reactions.*

Initial approval was based on two Phase III studies, the former enrolling 553 Ps patients (367 treated with 7.5 mg IV), the latter performed in 449 Ps patients (173 with 10 mg IM, 166 with 15 mg IM). The 10 mg dose resulted not significantly effective.

The safety profile included total lymphopenia (10 % of IM recipients, 29 % of IV recipients), mostly as CD8+ (42 IM; 59 % IV), persisting (21, and 36 %) at least up to 3 months. Malignancies were observed in 1.3 % of treated patients (0.5 % in controls) during the first 24 weeks of observation. When evaluated on a database of 1,869 patients, 63 treatment-emergent malignancies were reported in 43 patients, including skin cancer (20 BCC and 26 SCC in 27 patients), melanoma (3), solid organ tumors (12 in 11 patients), and lymphomas (3 NHL, 2 HL, 1 cTCL).

Serious infections during the first 24-week treatment were 0.9, and 0.2 % in placebo. They included cellulitis, abscess, wound infections, toxic shock,

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pneumonia, appendicitis, cholecystitis, gastroenteritis, and herpetic infections. Hypersensitivity reactions evaluated on 1,869 patients were 0.2 % as angioedema, and <1 % as urticaria. Hepatic enzymes elevations (ALT/AST) were observed in 1.7 % of treated patients and 1.2 % of controls. Injection site reactions after IM were 16 % (8 % in controls), and usually mild. Anti-alefacept antibodies were detected in 3 % of cases in an ELISA testing, and in 72 % of cases in a dual specificity testing [1].

The postmarketing setting received reports on malignancies (cutaneous, solid organ, lymphomas, leukemia), serious infections, and hepatic toxicity signs.

An important additional limitation of alefacept was the low number of patients that respond to treatment (about 30 %), and a long induction phase (about 6 weeks) after treatment completion before reaching maximal levels of efficacy. Nonetheless, attempts to individuate susceptible patients [2] or to combine alefacept with UVB therapy to accelerate the alefacept effects [3] have been recently proposed.

Over 42 trials were launched for alefacept. At present, 26 of them result completed, 7 terminated, 2 suspended, and 1 withdrawn. Three studies are registered as active (lymphoma, GVHD, Type 1 diabetes), and three are referred as recruiting (2 on GVHD, and the PSOLAR Registry).

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Belatacept

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Belatacept (Nulojix®, Brystol-Meyers Squibb) is a fusion protein binding to CD80 and CD86, thus blocking their interaction with CD28 receptor and the consequent costimulatory signal to activate T lymphocytes. The European CHMP recommended the granting of the marketing authorization for belatacept in April 2011. FDA granted approval on June 15, 2011 for the prophylaxis of organ rejection in EBV seropositive adult patients receiving a kidney transplant, in combination with basiliximab induction, mycophenolate mofetil, and corticosteroids. The EMEA approval followed on June 17, 2011 for the prophylaxis of graft rejection in adults receiving a renal transplant, in combination with corticosteroids and a mycophenolic acid, along with a recommendation for addition of an interleukin receptor antagonist (IL-2Ra) to the indicated regimen. In February 2012, belatacept received the marketing approval by the Australian TGA, and by the Authorities of Argentina, Brazil, Colombia India, Russia, and Switzerland.

Pivotal studies for approvals were mainly based on IM103008 (BENEFIT) Phase III trial (666 patients), IM103027 (BENEFIT-EXT) Phase III trial (543), and IM103100 (218), for a total of 1,427 (947 exposed) renal transplanted patients. A number of supportive studies included Study IM103045 on 250 liver transplants (which was also considered for general safety evaluations), IM103010 (maintenance study on 171 -83 exposed- patients), IM103034 (steroid avoidance study on 93 -62 exposed- patients), two investigator studies (IM103030, IM103036) on renal transplants, and one on pancreatic islets transplants (IM103058). Preliminary investigations on non-transplanted individuals included three studies on IV belatacept administration IM103001 and IM103024 on 70 healthy subjects; study IM103002 on 214 - 92 exposed RA patients), two studies on SC administration (IM103029, IM103038 on 33 -24 exposed- healthy subjects), and one study on both routes (IM103046) on 47 -41 exposed healthy subjects.

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It must be noted that initial submission to FDA CDER started in October 2000, followed by a number of reassessments; the final application, submitted in 2009 to FDA and EMEA, included only 24 months data from the pivotal trials, which was considered insufficient for this kind of studies. Therefore, both agencies raised significant questions and required the submission of the 36 months follow-up data, which was taken as the final endpoint for efficacy and safety evaluations [1–5].

At present, 25 trials with belatacept have been launched, 5 are competed/terminated, 14 are active, and 5 are recruiting.

44.1 Mechanism of Action

CD80 (B7-1) and CD86 (B7-2) are the ligands of CD28 and of CD152 (CTLA-4), all members of the same immunoglobulin superfamily. These ligands are expressed at the surface of antigen-presenting cells (APC), while the latter are T cell surface proteins. Upon B7/CD28 binding, a costimulatory signal necessary for T cell activation is generated, in concurrence with the pivotal binding of the T cell receptor (TCR) to the antigen-MHC complex situated on APCs (dendritic, monocyte/macrophage, thymus APC, and B cells). In contrast, the B7/CTLA-4 binding or the CTLA-4 crosslinking generate negative signals, which terminate T cell activation. In fact, CD28 is constitutively expressed on naive T cells, while the CTLA-4 expression on lymphocytes follows their activation (24–48 h), and strongly competes with CD28 because of a higher affinity (about 20-fold) for B7 ligands. Interestingly, memory T cells are not entirely dependent on CD28 costimulatory signals for activation. Other costimulatory signals are generated between T cells and APC cells by the CD40/CD40L binding, and through 4-1BB (CD137)/4-BBL interactions. Notably, CD137 is expressed on T cells (mainly CD8 +), dendritic cells, NK cells, granulocytes, and endothelia at sites of inflammation. CTLA-4 is also expressed by fetal cells, and by placental fibroblasts, indicating a potential role in the induction of maternal-fetal tolerance during pregnancy.

Belatacept (LEA29Y, BMS-224818, CTLA4-Ig) is a soluble recombinant fusion protein combining a human IgG1 Fc portion to the human cytotoxic T lymphocyte antigen-4 (CTLA-4). The CTLA-4 portion binds with higher avidity to CD80 (B7-1) and CD 86 (B7-2) molecules expressed on antigen-presenting cells (APC), thus physically interfering with their binding to the natural receptor CD28, and therefore blocking the costimulatory pathway activating T lymphocytes. However, belatacept is significantly more potent in inhibiting CD80 costimulatory signal than that of CD86.

Abatacept is the parental antibody of belatacept; it differs only in two amino-acids (L104 to E and A29 to Y), but has a higher avidity for the same targets (see abatacept, Chap. 41). In particular, it binds four times more avidly to CD86 and two times more avidly to CD80 than abatacept. Saturation concentration of

belatacept is also different, given that saturation of CD86 is 10-fold higher than that of CD80.

The binding to CD80/86 is reversible and is not species specific, although showing a reduced activity on some species (mouse, rat, rabbit) and a higher activity on Cynomolgus monkey, compared to abatacept. The Fc fragment fused in both agents is identical and was genetically modified at the hinge region to greatly reduce the binding capacity to FcRIII and FcRI receptors, thus inhibiting the activation of Fc-mediated immune activities, such as CDC and ADCC. Both products reduce T cell proliferation and survival, and also cytokine production (TNFα, IFNγ, IL-2, IL-4).

Moreover, belatacept seems to inhibit both CD4+ and CD8+ T lymphocytes, although the latter is less dependent on CD28 pathway, while abatacept inhibits only the proliferation of the former cells. Overall, these molecular improvements produced a 10-fold more potent inhibition of T cell activation.

In addition to the principal action on the B7/CD28 costimulatory pathway, abatacept (and presumably belatacept) has been found to exert inhibitory effects on intracellular signaling mediated by the same pathway in APC cells. For example, intracellular signaling events can be activated after the B7/CD28 binding on B cells, which increase immunoglobulin production while exerting inhibiting effects on T cell proliferation via p38MAPK and NF-kB pathways. However, belatacept had no effect on B cell proliferation, suggesting that it cannot initiate reverse signaling on these cells. Moreover, the blocking of CD28 will predominantly prevent naive T cell activation, since memory T cells—especially memory CD8+ T cells—are not entirely dependent on CD28 costimulatory signal for activation.

T cell activity is crucial for graft rejection and involves both CD4+ and CD8+ lymphocytes, which must recognize allogenic donor antigens presented to the TCR, in the contest of MHC on APCs, and receive the costimulatory signal via CD28. The latter signal results in the production of IL-2 stimulating T cell proliferation/differentiation, as well as in lowering the threshold of T cell activation. In contrast, Treg cells are important in downregulating immune rejection responses. Importantly, the belatacept blockade should not interfere with the activity of these lymphocytes, although some recent data are contradictory. Moreover, other immune and non-immune mechanism are involved in graft rejection, and therefore the CD28-mediated belatacept immunosuppression is not sufficient to fully control graft rejection, especially acute graft rejection [1–7].

44.2 Immunogenicity

The rate of anti-belatacept antibodies was 4 % (34/857 tested patients) after IV belatacept in two Phase III core trials and in one Phase II long-term extension cohort. They were increased at 5.6 % after treatment discontinuation. Cumulative antibody prevalence was 5.3 % during treatment and 6.5 % after belatacept discontinuation, and up to 7.8 % after IV administration. Neutralizing activity was

found in 27.6 % of cases. Anti CTLA-4 antibodies were detected as 4.4-6.2 %. The overall incidence was calculated as 2 per 100 P/Y, and did not increase after prolonged exposure. Titers were usually <1:20. No associations with graft rejection and with peri-infusional events were observed. Autoimmune events developed in 3 out of 65 (keratoconjunctivitis, GBS, psoriasis) anti-belatacept positive patients (38 had anti-CTLA-4 antibodies). A low incidence of anti-donor HLA antibodies was also reported in some belatacept arms, but no correlation with graft rejection was observed [1–5].

44.3 Adverse Events

The main safety population was based on 1,425 kidney transplanted patients enrolled in the three main trials. (BENEFIT, BENEFIT-EXT, and IM103100). Namely, 477 patients received the high regimen in study (more intense, or MI dose), 472 received the lower regimen (less intense, or LI dose), and 476 received CsA. The LI dose was the only approved regimen.

The BBW in the prescribing information included *post-transplant lymphoproliferative disorder* (PTLD), other *malignancies*, and *serious infections*. Additional warnings include *PML, acute rejection/graft loss,* and risk in use of live vaccines.

It must be noted that the general safety profile in the label mainly refers to two studies (IM103008, IM103027) for a total of 806 transplanted patients (401 treated, 405 controls) receiving the LI approved regimen, and evaluated at 1 and 3 years post-transplant, while the third study (IM103100) was considered only for some relevant AEs. The safety profile reported below refers to the extended evaluations performed by Agencies on all supportive data of both MI and LI treatments, in order to better define a comprehensive safety framework [1–5].

PTLD is a life threatening complication following organ transplantation in high-risk patients receiving immunosuppressive therapy (0.75 %). The disorder develops as B cell (CD20+) hyperplasia or neoplasia, usually associated with an uncontrolled EBV infection. Non-exposure related risk factors are EBV seronegativity, and CMV seronegativity as an additional risk factor in EBV seropositive patients. In fact, the rate of PTLD in EBV+/CMV+ subjects was approximately 0.3 %, and in EBV+/CMV- subjects was 1.9 %.

The primary PTLD risk with belatacept is characterized by the predominant involvement of CNS. According to pooled data from the three core trials, 14 cases of PTLD ($\approx$50 % EBV +) were observed on 1,425 (1,357 EBV tested) belatacept treated patients, including 9 cases of CNS involvement in the 3-year follow-up. Notably, only 11 % of the population in study was EBV-, while 50 % of PTLD were EBV-. Eight cases were reported in MI regimen and 6 cases in LI regimen. Therefore, the absolute risk of PTLD in the approved LI regimen is approximately 1 %. Three PTLD cases developed also in controls receiving CsA (one case after 4 years), but did not show CNS involvement. Most cases in the belatacept group (13/14) occurred within 18 months post-transplant. Fatal cases of PTLD were 8 in

belatacept treated groups (6 CNS-PTLD, 3 renal-PTLD), and 3 in controls. Two additional cases of PTLD occurred in liver transplant recipients (IM103045). Overall, the cumulative frequency of PTLD in the pivotal trials was 1.7 % in MI regimen, 1.3 % in LI regimen, and 0.6 % in the CsA control group.

Overall malignancies were reported up to 36 month in 8.6 % in MI regimen, and in 5.7 % in LI regimen (7.1 % in controls). NMSC respective frequencies were 4.2, 1.5, and 3.6 %. Once PTLD and NMSC cases were excluded, the frequencies of malignancies were 2.7, 3.2, and 3.4 %, respectively.

Cumulative rates of *infections* up to 3 years post-transplant were 79.2 % in the MI group, 82 % in the LI group, and 80.7 % in controls. Serious infections were 35.8, 33.5, and 37.8 %, respectively. The most common infections (>10 %) were UTI, URTI, nasopharyngitis, CMV infections, bronchitis, and influenza. The most common serious infections (>2 %) were UTI, CMV infections, pyelonephritis, gastroenteritis, and pneumonia.

Overall, this safety profile was comparable in all groups, with a tendency of serious infections to be lower in the LI group compared to MI group and controls. However, TB infections (13 cases vs. 1 in controls) were considerably higher in the study groups (1.5 % in MI, 1.3 % in LI), than in controls (0.2 %). Most of these patients pertained to countries with high prevalence of tuberculosis.

Progressive multifocal leukoencephalopathy (PML), which is usually fatal and associated with JCV reactivation, has also been observed in abatacept-treated patients. PML has been reported in patients under immunosuppressive therapies. Overall, CNS infections were more frequent in the MI group (3 cryptococcal meningitis, 1 West Nile virus infection, 2 fungal infections, 1 PML, and 1 facial nerve herpes zoster infection), than in the LI group (2 cryptococcal meningitis), and controls (1 meningococcal encephalitis). Two fatal PML cases occurred in MI treated subjects; one was a renal transplant recipient (polyoma JCV positive) after 2 years treatment, and the other was a liver transplant recipient enrolled in Study IM103045. No cases of PML were observed in the LI regimen. In addition, other polyoma virus infections (mostly BK virus) associated with nephropathy, which may cause kidney graft loss, were reported. During the 3 years follow-up, 6 cases of BK virus-associated nephropathy were observed in patients treated with MI abatacept (4 with graft loss), 3 cases in LI regimen, and 6 cases in controls treated with CsA (no graft loss). Overall, polyoma virus infections were higher in the MI group (6.3 %) than in LI group (3.8 %) and controls (5.7 %).

Acute *infusion reactions* in the pooled population were reported as 26 % in the MI group, and as 21 % in the LI group. Post-infusion events were observed in approximately 50 % of subjects of both groups. They were usually mild and associated with arterial pressure imbalance and nausea. No serious events or cases of anaphylaxis/anaphylactoid reactions were reported up to 3 years follow-up.

Belatacept was not associated with significant clinical *laboratory abnormalities*. Hypophosphatemia was the most frequent and non-serious imbalance in the study groups (17 % MI, 21 % LI, 13 % controls), usually occurring as early event (within 3 months of treatment).

The rate of *autoimmune events* at the 3-years endpoint was low in belatacept groups (1 %) compared to CsA (3 %). Notably, the total immunoglobulin levels were lower than baseline levels during treatment in belatacept and control groups.

AEs-related *discontinuation rates* in the three pivotal studies after 3 years posttransplant were 13.8 % in MI regimen, 15 % in LI regimen, and 18.7 % in controls, with a slight tendency to increase when compared to levels observed at 1 year follow-up.

Overall, the LI regimen (which is the one approved) showed a safer profile than MI, including for serious infections, CNS infections, polyoma virus infections, malignancies, and CNS-PTLD. This profile was typical of immunosuppressive treatments and was considered acceptable and manageable. The main safety concerns were about PTLD and serious infections, including PML. However, no cases of PML occurred in this group. Although the dimension of the observed population was not sufficient to allow definitive conclusions on autoimmune diseases, they remain an additional concern associated with belatacept long-term therapy [1–7].

Recently, updates of the Phase II (IM103010) extension study on 162/173 patients switching from CsA or Tacrolimus to belatacept reported a safety profile in line with previous experiences, with and increase of mucocutaneous fungal infections (17 vs. 4 % in controls). One TB was observed among the treated group and resolved without discontinuing belatacept. No cases of PML or PTLD were observed. SAEs were 37 % in the study group and 33 % in controls [8].

Although data from MI and LI regimen experience in core trials indicated an elevated risk related to higher exposures to belatacept, it was not clear whether the efficacy and safety were influenced or not by dose range, changes, or intervals during the study. A recent detailed analysis on all-phase trials with belatacept indicated that lower exposures did not substantially compromise efficacy, while higher doses were associated with an increase of serious AEs measured as serious infections and CNS system events [9].

Noteworthy, some cardiometabolic parameters appeared improved during LI belatacept studies, mainly as arterial blood pressure, dyslipidemia, and new onset diabetes after transplantation (NODAT), compared to CsA treated controls. In particular, NODAT were 7 % in LI group and 11 % in controls, systolic blood pressure was reduced in LI regimen by approximately 6–8 mmHg, and diastolic blood pressure lowered by approximately 3 mmHg, Similarly, mean non-HDL cholesterol level and triglycerides level were lower in the belatacept group, and increased in the CsA group. On this basis, by applying a predictive model for major adverse cardiovascular events (MACE), a better outcome—both for MACE insurgence and death—was estimated in patients undergoing long-term belatacept treatment [10].

44.4 Off-Label Experience and Postmarketing Surveillance

Due to the recent admission to the market, off-label experience and postmarketing reporting on belatacept are limited.

Among 25 off-label trials, one was conducted on pancreatic islets transplants, 2 on diabetes mellitus, one on liver transplant, and one on healthy volunteers.

In the FAERS database 474 reports include infections (≈16 % of reports), renal disorders (4 %), arrhythmias (2.8 %), skin neoplasms (2.8 %), and cardiac arrest (1.5 %). Most frequent infections were UTI (2.8 %), sepsis (2.6 %), and pneumonia (2 %).

In the EUV dataset there are 60 reports on serious events, including PTLD (1) and two other malignancies (1 melanoma), infections (2 sepsis, 2 pneumonia, 1 TB, 1 BK virus infection, 1 fungal infection), GI perforations (2), cardiac events (1 infarction, 1 CHF, 1 cardiac arrest), and hematological disorders (6 thrombocytopenia, 2 pancytopenia, 1 anemia).

44.5 Remarks

All adverse events appear related to the immunosuppressive mechanism of action of belatacept. Major concerns are about infections, especially CNS infections, and malignancies. The increase of CNS infections is intriguing, and some of them seem more typical of belatacept than of other similar agents. Apparently belatacept does not cross the blood brain barrier when administered as monotherapy or in various combinations, as experienced in Cynomolgus monkeys, and does not interfere with cells expressing CD80/CD86 in the brain. Therefore, there is no apparent evidence suggesting a direct pharmacological effect on the CNS. However, PML is related to JCV reactivation (as PTLD to EBV reactivation), occurs during other immunosuppressive therapies, and in the transplant population in particular (4/100.00 P/Y for PML), indicating that the induced general immunosuppressive state may be sufficient to trigger latent pathogenetic agents not exclusively located within CNS.

The risk for PTLD can be reduced by selecting EBV-positive transplant recipients. However, even among the EBV+ population belatacept may produce additional cases of PTLD. It has been calculated that such additional cases can be expected as 1:150 EBV+ kidney transplant patients, and that the majority of them would involve the CNS. Therefore, belatacept appears to exert an important impact on the CNS immune environment, as evidenced by the higher frequency of localized SAE triad PML, CNS-PTLD, and infections. Therefore, a Registry was recommended to monitor the incidence of postmarketing PTLD in the long term. Although in a limited experience, PML appears more frequently, yet for unknown reasons, in liver transplant recipients.

A possible additional concern may be related to autoimmune disorders, which may need longer observations to be detected. Such expectancy is based on theoretical assumptions and on preclinical data. In previous studies on animal models, thyroiditis (6 %) and pancreatic insulitis (18 %) were observed in rats, but not in Cynomolgus monkeys. The interference with downregulatory signals of CTLA-4, and the presence of anti-CTLA-4 antibodies have been associated with

autoimmunity. The blocking of CD28-CD80/86 on APC in the thymus may lead to the escape of self-reactive T cells, or to the lack in Tregs development, thus facilitating autoimmune processes. However, autoimmune underlying phenomena may be masked by the immunosuppressive activity of belatacept, and appear after therapy discontinuation [1, 2, 5].

Interestingly, belatacept reduced some adverse events, such as hypertension, dyslipidemia, hyperglicemia, and NODAT onset, thus offering a potential reassuring profile for long-term control of transplant recipients compared to CsA [10–12].

Finally, an important concern is about the higher frequency of acute graft rejections (AR) during belatacept combined therapy, which poses important implications on the role of CD28 pathway blocking in AR and/or on additional effects of the agent on immune homeostasis. Recent experimental data have shown that belatacept impairs both memory T cells and Treg cells in Rhesus monkeys, in contrast with previous data. Therefore, costimulation blockade may differently act on enhancing and suppressive immune cell compartments, yet rendering less effective the overall immunosuppressive action of this drug class. Additional recent information indicates also the possibility that CTLA4-Ig can accelerate AR by exerting deleterious actions on Treg cells [13, 14].

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Etanercept

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Etanercept (Enbrel®, Immunex, Amgen, and Pfizer) is a fusion protein combining the Fc domain of human IgG1 to the extracellular portion of the tumor necrosis factor receptor-2 (TNFR2, p75). TNFR2 binds to soluble TNFs (TNFα, TNFβ -LTα-), thus blocking the interaction with their natural receptors and interrupting the activation of TNF-related proinflammatory pathways.

In October 1998, FDA granted the first approval for the treatment of moderate to severe active rheumatoid arthritis (RA), and subsequently extended the indication to polyarticular-course juvenile rheumatoid arthritis (JRA; now JIA patients 2 years or older) in 1999, to active psoriatic arthritis (PsA) in 2002, to ankylosing spondylitis in 2003, and to chronic moderate to severe plaque psoriasis (Ps) in 2004.[1]

EMEA granted approval for the treatment of RA and for polyarticular-course juvenile chronic arthritis (JCA) in 2000, for plaque psoriasis (Ps) in 2004, for pediatric Ps from the age of 8–17 years in 2008 and from the age of 6 years in 2011, for Ps from the age of 6 years and for JRA from the age of 2 years in 2011. In 2012, the same Agency approved the indication for the treatment of children (from the age of 2 years) and adolescents with extended oligoarticular juvenile idiopathic arthritis (JIA), with enthesitis-related arthritis (ERA) from the age of 12 years, or with PsA from the age of 12 years. The product was also approved in Canada, Australia, Japan, and Switzerland starting from 2000, with similar prescriptions. However, the indication for pediatric Ps initially approved from FDA and Health Canada was withdrawn in 2009 (due to lack of postapproval studies).

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[1] JRA (commonly used in USA) and JCA (used in EU) are older terms replaced and included in JIA, which also encompasses other forms of idiopathic arthritis in childhood and adolescence. JRA includes systemic onset (sJIA), oligo/pauci-articular (oJIA), and polyarticular (PIJA or simply JIA) subgroups of arthritis. ERA and PsA refer to other clinical subgroups linked to AS and to Ps, respectively. Therefore, JIA describes a clinically heterogeneous group of arthritides.

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Since 1998, when pivotal trials for etanercept approval in RA took place, additional studies have followed up to 2013, for supporting extensions to JRA, PsA, Ps, and pediatric extensions to JIA, Ps, and PsA, as listed below:

Rheumatoid Arthritis: Pivotal Phase III study (16.0009) on 234 (134 exposed) patients evaluated for 26 weeks; supportive Phase II study (16.0004) on 180 (136 exposed) treated for 12 weeks, and Phase II study (16.0014) on 89 (59 exposed) patients treated for 24 weeks. Additional controlled studies include pilot Phase I study (16.0002 on 15 patients), Phase III study (16.0012) comparing etanercept to MTX in 632 (415 exposed) RA and MTX-naive patients for 52 weeks, and its extension (468) lasting up to 10 years; Study 0881A-308-EU on 682 RA patients who had failed previous DMARDs other than MTX, receiving etanercept in combination with MTX from 6 months up to 20 years (522 of them completed 52 weeks); Study 300-EU on 559 patients treated in combination with MTX.

Additional open-label studies were 16.0008, 16.0018, 16.0019, 16.0023, and 301-EU. All patients had failed at least one DMARDs treatment before enrollment, except for Study 16.0012. Overall, evaluations were mainly based on 1,694 RA patients (1,218 treated with 25 mg standard etanercept SC weekly dose). The higher dose (50 mg) was experienced in Study 16.0036 on 420 (367 exposed) RA patients for 16 weeks.

Psoriasic Arthritis (PsA): Phase III 16.0030 on 205 (101 exposed) and Ps patients (101 exposed) evaluating etanercept combined with MTX (part I, controlled) for 24 weeks, followed by etanercept for other 24 weeks (part II, open-label); Phase II Study 16.0012 including 60 PsA patients similarly treated for 12 weeks.

Juvenile idiopathic arthritis (JIA/JCA/JRA): Phase II–III Study 16.0016 on 69 children (JCA) treated with 0.4 mg/kg etanercept (max 25 mg) for 3 months, then (51 children) continuing treatment (25) or placebo (26) for 4 months; Long-term Study 20021618 (formerly 16.0018) in 54 MTX refractory/intolerant JIA (polyarticular, oligo/pauciarticular and systemic) patients 4–17 years old treated with etanercept as monotherapy; Study 0881A1-3338-WW (referred as 3338) including 127 children with extended oligoarticular JIA from the age of 2 years (60), and ERA patients (38) from the age of 12 years treated with 0.8 mg/kg etanercept (max 50 mg) for 12 weeks and extended up to 96 weeks (ongoing, recruiting, and including patients from the previous study); Open-label Registry 20021626 (formerly 16.0026) for children 2–18 years old with polyarticular-course JIA for long-term evaluation of etanercept with or without MTX.

Pediatric Psoriasis: Study 20030211 on 210 patients (106 treated), and Study 20050111 including 185 (90 exposed) patients 8–17 years of age, who had participated to the previous study and were followed for 96 weeks to support the treatment extension to children from 6 years of age, and for 264 weeks to fulfill the requested variation. Twenty-eight of these patients remain in follow-up to 18 years of age.

Plaque Psoriasis: Study 20021632 on 112 chronic Ps patients treated (57) with etanercept 25 mg as monotherapy for 24 weeks. Dose-ranging (25–50 mg) Study

20021639 in 652 (486 exposed) Ps patients (573 completed the study) for 24 weeks (part I), followed by selection of responders (409) versus incomplete responders (160) for retreatment for additional 24 weeks (Part II), of whom 203 responders and 160 incomplete responders completed the respective 24 weeks regimen; Study 20021642 on 583 (390 exposed) patients receiving at least one dose of 50 mg etanercept for 12 weeks (559 completed Part I), followed by 25 mg planned for 48 weeks and closed at week 36 to expedite enrollment of open-label Study 20030115 including also Study 20021639 fo a total of 912 exposed patients. Overall, evaluations were performed on 1,347 patients from the three trials, 1,126 receiving treatment for 6 months, 289 for 48 weeks, and continuing the long term up to 72 weeks.

The long-term Phase III extension studies 20030115 (391 patients) and 20030117 (600 patients) were planned to evaluate the 50 mg regimen up to 96 weeks.

Ankylosing spondylitis: Pivotal Study 16.0037 on 277 patients (138 exposed) evaluated for 24 weeks, and its extension (257 patients) up to 6 years; Phase III 311-EU (0881A3-312) on 84 AS patients (45 exposed) from previous Study 0881A3-311-EU treated for 12 weeks, who continued treatment up to approximately 96 weeks; Early Phase II Study 016.0626, a proof of principle study on 40 patients (20 exposed) treated for 16 weeks.

Overall, etanercept has been extensively analyzed both for efficacy and safety aspects, more than other agents of this drug class, and represents a paradigmatic example for the whole biomedicines' setting [1–11].

At present, 313 trials on etanercept are registered, including studies on RA (117), Ps (83), spondyloarthritis (55), AS (31), PsA (22), and JIA (13).

45.1 Mechanism of Action

The tumor necrosis factor (TNF) family is a group of 19 cytokines mainly involved in apoptosis, including TNFα and lymphotoxins (LTα, previously TNF β, and LTβ). They are homotrimeric (the former) or heterotrimetric (the latter) structures recognized by specific receptors (TNF-R1; TNF-R2; and LTβR). TNFα (also identified as TNF, being the pivotal molecule of the group) is expressed at the cell surface, mainly on activated macrophages and T lymphocytes, and can be cleaved by a converting enzyme (TACE) into a soluble form, which is considered the mature expression of this cytokine. However, the transmembrane precursor (tmTNF, 26 kDa) acts also as a bipolar molecule that transmits signals both as a ligand and as a receptor in a cell-to-cell contact fashion, while the soluble form (sTNF, 17 kDa) acts at distance by interacting with its receptors. Both soluble and transmembrane TNF can bind to TNFR1 and TNFR2, and are bioactive. However, sTNF binds to TNFR1 with a 30-fold faster dissociation rate than TNFR2. Therefore, much of the sTNF linked to the latter is promptly released and possibly captured by TNFR1.

Moreover, shedding of both receptors, mediated by TACE (TNF-alpha–converting enzyme), releases molecules capable of neutralizing TNF in solution, thus acting as potential natural TNF antagonists. TACE inhibitors, mainly active on metallopeptidase-3 (MMP3), this effect. TNFR1 is constitutionally expressed and ubiquitous (except for RBC), whereas TNFR2 is generally inducible and is preferentially expressed on endothelial and hematopoietic cells.

Macrophages, T and B cells, NK cells, neutrophils, endothelial cells, smooth muscle cells, osteoclasts, and fibroblasts produce TNF as a result of innate and adaptive immune responses. However, the primary source of TNF in immunoinflammatory processes is the monocyte/macrophage lineage. The production of TNF is regulated by feedback loops initiated by TNF-induced factors. In particular, IL-1, IFNγ and IL-2 induce TNF production, while IL-10, prostaglandins, and corticosteroids downregulate their production by inhibiting transcription of TNF mRNA. Exogenous molecules from bacteria (LPS, etc.), viruses, immune complexes, hypoxia, and trauma can also activate these cells. TNF release, in turn, stimulates the secretion of cytokines (IFNγ, IL-1, 6, 8,17, G-CSF, etc.) chemokines (MCP-1), adhesion molecules (ICAM-1, E-selectin), and inflammatory proteins (MIP-1 and 2), which are also involved in leukocyte mobility and endothelial permeability. Therefore, TNF is a key proinflammatory cytokine with a central role in inflammatory processes. TNF plays a vital role also in granuloma formation and maintenance.

LTs are similar to TNF. LTα is found as soluble homotrimer (LTα3) similar to sTNF, binding to TNFR1 and TNFR2 with comparable affinities. However, LTα3 does not rapidly dissociate from TNFR2, thus making the switch of the ligand to TNRF1 unlikely. LT-β captures LTα, thus forming LT$\alpha1\beta2$ (predominant) or LT$\alpha2\beta1$ heterotrimers at the cell surface. Both forms primarily interact with a specific receptor (LTβR), but the latter heterotrimer binds also to TNFR1 and TNFR2, albeit with less avidity. While LT$\alpha\beta$ complexes are expressed/induced on T and B cells, the respective LTβR receptor is constitutively expressed on fibroblasts, epithelial cells monocytes/macrophages, dendritic cells, and mast cells. Therefore, the activation of this signal pathway (via TRAF family transduction members) can be induced after cell-to-cell contact between lymphocytes and stromal/resident cell components. Notably, LTβRs do not exert apoptotic signals, but induce the expression of intercellular, vascular, and mucosal adhesion molecules, as well as a number of chemokines influencing the homing of lymphocytes and granuloma formation.

TNF and LT play a pivotal role in defending from intracellular bacteria such as mycobacterium or listeria, and consequently in granuloma formation and maintenance. In healthy humans, circulating TNF is hardly detectable. However, in patients with acute infections, septic shock, or chronic inflammatory diseases such as RA, PsA, AS or Crohn's Disease (CD), TNF levels are rapidly and consistently increased, becoming detectable in serum, stools, and synovial fluid. Interestingly, binding of TNFRs or TNF antagonists to tmTNF can induce reverse signaling through this membrane-anchored ligand, which can trigger cell activation, cytokine suppression, or apoptosis of the tmTNF-bearing cells. This peculiarity may be also relevant for AEs induction.

Etanercept is a recombinant fusion protein combining the extracellular portion of TNFR2 (p75) with a human IgG1 Fc portion containing the hinge region, CH2, and CH3 domains. This modified Fc fragment has a greatly reduced binding capacity to FcRIII and FcRI receptors, thus being unable to exert Fc-mediated immune activities such as CDC and ADCC, yet able to increase the plasma half-life of the fusion complex.

Etanercept binds to TNF and to LT acting as a decoy soluble receptor for both ligands, thus inhibiting their proinflammatory action, including the modulation of biological responses (cytokines, IL-6, adhesion molecules, etc.) induced by TNF. Etanercept has an affinity in the range of the natural TNFR; it is able to neutralize lethal doses of exogenous TNF, and to ameliorate the induced shock-like syndrome. Etanercept does not cross the blood–brain barrier (BBB).

Compared to antiTNF monoclonals, etanercept is the sole that: binds to both TNF and LT molecules; preferentially binds with both receptor arms to a single tmTNF trimer (monoclonals can crosslink two tmTNF); exerts persistent activity and possible competition between TNF and LT binding at lower concentrations; possibly interferes/reduces memory B cells; reduces follicular dendritic cells and germinal centers in tonsils (possibly related to LT inhibition); reduces LPS-induced apoptotic factor (death factor X), but is ineffective on LPS-induced production of TNF, IL1β, IL10, and IL12 in vitro.

These peculiarities may explain the different therapeutic efficacy of antiTNF inhibitors in various diseases, and the possibility of overcoming the resistance to treatment by switching among them. Moreover, the overall differences in the mechanisms of action of this drug class may be of relevance in the expression of the respective safety profiles, although this aspect is much less investigated.

Lenercept, a fusion protein combining two extracellular domains of the p55 TNFR to one IgG1 heavy chain, showed no efficacy or safety problems, but raised IgG and IgM antidrug antibodies, the former being dose dependent and the latter being correlated with the RF. Surprisingly, they were found to rather bind to Fc receptors at human cells surface than to TNFR. The development of this etanercept similar fusion protein was discontinued.

In RA, etanercept treatment reduces infiltration of inflammatory cells into active areas of the joints, as well as the expression of adhesion molecules. It also reduces chemotaxis and lowers tissue degradation. In Ps, the treatment is able to decrease epidermal inflammation and induces normalization of keratinocytes in psoriatic plaques. In PsA, short-term treatment reduces T cells and neovascularization in synovium and psoriatic skin.

The pivotal role of IL-6 in inflammation and autoimmunity has been proved in different experimental and clinical situations, and is sharply decreased by etanercept. IL6 levels are elevated in RA, JIA, and in many other autoimmune diseases. At articular level, IL-6 can stimulate pannus formation through VEGF-induced angiogenesis, and increases bone resorption as a result of osteoclastogenesis. The marked increase of acute phase proteins has been also related to IL-6 upregulation [12, 13].

Recently, it has been observed that a series of genes of numerous proinflammatory cytokines are overexpressed in RA patients, including IL-1β, TNF, and IL-18, which products contribute to synovial deterioration. In particular, they are overexpressed in unstable RA, and are downregulated in stable RA and during therapy. However, anti-inflammatory cytokines such as IL-10, IL-1Ra, and TGFβ1 are also increased, possibly as a homeostatic response to mitigate the inflammatory process [14]. Similarly, TL1A, a new TNF-like cytokine member of the TNF superfamily, and its receptor DR3 are increased in AS, Ps, and IBD [15].

45.2 Immunogenicity

Anti-etanercept antibodies are not frequent, and they are usually transient and nonneutralizing. Cumulative rates at one year are 6 % in RA, 7.5 % in PsA; 7 % in Ps; 9.7 % in pediatric Ps; 4.8 % in JIA, and 2 % in AS. In one study on pediatric PsA, early positivity (within 16 weeks) was found in 7/127 patients (5 ERA and 2 PsA patients). There is a general slight trend of these antibodies to increase over time, usually leveling up to 7 %. In one long-term study (8 years) in Ps patients, levels reached 9 %. In another study on pediatric Ps, baseline levels were 10.7 % and reached 44 % of transient nonneutralizing positivity. In these patients, ANA at baseline were 6.1 % with two patients remaining positive up to week 264. New ANA positivity (over 1:40) was 11 % versus 5 % in controls. Positivity for dsDNA occurred in 15 % versus 4 % in controls [9, 10].

However, in a recent review of 2,082 studies, and a meta-analysis performed on 17 of them to assess the immunogenicity of anti-TNF therapy on 865 patients, no anti-etanercept antibodies were detected. The absence persisted for 3 months after therapy withdrawal [16]. Interestingly, these data are consistent with the higher drug survival rate reported for etanercept in comparison with infliximab or adalimumab.

45.3 Adverse Events

The principal database for establishing the safety profile of etanercept included 1,381 subjects treated with etanercept (1,039 with RA). At initial submission for RA and JCA there were 849 patients from 32 studies, including 733 patients treated with etanercept for 6 months and 194 patients treated for 12 months. Among them, there were 531 arthritic patients (477 RA, 54 JCA) and 108 non-RA patients, including healthy volunteers and a small trial on sepsis. The entire cohort was enlarged to 1,039 subjects in July 1998, and subsequently up to 1,952 RA patients (5,832 P/Y).

However, basic safety data came from 531 RA patients of the original submission, treated with SC administrations of etanercept. In the Phase II study on patients with septic shock, etanercept was administered as IV single dose, and

caused a dose-dependent increase of mortality (30–53 % vs. 30 % controls) at 28 days after treatment. The major death cause was sepsis. Safety data from volunteers came from five studies, where no deaths and no serious events had been observed.

Additional safety data came after subsequent extensions of treatment to other rheumatic and nonrheumatic diseases. Study 20000125 was dedicated to investigate the association of etanercept with anakinra in 242 RA patients, who subsequently entered the long-term Study 20000223 evaluating safety on total of 558 patients up to 5 years. The AS safety profile was evaluated in 401 patients from three studies, including 201 subjects treated for 12–24 weeks.

The safety profile for Ps was essentially based on 1,347 patients (1,261 receiving at least one dose of etanercept, 1,204 patients exposed for 3 months, 831 for 9 months, and 455 for 12 months).

Safety of PsA was evaluated in 265 patients in two studies, including 101 patients treated up to 48 weeks.

The safety on the pediatric population included 196 subjects (60 JIA, JCA 69, 38 ERA, and 29 PsA). Safety for pediatric Ps was evaluated in 210 patients from two studies, including 106 treated (90 patients up to 96 weeks) and in long-term studies up to 264 weeks. However, for some patients, such as those enrolled in Study 20021618 on 54 JIA, AEs were reported only during the first year of treatment.

Etanercept has an established safety profile, mainly on RA patients, due to an over 15 years experience in thousands of patients. The general profile for the group of diseases officially admitted to etanercept treatment is based on such experience, with additional safety data derived from trials submitted for indication extensions, which contributed to better define the specific safety profiles of these diseases within the general framework depicted from the initial RA studies.

In the last label, the BBW includes *serious infections* and *malignancies.* The latter warning was included in 2009, due to postmarketing observations and studies involving children and adolescents treated with TNF blockers, mainly in relation to increased rates of *lymphomas* in these patients. Among the former, *active TB*, *fungal infections,* and other bacterial and viral *opportunistic infections* were the most concerning drug-related complications. Other AEs categories were *hypersensitivity reactions, immune and autoimmune responses, HBV reactivation, nonmalignant hematologic disorders*, *and neurologic events.* Overall, such safety profile can be considered as a drug class characteristic, although not necessarily occurring with similar frequencies in each TNF-inhibitor therapeutic regimen.

Serious infections in all rheumatic disorders treated with etanercept occurred in 6.3 % of cases followed up for 48 months. These values were comparable to the relating controls, except for the combined therapy with MTX. In Ps, serious infections were estimated as <1 % and were comparable to controls (at 24 weeks endpoint), and to the study population. Opportunistic infections were estimated as 0.09 % (0.06 per 100 P/Y) on a population of 15,402 treated patients. They included a variety of microbial agents, and tended to disseminate in various organ systems. Among them, TB, new or reactivated, was observed, also in patients with

prior negative testing. Fungal infections represented more than 50 % of fatalities, being pneumocystis and aspergillus the most common infectious agents. Bacterial infections included also Listeria, Legionella, and atypical Mycobacteria. In the postmarketing setting, HBV reactivation (<0.01 %) after etanercept was also observed.

Overall, the adult safety profile of RA, PsA, AS, and Ps included URTI, sinusitis, and influenza as the most common infectious events, with a mild/moderate outcome.

In the RA population, the overall safety profile—provided by over 10 years of observations and therapy in controlled studies—registered 19 serious infections, including one death for staphylococcal septicemia, in 1,039 treated patients (1.8 %).

Malignancies were mainly represented by lymphomas (50 %). Acute leukemias were rare among adult patients (RA, PsA, AS) and more frequent in young patients (JIA/JRA, pediatric Ps, and PsA), mainly in the postmarketing setting. Notably, in both adult and pediatric controlled trials with etanercept, no cases of hematologic malignancies were observed. Solid tumors, and in particular melanoma and NMSC, were also observed with an increased frequency compared to the relative control patients. A higher number of noncutaneous solid neoplasms were observed in patients with Wagener's granulomatosis (5.6 %).

Estimated rates for melanoma (0.043 100 P/Y) and NMSC (0.41 100 P/Y) among the pooled RA, PsA, and AS cohorts of patients were in the range of the study population. However, the rates of NMSC in adult Ps patients were higher (3.54 per 100 P/Y; 1.28 per 100 P/Y in controls). In long-term studies on RA lasting up to 6 years, 129 malignancies were observed among a pooled population of 4,114 treated patients (3.1 %), including a minor group treated also with MTX (231 patients). Similarly, 2/240 (0.8 %) malignancies were detected in PsA patients, and 6/351 (1.7 %) in AS patients. In the Ps adult population, 43 NMSC cases were registered among 2,711 treated patients (1.6 %). Overall cases of lymphomas in RA, PsA, AS, and Ps pooled data were 18 out of 7,416 treated patients (0.24 %).

Nonmalignant serious *hematologic events* were reported as rare (<0.1 % pancytopenia) or very rare (<0.01 % aplastic anemia).

Injection site reactions in pooled rheumatic diseases occurred in 36 % versus 9 % in controls, and were usually mild.

Autoimmune disorders including autoantibody formation (ANA: 11 %; dsDNA: 15 %), lupus like syndrome (<0.1 %), and autoimmune hepatitis (<0.1) were also observed.

Overall *neurologic events* were rare (<0.1 %), and included central and peripheral demyelinating disorders, exacerbation or new (MS, ON, transverse myelitis, GBS, and PNP). However, in RADIUS Registries collecting about 10,000 reports, demyelinating disorders were observed in 0.5-1 %.

Cardiac failures, mainly as CHF exacerbation and rarely as new onset, have been reported in trials and in the postmarketing reporting.

Major *respiratory disorders* were not observed in controlled studies, but cases of interstitial lung diseases were reported in the postmarketing setting.

Allergic reactions associated with etanercept administration in clinical trials have been reported as <2 %. Pruritus is the most common event, while rash, urticaria, and angioedema are uncommon. Angioedema, bronchospasm, vasculitis (including leukocytoclastic vasculitis), erythema multiforme and Stevens-Johnson syndrome are rare, and toxic epidermal dermolysis is very rare. Four cases of macrophage activation syndrome (MAS) have been reported in JIA studies. The overall discontinuation rate in adult patients was approximately 4 % [1–11].

Within the depicted framework, differences in frequency and grade of severity of various AEs were observed among the cohort of patients, in relation to the underlying disease under treatment. Major differences emerged when comparing pediatric versus adult patients with rheumatic immune disorders and Ps in controlled trials. Due to differences in study design and in treatment regimens, these data must be considered as indicative of a general trend in different diseases.

45.3.1 Additional Adult Safety Profiles (Ps, PsA, AS)

Among adult populations with different underlying diseases, differences in malignancies, infections, and immunogenicity were mainly observed between the groups of psoriasic patients (Ps, PsA) and rheumatic diseases.

Plaque Psoriasis: In 1,347 patients followed-up to 48 weeks, (45–56 % vs. 51 % in controls) developed at least one AE. Injection site reactions were the most common event (11–16 % vs. 6 % in controls) up to 24 weeks of treatment—with a slight increase related to etanercept dosage—and tended to decrease over time, reaching controls. SAEs occurred in similar proportion within groups, including in controls. They included one demyelinating disease, cystitis, gastroenteritis, lymphadenopathy, lymphoma, pancreatitis, papillary thyrocarcinoma, pneumothorax, psoriatic arthritis, pulmonary emboli, and worsening of psoriasis.

Serious infections were rare (<0.1 %) and included cellulitis (6), pneumonia (5), abscess (2), forunculosis, pharyngitis, cholecystitis, osteomyelitis, and gastroenteritis (1 each). No reports of opportunistic infections or tuberculosis occurred in any study. The demyelinating disorder appeared as neuropathy of upper extremities, and revealed a MS-like demyelinating plaque. Usually, after withdrawal of etanercept and proper supportive therapy patients markedly improved.

As for malignancies, 23 occurred in 21 treated patients, and 2 occurred in controls, on a population of 1,038.7 P/Y. They included 10 noncutaneous neoplasms (3 prostate cancer, 1 bladder carcinoma, 1 pancreatic carcinoma, 2 breast carcinoma, 1 papillary thyroid carcinoma, 1 lymphoma, and 1 oligodendroglioma). Lymphoma and thyroid carcinoma were considered drug-related; both were in the 50 mg × 2 weekly regimen. Thirteen cutaneous neoplasms occurred in 11 patients (8 BCC, 5 SCC). One lentigo maligna (precancerous) occurred, and no malignant melanomas were reported. Only two cases of BCC were considered drug-related.

These data are higher than frequencies occurring in the general population. However, the risk of the psoriatic population exceeds that of normal population, ranging between 1.3 and 1.78 P/Y. Therefore, data observed in etanercept-treated groups resulted to be higher than frequencies occurring in population with moderate psoriasis (1.8–2.1) and lower than the severe psoriasis population (2.3–3.6). Notably, the rates of cutaneous malignancies in etanercept-treated groups were lower than expected compared to the psoriasis population.

In 2,711 patients followed up to 2.5 years, 60 malignancies were observed (2.2 %). Non-neutralizing antietanercept antibodies were detected in 7 % of tested cases [9, 11].

Psoriasic arthritis: Patients with at least one AE were 51 %, mostly being non-dose related, and appeared during the first 12 weeks of treatment. Injection site reactions were the most common event (36 % vs. 9 % in controls) up to 24 weeks of treatment. In longer observations, most common AEs were injection site reactions (11–16 % vs. 9 % controls), URTI (21 %), UTI (6 %), cephalea (8 %), sinusitis (6 %), and rash (5 %). Notably, injection site reaction was the only event significantly increased compared to controls, and tended to decrease (4–7 %) over time.

In the pivotal Phase III study during maintenance and open-label exposure 18 SAEs occurred, including 2 prostate carcinomas, one skin carcinoma, one metastatic carcinoma, one pneumonia, 2 COPD, and 5 cardio/neuro-vascular disorders [4, 9, 10].

Ankylosing spondylitis: In clinical studies on 351 patients followed up for 2 years, 6 malignancies were observed (1.7 %). Non-neutralizing antietanercept antibodies were 2 % [3, 9, 10]. In registry-based studies, there was a significant high outcome of uveitis during etanercept treatment compared to infliximab [17].

45.3.2 Pediatric Additional Profiles (JIA, Ps, PsA)

In JCA patients, infections were 60 % (31 % in controls). In fact, several adverse events were more commonly reported in 69 juvenile chronic arthritis patients after 12 weeks of treatment with etanercept, than in the 349 adult RA patients treated for longer periods. Most common events included cephalea, nausea, abdominal pain, and vomiting. SAE included varicella with aseptic meningitis (no sequelae), gastroenteritis, depression, cutaneous ulcer, esophagitis/gastritis, group A streptococcal septic shock, Type 1 diabetes mellitus, and soft tissue and postoperative wound infections [5, 9, 11].

In polyarticular JIA, SAEs were approximately distributed as 1/patient. Serious infections were 13.8 %, systemic disorders were 8 %, and gastrointestinal disorders were 3.4 %. Urogenital, cutaneous, and nervous events were detected in single patients (1.7 %).

In long-term study lasting 10 years on 58 JIA patients, SAEs were approximately 13 % and included serious infections (3.2 %), but no malignancies (including lymphoma) or deaths were registered. In a subsequent review of these

data, 2 events (1 JRA flare, and 1 infection), not detected before, were added to the 7 events previously recorded in a total of 5 patients as 2 purpura fulminans (1 associated with sepsis, shock, and coagulative disorder), 4 infections, one skin disorder, and one meningitis. One case of mild uveitis was also observed in a pauciarticular JIA patient, who remained in therapy for 3 years. Four cases of varicella (2 serious) also occurred, but were not considered opportunistic AEs.

The rates and types of SAEs and serious infections remained relatively constant over approximately 10 years of follow-up. No relevant respiratory, cardiovascular, demyelinating, and muscular disorders were observed. No malignancies and deaths were registered. No previously unknown safety risks were identified and the overall safety profile in pediatric patients with DMARD-refractory JIA was considered acceptable.

Overall, the JIA profile was similar to that of the adult RA profile in typology, except for an increased rate of infections. In fact, the most common events observed in controlled studies during the first 48–52 weeks of treatment in the range of 2–18 years patients were infections (over 60 %), which occurred as mild/moderate. Other frequent events were cephalea (19 %) and vomiting (13 %). However, in the long-term observation on a more consistent sample of JIA patients (594) of the same age range, infections leveled to 3.8 % versus 2 % in controls. Notably, four cases of MAS, IBD associated disorders, and uveitis were reported in case studies and in postmarketing settings, and some of them reactivated after rechallenge with etanercept. The average rate of anti-etanercept antibodies was 4.8 % [2, 5, 8, 10, 11].

A long-term (10 years) evaluation in 346 *adult patients receiving etanercept in childhood* for various forms of JIA was performed on the basis of data reported in the JuMBO Register. During the observation period (598 P/Y), SAEs in treated patients (5.7 per 100 P/Y) included 7 infections (2.1 per 100 P/Y), 6 new outcomes (2 IBD, 2 uveitis), and 1 death (suicide). No malignancies or TB were observed. Among controls, new onsets included 2 uveitis, 2 psoriasis, 1 optic neuromyelitis, and 1 LES. The majority of overall patients reported fatigue, but the general quality of life was improved. Overall, autoimmune events (10) were equally distributed; IBD and uveitis were mostly present in ERA patients, as expected. Overall, the safety profile after 10 years of treatment was reassuring, although investigators did not exclude a possible underreporting of AEs and SAEs due to study typology [18].

In *pediatric Ps*, at least one AE occurred in 89 % of patients. SAEs were 3.9 %. Noninfectious SAEs were 2.8 %, and serious infections were present in two patients (1.1 %) as cellulitis and mononucleosis. The most frequent events were infections (over 75.7 %; 0.76 AEs/P), which remained stable over time (77.3 %; 0.78 AEs/P at 264 weeks). All common events included URTI (37.6 %), nasopharyngitis (26.0 %), cephalea (21.5 %), acne (18.2 %), pharyngitis streptococcal (14.9 %), sinusitis (13.3 %), skin papilloma (13.3 %), cough (12.2 %), influenza (11.6 %), and oropharyngeal pain (11.0 %). Laboratory abnormalities were also mild/moderate. No opportunistic infections, malignancies, or deaths occurred

during the study. None of these events occurred during the off treatment period. Discontinuation rate was 3.3 %.

Long-term studies up to 96 weeks on 210 patients, including previous 181 patients, reported a set of AEs comparable to the 48 weeks profile. Notably, in the 6–7 years old subgroup, infections were lower than the overall population of 8–17 years (58.7 vs. 84.9 100 P/Y). No injection site reactions, no malignancies or death, no effect on patient's growth rates, and no new signals occurred in this study subgroup. However, data on the overall pediatric Ps population were limited and therefore a Postmarketing Registry was requested in EU (PURPOSE) study to enroll 100–200 Ps patients <17 years old in 9 years, up to 2018.

Overall, the safety profile of pediatric Ps remained in the range of adult Ps treated with the same biomedicine, with an increase in the rate of infections observed for the whole pediatric cohort compared to adults [6, 7, 11].

In *oligo/pauci Ps, ERA, and PsA* in patients 2–17 years old, the safety profile was similar to the general JIA profile [10, 11].

45.3.3 Safety Profiles in Other Studies

Etanercept was experienced in *therapeutic associations*. With anakinra in Study 20000223 and 20000125 on 242 RA patients. Out of 204 subjects completing therapy, 25 had SAEs, and 13 discontinued treatment because of AEs, without efficacy improvement. Therefore, the association was dismissed and contraindicated in official product information records.

Recently, three additional studies have added more comparative information on efficacy and safety profiles among various TNF inhibitors, including etanercept. In the first head-to-head study (2006-006275-21/GB, RED SEA), etanercept (60 patients) was compared to adalimumab (60) as first-line therapy designed to reflect real clinical practice in active RA for 2 years. Among 14 observed SAE, 7 occurred in the etanercept group and 6 in the adalimumab group. One death (cardiac failure) occurred in the former group soon after drug withdrawal for skin rash, and was considered drug-related. Two deaths (cardiac ischemia) occurred in the adalimumab group.

Malignancies occurred as AML in etanercept and as ovarian cancer in adalimumab, and were considered nondrug-related. Events by body system with higher frequency (more than 2) in etanercept included constitutional and allergic signs (18 events vs. 8 events in adalimumab), injection site reactions (19 vs. 9), ear/nose/throat disorders (10 vs. 5), urinary disorders including UTI (4 vs. 0), and laboratory abnormalities (6 vs. 3). The more represented events in the adalimumab group were fatigue (5 vs. 1) and gastrointestinal signs (11 vs. 9). One case of cellulitis occurred also in the latter group [19].

In the ESTHER (NCT00844142) comparative trial of etanercept versus sulfasalazine in 76 subjects with early axial AS, 321 AEs occurred in 71 patients (93 %), of which 167 occurred in 39 etanercept-treated patients (55 %; 4.3 AEs/P),

and 154 in 32 subjects in the comparative group (45 %; 5.3 AEs/P). The most common events were URTI. Among 7 observed SAEs, 3 were in the etanercept group (1 treatment related), and 4 in the sulfasalazine group (2 treatment related). No details were provided on these events, and presumably no new signs were observed during the study [20].

Dosage lowering, or dose interval prolongation may modify the overall AEs profile, and possibly reduce their incidence, provided that the efficacy of treatment is maintained for long-term disease control. The PRESERVE trial focused on 604 patients with moderate RA. In particular, the study investigated the possibility of lowering dosage (25 mg) and evaluated withdrawal effects of etanercept in combination therapy with MTX.

In the open-label phase, 5 % of patients had SAEs, including pneumonia (1 %), cellulitis, and acute pyelonephritis (<1 % each). Two cases of BCC were observed. Less than 3 % of patients were withdrawn because of them. No unexpected safety or tolerability signs were observed. The most frequent events were cephalea (6 %) and nasopharyngitis (5 %). In the double-blind phase, 58 % had treatment related events, including nasopharyngitis (8 % in 50 mg standard dose; 5 % in the reduced 25 mg dose, as in controls), and bronchitis (6, 5, and 3 %, respectively). SAEs were 6 %, including sepsis (1 % in 50 mg and in placebo groups). Two cases of malignant melanoma (1 each in 50 mg and in placebo) were observed. Two deaths occurred in the standard dose group, due to pulmonary embolism and septicemia. The withdrawal of etanercept produced reactivation of disease (over 50 % of cases).

Overall, the study did not show a substantial reduction of AEs in frequency or typology as consequence of the reduction of etanercept dosage [21].

Another study on 78 AS patients, receiving 50 mg etanercept weekly or every other week, reported similar results [22].

In the first systematic review analysis on the effects of off-label dosing, including escalation, reduction, and interruption of biomedicines (antiTNF agents and antiIL-12/23 agent) in psoriasis, most relevant safety data on etanercept associated with dose escalation revealed an increase of serious infections (1.9 events 100 P/Y), and myocardial infarction (2 cases in high weekly dosage group). Two more serious infections, 12 malignancies (9 NMSC, 3 internal), and 5 cardiovascular serious events (3 CHF, 2 ischemic) occurred after withdrawal/retreatment in the high dosage groups [23]. The safety profile in elderly patients (over 65 years) remained in the range of adult Ps and PsA outcomes [24].

Taken together, these data showed that the safety profile of etanercept was rather stable within the range of experienced treatment variations, with a trend of some AEs, including serious infections, to increase with dose. Moreover, long interruptions produced disease reactivation, and retreatment induced a substantial number of drug-related adverse events.

In the APPEAL open-label trial (NCT00422227) on 300 Asian RA patients, the combination of etanercept and MTX was compared to DMARD/MTX standard 16 weeks therapy. Similar TEAEs occurred in the two groups (68 % vs. 77 % respectively), as for SAEs (3 % vs. 2.9 %), which included cardiac, gastrointestinal,

and constitutional disorders (0.5 % each in the study group; 0.5 % in control). Infections were slightly more frequent in the study group (1.5 % vs. 1 %), while ALT/AST increase was present only in the control group. Three opportunistic infections (2 Herpes zoster, 1 serious, and 1 Pneumocystis pneumonia) were observed. Twelve latent TB remained silent and no new TB infections were reported. Overall, the safety profile was similar to nonasiatic populations and no new signals emerged [25].

A number of recently published studies and case reports related to etanercept and to other TNF inhibitors appear of interest as potential alert for new signals, either as single or drug class events of special interest.

Cutaneous nontumoral disorders have been reported during etanercept therapy; they include erythema multiforme, eczematous and vascular lesions, urticaria-like reactions, lupus erythematosus, alopecia areata, and lichenoid eruptions. As for the latter, which usually follows infliximab therapy, one case was recently reported after etanercept therapy [26].

Cutaneous, uveal and diffuse sarcoidosis has been reported as well. Notably, 16/28 cutaneous cases reported in the literature occurred in etanercept treated patients (57 %), and 4 were localized at cutaneous level without the involvement of other organs [27]. Sarcoid uveitis (7 cases) after antiTNF therapy was also recently reviewed [28]. Psoriasic eruptions (8 cases of palmoplantar pustular Ps) were also reported during etanercept therapy for other underlying diseases, including CD and RA [29, 30].

Hematologic disorders have not increased in clinical studies. However, pancytopenia (6 cases), and one life-threatening diffuse alveolar hemorrhage have been recently reported in RA [31, 32].

The first case of Schönlein-Henoch purpura has been recently described in AS, after commencing etanercept therapy [33].

Finally, a few cases (5) of AML following etanercept therapy have been described, including one case evolving from previous MDS and one case with cutaneous infiltrates in chronic lymphocytic vasculitis [34]. A number of additional AML have been reported in the postmarketing setting (41 cases in EV database by the end of 2012). Overall, these data suggest some caution for hematologic risks, including acute malignancies, not clearly emerging from previous clinical trials, at least in patients with previous hematological abnormalities.

New onsets of CD have been signaled after etanercept therapy in 10 AS patients, including one recent case responding to standard therapy after etanercept discontinuation and switching to infliximab [35], in two pediatric Ps [11], and in JIA [36]. In the EV postmarketing setting, 205 cases of CD, 108 UC, and 21 IBD were registered, thus posing renewed attention on the possible causative relation of etanercept administration with exacerbation/induction of these immune-mediated diseases.

One study individuated 8 HBV reactivations in 468 patients undergoing treatment with antiTNF inhibitors (1.7 %). Seven of these cases were in the group of 269 patients treated with etanercept (2.6 %), as compared to 1/95 treated with adalimumab, and none of the 100 patients treated with infliximab. Notably, these

cases were individuated among HBsAg-negative and HBc-positive patients (occult carriers) [37].

An interesting study on 3 cases of unilateral or bilateral scleritis insurgence during etanercept SC treatment for RA raised, some concern about the possibility of a paradoxical inflammatory effect of etanercept at ocular level. All cases improved after drug discontinuation. In the literature search, 4 more cases of scleritis and 14 cases of uveitis were identified as related to etanercept treatment [38].

Finally, evaluation of allover mortality rates in antiTNF therapy on a large population of autoimmune disorders did not show increased values, compared to patients treated with nonbiologic therapy [39]. However, a study on mortality rates among three TNF inhibitors in the Swedish ARTIS Register found an increase in the relative drug-specific risk for etanercept (2.34) compared to adalimumab (2.04), and to 0.62 for infliximab on 6322 RA patients. Although overall differences did not reach statistical significance and the follow-up was limited, the study is relevant since it compared individual biomedicines to the rates of the RA population, instead of evaluating mortality for the whole drug class, and showed significant differences when single agents and particular patients' subsets, or comorbidity factors were considered individually [40].

45.4 Off-Label Experience

Among 313 trials launched for etanercept investigations, some are dedicated to potential new indications, such as Type 1 diabetes/islet transplantation (11 studies), GVHD (5), panuveitis (5), myelodysplastic syndrome (5), Alzheimer' disease (4), hidradenitis suppurativa (3), WG (2), and Behçet's (2). Other off-label investigations mostly relate to a broader spectrum of rheumatic disorders, such as oligoarticular arthritides and spondyloarthropathies, cutaneous disorders, bone disorders, uveitis/panuveitis, or to variations over the indicated regimens for in-label diseases. [23, 41].

A recent review on persistent oligoarticular *juvenile idiopathic arthritis* (oJIA) patients included in the Dutch Arthritis and Biologicals in Children Register, and refractory to previous nonbiologic treatments, found 16 cases treated off-label with etanercept (14) or with adalimumab (2 cases with associated uveitis), with an exposure for the former of 16 P/Y. Two SAEs (perforated appendicitis and restrictive pulmonary function) occurred only in the etanercept group. No other safety signals were reported, and the treatment was judged as effective [42].

The use of etanercept in *uveitis* is mostly related to the presence of an underlying rheumatic disease. However, a larger experience has accumulated on *Behçet's disease*. A review of the literature found 37/268 cases treated with etanercept up to 2011, including one small trial. The overall safety profile, mainly based on infliximab-treated patients (83 %), was reported as acceptable and comparable to previous experience in other diseases. Notably, no demyelinating events were observed [43].

In pediatric Behçet's disease, in which uveitis is less common, six cases in the literature (four included in the previous review) showed one bacterial endocarditis, fatigue and pyrexia (one case each) [44].

Etanercept has been used in a number of *cutaneous disorders*, such as pyoderma gangrenosum (15 cases in the literature) [45], hidradenitis suppurativa (20) [46], vitiligo (2) [47], and pemphigoid (four cases associated with psoriasis) [48], without relevant or new safety signals.

However, in *ANCA-associated vasculitides* (AAV, including WG investigated in the WGET trial, and Churg–Strauss syndrome) etanercept administration raised substantial concern due to an increase in the incidence of various solid malignancies, mainly of intestinal origin. In particular, six malignancies occurred during the WGET trial, all in the group receiving etanercept (SIR 3.8 vs. 1.9 of the normal population), and the risk remained increased (SIR 3.92 vs. 2.89 in placebo) in the 5 years follow-up, showing eight new malignancies in the treated group (5 in controls) compared to normal population, but not to the respective control. Since these patients have an increased risk of malignancy per se, and for other therapies (cyclophosphamide), etanercept was considered a potential enhancer of overall risks of malignancy, especially in long-term therapy [49].

Etanercept treatment for 52 weeks of dermatomyositis evaluated in 16 patients (11 exposed) caused 6 severe AEs in three patients of the study group (3 in controls), including UTI, pyrexia, postherpetic neuralgia, psychosis (two patients), and miscarriage. ANA were present in two treated patients at baseline (0 in controls), and two patients became positive at week 52 (1 new case in controls). None developed dsDNA antibodies [50].

Recent investigations are evaluating the therapeutic potential of etanercept in *acute and chronic cardiac, cerebrovascular, and respiratory disorders*. Treatment with intraspinal etanercept (the drug does not cross BBB) in three patients with chronic stroke was based on the assumption that inflammatory microglial activation may produce an excess of TNF, which may be blocked by the drug in study. Notably, patients had a rapid neurological improvement, years after the acute stroke episode, without additional adverse events [51].

Treatment of CHF with etanercept was based on the assumption that elevated levels of TNF are with the disease, and that antiTNF therapy reduces cardiac events in patients treated for rheumatic disorders. However, in contrast with previous observations, efficacy was not confirmed, and additional cardiac toxicity due to therapy, including etanercept, was observed [52].

Treatment in acute COPD exacerbation with etanercept was based on the inflammatory nature of acute episodes associated with a considerable local increase of TNF (and IL-6) during the acute phase. In a trial enrolling 81 subjects (41 exposed), SAEs were more frequent in the control group treated with prednisone than in the study group (7 vs. 4 respectively), including pneumonia (3 vs. 2). One pneumothorax, 3 sinus/pharyngitis, and 1 death were present only in the study group (cardiopulmonary arrest), the latter occurring 3 days after entering the trial [53].

Finally, some interesting case reports related to the use of etanercept in unresponsive/intolerant *familial mediterranean fever*, with the attempt of reducing arthritic signs showing similarities with spondyloarthropathies, and preventing febrile episodes and consequent cytokine-induced, including TNF, production of amyloidosis (see CAPS, Chap. 12, 46, 51). A retrospective analysis individuated 59 cases (21 treated with etanercept) with no adverse events even in patients with compromised renal function [54].

Overall, the off-label use of etanercept did not show substantial modifications of the safety profile and did not produce new safety signals. In off-label variations of dosage/exposure in approved diseases treatment, no substantial reduction of AEs was observed by lowering etanercept single or weekly dosage, while a trend to AEs increase was observed by increasing standard regimens.

45.5 Postmarketing Surveillance

In the FAERS database, over 135,000 reports indicate injection site reaction (21 %), cutaneous reactions (6.3 %), infections (6.3 %), respiratory (3.5 %), and musculoskeletal disorders (3.2 %) as the most common categories of encountered AEs during etanercept treatment. A total of 6,312 malignancies were reported, being the most frequent skin (951), breast (892), gastrointestinal (758), and respiratory tumors (756).

In the EUV database, among 187,175 reports (42 % serious; 2.5 AEs/P), the most frequent were muscular disorders (11 %), infections (9.7 %), skin (8 %), nervous (5.8 %), respiratory (4.9 %), and gastrointestinal disorders (4.9 %). The most common infections were nasopharyngitis (6,117), sinusitis (4,233), pneumonia (2,907), bronchitis (2,043), influenza (2,018), and cellulitis (682). Most frequent neoplasms were breast cancer (566), lymphoma (339), melanoma (329), BCC (303), SCC (171), prostate cancer (241), colon carcinoma (153), and bladder cancer (107). In a Japanese postmarketing dataset on 7,091 patients treated with etanercept, SAEs were 5.7 %, including infections as 1.9 % of the total population.

45.6 Remarks

Etanercept has a well-established safety profile, mainly on RA patients, due to over 15 years of experience in thousands of patients. The general profile for the group of diseases officially admitted to etanercept treatment (RA, JIA, PsA, AS, and plaque Ps) is based on such experience, with additional safety data derived from trials submitted for indication extensions. Moreover, etanercept is the most representative of anti-TNF inhibitors and shows paradigmatic features for the whole biomedicines' class setting.

The overall experience with TNF antagonists in a number of inflammatory and immune-mediated diseases represents also a proof of concept of the role of the TNF family in their pathogenesis. However, not all TNF antagonists have identical mechanisms of action. These differences may explain in part some diversity in clinical response and in some drug-related AEs, including cases of resistance/intolerance to one of them showing a good response after switching to another TNF antagonist.

The most relevant AEs related to etanercept administration are infections and malignancies. As for serious infectious events, combined data from randomized controlled trials, safety registries, and postmarketing reporting indicate an overall increased risk of local and systemic events caused by intracellular microbes, including TB and opportunistic agents. However, they appear to be less frequent and delayed than other TNF inhibitors, such as infliximab or adalimumab, and the risk of TB unmasking was lower with etanercept [12].

As for malignancies—the second major concern—lymphomas are the most frequent events compared to matched controls, to background levels in healthy population, and to the relative diseased populations where overall rates of malignancies are increased regardless of the treatment. The increased risk results more evident when younger populations, or special subsets of patients, are considered.

The risk for solid tumors remains questionable, except for NMSC, since in most relevant studies the increased values did not reach statistical significance. Dose-ranging studies did not show a clear dose-related risk for overall AEs, although a trend to increase of frequency and severity of some events was observed at higher doses ($\geq$50 mg). The overall long-term studies are reassuring and confirm a rather stable incidence of AEs, including SAEs, over 10 years follow-up [9–11].

The off-label experience with etanercept is frequent, although not particularly successful, but no new relevant safety signs have emerged.

Recently, some concern have been raised about hematologic disorders, including AML, mainly in relation to some subsets of patients with previous blood dyscrasias related to bone-marrow depression [34].

The immunogenicity of etanercept is considerably and consistently low, during treatment and after therapy, at least up to 3 months after withdrawal [40]. Interestingly, this peculiarity may be related to the blocking of LTs and the subsequent interference with T and B cell interactions and function.

Renewed attention has been given to two possible concerns specifically related to etanercept mechanism of action, which may appear also as paradoxical, namely the possible reactivation/induction of immune mediated IBD, and of granulomatous sarcoidotic lesions. It must be noted that etanercept is the only TNF inhibitor acting also on LTs, which are involved in the organization of germinal centers, in the homing of lymphoid components, and in the local organization of granulomatous processes [12]. Nonetheless, etanercept seems ineffective in granulomatous disorders, such as CD, WG, and sarcoidosis.

In a recent study more cases of sarcoidosis were reported as a consequence of etanercept therapy, while no peculiar AEs could be referred to the LTs blocking, so far. It seems, therefore, that such blocking is nonsignificant both for efficacy and safety aspects. However, another possible explanation of inefficacy has been related to the different binding peculiarities of etanercept (i.e., it binds to 2/3 TNF binding sites, while infliximab binds to all three sites), or to an inferior binding stability, or to the lack of antiapoptotic capacity. These factors may also support the potential capacity of etanercept in developing IBD (mainly in JIA patients) or in triggering new CD onsets [55]. Nonetheless, the potential risk of increasing of other autoimmune processes, such as SLE or demyelinating disorders remains low for etanercept compared to other TNF inhibitors. It has been postulated that blocking of TNF activity may result in an increased activity of T and B cells that react with autoantigens. In this case, etanercept may partially counteract this action by downloading T and B cells activity, due to LTs blocking, a function lacking in other agents of the same drug class. In fact, new onsets of autoantibodies such as ANA and dsDNA have been reported after treatment with TNF antagonists, although less frequently l with etanercept.

Another peculiarity of etanercept with respect to other TNF inhibitors emerged from studies on AS, where anemia is frequently associated. Patients treated with infliximab and adalimumab showed levels of hemoglobin significantly improved, while such effect was not significant in etanercept-treated patients [56]. The difference was attributed to the etanercept lack of binding to tmTNF and of apoptotic capacity.

The problem of resistance to TNF therapy remains a general concern, including the risk of exposure to unbalanced AEs, and the reason of its overcoming by switching to another TNF inhibitors acting of the same targets remains unexplained. Part of such resistance is attributed to the heterogeneity of the population with rheumatic disorders. Recent information indicates that susceptibility to this drug class may be predicted by assessing the presence of some IL-6 gene isoforms (−174 G/C), that resulted present in over 96 % of responsive patients [57].

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Rilonacept

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Rilonacept (Arcalyst®, Regeneron) is a dimeric fusion protein combining the extracellular portion of human interleukin Type I receptor (IL.1RI) and the IL-1 receptor accessory protein (IL-1RAcP) with the Fc portion of human IgG1. Acting as a decoy receptor for both IL-1α and IL-1β, rilonacept inhibits their interaction with the natural receptors and therefore the expression of the consequent bioactivity of the bound cytokines.

FDA granted approval in 2008, after recognition of rilonacept as orphan drug in 2004, for the treatment of Cryopirin Associated Periodic Syndromes (CAPS), including Familial Cold Autoimmune Syndrome (FCAS), and Muckle-Wells syndrome (MWS) in adults and children 12 and older. On January 9, 2013 a request for designation extension as orphan drug to Familial Mediterranean Fever (FMF) was submitted, and is pending.

EMEA recognized rilonacept as orphan drug in 2007 for the treatment of CAPS, including Familial Cold Urticaria Syndrome (FCUS), Neonatal Onset Multisystem Inflammatory Disease (NOMID), Chronic Infantile Neurological Cutaneous Articular Syndrome (CINCA), and MWS. In 2009, the European Agency approved the marketing for the same indications. However, in October 2012 the marketing authorization was withdrawn in consequence of a previous request of the manufacturer. By that time the product had never been placed on the market in any EU country.

The pivotal Phase III trial (IL1T-AI-0505) included 89 CAPS patients. Due to the rarity of the disease the study was planned in two subsequent randomization stages, in order to evaluate safety and efficacy of SC doses (160 mg) in adult patients for 6 weeks, followed by withdrawal and readministration of rilonacept in all patients for 18 weeks. An open-label extension was planned, and included pediatric patients receiving 2.2 mg/kg up to week 88, with a 42 days additional follow-up.

Preliminary studies included seven Phase I studies (IL1IT-AI0406, IL1IT-RA-0401, 0402, 0111, 0504, 0608, one unspecified) enrolling 210 subjects (131 healthy volunteers, 30 RA, 10 autoinflammatory diseases, 10 chronic active gout, 6 unspecified), and eight Phase II studies (IL1IT-RA-0102, 0004, 0408, 0409, 0404,

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0425, ILIT-CV-‘503, one unspecified) enrolling 496 subjects (364 RA, 79 osteoarthritis, 35 atherosclerosis, 14 unspecified). Overall, 600/790 subjects received single or multiple doses of rilonacept up to week 26, while two ongoing studies, including the pivotal trial, were planned for 88–105 weeks final observation.

Subsequently, the extension for prevention of gout flares in patients initiating uric-acid lowering therapy was submitted to FDA in 2011, on the basis of additional Phase III studies (IL1T-GA-0810, and 0816) on 488 patients. However, on May 2012 the FDA Arthritis Drug Advisory Committee (ADAC) unanimously recommended against the approval for such indication due to inadequacy of the risk/benefit balance, and to the short observation period (16 weeks). The final FDA decision is still pending [1–4].

At present, 17 trials have been launched on rilonacept including 10 completed, 2 active, 2 recruiting, 1 not yet recruiting, and one withdrawn.

46.1 Mechanism of Action

The group of IL-1 (eleven proteins, seven with proinflammatory activity, including the major representative IL-1α and IL-1β) is involved in the inflammatory response, being also identified as endogenous pyrogens, inducers of prostaglandin, collagenase releasers, promoters of the expression of adhesion molecules on endothelial cells, and of transmigration of leukocytes. Inflammation is mostly influenced by the relative amounts of IL-1α and IL-1β, both interacting with ubiquitous Type I receptor (IL-1RI) and Type II receptor antagonist IL-1Ra, acting as a competitor and regulator of IL-1 signaling. All IL-1 cytokine precursors, except for IL-1α, must be cleaved by intracellular caspase-1 or extracellular proteases to become active before binding to respective receptors and triggering transduction activating signals.

The IL-1α precursor is associated with microtubules in endothelial, epithelial, and parenchymal cells. When activated by membrane-associated calpain proteases, IL-1α is expressed at cell surface and interacts with IL-1 receptors expressed on adjacent cells, or is released with membrane fragments (apoptotic bodies) and activated by extracellular neutrophil proteases.

IL-1β is not constitutively expressed; its precursor is inactive and must be cleaved by caspase-1 that removes amino-terminal amino acids. IL-1β transcription is triggered by exogenous (microbial) or endogenous factors (TNF, IL-18), or by autostimulation from the same IL-1α and IL-1β molecules. Therefore, the former stimuli may trigger septic inflammation, while the latter ones induce sterile inflammation or autoinflammation.

The active soluble form of IL-1β is a potent pro-inflammatory cytokine produced by various cell types, including monocytes, macrophages, mast cells, dendritic cells, endothelia, keratinocytes, fibroblasts, microglia and astrocytes, neuronal and Schwann cells. Interestingly, some of these cells, such as keratinocytes, produce the IL-1β precursor, but are not able to process it to the active

form subsequently implemented by external proteases. IL-1β stimulates thymocyte and T lymphocyte proliferation by inducing IL-2 release, maturation and proliferation of B cells and of some dendritic cells, and mobilization of neutrophils and platelets from bone marrow. The synthesis and release of IL1β require two distinct signals (for synthesis and assembly), which are normally initiated by pathogen-associated molecular patterns (bacterial RNA, lipopolysaccharides), but also by cytokines and endogenous irritants (uric acid or heat shock proteins). Therefore, this cytokine is implicated in inflammatory processes after injury and infections, but also in acute and chronic autoimmune diseases, pain, and neurological disorders.

CAPS are a group of rare autosomal hereditary periodic fever syndromes associated to CIAS1 gene mutations, resulting in overproduction of IL-1β. They are defined also as autoinflammatory diseases caused by the CIAS1 encoded cryopirin (or NALP3), a component of the inflammasome controlling the activation of IL-1β. In CAPS there is over-secretion of IL-1β (up to 5-fold higher than in healthy subjects) and an increased expression of IL-1Ra, but not enough to counteract IL-1β activity. Both IL-1 s are also involved in other pathologic inflammatory processes such as acute ischemic diseases, chronic heart failure, osteoarthritis, gout, RA, diabetes (IL-1β is toxic for beta-pancreatic cells), and in chronic systemic inflammatory diseases such as Still's disease, Schnitzler syndrome, and MAS (see Chap. 3).

Rilonacept (IL-1 Trap) is a dimeric fusion protein combining the extracellular portion of human interleukin-1 receptor (IL-1RI) and the IL-1 receptor accessory protein (IL-1RAcP) to the Fc portion of human IgG1. Therefore, it acts as a high affinity trap-receptor for soluble IL-1α, IL-1β, and IL-1Ra, thus inhibiting the pro-inflammatory mediators which are induced by the IL-1 downstream. Among these mediators are IL-1β itself and acute phase proteins, such as serum amyloid A (SAA) and C-reactive protein (CRP). Rilonacept also blocks the antagonist (anti-inflammatory) activity of IL-1Ra.

Rilonacept is though to bind to both IL-1RI and IL-1RAcP extracellular chains of the IL-1R cell surface complex. Noteworthy, the anti IL-1 canakinumab binds to IL-1β outside the IL-1β/IL-1R interface, and does not interfere with IL-1α or IL-1Ra function. This peculiarity may be relevant both for efficacy and safety.

CRP and SAA are known indicators of inflammatory activity, and are elevated in CAPS. CRP levels were reduced by rilonacept administration in RA patients on whom had been conducted preliminary pharmacodynamic studies. Elevated SAA has been also associated with the development of systemic amyloidosis in these patients. It has been suggested that similar pro-inflammatory mechanisms induce acute attacks in uric-acid deposition-related inflammatory arthritis (gout flares).

The half-life of rilonacept is approximately one week, allowing long administration intervals compared to other agents of the same drug class [5, 6].

46.2 Immunogenicity

Anti-rilonacept antibodies directed to the receptor domains were detected in about 35 % of treated patients, after at least 6 weeks treatment, and persisted for 18–24 weeks (13 %). Five patients (9 %) had neutralizing antibodies [1–4].

46.3 Adverse events

The initial safety profile of rilonacept was based on 600 treated subjects, including RA patients (292), CAPS (99), and healthy volunteers (91). A more detailed analysis was conducted on the 89 CAPS patients enrolled in the pivotal Study IL1T-AI-0505, all treated in the two-stage (part A, and B) re-randomization controlled study. The safety analysis by EMEA included a total of 614 treated patients: 64 of them had CAPS and 45 subject subsequently entered in the open-label phase, including four children aged 12–17 years.

The last prescribing information of rilonacept does not include a BBW, but indicates as major adverse events *infections*, serious and life threatening, and *hypersensitivity reactions.* Among them, the most common events were *URTI* and *injection site reactions*.

At the first observation endpoint after 6 weeks in naive CAPS patients treated with rilonacept (part A, conducted in Winter), AEs were more frequent in the study group than in placebo (74 vs. 54 %), and included URTI (26 vs. 4 %), sinusitis (9 vs. 4 %), cough (9 vs. 0 %), and hyperesthesia (9 vs. 0 %). Injection site reactions were the most frequent event (48 vs. 13 %), but were mild and did not cause therapy discontinuation. No severe AEs were reported in the first part of the study. During part B observation, conducted in Summer, infections lowered toward control levels (18 vs. 22 % in controls).

Two severe AEs were reported (migraine, bronchitis), and one withdrawal occurred due to joint pain. No deaths occurred in both study parts, up to 24 weeks of observation, and two deaths occurred in the long-term open-label extension (pneumococcal meningitis, myocardial infarction). In off-label studies, 2 serious infections caused by Mycobacterium intracellulare and by Streptococcus pneumoniae meningitis (fatal) were recorded, and one non-infectious neutropenia also occurred. Overall, 6 SAEs were observed in 4 patients, including the previously reported infections, one sinusitis/bronchitis, and one colitis with gastrointestinal bleeding. Among laboratory parameters, a decrease in lipid profile was also reported, *i.e.* the mean total cholesterol, HDL, LDL, and triglycerides [1–4].

Recently, additional long-term data (up to 96 weeks) from the pivotal Study IL1T-AI-0505 (NCT00288704) on 101 CAPS patients have been published. Overall, the safety profile remained generally well tolerated and comparable to the initial depicted framework, both in adult and pediatric patients. Any AE (89 %) included injection site events mostly limited to local erythematosus reactions (32 %), and infections mainly represented by URTI (9 %) and UTI (8 %) as the

most common ones. Notably, anti-rilonacept antibodies detected during the first 24 weeks of study (24 %) did not decrease over time up to week 96, both in frequency and titer. Overall, 9 SAEs were recorded in 7 patients during the study, including 2 deaths [7].

46.4 Experience in Gout Studies

Studies submitted in 2011 for treatment extension to prevention of gout flares, in patients initiating uric-acid lowering therapy, received a negative unanimous response from ADAC members, and a final decision is still pending. Trials consisted in the pivotal Study IL1T-GA-0810 (161 treated, 79 controls), and on Study IL1T-GA-0816 (166 treated, 82 controls). Patients were treated with weekly SC doses (80 or 160 mg) of rilonacept for 16 weeks. A larger dataset used for safety evaluations included 1,886 (1,353 exposed) patients from the pivotal Phase III studies, from a previous Study IL1T-GA-0815, and from IL1T-GA-0619 Phase II study.

Any TEAE (at least one/patient) were 66 % in pooled treated patients, and 60 % in placebo. Respective drug-related events were 27 versus 13 %. Most common infections (20–23.5 vs. 21 % in placebo) were nasopharyngitis (4 %), URTI (4 %), and influenza (3 %). Three severe infections (recurrent liver abscess, appendicitis, sepsis/bacterial arthritis) occurred in the study groups, and 2 (cellulitis, viral meningitis) in the placebo group. Injection site reactions were remarkably increased in the study groups (10.5–15.5 vs. 2.6 % in placebo) and showed a higher incidence in the high dose group.

All SAEs were equally distributed in all groups and were not related to the administered rilonacept dosage (3.2–4.9 vs. 4.1 % in placebo). However, a greater incidence in the study groups was observed for cardiac events (0.6 vs. 0.2 %), nervous disorders (0.4 vs. 0.2 %), and neoplasms (0.5 vs. 0 %). Discontinuation rates were approximately 4 % in the study groups and 3 % in controls, being injection site reactions the most common cause of interruption. Discontinuation rates due to hypersensitivity were low (0.1–0.2 %).

There was no difference in death rates among all groups. Severe neutropenia occurred in <1 % of cases and was not associated to infections. A small mean increase in triglycerides was associated with a reduction in CRP levels of treated patients, and minimal effects on cholesterol.

Overall, the safety profile was in the expected range of previous experiences with rilonacept, except for neoplasms. However, 6 patients had malignancies and 3 developed benign neoplasms in the study groups. The former included prostate cancer (3), breast cancer (1), gastric cancer (1), and oropharyngeal cancer (1), all observed in rilonacept-treated subjects, and 5 out of 6 in the high dose group. One BCC was observed in the placebo group and was not considered serious.

Three benign neoplasms were observed only in the high dose group. Although the sponsor did not attribute the encountered malignancies to the treatment, their presence was the main concern raised by ADAC, and the short observation follow-up

(16 weeks) was considered inadequate to assess the effective risk of cancer, and of infections in patients with comorbidities [4, 8].

Recently, additional data have been provided by Study IL1T-GA-0814 (NCT00855920) on 225 randomized patients, who were treated during the first 48 h of an acute gout attack with a single SC dose of rilonacept and indomethacin. Any AE were 36 % in the rilonacept-treated group, and about 47 % in the therapy combination, compared to 30 % in controls receiving only indomethacin. SAES (4 %) were only present in the combination therapy group, but were considered not related to the treatment. AE-related withdrawals were similar across all groups [9].

46.5 Off-Label Experience

Among 18 trials evaluating rilonacept efficacy and safety there are six studies on gout, two on FMF, and single studies on Type 1 diabetes, systemic sclerosis, JIA, chronic kidney disease, atherosclerosis, and Schnitzler's syndrome. Additional small studies and case reports have been published on chronic renal disease, cardiovascular/atherosclerotic disease prevention, Type 2 diabetes, Still's disease, and Schnitzler syndrome [10].

Although not officially enclosed in recorded indications of rilonacept, *FMF* pertains to the group of hereditary periodic fever autoinflammatory diseases associated to mutations of the MEFV gene. At present, the request presented to FDA for extending the indication to FMF is still pending.

In a recent small trial (NCT00582907) on 14 FMF patients, 11 of them completed the 12 months study. AEs (109 events) were mostly observed in rilonacept-treated patients (67 %), including infections in 5 subjects. Five SAEs were observed in 3 treated participants, including one case of pneumonia. Injection site reactions were observed only among rilonacept-treated patients (1/patient/month vs. 0 in controls). No opportunistic infections or discontinuations were recorded. The study was in line with previous case reports experience, and showed that some patients were resistant to therapy, possibly in relation to the type of MEFV gene mutation [11].

Still's disease occurring in JIA or in adults is considered an autoinflammatory disorder. Improvements have been recently reported in 3 patients who switched to rilonacept from previous therapy with anakinra and/or abatacept without any reported safety signs [12].

However, in a previous experience on 5 patients resistant to different therapies, including anti-TNF inhibitors and anakinra, AEs included 1 MAS and 2 mycobacterial infections. Interestingly, patients showed increased levels of IL6, TNFα, and IL18, the latter appearing as a potential biomarker for predicting treatment response [13].

Schnitzler syndrome is another possible autoinflammatory disease, which appears as chronic urticaria associated with a monoclonal (mainly IgM) gammopathy. Previous case reports and a recent small open-label trial (NCT01045772)

on 8 patients showed an expected safety profile including 13 AEs, mostly mild/moderate, and three infections. No serious treatment-related events occurred. Five AEs were related to skin disorders, including 1 BCC and actinic keratosis in a predisposed subject [14].

46.6 Postmarketing Surveillance

By September 2011, a total of 196 patients treated with rilonacept had been followed. Seventy-seven had continuous therapy (68 in clinical trials) from May 2008 with no new signaled signs. Twelve spontaneous reports included 2 serious infection (diverticulitis, viral infection), 5 non-serious injection site reactions, and 1 lung cancer (fatal). This was the only additional death reported in the post-marketing setting up to September 2011.

46.7 Remarks

Experience with rilonacept is limited because of the rarity of CAPS and of the recent release in the market.

The product has shown a high rate of injection reactions, although usually mild/moderate, and a consistent immunogenicity directed to the receptor domains, which appears rather persistent over time and in part produced by neutralizing antibodies. Infections are common, mainly as upper airways viral infections. However, some serious events caused by streptococcal and mycobacterial agents have been observed. The major concern emerging from the few short-term studies in acute gout is about the potential capacity of inducing malignancies, although this risk needs to be confirmed.

A few off-label reports did not add further information to the safety profile, and no new signs were recorded. At present, the capacity of this recombinant decoy receptor to offer consistent advantages on other IL-1 blockers, both in safety and efficacy, still needs to be clarified.

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Romiplostim

47

Romiplostim (Nplate®, Amgen) is a non-glycosylated fusion protein containing two thrombopoietin receptor binding domains covalently linked to an Fc portion of a human IgG1, and therefore acting as an analogue of thrombopoietin (TPO) by binding to TPO receptor (TPOR) on megakaryocytes, thus stimulating the production of platelets.

In August 2008, FDA approved the use of romiplostim for the treatment of chronic immune thrombocytopenia (ITCP) in patients who have had an insufficient response to corticosteroids, immunoglobulins, or were splenectomized. In March 2009, EMEA approved the use of romiplostim for ITCP in splenectomized adult patients, and as second line treatment of non-splenectomized patients where surgery was contraindicated. Both Agencies had previously designated the biomedicine as an orphan drug, in 2003. At present, romiplostim is also approved in Canada, Australia, New Zealand, Mexico, Central and South American countries, and Japan.

Pivotal trials submitted for approvals were conducted on ITCP patients, either splenectomized (Study 20030105) or non-splenectomized (Study 20030212), and enrolled 63 (42 treated with drug in study) and 62 (41 treated) subjects, respectively. All patients had inadequate platelet response to standard care treatments.

Overall, there were 10 clinical studies on ITCP patients, one (Study 20050159) on 44 patients with myelodysplastic syndrome (MDS), one (Study 20050144) on 21 patients with chemotherapy-induced thrombocytopenia (CIT), and 2 pharmacological studies in healthy volunteers, including one on 48 predominantly Caucasian subjects (Study 20000109), and one on 30 Japanese subjects (Study 20040134).

One open-label extension study (20030213) provided long-term safety experience on 143 patients, who had been enrolled from the two pivotal studies [1–4].

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At present, 33 trials have been launched on romiplostim, including 18 studies on ITCP, 5 on MDS, 3 on pediatric ITCP, 5 on CIT, one on multiple myeloma, and one on HCV- positive ITCP patients. Twenty-three of these studies are completed.

47.1 Mechanism of Action

TPO is the major soluble regulator of megakaryocytes differentiation and platelet formation, and is the natural ligand of the receptor c-Mpl (or CD110) expressed on these cells. The receptor consists of two extracellular domains and one intracellular portion. Upon TPO binding, homodimerization triggers intracellular signals involving JAK2 and MAPK pathways, and STATS protein activation.

Homeostatic downregulation is predominantly performed by internalization and degradation of TPO-stimulated c-Mpl. Excessive TPO signaling usually caused by mutations of c-Mpl, results in myeloproliferative disorders.

Romiplostim (AMG531) is a non-glycosylated fusion protein containing an Fc carrier portion of a human IgG1 covalently linked to two TPOR binding domains, which stimulate the production of platelets by binding to c-Mpl on megakaryocytes.

Romiplostim competes with endogenous TPO for the binding to the same receptor, but has no homology with the natural ligand and does not interfere with its biologic function, thus avoiding the potential induction of cross-reactive antibodies. In fact, initial attempts to introduce recombinant human TPO in therapy were unsuccessful because of a high incidence of cross-reactive neutralizing antibodies against endogenous TPO. However, ITCP patients have preexisting antibodies directed to TPO, and may show non-cross-reactive antibodies directed to romiplostim, preexisting or newly formed as a consequence of therapy. Nonetheless, the potent stimulatory effect of romiplostim on c-Mpl is apparently overwhelming the potential negative effects produced by the formation of autoantibodies and anti-drug antibodies. Recent observations have also indicated that TPOR agonists improve the activity of Treg and production of TGF-β1, which may contribute to upregulate protective tolerogenic signals.

Eltrombopag is a non-biological TPOR agonist with similar properties. Despite its binding site is different from that of romiplostim—being the former situated in the inner part and the latter in the outer part of the extracellular portion of c-Mpl—some AEs are common to both molecules.

Treatment-induced overstimulation, especially in dysproliferative bone marrow disorders MDS, increases the risk of progression to AML [5–8].

47.2 Immunogenicity

About 8 % of patients (235 tested) had preexisting anti-romiplostim antibodies, and 5 % had anti-endogenous TPO. Preexisting neutralizing antibodies were present only for TPO (0.4 %). No cross-reactivity was detected between the two types of antibodies.

Newly formed antibody rates during treatment was 6−10 and 4−5 %, respectively. New neutralizing anti-romiplostim antibodies were rare (<1 %) and transient. None of such antibodies was directed to TPO.

Since romiplostim is produced by microbial fermentation in an *Escherichia coli* setting, the use in patients with known hypersensitivity to bacterial-derived product has been contraindicated [4, 7].

47.3 Adverse Events

The clinical safety profile of romiplostim was based on 14 clinical studies, including 10 studies on ITCP, one study on MDS, one study on CIT, and two studies on healthy volunteers, for a total of 451 subjects (392 treated), among whom there were 308 ITCP patients (271 treated).

The initial safety evaluation identified five major concerns consisting in *risk of bone marrow fibrosis*, *risk for malignancy* or progression of malignancy, *thrombotic events*, *alteration of endogenous TPO,* and *worsening of ITCP* after discontinuation, and *immunogenicity*.

Major warnings in the last romiplostim prescribing information included the risk of *progression to AML* in MDS patients*, thrombotic/thromboembolic complications* including portal vein thrombosis, and *worsening of thrombocytopenia* after discontinuation of therapy.

The overall incidence of AEs in ITCP patients treated with romiplostim was 91.5 %. Most common events occurring at higher frequency in pivotal studies were cephalea (35 % vs. 32 % in controls), fatigue (33 % vs. 29 %), epistaxis (32 % vs. 24 %), arthralgia (26 % vs. 20 %), dizziness (17 % vs. 0 %), diarrhea (17 % vs. 15 %), URTI (17 % vs. 12 %), insomnia (16 % vs. 7 %), pain (muscular, shoulder, extremities) and abdominal pain (11−14 % vs. 0−5 %), dyspepsia (7 % vs. 0 %), and paresthesia (6 % vs. 0 %). The majority of these events were mild to moderate. Seven treated patients experienced renal dysfunctions, which in one case led to fatal renal failure.

In one extension single-arm study (20030213) on 142 ITCP patients, the overall pattern of AEs was similar. Treatment-related SAEs were 8.5 %, with 25.5 % of patients reporting at least one serious event (1.65 AEs/P). Among them, there were 846 *bleeding episodes* (41 serious), and 21 *thromboembolic events* (13 in treated patients). Importantly, their incidence did not differ between treated (57 %) and placebo (61 %) groups, but severity was higher in the latter cohort, and platelet counts were significantly increased dose-dependently. Moreover, the overall platelet response was similar between splenectomized and non-splenectomized patients (79 % vs. 88 %), but suggested a more durable response in the latter group (38 % vs. 61 %, respectively). Bleeding was reported as 15 % in exposed patients vs. 34 % in controls (serious 6 % vs. 9.8 %).

Deaths (11) in ITCP studies were less frequent in the study group (8, 2.5 %) than in placebo (3 cases, 6.5 %). In the study group, deaths were caused by intracranial hemorrage, hemorrage, aplastic anemia, cardiac arrest, renal failure,

intestinal infarction, pneumonia, and ARDS. The three cases in the control group were caused by pneumonia, pulmonary embolism, and cerebral hemorrage. Romiplostim discontinuation caused a severe *worsening of thrombocytopenia* and an increased risk of bleeding in 4 patients in clinical studies (7 %), which resolved in 2 weeks [1–4].

Overall, special events—observed in 219 patients receiving at least one dose of romiplostim—consisted in: an increase of reticulin formation and bone marrow fibrosis detected in about 4 % of cases (3.7 per 100 P/Y); an increase of thromboembolic events (5.9 %; 7.8 per 100 P/Y); an increased risk of progression to AML observed in MDS patients.

In a controlled study on 219 MDS subjects (147 treated; 72 placebo), *progression to AML* was observed in 9 cases in the study arm and 2 in the placebo, with an increase (>10 %) of circulating myeloblasts in 25 treated patients, and in 3 cases among controls. In 4 of the treated cases, myeloblasts lowered to baseline levels after drug discontinuation. In an additional single-arm trial on 72 patients with thrombocytopenia in MDS, 11 % had signs of disease progression and three of them developed AML. These studies conducted to raise a warning against the use of romiplostim in MDS and to the restriction of the indication only to ITCP [4].

The incidence of other malignancies in ITCP patients treated with romiplostim was 6.8 % (15 patients) and included various solid tumors (11), one melanoma, and one B cell lymphoma. Among these, there was one case of myelofibrosis [2].

Experience in *clinical practice* (NCT010113181) in 72 patients treated for 2 years showed arthralgia (26 %), fatigue (13 %), and nausea (7 %) as the most frequent AEs. Transient thrombocytosis occurred in 19 % of treated patients, and in 3 % of controls. Importantly, patients with specific comorbidities potentially causing exclusion from clinical trials showed a similar safety profile. However, 2 cases of TIA without sequelae were observed, and bone marrow assessment for fibrosis was not performed. Interestingly, the bleeding score was associated with the presence of anti-platelets antibodies, which could predict resistance to TPO-mimetics [9].

In a previous study (20060131) and its subsequent extension up to 277 weeks on a total of 292 adult patients, the safety profile was similar and remained stable over time. AEs were reported in 98 % of cases, with cephalea (38 %), nasopharyngitis (34 %), and fatigue (32 %) as the most common and mild events. Eleven patients showed increased bone marrow reticulin. Notably, one MDS and one lymphoma were reported in the standard of care treatment arm. Two deaths (angina, myocardial infarction) out of 16 were considered treatment-related.

Overall, AEs occurred in nearly all patients, but they were generally mild to moderate, and often related to the underlying disease. Bleeding episodes were more frequent in controls, while thrombotic events were initially equally distributed, although in the long-term they tended to increase more in the study group than in controls (8 per 100 P/Y vs. 6 per 100 P/Y). An increase of all-grade (75 % over grade 3) bone marrow reticulin was found in 8/8 tested patients, and decreased after treatment discontinuation [7, 10, 11].

One study (NCT00603642; 20060216) evaluated the safety and efficacy of romiplostim in 34 adult Japanese ITCP patients treated for 100 weeks (median). An extension study, enrolling patients from this trial and normal subjects from the previous PK/PD study (20040134), followed 44 patients for 3.5 years.

In the first evaluation on 34 ITCP patients (22 treated for 12 weeks and followed up) a similar proportion of subjects (91 % vs. 92 % in placebo) experienced at least one AE.

Serious/severe events were 9.1 and 8.3 %, respectively. Most common events were nasopharyngitis (41 % vs. 17 %), cephalea (32 % vs. 17 %), peripheral edema (18 % vs. 0 %), pain (14 % vs. 0 %), nephrocalcinosis (9 % vs. 0 %), thrombocytopenia (9 % vs. 0 %), and fatigue (9 % vs. 0 %). Three events of thrombocytopenia occurred after romiplostim discontinuation. Interestingly, no bone marrow reticulin or thrombotic/thromboembolic events occurred. No neutralizing antibodies were detected, but no data were provided on the whole anti-romiplostim antibodies. The long-term extension study on a different population of 44 patients (5 discontinued) has showed that AEs did not increase over time, up to 3.5 years. One thromboembolic event occurred in a patient at risk. No new signs or malignancies were observed.

Since racial differences were expected, these outcomes are of relevance. In fact, two previous pharmacokinetic studies with eltrombopag had showed a higher plasma concentration (up to 55 %) exposure in East-Asian ITCP patients, and a consequent need of reducing the initial dose [12, 13].

47.4 Off-Label Experience

Off-label studies mainly focused on the treatment of *thrombocytopenia in MDS* and on the treatment of *pediatric ITCP*. Part of MDS data were also considered in the general safety profile previously examined, on the basis of studies included in the initial request for ITCP romiplostim treatment approval.

In a dose response study on 44 MDS patients, a linear but variable effect of romiplostim on the increase of platelet was found, as in previous observations on MDS and ITCP. However, platelet survival was shorter in these patients (42 h) compared to healthy subjects ($\approx$11 days), and 22 % of patients were non-responders. No adverse events were reported [14].

In the mentioned Phase II study (20050159) on 28 thrombocytopenic low risk MDS patients, treated with SC and IV injections of romiplostim for 8 weeks, AEs were present in 93 % of cases. Eleven patients entered a 1-year extension phase. No placebo controls were planned.

The most common events were cephalea and fatigue (18 % each). Several SAEs occurred in 5 patients (cardiac arrest, cerebral infarction, chest pain, coronary artery dissection, febrile neutropenia, HZV infection, pneumonia, mucosal inflammation, rectal hemorrage, acute renal failure, staphylococcal infection, and fatal. After IV administration, one hypersensitivity reaction was observed; discontinuation could

be avoided switching to the SC route. Three episodes of neutropenia, one leukocytosis, and leukopenia occurred. TEAEs were 18 %. Serious adverse events were attributed to underlying diseases. However, 6 patients discontinued the study before the extension phase, and only some discontinuations were attributed to disease progression. No patients developed myelofibrosis, thromboembolic events, or neutralizing antibodies.

Interestingly, weekly SC injections reached the highest mean drug plasmatic concentration of the drug in study. Noteworthy, two patients experienced an increase in circulating blasts, and one progressed to AML. The overall safety profile was similar to previous MDS experiences [15].

Another Phase II study (NCT00418665; 20060102) was conducted on 39 patients with low/intermediate MDS, and 27 of them were treated with lanidomide—which is known to induce thrombocytopenia—and with two alternative doses (500 or 750 μg) of romiplostim. All patients had adverse events (89 % in placebo). Serious events tended to be more frequent in the study groups (29−14 % vs. 11 % in controls), but their incidence was not dose-related. In fact, only one case of serious worsening thrombocytopenia was related to romiplostim treatment. The AEs more frequently observed in groups in combined therapy were fatigue (50−21 % vs. 11 %), rash (36 % vs. 22 %), nausea (29−36 % vs. 0 %), thrombocytopenia (21−7 % vs. 0 %), anemia (7−14 % vs. 0 %), back pain (14−0 % vs. 0 %), febrile neutropenia (7 % vs. 0 %), and hyperkalemia (7 % vs. 0 %). During the extension period the safety profiles were similar. One case of leukocytosis was considered related to treatment. One patient died after intestinal obstruction, but was not considered as drug-related.

No patients developed neutralizing antibodies. No clinical differences were detected in bone marrow reticulin/collagen formation among all groups. No drug-related progression to AML was observed. However, 2 patients had an increase in circulating blast and were suspected of progression, but did not meet diagnostic criteria for AML (lack of repeated bone marrow biopsies) [16].

Overall, the safety profile was similar to the previous studies on MDS, including the potential risk of blasts increase and disease progression to AML.

A similar Phase II trial (NCT00321711; 20050232) on 29 low/intermediate MDS receiving decitabine and romiplostim (15 in treatment combination in the controlled part; 2 entering the extension part) showed AEs equally distributed among treated and placebo groups (14 and 3 patients, respectively). Two serious treatment-related events occurred, one was pulmonary artery embolism in the study group during the controlled phase, and one was DVT occurred during the extension part (and leading to discontinuation).

Among the 3 deaths observed during the whole study period, one occurred in the study group, and none was attributed to treatment. Neither signs of bone marrow reticulin increase nor neutralizing antibodies were detected. Notably, progression to AML was reported in 2 patients receiving romiplostim.

Overall, these results were in line with previous observations, and no new signs emerged from the combined therapy of romiplostim with hypomethylating drugs.

Nonetheless, the incidence of progression to AML confirmed the previous range of 5−7 % of patients receiving romiplostim.

Taken together, studies on MDS have confirmed a generally acceptable safety profile, yet with an additional serious risk of AML progression, related to the underlying disease and increased by treatment with the TPO-mimetic [17].

During these studies, as well as in one case of *autoimmune lymphoproliferative syndrome*, rash was more frequently observed after the administration of romiplostim at high dosage (≥500 μg) [18].

Recently, results on safety and efficacy of the short-term romiplostim treatment (NCT00515203; 20060195) of 22 unresponsive chronic ITCP *pediatric patients* (17 treated), receiving weekly doses for 3 months, have been published.

At least one AE was present in 94 % of cases. Only one SAE occurred in the study group, but was not attributed to therapy. The most common event in the study group was epistaxis (35 % vs. 20 %). Pyrexia (24 %), oropharyngeal pain (24 %), upper abdominal pain (18 %), rash (18 %), and nasopharyngitis (12 %) were only present in the study group, while cephalea, confusion, cough, vomiting, petechiae, URTI, and pain (including abdominal pain) were predominant among controls. No discontinuation or deaths were observed. An extension of the study was planned due to the encouraging results in efficacy and safety [19].

Another small case series evaluated the safety and efficacy of short-term romiplostim treatment in 8 ITCP pediatric patients for 1−22 weeks (median 12 weeks). Adverse events were infrequent and mild. They included one case of epistaxis, 2 cases of afebrile nasopharyngitis, and one case of bronchitis. Interestingly, two patients initially improved but the effect was transient, even after dose escalations up to 7 μg/kg. The lost response was attributed to the formation of anti-TPO antibodies, but no data were reported [20].

47.5 Postmarketing Surveillance

In the FAERS database on 11,000 reports, most common AEs related to hematological abnormalities (7.5 %), fatalities (3.7 %), leukemias (3.5 %), infections (3.5 %), dermatological disorders (2.5 %), thromboembolic events (2.2 %), and respiratory disorders (2 %). In particular, 579 MDS, 348 DVT, 221 AML, and 341 cases of pneumonia have been reported.

In the EUV database on 4,077 reports, AEs mostly relate to hematologic disorders (21 %), vascular disorders (11.5 %), infections (13 %), skin disorders (6.7 %), and thrombo/embolic events (3 %). In particular, 150 MDS (4 transformed), 73 AML, myelofibrosis (30), and 5 cases of pneumonia were reported. Four cases of anaphylactic reactions and 5 drug-related hypersensitivity reactions were also signaled.

47.6 Remarks

Assessing safety and efficacy of a new therapeutic agent after a few years of experience is rather difficult and inappropriate. A recent accurate review from Cochrane on six trials enrolling 808 patients treated with the two TPO-mimetics, brought to conclude that there is no evidence supporting the efficacy in ITCP, since such treatments did not reduce bleeding events—although an increase in platelets counts could be observed—nor improve survival [21].

Total AEs were not significantly different among study groups, SOC therapy, and placebo (RR 1.04 vs. 0.97 vs. 0.75, respectively). Interestingly, total SAEs rose when TPO agonists were associated with SOC, but not when they were associated with placebo. Both issues have been subsequently criticized by other experts supporting therapy efficiency, although expressing some concern about treatment-related risks [22, 23].

While waiting for additional results and long-term experience, a preliminary analysis of the present safety profile of romiplostim may be attempted. Overall, the major drug-related AEs seem essentially referable to the mechanism of action of this fusion protein. In fact, major concerns are about bone marrow induced abnormalities, such as myelofibrosis, progression of myelofibrosis, or progression to AML, which may be directly related to the overstimulation of c-Mpl. Indirect effects of overstimulation, such as thromboembolic events, are conceivably related to overproduction and activation of platelets, the latter being more attributed to romiplostim than to eltrombopag.

Stimulation of reticulin and of Type 1 collagen production have been related to stimulating factors, such as TGFβ, produced by overactivated megakaryocytes. The presence of c-Mpl was not detected only on mature megakaryocytes, but also on precursors, thus exposing them to overstimulation, which may lead to dysproliferative stages and to AML [24].

Notably, excessive TPO signaling caused by mutations of c-Mpl results in myeloproliferative disorders, which may be reasonably comparable to the action of TPO-mimetics. However, prompt discontinuation of therapy allows partial recovery of the disorder, although long-term observations are still lacking.

Similar AEs, including bone marrow fibrosis, thromboembolic complications, and progression to AML, are also induced by eltrombopag, a completely different non-biologic molecule exerting overstimulation by binding to c-Mpl at a different site. A thrombocytopenic rebound after discontinuation has been observed with both TPO-mimetics. By contrast, eltrombopag expresses a considerable hepatotoxicity compared to romiplostim.

Additional AEs such as infections, constitutional signs, renal function abnormalities, and renal failure, are of more uncertain origin, as for the potential mechanisms of resistance to TPO-mimetics therapy.

Neglectable adverse effects seem related to anti-drug antibodies induced by romiplostim that are infrequent and mostly lacking of neutralizing activity.

More consistent conclusions on this fusion protein safety profile demand a larger dataset, especially considering that romiplostim has a novel mechanism of action.

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Part IV
Cytokines

Cytokines

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Cytokines are a large and a heterogeneous group of extracellular peptides binding to specific receptors on targeted cell surface, thus starting an intracellular signaling cascade exerting multiple effects. They are secreted by immune and nonimmune cells and regulate both innate and adaptive immune response, cell movement, and communication inside and beyond the immune system. Their action is similar but not identical to that of hormones, since they predominantly act in a short range via autocrine or paracrine signals at cellular level, instead of sending long-range signals affecting distant organs. Moreover, cytokines are typically pleiotropic molecules produced simultaneously by different ubiquitous non-specialized cell types, while hormones are selective in their function and are secreted by highly specialized cells.

The classification and denomination of over a hundred of these proteins is complex and confusing.

Interleukins (over 30 members in humans) initially indicated the cytokines providing a cross talk at leukocyte level, but the term is now more widely used and somehow overlaps the cytokine definition. They are usually identified by the IL acronym followed by an identification number (i.e., IL-1, IL-2, etc.), which usually identifies their discovery chronology. Two families of receptors (ILRs), have been identified (Type 1, and Type 2). The first group includes receptors of most interleukins (IL-2, 3, 4, 5, 6, 7, 9, 11, 12, 13, 15, 21, 27), while Type 2 receptors include those specific for a smaller group (IL-10, 20, 22, 28). Moreover, two additional receptors are structurally different from the others, namely IL-18R is part of the immunoglobulin superfamily, and IL-8R belongs to the chemokine receptor family.

The *tumor necrosis factor family* (TNF) includes 19 members, among which TNFα, LTα (TNFβ) and LTβ (TNF-C) are the most representative. They induce cell death by apoptosis. TNFα is also a pyrogen and stimulates cell proliferation. The other members of the group include various ligands (CD27L, CD30L, CD40L, 4-1BBL, and OX40L) involved in T- and B-cell activation, or act as additional inducers of apoptosis (FASL, TRAIL). Most TNF members are transmembrane homotrimeric structures (LTs are heterotrimetric), transmitting intracellular

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signals upon binding the respective ligands, and can be cleaved from cell surface, thus becoming soluble receptor acting as competitors involved in homeostatic regulation of this class of cytokines.

The *Interferon family* (IFN) in humans includes 17 glycoproteins, among which the most relevant molecules are grouped in Type 1 (IFNα, IFNβ, and IFNω) and Type II (IFNγ) subgroups. They are coded by distinct genes and differ both in structure and antigenicity. Type I consists of 13 different molecules among which IFNα-2a, IFNα-2b, IFNβ-1a, and IFNβ-1b are the most representative. IFNγ is the only member of the Type II subgroup and pertains also to the macrophage activating factors family (MAFs), which includes IL-4 and Gc-MAF.

IFNα and IFNβ primarily exert antiviral and antiproliferative effects, whereas IFNγ acts also as an immunoregulatory cytokine. In contrast with other interferons, which can be expressed by virtually all cells, IFNγ is produced by CD4+ Th1 helper lymphocytes, CD8+ cytotoxic T cells, and NK cells, and promotes MHC expression, antigen presentation, Th1 differentiation, macrophage activation, production of phagocyte superoxide, and the synthesis of antiviral molecules.

IFNs are antiviral agents secreted by infected cells, which also activate non-infected neighboring cells to produce a series of proteins able to reduce intracellular protein synthesis, thus destroying both the virus and the infected cell. IFNs upregulate the MHC complex, increasing presentation of viral antigens to cytotoxic T cells and NK cells. An additional exclusive property of IFNγ consists in direct activation of macrophages and NK cells, thus expressing an enhancing effect on immune response (immune interferon). IFNs production is also stimulated by bacterial pathogen associated molecular patterns (PAMPs), by other cytokines (IL-1, IL-2, IL-12, TNFs, and CSF) and by Toll-like receptors of innate immunity. In particular, some of the latter, such as TLR3, induces IFNs production after binding to viral dsRNA.

Hemopoietic Stimulatory Factors essentially promote growth and commitment of stem cells and of differentiating cells of all hemopoietic cell lines. In this case, nomenclature of some factors indicates the initial discovered function than the overall activity of the molecule. For example, the GM-CSF (also called CSFα) indicates the capacity of stimulating the growth of granulocytes and macrophage colonies in experimental animals and in vitro, but identifies a multipoietin acting on all bone marrow cell lines, and on dendritic cells, not only as growth/differentiation inducer, but also as a chemokine for eosinophils. Moreover, GM-CSF synergizes with other cytokines, including IL-1, IL-3, and G-CSF in inducing cell maturation.

Transforming Growth Factors (TFGα, TGFβ) are non-hemopoietic polypeptides originally identified as inducers of oncogenic transformation of fibroblasts in vitro, which acquire unlimited proliferation and loose cell contact inhibition. TFGα induces epithelial and neuronal cell growth, is produced by macrophages and keratinocytes, and is upregulated in some human cancers. TFGα is also considered a member of the *epidermal growth factors* (EGF) family, which are active in wound healing processes. Three TGF isoforms of the Beta type (TGFβ-1, TGFβ-2, TGFβ-3) have been identified as inducers of cell differentiation,

embryonic development, but also as immune regulators. Overall, TGFβ factors are considered local homeostatic regulators, with anti-inflammatory activity, and inducers of wound repair and fibrosis, together with another growth factor, the *Platelet-Derived Growth Factor* (PDGF), which shows also angiogenetic properties, but is deprived of transforming capacity.

Cytokines are also classified according to their function, as *Inflammatory* (IL-1 α/β, IL-6, 8, TNFα, LT α/β, as the most representative), *Pro-inflammmatory* (IL-12, 17,18,23,27), *Anti-inflammatory* (IL-4, 10,13,21,27, and TGFβ), *T cell growth factors* (IL-2, 4,7,9,12,15,21), *B cell growth factors* (IL-2, 4,5,6,7,13,14,21), *Hemopoietic* stimulatory factors (IL-3, 6,9,11, M-CSF, GM-CSF), and *Interferons* (IFNs) expressing anti-viral and immunomodulatory activities.

It must be noted that due to their pleiotropic activities the cytokine grouping refers their respective predominant function, and that the same agent may be located in more than one functional group. For example, IL-6 elicits pro- and anti-inflammatory signals; IL-8 has chemiotactic properties, induces phagocytosis and angiogenesis. Moreover, some molecules were initially attributed to one cytokine class, and subsequently moved to another in consequence of the discovery of additional functions. For example, IL-4 is identified also as a member of MAF family; IL-16 has chemokine-like functions and was initially identified as lymphocyte chemoattractant factor (LCF), being active on T lymphocytes and on other cells, such as eosinophils, monocytes and dendritic cells expressing the CD4 surface molecule, which is considered its natural receptor.

Chemokines refer to a structurally different class of cytokines mediating chemotaxis and chemokinesis, and are divided into four groups. *Alpha-chemokines* (at least 17 members) are identified by the CXC acronym followed by L (for ligand) or R (for receptor) and an identification number (i.e., CXCL1, etc.). *Beta-chemokines* (at least 27 members) are identified by the CCL or CCR acronyms followed by an identification number. *Gamma-chemokines* (2 members) are identified by the XCL acronym. *Delta-chemokines* (1 member so far) are identified by the CX3CL acronym. The C letter in all acronyms stays for Cysteine, while the X letter indicates the number of aminoacids interposed between cysteines (1 or 3) at the N-terminal. All these molecules induce leukocyte cells extravasation and attraction on a gradient concentration basis. CCL chemokines attract monocytes (CCL2), T cells, NK cells, eosinophils, basophils, and dendritic cells expressing the respective receptors. CXC chemokines include two subgroups attracting neutrophils or lymphocytes, respectively. XCL chemokines attract T-cell precursors in the thymus. The only identified CX3CL1 chemokine is a chemoattractant and cell adhesion molecule expressed as soluble or surface-linked signaling molecule. It must be noted that IL-8 is now considered a α-chemokine (CXCL8).

Cytokines are usually not constitutively expressed or secreted, but are produced upon stimulation by a variety of exogenous (microbial) and endogenous (including cytokines) agents, thus contributing to innate and adaptive immune response, mainly as inducers of inter-cell communication among cell types that cooperate in the modulation of the immune response.

Binding of cytokines to their specific cell surface receptors initiates intracellular signaling promoting a variety of functions related to the specifically targeted cell.

These structures are composed of several membrane-associated subunits, which may be shared by different cytokine receptors, and modulate the affinity for the respective ligands. For example, three assembled subunits (α,β,γ) confer maximal affinity to IL-2R for IL-2, two subunits (β,γ) show an intermediate affinity, and one subunit (γ) exerts low affinity for the same ligand.

Cytokine receptors are grouped into six classes, according to their molecular structure. *Type I receptors* are multichain transmembrane structures with conserved motifs in the extracellular portion, and may share some subunits with other receptors. They include interleukin receptors (IL-xR), and hemopoietic growth factor receptors (GM-CSF, EPO, etc.). *Type II receptors* are specific for IFN molecules, and for IL-10, IL-20, IL-22, and IL-28. The third class of receptors consists of Ig-like structures of the *Immunoglobulin Superfamily* including IL-1R, IL-18R, and CSF-1R. The fourth class pertains to the *TNF Receptor Family*, showing a cysteine-rich common extracellular binding domain, and includes TNFR Type I and II (CD120a, CD120b) cell receptors, CD27, CD30, and CD40 cell surface ligands expressed on monocytes, granulocytes, T and B cells. The fifth class identifies the *Chemokine Receptors Family,* structured as 7-loop transmembrane helix coupled to G-protein, and including CCR1, CXCR4, CCL2R, and CXLC8R. Finally, the sixth class includes two types of *Transforming Growth Factor* receptors (TGFβ1R, TGFβ2R) binding several isoforms of TGFs. Both receptors share a high affinity for TGFβ1 and low affinity for TGFβ2.

Along with the discovery of cytokines and identification of their functions, recombinant gene technology allowed production of factor analogues suitable for human therapy. Recombinant erythropoietin, thrombopoietin, G-CSF, and GM-CSF, rapidly entered in supportive treatment of anemia, thrombocytopenia, and leuco/pancytopenia. Natural and recombinant TNFα were approved for the treatment of HBV and HCV hepatitis, as well as in HCL, CML, and other hematologic and solid tumors. Natural and recombinant IFNβ were approved for the treatment of severe viral infections (HZV, HSV) and for some recurrent relapsing forms of multiple sclerosis (MS).

As for other cytokines, their clinical application as immune activators is more limited and not particularly successful. IL-2 has been approved for metastatic renal carcinoma, metastatic melanoma, cTCL, HCV hepatitis, and in HIV-related CD4+ T cell loss. Mostly, it has been used in concomitance with other activation factors, to expand in vitro activated killer lymphocytes (LAK) or T cells infiltrating the tumor (TIL) to be reintroduced (also in concomitance with IL-2) in the same patient. TNF, IL-1, IL-2, IL-12, and IL-18 have been experienced in clinical trial for a number of malignant tumors with limited results and rather severe adverse effects. So far, the best results from these products have been obtained with hemopoietic growth factors, as supportive treatment of hematological toxicities related to other chemo- or biological therapies.

In contrast, immunosuppressive cytokines, receptor antagonists, cytokine-Traps, and receptor blockers have rapidly and successfully expanded to control

Table 48.1 Cytokines and cytokine receptors in human therapy

INN	Trade name Company	Target Type	Indications FDA and/or EMEA	Approval[a] FDA/EMEA
Aldesleukin	Proleukin Bayer	IL-2R rHuIL-2	RCC, MM	1992/NA
Denileukin-diftitox	Ontak Seragen, Aisai	IL-2R rHuIL-2-DT	cTCL	1999/NA,OD
Oprelvekin	Neumega Wyeth, Pfizer	IL-11R non-glycolylated rHuIL-11	CIT	1997/NA
Anakinra	Kineret Amgen, Sobi	IL-1 rHuIL-1R	RA, CAPS	2001/2002
IFN-α2a	Roferon Hoffman-Laroche	IFNAR rHuIFN	HCV/HBV hepatitis, RCC, HCL, cTCL, CML, KS	1986
Peg-IFN-α2a	Pegasys Hoffman-Laroche	IFNAR rHuIFN	HCV/HBV hepatitis	2002/2002
IFN-α2b	Intron A Schering-Plough	IFNAR rHuIFN	HCL,MM,FL,KS, HCV/HBV hepatitis Condyloma acuminata	1986/2000
Peg-IFN-α2b	Pegintron/ sylatron Schering	IFNAR pegylated-rHuIFN	HCV/MM	2001/2000
IFN alfacon-1	Infergen Boerhinger	Synthetic IFN	HCV/HBV hepatitis,	1997/1999
Peg-IFN-α2a	Pegasys Hoffman-Laroche	IFNAR rHuIFN	HCV/HBV hepatitis	2002/2002
IFN-β1b	Betaseron, Avonex, Rebif Extavia[b] Bayer, Biogen Serono Novartis	IFNAR rHuIFN	MS	1993, 1996, 2002. 2009/ 1995, 1997, 1998, 2008
IFN-γ1b	Actimmune Intermune	IFNGR rHuIFN	CGD, OP	1999, 2004/OD
FN-αN3	Alferon Interf. Sci., Hemispherx	IFNAR natural IFN	Condyloma acuminata	1989/NA
Epoetin-α	Epogen, Procrit Amgen	EpoR rHuEPO	CRF, CKD	1989/NA[c]

(continued)

Table 48.1 (continued)

INN	Trade name Company	Target Type	Indications FDA and/or EMEA	Approval[a] FDA/EMEA
Epoetin-β Peg-Epoetin-β	Neorecormon, Mircera^ Roche	EpoR rHuEPO, peg-rHuEPO	CRF, CKD, Cancer	2007/1997, 2007
Darbepoetin-α	Aranesp Amgen	Hyperglycosylated rHuEPO EpoR	CKD, non-myeloid cancer	2001/2001
Filgrastim Peg-filgrastim	Neupogen, Neulasta Amgen	rHuG-CSF	Neutropenia, HSCT, Cancer	1991, 2002/ 1991, 2002
Sargramostim	Leukine Berlex, Bayer, Sanofi-Aventis	rHuGM-CSF	Neutropenia in AML, HSCT	1991/NA
Ancestim	Stemgen Amgen	rHuCSF	Ex vivo PBPC mobilizer	In vivo 1999 in other countries
Palifermin	Kepivance Amgen	Epithelia rHuKGF	Oral mucositis	2004/2005
Becaplermin	Regranex Ortho, Janssen, J & J	Epidermis rHuPDGF	Diabetic ulcer (topic)	1997/NA

[a] initial approval date. Some of the reported indications were approved at a later time. ^only pegylated formulation approved by FDA
[b] status to be determined in US. [c] biosimilar formulations approved. For targets and therapeutic indications acronyms see text and list. NA: not approved. OD: orphan drug

transplant rejection, GVHD, autoimmune diseases, chronic inflammatory diseases, and autoinflammatory disorders. These new biomedicines have been mainly constructed as monoclonal antibodies directed against various ligands and receptors, or as fusion proteins combining the binding site of some cytokines to an IgG Fc fragment carrier, such as etanercept containing the extracellular portion of TNFR2, and rilonacept utilizing the IL-1R1 as decoy receptor. Anakinra is a recombinant human IL-1 receptor antagonist, competing with IL-1α and IL-1β for the same cell surface Type I receptor (IL-1R1).

Therefore, understanding the individual capacity of natural and recombinant cytokines in inducing AEs during human therapy, not only completes the safety spectrum of biomedicines examined in this volume, but also is instructive for evaluation of pathogenetic routes of adverse events of the whole drug class, when interfering with specific cytokine ligands or receptors [1–3].

Table 48.1 reports the recombinant cytokines and cytokine receptor analogues in human therapy analyzed below. Possibly, their number and properties do not give an idea on their future clinical potential. At present, more than 120 companies are developing over 270 products for cytokine-based therapy, inclusing mimic

cytokines, cytokine inhibitors and receptors. Among them there are also hemopoietic growth factors inhibitors (CAM-3001, AZD2423, AMMG 761, and PRO140) and two chemokine inhibitors (CXCR4 inh and CCR5 co-receptor antagonist), which may lead the way to new drug classes of biomedicines [4].

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Interleukins

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Only a few *interleukins* have been approved for human therapy, mostly because of the severe selection determined by the burden of adverse events observed since the first clinical experience with IL-1. Other recombinant products, such as rHuIL-4, and rHuIL-10 were tested in Phase I-II trials with insufficient benefit and therefore their development was halted. Similarly, a rHuIL-6 was used in acquired aplastic anemia in a Phase I–II study, with no efficacy and some AEs (cephalea, hypertension, tachycardia, arthralgia), and an increase of acute-phase reactants in all patients, leading to an anticipated discontinuation. More recently, a preliminary study on rHuIL-7 (CYT107) has shown to promote T (CD4 and CD8 subsets) cell recovery after allogenic stem cell transplantation, showing some effect, without evidence of relevant AEs [1].

Nonetheless, important systemic reactions occur also with the admitted products, such as the *capillary leak syndrome* (CLS), the proinflammatory *cytokine release syndrome* (CRS), the *flu-like syndrome* (FLS), most probably caused by the specific mechanism of action of the administered cytokine or to indirect imbalance effects produced on the overall cytokine homeostatic system, as observed during the CRS "*cytokine storm*" (see Chap. 3). More complex and less understood consequences mainly observed after prolonged administration include thyroid disorders, systemic lupus erythematosus and diabetes, to quote some examples.

Inhibitors of interleukins found a wider and significant clinical application as monoclonal antibodies or fusion proteins directed against various ligands and receptors, as previously described in the respective sections. For example, among the former adalimumab, basiliximab, brentuximab, certolizumab, denosumab, golimumab, and infliximab, are blockers of different members of the TNF family; canakinumab neutralizes IL-1β, basiliximab and daclizumab are directed against IL-2R, tocilizumab is directed against IL-6R, and ustekinumab binds to a subunit shared by IL-12 and IL-23. As for the latter, anakinra contains an IL-1 receptor

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antagonist, etanercept contains the extracellular portion of TNFR2, and rilonacept utilizes IL-1R1 as a decoy receptor.

Other experiences were not successful. For example, MRA, a humanized anti-human IL-6R monoclonal antibody, and HuMax IL-15, a high affinity humanized IgG1 mAb raised safety concerns and lacked efficacy. MRA was experienced in rheumatic diseases (RA, JRA, Castleman's, and Still's disease), where some serious events occurred (EBV fatal reactivation, allergic pneumonitis, infection). HuMax was meant to interfere with IL-15 mediated T cell recruitment and activation, survival of T memory cells, activation of neutrophils, fibroblasts, and endothelia survival, but showed a low efficacy profile. Mepolizumab, an anti IL-5 mAb intended for the treatment of asthma and tried for the treatment of eosinophilia (hypereosinophilic syndrome, eosinophilic esophagitis), in which high levels of IL-5 had previously detected, showed poor efficacy with apparent tolerability. Similarly, CAT-354 a human IgG4 anti IL-13 intended for the treatment of airways heperresponsiveness, did not reach its goal. Finally, CAT-213, another human IgG4 mAb directed to the chemokine eotaxin-1 exibiting attraction for eosinophils, did not proceed in clinical experimentation.

49.1 Interleukin-1 (IL-1)

The group of IL-1 ligands (11 proteins, 7 with proinflammatory activity, including the major representative isoforms IL-1α and IL-1β) is involved in the inflammatory response, acting as endogenous pyrogens, inducers of prostaglandin, collagenase releasers, promoters of the expression of adhesion molecules on endothelial cells, and of transmigration of leukocytes. Inflammation is mostly influenced by the relative amounts of IL-1α and IL-1β, both interacting with ubiquitous Type I receptor (IL-1R1) and Type II receptor antagonist IL-1Ra, a competitor and downregulator of IL-1 signaling. IL-1R1 associates with a receptor accessory protein (IL-1RAP) to form a transmembrane complex that initiates IL-1-dependent intracellular signaling. However, a second receptor (IL-1R2) serves as inhibitor of such signaling, both as membrane-bound or soluble exogenous and endogenous decoy receptor. IL-1R2 is active as single molecule or in association to IL-1RAP that enhances affinity for IL-1, which is 100 times more elevated for IL-1β with respect to IL-1α [2].

All IL-1 cytokine precursors, except for IL-1α, must be cleaved by intracellular caspase-1 or extracellular proteases to become active before binding to respective receptors and triggering transduction activating signals. The IL-1α precursor is associated to microtubules in endothelial, epithelial and parenchymal cells. When activated by membrane-associated calpain proteases, IL-1α is expressed on cell surface and interacts with IL-1 receptors expressed on adjacent cells, or is released with membrane fragments (apoptotic bodies) to be subsequently activated by extracellular neutrophil proteases. IL-1β is not constitutively expressed; its precursor is inactive and must be cleaved by caspase-1 that removes some amino-terminal

amino acids. The activation of IL-1β can be downregulated by endogenous soluble IL-1R2, which binds the IL-1β precursor, thus blocking its caspase-1 cleavage.

IL-1β transcription occurs after exogenous (microbial) or endogenous factors (TNF, IL-18) triggering, or by autostimulation from the same IL-1α and IL-1β molecules. Therefore, the former stimuli may trigger septic inflammation, and the latter ones induce sterile inflammation or autoinflammation. The active soluble form of IL-1β is a potent pro-inflammatory cytokine produced by various cell types, including monocytes, macrophages, mast cells, dendritic cells, endothelia, keratinocytes, fibroblasts, microglia and astrocytes, neuronal and Schwann cells. Interestingly, some of these cells, such as keratinocytes, produce the IL-1β precursor but are not able to process it to the active form, which is subsequently implemented by external proteases. IL-1β stimulates thymocytes and T lymphocyte proliferation by inducing IL-2 release, maturation and proliferation of B cells, of some dendritic cells, and induces mobilization of neutrophils and platelets from bone marrow. The synthesis and release of IL1β requires two distinct signals (for synthesis and assembly), which are normally initiated by PAMPs, such as bacterial RNA and lipopolysaccharides, but also by cytokines and endogenous irritants (uric acid or heat shock proteins). Therefore, IL1β is implicated in inflammatory processes after injury and infections, induction of pain and pyrexia, in acute and chronic autoimmune diseases, and in autoinflammatory diseases.

Clinical trials of IL-1α and IL-1β during the early 1990s soon established a heavy common safety profile for this cytokine, associated with a low antitumoral effect and a certain stimulatory capacity on the stem cell compartment, which induced to discontinuation of in vivo investigation and proceeded to ex vivo applications as bone marrow cell expander after harvesting of hematopoietic stem cells, in combination with other cytokines [3].

FLS with the entire cohort of signs and symptoms (pyrexia, chills, fatigue, nausea/vomiting, cephalea, tachycardia, malaise, arthralgia, and myalgia) developing within a few hours after administration was constantly present. The most concerning sign was a profound and prolonged dose-dependent hypotension resistant to indomethacin. The clinical feature reminded that of septic shock ascribed mainly to the insurgence of CLS, and to a possible central toxic effect on cerebral vasculature and on hypothalamic thermoregulatory centers, since IL-1 can pass the blood–brain barrier (BBB). This double action of IL-1 was substantiated by the rapidity of symptoms insurgence in animal models (about 20 min in mice), which could not be explained only by prostaglandin and other pyrexia mediators' synthesis and action [4]. At higher doses, confusion, somnolence, dyspnea, edema and prerenal azotemia appeared. Cardiovascular events were the most worrisome (hypertension, hypotension, myocardial dysfunction including tachycardia). Most IL-1 related events were resistant to indomethacin. Tachyphylaxis also developed during prolonged administration and therefore the clinical trials were halted at Phase II level. while major attention was given to therapeutic applications of IL-1 blocking (see anakinra, canakinumab, rilonacept, Chap. 12, 46, 51 respectively). No differences were identified between IL-1α and IL-1β safety profiles, which in fact bind to the same receptor and share similar biological properties.

49.2 Interleukin-2 (IL-2)

IL-2 is a tetra α-helical protein of 15,000 kDa secreted mainly by activated CD4+ T cells, and to a lesser extent by activated CD8+ T cells, NKT cells, NK cells, dendritic cells, and mast cells. The production in the former pivotal cells is triggered rapidly after TCR antigen binding and activation of costimulatory signals (CD28), but is quite so inhibited by IL-2 gene silencing and degradation of IL-2 mRNA. The TCR transcription induction follows the NF-kB downstream pathway, and subsequent gene silencing is mediated by factors (ZEB, CREM, BLIMP1) inhibiting IL-2 gene transcription, and via a STAT5-dependent feedback auto-regulatory IL-2 loop (autocrine regulation) by which soluble IL-2 inhibits its own production, after binding to its receptor (IL-2R).

IL-2R consists of three subunits (α, β, γ or CD25, CD122, CD132, respectively). CD122 and CD132 form a dimeric transmembrane low-affinity receptor, which activates downstream intracellular signaling. This receptor is poorly expressed on naive CD4+ T cells, is expressed on CD8+ T cells, memory CD4+ T cells, and highly expressed on CD8+ memory T cells and NK cells. The trimeric receptor associates also CD25 (IL-2Rα), which increases affinity (10–100 fold) of the complex, but does not participate to signal transduction. The high affinity receptor (IL-2R$\alpha\beta\gamma$) is transiently found on T cells following TCR-mediated activation. CD25 is constitutively expressed only on Treg cells, together with intermediate levels of the dimeric receptor.

Following IL-2/LR binding, the tetracomplex is internalized and degraded except for CD25, which can be recycled to the cell surface. The signal transduction follows three main activating pathways (JAK-STATS, PI3K-ACT, MAPK).

Noteworthy, CD132 is shared by IL-4, IL-7, IL-9, and IL-21, while IL-15 shares the whole heterodimer receptor (CD122–CD132).

The paracrine steady-state activity of IL-2, triggered by exogenous (microbial) or endogenous (self-peptide/MHCII) background stimulation, involves mainly the homeostasis of neighbouring T cells (CD4+, CD8+, Treg), which are further expanded during stronger antigenic exposure by activation of dendritic cells and overproduction of IL-2 in peripheral lymphoid organs and tissues.

IL-2 optimizes CD8+ T cell expansion, response and memory acquisition, and is crucial for the balance of Th-17/Treg cell compartment, by increasing the latter and decreasing IL-17, which contains autoreactive and pro inflammatory cells. The effect on Th-17 cells is mediated by inhibition of IL-6Rβ portion of IL-6R receptor, thus reducing the IL-6 activation STAT3 pathway, necessary for Th-17 cells proliferation and maturation. IL-2 inhibits also the generation of follicular helper T cells, a subset of CD4+ lymphocytes residing in germinal centers and providing a specialized help to antibody-producing B cells [5–8].

Because of these functions, and in particular of the capacity to stimulate CD8+ cytotoxic T cells and NK cells, IL-2 has been identified as a candidate for tumor immunotherapy both in vivo or for ex vivo production of autologous lymphokine activated killer cells (LAK) to be reinjected in vivo. In fact, IL-2 has

been the first recombinant cytokine biomedicine, and has been used as booster of the CD4+ T cell compartment in advanced HIV infections. However, stumbling limitations came from the short activity of IL-2 and mostly from the insurgence of serious AEs.

CLS is the major adverse event induced by high dose IL-2 IV administration, characterized by an immediate increase in vascular permeability resulting in severe hypotension (within 2–12 h), hypoperfusion, tissue accumulation of fluids, edema, effusions (including pleural and pericardial) and, ultimately, multiple organ failure. The syndrome is reversible and controlled by vasopressor therapy and judicious fluid replacement.

At lower doses FLS is common. However, longer treatments showed additional signs such as diffuse edema, chronic arthritis, myositis, thyroid manifestations, hypersensitivity reactions, including anaphylaxis, angioedema, allergic cutaneous manifestations, and impairment of positive chemotaxis neutrophil function. On this basis high dose treatments were gradually abandoned, while low-dose treatments continued, mainly in autoimmune diseases with IL-2 conjugated to proteic carriers which prolonged its half-life, and could stimulate Treg cells while expressing a milder safety profile. Occasionally, a high IL-2 dose was associated with denileukin-diftitox, a IL-2 molecue fused to diphteria toxin (Chap. 50).

Aldesleukin (Proleukin®, Bayer, Novartis, Chiron, Prometheus) is the only recombinant IL-2 approved by FDA in 1992 for the treatment of metastatic renal cell carcinoma (RCC) with IV high dose therapy. In January 1998 the indication was extended the same high dose treatment to metastatic melanoma with a commitment for the manufacturer to obtain data on the use of lower dose regimens as monotherapy and/or in combination with chemotherapy. EMEA granted the orphan designation for the treatment of RCC in 2003, but was withdrawn in 2006 on request of the sponsor.

Safety analysis was mainly based on 255 patients with metastatic RCC, and on 270 patients with metastatic melanoma receiving 18–20 IV doses of aldesleukin every 8 h for up to 5 days for a maximum of 14 doses.

The safety profile of aldesleukin includes a series of specific *contraindications* consisting in cardiac disorders (sustained ventricular tachycardia, cardiac arrhythmias resistant to standard therapy, ischemia/infarction, tamponade), renal failure, gastrointestinal disorders (bleeding/ischemia/perforation), coma or toxic psychosis, repetitive seizures, and recent intubation.

The *most common* AEs included hypotension (71 %), diarrhea (67 %), oliguria (63 %), chills (52 %), nausea/vomiting (35–50 %), dyspnea (43 %), rash (42 %), confusion/somnolence (22–34 %), pyrexia (29 %), pruritus (24 %), asthenia/malaise (23–27 %), tachycardia (23 %), stomatitis (22 %), anorexia (20 %), exfoliative dermatitis (18 %), respiratory and lung disorders (11–24 %), edema/weight gain (15–16 %), vasodilatation (13 %), infections (13 %), cardiovascular disorders (11 %), pain/abdominal pain (11–12 %), arrhythmia (10 %), dizziness/anxiety (10–11 %), cough (11 %), and rhinitis (10 %). Laboratory abnormalities included bilirubinemia (40 %), thrombocytopenia (37 %), creatininemia (33 %),

anemia (29 %), AST increase (23 %), leukopenia (16 %), electrolytes decrease (11–12 %), ALP increase (10 %), and acidosis (10 %).

The *most serious events* included anuria/oliguria (5–6 %), hypotension (3 %), acute respiratory disorders (3 %), and coma (2 %). Cardiac disorders, including infarction and cardiac arrest, psychosis/confusion, acute kidney failure, apnea/dyspnea, and laboratory abnormalities were each observed in 1 % of cases [5, 6]. Serious eosinophilia with infiltraton of cardiac and pulmonary tissues was also observed.

Among events observed also in the postmarketing experience there were cases of new or exacerbated autoimmune disorders, mostly represented by hypothyroidism (about 13 %). Thyroid dysfunction occurred after 2–4 month of treatment, the majority being associated with organ specific autoantibodies and was usually reversible after treatment discontinuation. However, the incidence increased over time and in combined therapy with IFNα, reaching 100 % of cases in some studies. Exacerbation of Crohn's disease, RA, pemphigus, psoriasis, scleroderma, epidermal necrolysis, erythema nodosum, vitiligo, nephropathies, demyelinating neuropathy, fatal infections and hemorrage were also reported.

The presence of non-neutralizing anti-aldesleukin antibodies was frequent (66–74 %) among RCC and melanoma patients.

Interestingly, the administration of corticosteroids for acute life-threatening toxicities resulted in loss of efficacy of the drug in study.

Overall, the core of most common and serious experienced AEs can be referred to CLS acute and delayed consequences, and to a possible triggering of autoimmune states either new or latent [5–7].

Inleusin (3S BIO, Shenyang Sunshine Pharm., China) is a recombinant IL-2 for injection indicated for treatment of mRCC and MM in adults, and for cancer-induced pleural effusion and ascites. It shows a similar safety profile than aldesleukin.

Because of the limited experience with therapeutic interleukins and the diversity of their functions it is not possible to draw a drug class safety profile after in vivo administration. However, the most typical manifestation of reactivity to both IL-1 and IL-2 can be summarized in the *CLS, CRS, and FLS triad*, which can be ultimately ascribed to the exaggerated expression of the physiological mechanisms of action of these two interleukins. AEs to IL-11 are related to its peculiar activity on thrombocytes, and therefore they have unique characteristics, sharing more similarities with other hemopoietic stimulatory factors rather than with other interleukins, as described below.

Interleukin-3 (IL-3) also stimulates hemopoietic stem cells, acts on lymphoid precursors in conjunction with IL-7, and synergizes with other cytokines on further maturation steps of all myeloid lineages. This cytokine has been experienced mostly in ex vivo human cell manipulation for cellular therapy.

Alternative strategies using interleukins or their receptors as carriers or antagonists were more successful.

For example, daclizumab (Zenapax®, Hoffman-LaRoche) is directed to CD25 receptor component of IL-2R, acts as a receptor antagonist, thus blocking the action of IL-2.

A second approach consisted in constructing carriers of toxins targeting specific receptors, such denileukin-diftitox (Ontak®, Seragen, Eisai), a recombinant IL-2 designed to direct the cytocidal action of diphtheria toxin to IL-2R expressing cells.

A third approach consisted in structuring receptor analogues for interleukins, such as anakinra (Kineret®, Amgen), a recombinant human IL-1Ra which antagonizes the natural IL-1R in binding IL-1. A similar function is expressed from the fusion protein rilonacept. However, the latter acts as a competitor for both IL-1R and IL-1Ra receptors, since it binds directly to soluble IL-1 before its binding to either receptors. Both biomedicines function as decoy receptors defined also as "molecular traps." Similarly, the recently approved aflibercept acts as more complex and potent VEGFs trap, since includes two different VEGF receptor portions showing an high binding affinity (exceeding the affinity of the natural receptor), and inhibiting activity for two VEGF isoforms.

A detailed description of daclizumab is reported in Chap. 16, and of rilonacept and aflibercept in Chaps. 42 and 46, among other mAbs or FP, respectively. Denileukin-diftitox and anakinra descriptions are provided are provided in the following Chaps. 50 and 51.

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Denileukin-Diftitox

50

Denileukin-diftitox (Ontak®, Seragen, Eisai) is a recombinant protein consisting of interleukin-2 (IL-2) fused with diphtheria toxin (DT). The IL-2 portion binds to cells bearing its receptor (IL-2R); the complex is endocytosed and the toxin exerts its potent targeted cytocidal action within the cytoplasm. It is the first recombinant ligand toxin fusion protein used for human therapy.

FDA granted accelerated approval in 1999 for the treatment of recurrent cutaneous CD25+ T-cell lymphoma (cTCL), and the indication was confirmed in 2008. EMEA designated it as orphan drug for the same treatment in 2001.

The basis for initial approval was the pivotal Phase III study (93-04-14) on 71 pretreated patients with Mycosis Fungoides (MF) and Sèzary syndrome (SzS) forms of cTCL. Two subsequent supportive Phase III studies (93-04-11; 93-04-14) followed, including 236 patients. Previous Phase I and II studies evaluated the clinical response in B cell NHL, HD, and cTCL in 88 patients.

Overall, 263 patients with CD25+ cTCL at various stages were treated with a maximum of eight cycles of two different doses of denileukin, while 44 patients received a placebo [1, 2].

50.1 Mechanism of Action

IL-2R consists of three subunits: α, β, γ (or CD25, CD122, CD132, respectively). CD122 and CD132 form a dimeric transmembrane low-affinity receptor (IL-2R$\beta\gamma$) that activates downstream intracellular signaling. This receptor is poorly expressed on naive CD4+ T cells, is expressed on CD8+ T cells and memory CD4+ T cells, and highly expressed on CD8+ memory T cells and NK cells. The trimeric receptor also associates CD25 (IL-2Rα), which increases affinity (10-100 fold) of the complex, but does not participate to signal transduction. The high affinity

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receptor (IL-2R$\alpha\beta\gamma$) is transiently found on T cells following TCR-mediated activation. CD25 is constitutively expressed only on Treg cells, together with intermediate levels of the dimeric receptor.

Following the IL-2/IL-2R binding, the tetracomplex is internalized and degraded, except for CD25 that can be recycled to the cell surface. The signal transduction follows three main activating pathways (JAK-STATS, PI3K-ACT, MAPK).

Noteworthy, CD132 is shared by IL-4, IL-7, IL-9, and IL-21, while the whole heterodimer receptor (CD122-CD132) is present in the IL-15 molecule.

Denileukin-diftitox (DAB389IL, E777) is an immunotoxin developed to direct the DT cytocidal action against IL-2R expressing cells, which internalize the entire complex thus allowing DT to express its toxic action.

The truncation of a previous prototype (DAB486IL) improved activity and prolonged the half-life of the molecule. This has led to a final fusion protein that contains both fragment A and B of the toxin, followed by the human IL-2 sequence, meaning that the receptor binding domain of DT was substituted with IL-2. Therefore, denileukin can bind to any of the receptor components, but induces internalization of the receptor-bound complex only in cells bearing the high affinity isoform of IL-2R (α, β, γ) and the intermediate isoforms of the receptor, although with 100-fold lower binding capacity. However, it does not trigger IL-2R intracellular signals.

After internalization (lasting about 10 min) through clathrin-coated pits into an endosome, where the low pH induces unfolding of the protein, fragment A is cleaved and translocated into cytoplasm where inactivates the diphthamide residue of elongation factor 2 (EF-2), thus inhibiting protein synthesis within 6 h. The cell dies within three days.

It has been estimated that approximately 50 % of cTCL cases express IL-2R, and consequently cTCL positive cells are promptly killed by denileukin-diftitox. In some cases of MF, only CD25 is available for the drug binding. Due to the high cytocidal effect on the Treg subtype, this fusion protein is also evaluated in other hematologic malignancies and solid tumors, in combined anti-neoplastic therapies, and for depletion of Treg cells prior to immunotherapy [3–5].

50.2 Immunogenicity

In Study 93-04-10 on 71 patients, and in two pharmacokinetic studies (93-04-12, 92-04-01) on a total of 128 patients, anti-drug antibodies were reported very frequently. In the pivotal Study 93-04-14, 32 % of subjects had previous anti-DT antibodies, and all but one developed moderate levels of neutralizing antibodies, after completion of two courses of treatment. Although their titer did not change over time, neutralizing antibodies clearly produced a marked reduction (about ten fold) of serum concentration of the drug in study. Nonetheless, their presence did not interfere with treatment.

Interestingly, anti–IL-2 antibodies were present in 56 % of cases. Both titers did not correlate with AEs [6, 7, 10].

50.3 Adverse Events

Safety data are based on the three mentioned studies on 234 patients receiving two different doses of denileukin: 9 or 18 μg/kg [1].

The *most common adverse events* reported in patients treated with the respective two doses versus placebo were: pyrexia (64 %; 49 %; 16 %), nausea (60 %; 47 %; 23 %), rigors (47 %; 42 %; 20.5 %), fatigue (44 %; 47 %; 32 %), vomiting (34.5 %; 13 %; 7 %), cephalea (25.5 %; 29 %; 18 %), peripheral edema (25.5 %; 20 %; 23 %), diarrhea (22 %; 22 %; 9 %), anorexia (20 %; 9 %; 4.5 %), rash (20 %; 24 %; 4.5 %), myalgia (20 %; 18 %; 4.5 %), cough (18 %; 20 %; 7 %), pruritus (18 %; 16 %; 9 %), lumbalgia (18 %; 16 %; 2 %), asthenia (18 %; 18 %; 4.5 %), hypotension (16 %; 7 %; 2 %), URTI (13 %; 13 %; 11 %), dizziness (13 %; 11 %; 11 %), arthralgia (13 %; 16 %; 11 %), pain (13 %; 11 %; 7 %), chest pain (13 %; 4 %; 2 %), dysgeusia (11 %; 0 %; 2 %), and dyspnea (11 %; 13 %; 4.5 %).

Lymphopenia, the most common hematologic abnormality, was observed in approximately 70 % of the patients, but was transient and recovered within two weeks. Anemia, leukopenia, and thrombocytopenia were mild and usually did not require treatment.

A *BBW* warning includes CLS (11 %) *serious infusion reactions* (8 %), and *loss of visual acuity* (4 %). An additional alert was included for hypoalbuminemia to be monitored during treatment.

The *most common serious events* were *CLS* (11 %), *infusion reactions* (8 %), and *loss of visual acuity* (4 %). Discontinuation rates related to AEs were consistent (28 %).

During the first pivotal trial, two deaths were attributed to the drug in study (1 sepsis in an inappropriately enrolled patient with pancytopenia; 1 myocardial infarction in a coronary bypass implanted patient).

Approximately 90 % of AEs appeared during the first course of treatment. Constitutional and gastrointestinal signs were over 90 %, and FLS occurred in about 85 % of cases. Infections, in particular septic ones, were of staphylococcal origin, which is common in patients with relevant skin lesions such as cTCL [1, 2, 6].

The second pivotal study allowed to better compare the incidence of denileukin-related AEs with those emerging from the underlying disease or unrelated to treatment, and with the placebo group (100 patients treated, 44 controls). Overall, drug-related reactions usually occurred during the first two courses of treatment. Interestingly, no difference in the rate of infections was reported, and sepsis resulted to be unrelated to the therapy in study. Drug-related lymphopenia (22 %) appeared during initial treatment and then rapidly improved. CLS (10 %) was usually mild, yet two patients had a severe response. Therapy-related discontinuations occurred in 17 % of cases. ALT/AST increases were detected in 84 % of cases and were usually transient. Creatinine increase was estimated as 12 %. Continued treatment did not cause worsening of laboratory parameters and was not associated with liver or renal toxicity [8].

Recently, an *alternate dosing regimen* was experienced in eight patients with persistent or recurrent cTCL, excluding MF and SzS cases. CLS appeared in 6 patients (75 %) only between day 1 and 10 of the first therapy cycle, and three were severe. Infusion reactions (back pain, nausea) occurred in four patients. Other AEs included vomiting (4), fatigue (1), transaminase (3) and creatinine elevations (1), and one thyrotoxicosis. Overall, CLS was the major observed drug-related event, in spite of premedication [9].

In an open-label Phase III trial on 20 *relapsed cTCL* patients, all experienced at least one AE after denileukin treatment, and it was drug-related in 55 % of cases. As expected, the majority of such events (nausea, pyrexia, fatigue, rigors) occurred during the first cycle of treatment, decreasing at subsequent cycles. Severe events were essentially represented by infections (2/15). Interestingly, no CLS and no deaths were observed in the study period. The particularly mild safety profile was attributed to the previous therapy with the same biomedicine [10].

Associated denileukin therapy was evaluated in 14 cases of MF, one case of nasal NK/T cell lymphoma and one of advanced erythrodermic cTCL, in combination with bexarotene. The basis for this approach was a potential drug synergism associated with a lower toxicity compared to the more aggressive chemotherapies. The overall profile did not differ from the conventional framework experienced in cTCL, showing that no synergistic effect occurred in AEs induction [11].

Finally, in a Phase II trial (CONCEPT), 49 patients with peripheral TCL (excluding MF and SzS) were treated with denileukin combined with CHOP therapy, receiving a median of six cycles and being followed up for 22 months. Five patients (10 %) discontinued therapy due to drug-related events (allergy, dyspnea and pneumonitis, febrile neutropenia, cardiac ischemia and TLS, and one unspecified death). The most frequent treatment-related AEs were lymphopenia (25 %), neutropenia/leukopenia (16 %), thrombocytopenia (12 %), febrile neutropenia (10 %), and anemia (8 %). Two patients had serious cardiac events.

Overall, the safety profile was similar to CHOP therapy associated with alemtuzumab, yet denileukin-CHOP combination showed a lower rate of infections (12 %) and absence of opportunistic ones [12].

50.4 Off-Label Experience

Denileukin-diftitox is being currently evaluated in combination with other therapies for the treatment of various cancers including RCC, B cell NHL, T cell NHL, CLL, melanoma, pancreatic cancer, ovarian cancer, and acute myeloid leukemia (AML).

Initial attention was given to *recurrent/refractory CLL* (48 % with CD25+ cell targets); patients showed only partial response and a number of AEs. In 28 enrolled patients, mostly pretreated with a mean of 3.4 cycles of fludarabine, receiving a mean of 4–5 cycles of denileukin, severe events included CLS (14 %),

neutropenia (32 %), infections (29 %), thrombocytopenia (25 %), fatigue (21 %), rash (18 %), dyspnea (18 %), and hypotension as the most common. Liver enzymes were often altered (32 %) and, less frequently, hypocalcemia was observed (10 %). Discontinuation rate was consistent (23 patients) and in about 50 % of cases it was attributed to AEs (3 CLS, 4 rash, neuropathy, fatigue/pain, pneumonia, pleural effusion, and 4 bacterial infections). The remaining halted cases were related to disease progression. Neither AEs nor response to therapy were found to be dose-related [13].

In a pilot study on 18 patients with *metastatic RCC*, denileukin was administered after a high dose of IL-2. The latter is an approved procedure for inducing cytotoxic T lymphocytes (CTL) in resistant or intolerant RCC cases. However, such treatment also stimulates Treg, which tend to suppress immune response, including anti-tumor immune response. Denileukin was therefore subsequently infused, in order to deplete CD25+ cells including Treg. The control group consisted in 15 melanoma patients treated only with IL-2.

CLS occurred in both groups (44 vs. 40 % in controls). Cardiac events (atrial fibrillation, infarction) occurred only in the study group (11 and 6 % respectively), as well as for malignant pleural effusion (6 %). The only unusual AE was the development in a single patient of transient acantholytic dermatosis (Grover disease) concurrent with CLS, which was related to the IL-2 treatment [14].

One case of cutaneous *anaplastic B cell lymphoma* resistant to radiation and different chemotherapies, including MTX, was treated with monthly cycles of denileukin. The patient achieved remission after eight cycles, and mild fatigue was the only encountered AE [15].

More recently, 23 patients with *naive B cell NHL* were treated with a combination of denileukin and rituximab. In this experience, the combination significantly increased the frequency of AEs, compared to rituximab alone. Severe AEs were detected in 57 % of patients (52 % related to drug in study). Serious reactions occurred in 30 % of patients. CLS was present in 26 % of cases and was associated with serious hypotension (grade 4–5) in two patients, causing a fatal refractory cardiogenic shock in one of them. Two patients had serious cardiac events (ischemia, rupture of mitralic chordae tendineae). Three additional severe events included myositis, thrombosis, and neutropenia in three different patients. Due to the high level of serious AEs the trial was discontinued [16].

Finally, in a Phase II trial 60 patients with *unresectable stage IV melanoma* were treated with 1–4 cycles of denileukin every 21 days. The most common AEs were nausea (38 %), fatigue (21 %), emesis (16 %), rash (15 %), and chills (10 %). Interestingly, 5 % of patients reported pain at tumor site, which was attributed to a drug-related inflammation. One patient developed vitiligo, as a result of denileukin administration, attributed to the presence of immune cross-reactivity against antigens expressed by melanoma cells and melanocytes [17].

50.5 Postmarketing Surveillance

Among 207 reports in the FAERS database, the most frequent events were CLS (28), pain (22), pyrexia (18), nausea/vomiting (17/18), hypotension (16), and dyspnea (14), and 3 cases of CRS.

Seven cases of retinopathy, three cases of hyperthyroidism, and one goiter were reported. In fact, a few cases of visual disorders, one case of retinopathy and a number of thyroid disorders were observed in clinical studies, and in approximately 3 % of the postmarketing reports. Moreover, five cases of TLS were also observed. In a different database (ehealthme.com) nine cases out of TLS 404 reports were registered.

Anti-TPO antibodies were also detected; some cases improved after denileukin discontinuation, but some others developed chronic hypothyroidism. The pathogenetic mechanism of these events remains unclear [18, 19].

50.6 Remarks

Denileukin-diftitox is the only immunotoxin so far approved for human therapy, yet additional products are currently under evaluation [20].

The overall safety profile indicates CLS as the event of major concern, although manageable, and in part preventable by proper premedication. Other serious events, such as infections, do not seem to be correlated with denileukin administration. Hematologic abnormalities, mainly consisting in lymphopenia, are usually transitory and tend to resolve spontaneously. The majority of constitutional signs, including FLS, tended to emerge during early phase of treatment and decrease over time. Nonetheless, discontinuation rates were consistent.

Two intriguing aspects relate to thyropathy and to visual loss/retinopathy. Visual loss has been reported in about 4 % of cases in clinical studies, while retinopathy is present in about 3.4 % in the postmarketing setting [9, 18, 19]. Part of the thyroid abnormalities start with hyperthyroidism and evolve in chronic hypothyroidism, which has been attributed to a local inflammatory process triggered by cytokines in the presence of denileukin.

The role of Treg depletion on activation of autoimmune retinopathy and of autoimmune thyroiditis [21, 22], as well as on induction of Type 1 diabetes in mice [23], has been demonstrated, and raises concerns for the possible consequences of similar treatments at human level. Overall, the pathogenetic mechanisms deriving from unchaining autoreactive cell clones due to Treg persistent depletion remains crucial for understanding the insurgence of autoimmune disorders in denileukin-treated patients.

It is unclear whether response rates and AEs profile are dependent by the presence of CD25+ targets. In fact, the depleting action on normal Treg cells may induce an enhanced immune response against CD25 negative/low affinity cells, and produce immune rebounds implicated in the genesis of some AEs. Interestingly, the

effect on Treg cells seems precocious and transitory, seeming to parallel some aspects of the safety profile of denileukin.

Moreover, steroid premedication may enhance the expression of CD25 on T cells that could be correlated with an increased therapeutic response, yet also modify the expression of AEs [23]. With this respect, it must be reminded that two over three IL-2R chain components are promiscuous transmembrane proteins. In particular, CD132 is shared by IL-4, IL-7, IL-9, and IL-21, while the whole heterodimer receptor (CD122-CD132) is present in the IL-15 molecule. Despite internalization and subsequent cytocidal activity are only managed by high and intermediate affinity IL-2R receptors, contrasting functional interferences after binding to promiscuous components of other receptors cannot be excluded, leading to potential consequences for efficacy and safety during denileukin therapy.

A particularly severe safety profile was experienced in one study on B cell NHL treated with a combination of denileukin and rituximab. This small experience does not allow definitive conclusions, yet raises further concern about the attempt of multitargeting therapy directed to transmembrane signaling structures [16].

One case of TLS was observed in a CHOP combined therapy study, and presumably was related to chemotherapy. However, a few other cases were reported in postmarketing settings, although the potency of massive cell destruction of this immunotoxin is limited, due to the relative low number of target cells expressing high affinity IL-2R [3].

Finally, it is difficult to separate DRAEs from underlying disease-related events, especially in rare pathologies were controlled studies are not easily programmable.

One recent placebo-controlled study on MF/SzS patients, receiving denileukin as monotherapy, revealed an acceptable profile of AEs related to disease. Peripheral edema occurred with equal frequency in treated and control groups (23 %), and serious infections were present in both groups, being sepsis predominant among placebo patients. However, two cases of rather severe CLS occurred in the study group. Importantly, this study evidenced spontaneous remission in untreated patients that may represent another relevant confounder in uncontrolled studies [24].

The overall experience from off-label treatments did not show new emerging safety signals, except for a general warning on higher toxicity observed in some combined therapies.

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Anakinra

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Anakinra (Kineret®, Amgen, Sobi) is a recombinant human interleukin-1 (IL-1) receptor antagonist (r-metHu IL-1ra), which competes with IL-1α and IL-1β for the same cell surface Type 1 receptor (IL-1RI), and thus inhibiting their biological proinflammatory activity. In November 2001, FDA granted approval for the reduction of signs and symptoms and for slowing the progression of structural damage in moderately to severely active rheumatoid arthritis (RA), in patients 18 years of age or older who have failed one or more disease modifying antirheumatic drugs (DMARDs). In December 2012, the indication was extended to the treatment of children and adults with neonatal-onset multisystem inflammatory disease (NOMID), an autoinflammatory disease of the cryopirin-associated periodic syndromes (CAPS) group. Anakinra was designated as orphan drug for NOMID in August 2010 by the same Agency. EMEA granted approval in March 2002 for the treatment of signs and symptoms of RA in combination with methotrexate (MTX), in adults with inadequate response to MTX alone [1–4]. An application for the use of anakinra in CAPS has been also submitted to EMEA in September 2013, and received a positive response from CHMP.

Pivotal studies for RA include Study 990757 on 1,399 subjects, of whom 1,116 were exposed to anakinra for 6 months, and Study 990145 providing a 6 months interim analysis on the first 501 subjects (250 treated).

Additional studies include the Phase II Study 560 on 473 randomized patients (352 treated); Study 960180, a dose-ranging analysis on 419 (345 treated) randomized patients; Study 960182 enrolling 141 patients (111 treated) for a 3-month dose–response to lower doses of anakinra (2.5–30 mg); Study 20000125 on 58 RA patients treated for 6 months, who had previously received etanercept for at least 3 months [1–4].

Pivotal study for NOMID was based on 43 patients from an ongoing open-label trial (NCT00069329, 03-AR-0298), treated with two doses of anakinra up to

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60 months. A subset of these patients (11) was studied after withdrawal of the drug in study [4–6].

At present, over 50 trials are investigating anakinra in various diseases including studies on RA (8), CAPS (3), and on various off-label investigations.

51.1 Mechanism of Action

IL-1 ligands (a group of 11 proteins, seven with proinflammatory activity, including the major representative isoforms IL-1α and IL-1β) are involved in the inflammatory response, acting as endogenous pyrogens, inducers of prostaglandin, collagenase releasers, promoters of the expression of adhesion molecules on endothelial cells, and of leukocytes' transmigration. Inflammation is mostly influenced by the relative amounts of IL-1α and IL-1β, both interacting with ubiquitous Type I receptor (IL-1R1) and with Type II receptor antagonist IL-1Ra, a competitor and downregulator of IL-1 signaling. IL-1R1 associates with a receptor accessory protein (IL-1RAP) to form a transmembrane complex that initiates the IL-1-dependent intracellular signaling. However, a second receptor (IL-1R2) serves as inhibitor of such signaling, acting as membrane-bound and as soluble decoy receptor. IL-1R2 is active as single molecule or in association with IL-1RAP, which enhances affinity for IL-1. Such affinity is 100-fold higher for IL-1β than for IL-1α [7].

All IL-1 cytokine precursors, except for IL-1α, must be cleaved by intracellular caspase-1 or extracellular proteases to become active before binding to respective receptors and triggering transduction activating signals. The IL-1α precursor is associated with microtubules in endothelial, epithelial, and parenchymal cells. When activated by membrane-associated calpain proteases, IL-1α is expressed at cell surface and interacts with IL-1 receptors expressed on adjacent cells, or is released with membrane fragments (apoptotic bodies) to be subsequently activated by extracellular neutrophil proteases. IL-1β is not constitutively expressed; its precursor is inactive and must be cleaved by caspase-1 that removes some amino-terminal amino acids. The activation of IL-1β can be downregulated by endogenous soluble IL-1R2, which binds to the IL-1β precursor, thus blocking its caspase-1 cleavage.

IL-1β transcription is either triggered by exogenous (microbial) or endogenous factors (TNF, IL-18), or occurs due to the autostimulation of IL-1α and IL-1β molecules. The former stimuli may trigger septic inflammation, while the latter one induces sterile inflammation or autoinflammation.

The active soluble form of IL-1β is a potent pro-inflammatory cytokine produced by various cell types, including monocytes, macrophages, mast cells, dendritic cells, endothelia, keratinocytes, fibroblasts, microglia and astrocytes, neuronal, and Schwann cells. Interestingly, some of these cells, such as keratinocytes, produce the IL-1β precursor but are not able to process it to the active form, which is subsequently implemented by external proteases. IL-1β stimulates

thymocytes and T lymphocyte proliferation by inducing IL-2 release, maturation and proliferation of B cells and of some dendritic cells. It also induces mobilization from the bone marrow of neutrophils and platelets. Synthesis and release of IL1β require two distinct signals (for synthesis and assembly), which are normally initiated by PAMPs, such as bacterial RNA and lipopolysaccharides, but also by cytokines and endogenous irritants (uric acid or heat shock proteins). Therefore, IL-1β is implicated in inflammatory processes occurring after injury and infections, in induction of pain and pyrexia, in acute and chronic autoimmune diseases such as RA, and in autoinflammatory diseases such as CAPS.

IL-1α and IL-1β are critical mediators of inflammation and of joint damage in RA. They are found in synovial fluid and in plasma concentrations that follow the activity of the disease. They induce cartilage degradation due to loss of proteoglycans, and stimulate bone resorption. Interestingly, intrathecal IL-1β concentrations were found markedly increased in RA patients, while IL-1Ra was decreased in their cerebrospinal fluid. Both factors contribute to generate systemic signals such as pain, pyrexia, and fatigue [8].

CAPS are a group of rare autosomal hereditary periodic fever syndromes associated with NLRP3 gene mutations, such as NOMID, resulting in overproduction of IL-1β. They are also defined as autoinflammatory diseases caused by the NALP3 encoded cryopirin (or CIAS1), a component of the inflammasome controlling the activation of IL-1β producing periodic pyrexia, rash, joint pain, and multiorgan inflammation.

In CAPS, there is over-secretion of IL-1β (up to fivefold higher than in healthy subjects) and increased expression of IL-1Ra, which apparently is not sufficient to counteract IL-1β activity. However, recent findings indicate that monocytes from CAPS patients show an impaired production of IL-1Ra [9]. In some patients, a genetic mutation in the IL1RN gene produces a deficiency of IL-1Ra (DIRA), leading to a complex pathology with exaggerated inflammatory response, skin pustulosis, joint and bone lesions, which promptly respond to therapy with IL-1Ra analogues, such as anakinra [10].

Both IL-1s are also involved in other pathologic inflammatory processes, such as acute ischemic diseases, chronic heart failure, osteoarthritis, gout, diabetes (IL-1β is toxic for beta-pancreatic cells); in chronic systemic inflammatory diseases, such as Still's disease and Schnitzler syndrome; and in the macrophage activating syndrome (see MAS, Chap. 3).

Anakinra is a recombinant non-glycosylated human IL-1Ra, which competes with IL-1α and IL-1β for the same cell surface receptor (IL-1RI), thus inhibiting their biological proinflammatory activity. It differs from the natural IL-1Ra in having an additional N-terminal methionine residue. After SC injection, maximal plasma levels are reached in 3–9 h. Anakinra has a short half-life (4–6 h), is eliminated more rapidly after IV injection (<3 h), and has an affinity for IL-1R1 similar to that of IL-1, which demands up to 1,000-fold excess dosage for an efficient blockade of IL-1 signaling [7]. Clearance is significantly impaired in patients with severe renal insufficiency.

Anakinra neutralizes the biological activity of IL-1α and IL-1β, but does not trigger IL-RI-mediated signals. In vitro, it inhibits the induction of nitric oxide, and the production of prostaglandin E2 and collagenase by synovial cells, fibroblasts, and chondrocytes [1–4, 11].

Anakinra was originally developed as a treatment for septic shock, but its beneficial effect has not been confirmed by subsequent studies.

51.2 Immunogenicity

In RA patients, anti-anakinra antibodies were detected in 49 % of exposed subjects in two pivotal studies. Neutralizing antibodies were detected in 2 % of cases on 1,615 tested subjects, in part persisting during the follow-up. No associations with adverse events were identified.

In NOMID patients the immunogenicity was not evaluated.

51.3 Adverse Events

Initial safety evaluation in RA patients was based on pivotal Study 560, Study 960180, and on their respective extension studies 0564 and 960181, for a total of 829 subjects exposed to anakinra, and 195 controls. Among them, 318 subjects were treated with anakinra for 6 months and 175 subjects were treated for 1 year, with daily doses $\geq$75 mg. Nine additional studies supported the safety analysis with 411 exposed patients and 48 controls, for a general total of 1,240 exposed subjects and 243 controls.

In NOMID patients, safety evaluation was based on 43 subjects exposed for up to 60 months. In particular, 23 of them completed at least 36 months and 20 patients reached 60 months of treatment. However, in a number of patients dose escalations were necessary in order to control severe inflammation, mainly during active infections or surgery, due to recurrence of flares.

In the last prescribing information, warnings have been issued for *serious infections*, *hypersensitivity reactions* (including anaphylaxis and angioedema), *risk of chronic infections and malignancies* related to the immunosuppressive action of anakinra, and *risk of neutropenia*, particularly when used in combination with TNF-inhibitors. The most common experienced event was *injection site reaction* (71 %, 3 % severe; 29 % in placebo). Hypersensitivity reactions were reported as rare (<0.1 %).

The incidence of *infections* was 39 versus 37 % in controls. During the first 6 months, serious infections (2 vs.1 %) did not significantly increase over time (3 vs. 2 % after 1 year). They mostly included bacterial events (cellulitis, pneumonia) and bone/joint localized infections that resolved in 73 % of cases allowing treatment continuation. No serious opportunistic infections were observed. However,

anakinra in association with etanercept induced 7 % of serious infections, and therefore such association was not recommended.

Malignancies observed in a database of 5,300 RA patients treated with anakinra included eight lymphomas (0.12 per 100 P/Y, 3.6-fold higher than the general population rate in the SEER database). However, these data were consistent with reported rates of the general population of RA patients, and did not increase over time.

Nonlymphomatous neoplasms (37) were mostly represented by breast cancer, respiratory, and digestive system tumors. In one pivotal study (960180 and extension 960181), three melanomas were also observed (threefold higher than the expected rate).

A wide analysis assessing the risk of malignancies in 29,423 RA patients treated with nine different biomedicines for at least 6 months did not show a higher cumulative incidence of tumors compared to DMARDs therapy or placebo. Possible exception could be suggested for lymphoma (Peto OR: 2.1) in patients receiving TNF inhibitors. Interestingly, anakinra showed a significant decrease in risk, when associated to MTX at 24 weeks (see also adalimumab Chap. 6).

Signs of hematological toxicity mainly consisted in neutropenia (8 vs. 2 % in controls) and were severe in 0.4 % of cases, increasing to 2 % when in combination therapy with etanercept, showing a differential increase of eosinophilia (9 vs. 2 %) and thrombocytopenia (2 vs. 0 %) [1–4, 12].

In one study (20000125) examining the combination with etanercept in RA, 19 % of patients discontinued the treatment due to AEs. Seven SAEs were reported, including four serious infections (two pneumonia, two cellulitis), for an estimated rate of 13.8 per 100 P/Y [1].

In NOMID patients infections were frequent, rather occurring during the first 6 months of therapy (2.3 P/Y) than later (1.7 P/Y). URTI, sinusitis, ear infections and nasopharyngitis were the most common infectious events. Their frequency was higher in patients <12 years of age. Three patients in the study group and two subjects in the placebo group had serious infections (1.8/patient), mostly consisting in pneumonia (five episodes in three patients) and gastroenteritis. No opportunistic infections were observed. Neutropenia was observed in two patients, and in one case was associated with infections (URTI, otitis media). Injection site reactions occurred in 10 patients (1.7/patient) during the whole 60-month study, and tended to decrease over time, with no reactions observed after 2 years of treatment. Most common events consisted in arthralgia, cephalea, pyrexia, URTI, nasopharyngitis, and rash. One case of angioedema and one case of MAS were observed in the low dose and high dose groups, respectively. No malignancies were observed and no discontinuations occurred along the study. Overall, 24 SAEs were reported in 14 patients (five related to study procedure lumbar puncture), and 6 of them were considered drug-related (two infections, MAS, gastroenteritis, hypopyon, vertigo) [4–6, 10, 11].

A number of studies on NOMID published before official treatment authorization were in line with the depicted safety framework [13]. Overall, general concern was about painful weekly injection site reactions imposed by the short

half-life of this biomedicine, indicated in children for permanent treatment of genetically determined diseases, where relapses could be experienced within 3–5 days from therapy discontinuation.

51.4 Off-Label Experience

Most of the off-label observations consist in small cohort studies and case reports focusing on non-RA rheumatic disorders, non-NOMID CAPS, chronic inflammatory diseases, and some severe cutaneous disorders. Moreover, some ongoing trials are investigating efficacy and safety of anakinra in cardiovascular and cerebrovascular disorders (4), Type 1 and 2 diabetes (6), cancer (3), JIA/JCA (2), polymyositis/osteoarthritis/joints (3), pain (2), and single investigations on amyotrophic lateral sclerosis, and on Behçet's, Still's and Sjögren's syndromes [10, 14].

Experience on Familial Mediterranean Fever (FMF), Muckle-Wells Syndrome (MWS), DIRA, and more recently on TNF Receptor-Associated Periodic syndrome (TRAP), have confirmed a safe profile. In some experiences, anakinra was effective in cases resistant to conventional therapies and to other biomedicines.

In *FMF* studies, among 30 cases treated with anakinra reported in the literature, painful injection site reactions were frequent; individual cases of interstitial pneumonia, neutropenia, haemophilus bronchitis, and viral diarrhea were also reported. One venous catheter infection and one hypertension were observed in the first case of kidney transplant FMF recipient [15]. In one case of severe FMF associated with amyloidosis and Behçet's disease, treated for 1 year with anakinra and colchicine, all disease signs improved, including proteinuria, and no significant AEs were reported. However, after 18 months of treatment proteinuria gradually increased, indicating that progression of kidney disease was less controlled than FMF attacks or mucocutaneous Behçet's type lesions [16].

In *MWS* experience on pediatric (5) and adult (7) patients, treatment with anakinra was considered safe, and was in line with previous experiences. Mild injection site reactions (42 %), mild infections (42 %), hyperactivity and weight gain (33 %) were observed. Hyperactivity was an unusual event observed in four children and was ascribed to treatment-related improvement of patient's energy. No serious events occurred during the study period. However, a dosage increase caused more local discomfort and pain at injection site in some children [17].

In seven *TRAP* patient records in the literature, injection reactions (71 %), 1 case of bronchopneumonia and one of pharyngitis were observed [18]. In a family of 15 members affected with TRAP, three patients received anakinra with no benefit and with strong injection site reactions, limb swelling, skin induration and fasciitis progressing down the limb. CRP raised in all patients. One patient developed concomitant multiple sclerosis [19].

Experience in 11 *DIRA* patients showed transient injection-site reactions in three of them, one anaphylactic reaction, and infections (bacterial cellulitis, pneumonia, and joint bacterial) in one case. Notably, in one patient treatment

discontinuation after 4 years of therapy led to a relapse and to the subsequent remission after therapy reconstitution [20–22].

After long-term treatment of *Still's disease* in 28 adult-onset patients (followed for 23 months), all of them showed mild injection site reactions and two of them had a SAE (severe rash at injection site) causing therapy discontinuation.

No severe infections were observed [23]. Recently, cases of Still's disease with serious complications have been positively controlled by anakinra without drug-related concerns. One case of suppurative necrotizing granulomatous lymphadenitis, and one case of life threatening parvovirus B19 infection and MAS in a suggestive Still's disease were successfully treated with anakinra, without reported AEs [24, 25].

In a double blind randomized trial, 26 patients with *Sjögren's syndrome* (13 exposed) suffering severe fatigue were treated with anakinra for 4 weeks, with partial response. The study was based on the assumption that increased levels of IL-1β in CNS fluids could be responsible of severe fatigue. Injection site reactions were reported in 54 % of patients in study, and in two subjects of the placebo group (15 %). Three cases of transient neutropenia, and two serious events (injection site reaction, which caused discontinuation and hospitalization for pyrexia, malaise and persistent skin changes; and one case of gastroenteritis) occurred. In the placebo arm, chest pain, diarrhea and neutropenia (one patient each) were recorded [26].

Experience in *Schnitzler's syndrome*, an autoinflammatory disorder characterized by chronic urticaria and paraproteinemia (usually IgM), is based on about 100 case reports. In a recent review on 26 patients, 20 had monoclonal IgM, one had polyclonal IgM, and three had monoclonal IgG gammopathy. Three patients developed Waldenstrom's disease, an event that is present in about 15 % of this class of patients, and no other relevant AEs were reported [27]. In a recent case associated with IgM gammopathy complicated by chronic pancreatitis, diabetes mellitus, peripheral neuropathy, a history of HCV hepatitis, and a family history of recurrent pancreatitis, anakinra was effective and no significant adverse events were reported, except for the expected injection site reactions. Interestingly, recurrent attacks of pancreatitis were also resolved by this therapy [28]. In another case of Schnitzler's disease associated with IgM gammopathy, anakinra was used daily as first-line therapy for 3 years in the absence of any side effects [29].

Finally, anakinra has been used in two patients with severe *delayed-pressure urticaria*, a disorder resistant to a number of treatments, including antihistamines, without adverse consequences [30].

A recent overview identified seven cases of a rare form of *cytophagic histiocytic panniculitis* (CHP) and/or *hemophagocytic lymphohistiocytosis* (HLH) treated with anakinra. These forms are often associated with infections, rheumatologic disorders including SLE, systemic onset JIA, SS, Sjögren's syndrome, or with lymphocytic malignancies. One case of CHP and 6 cases of severe life-threatening HLH developing MAS were controlled by therapy in the absence of relevant AEs [31]. In contrast, cases of MAS and HLH were observed in Still's disease and JIA after short-term anakinra treatment, suggesting some caution in such uncontrolled

therapeutic attempts [32, 33]. Noteworthy, a supportive study in 86 JRA receiving anakinra for 28 weeks was included in the initial application for the indication of RA treatment. A subset of these patients was treated in an extension study for one year. The study resulted in insufficient positive response to treatment, and therefore the sponsor did not request the indication for JRA and the indication was not recommended [1, 4].

Experience on some serious cutaneous disorders is even more anecdotal.

Single case report on *acrodermatitis continua of Hallopeau* showed an initial transient hypertriglyceridemia, but no other adverse effects from anakinra [34].

One patient with *pyoderma gangrenosum* in PAPA syndrome (pyogenic arthritis, pyoderma gangrenosum and acne) with elevated levels of IL-1 treated for 6 months with anakinra remarkably improved. Interestingly, no relapse was observed up to 3 months after therapy discontinuation, and no AEs were observed during the study [35].

Experience in *gout*, and in particular in acute attacks, is favorably considered because anakinra has the shortest half-life (4–6 h) among the IL-1β antagonists/blockers, and therefore can be used on demand, thus limiting treatment-related discomfort. In one open-label study, 10 patients resistant to conventional therapy and showing significant comorbidities were treated with three daily doses. No treatment-related side effects were observed during therapy and there were no infectious complications. However, the study excluded any patient who had proven or clinically suspected active infections [36, 37]. Similar data were reported in another study on three cases of acute gout. Injection site reactions were rare. No serious side effects were encountered. However, candidate patients at risk of infection were excluded also from this study. Overall, no infectious complications have been described for the use of anakinra in gout [38].

A double blind trial on 70 patients with *Type 2 diabetes* (34 treated daily for 13 weeks), subsequently observed for 39 weeks after treatment, reported injection site reactions in 50 % of treated patients in the first part of study, and none in the placebo group. Infections (UTI, URTI) in study group were transient and mild. AST elevations occurred in one treated patient. Interestingly, the beneficial effects of treatment on endogenous insulin production and on inflammation parameters were prolonged during the follow-up period without therapy, in the absence of any additional adverse events [39]. In another study assessing insulin sensitivity in 19 obese nondiabetic patients with metabolic syndrome treated with anakinra for 4 weeks (13 completed the study), 12 subjects had injection site reactions, which caused treatment discontinuation in two of them, and one subject had to withdraw because of infection (influenza). No other adverse events were reported [40].

Finally, one case of preterminal renal failure in a transplanted recipient with gouty polyarthritis treated with anakinra for 5 days, followed by discontinuation and re-administration for 15 days because of relapse, showed neutropenia and deterioration of renal function requiring resumption of hemodialysis. No infections were observed. This single report in the literature, and the observation of significant elevation plasma concentration of anakinra in patients with renal impairment

observed in PK/PD studies, suggest caution in treatment of such patients with anakinra [41].

51.5 Postmarketing Surveillance

By the end of 2012 there were 1,376 reports in the FAERS describing 4,186 AEs (3.0 AEs/P). The most common events included injection site reaction (12 %), cutaneous reactions (6 %), and infections (5.4 %). Ten cases of anaphylactic reactions were reported.

Infections included 17 cases of pneumonia, 16 cases of sepsis, and 10 cases of septic shock. Only one opportunistic infection was registered. Notably, 46 cases of hematophagic histiocytosis were also reported.

Malignancies reporting included 11 cases of malignant melanoma, 10 cases of HL, and 3 cases of NHL.

In the EUV database, 508 reports (483 serious) included 1,441 AEs (2.8 AEs/P). Most common events were infections (14.6 %), cutaneous (9 %), respiratory (7 %), nervous (5.6 %), and gastrointestinal disorders (5.2 %). In particular, 27 injection site reactions (1.7 % of reported AEs), 19 hypersensitivity cases (one drug-related), and 12 anaphylactoid reactions were recorded. Most common infections included pneumonia (18 cases), sepsis (17), septic shock (5), and TB (4). Moreover, 23 cases of hematophagic histiocytosis were registered. Among malignancies, 10 cases of HL were the most common reported events. Eight cases of renal impairment/failure were also reported.

51.6 Remarks

Anakinra has been used in over 150,000 patients in a wide spectrum of diseases and still leads the IL-1 blockers group of biomedicines, with an overall good safety profile. The major concern is about injection site reactions, which are frequent and painful. In fact, the short half-life and an affinity approximately equivalent to endogenous IL-1 of this biomedicine demand frequent injections and sustained dosage, since treatment discontinuation usually is followed by immediate relapse of most symptoms. Therefore, injection reactions, although rarely reported as severe, are of particular discomfort especially, in children. Moreover, the assessment criteria adopted in different case reports are difficult to compare. Pain is a consequence of IL-1 activity related to the peripheral production of nitric oxide, prostaglandins, and other factors at periphery, or to a direct production of IL-1 by microglia and astrocytes. Other immune factors, such as TNF and IL-6, may induce hyperesthesia. Nonetheless, the remarkable experience with anakinra in a number of pathological conditions stresses the major role exerted by IL-1 in causing this crucial symptom.

Experience of most studies in pediatric age is limited to small cohorts and case reports, due to the rarity of the investigated diseases. Overall, anakinra has accumulated a long experience, yet not in long-term and controlled studies, except for the treatment of RA.

Infections during treatment are frequent, especially in children <12 years of age, although not significantly higher than in controls, and usually resolve without treatment discontinuation. Serious infections are in the range of 2–3 %; they do not seem to increase over time and are mostly limited to the respiratory system (pneumonia is the most concerning event).

However, a general increased risk of infections has been attributed to the need of temporary dose elevations of anakinra due to the occurrence of active infections (not necessarily induced by the treatment) when patients developed flares.

Due to the lack of consistent long-term studies in non-RA patients, the risk for malignancies is still difficult to assess. In RA patients the incidence of lymphomas is increased and raises concern. In these patients anakinra, as other biomedicines employed in RA, may be acting as a supportive cause in a population already at risk for malignancy related to the underlying disease. In other experiences in pediatric age, the present data do not indicate an increased risk of malignancies. However, they are too limited and fragmentary to exclude the existence of such risk in long-term treatments, especially if moderate. Alternatively, preliminary epidemiologic data show an association between IL-1 expression and cancer progression, which may imply a potential higher risk for malignancies in all conditions where IL-1 and/or other proinflammatory cytokines are overproduced [12].

Long-term observation is also needed for a better evaluation of renal impairment and for tuning dosing management in long lasting therapy with anakinra. In fact, plasma clearance in mild/moderate renal insufficiency is reduced by 70–75 %, and renal damage related to some underlying conditions may be aggravated [41], or not ameliorated in the long run, in spite of an initial transient beneficial effect [16].

Neutropenia is the major representative of hematological toxicity. Although not particularly frequent or severe during treatment with anakinra, it can be considerably increased in combined therapy, as observed with etanercept and in some off-label case reports.

Although immunogenicity was detectable in almost 50 % of cases in RA patients, no data are available in NOMID and in other off-label investigations. However, hypersensitivity reactions, anaphylaxis, angioedema, and anaphylactoid reactions were occasional in studies, but not so infrequent in postmarketing settings. Contradictory observations are related to the insurgence and control of MAS obtained during anakinra pediatric administrations. While MAS was observed in association with anakinra treatment [4–6, 10, 11, 32, 33], in other experiences this biomedicine seemed to be beneficial on previously acting MAS [24, 25, 31, 33]. In the former case, MAS was postulated to rather represent an insufficient control of the underlying inflammation than a drug-related event [42].

Elucidation of this controversy seems crucial to confirm adequacy of anakinra treatment, particularly in pediatric in- and off-label indications.

Taken together, anakinra offers a safe profile in standard therapy up to a few years observation, but need confirmation for long-term treatments, especially in pediatric age and for rare in- and off-label disorders. Therefore, international registries and postmarketing surveillance are the crucial tools to confirm long-term safety.

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Interferons

52

The group represents the first relevant clinical drug development among cytokines, and include the natural and recombinant forms of three molecular classes. Interferons are indicated for a wide spectrum of disorders including the treatment of HBV and HCV hepatitis, hairy cell leukemia (HCL), cutaneous T cell lymphoma (cTCL), follicular non-Hodgkin's lymphoma (FL), chronic myelogenous leukemia (CML), malignant melanoma (MM), renal cell cancer (RCC), and multiple sclerosis (MS). A variety of products have been marketed since 1986. In particular:

IFN-α2a: as Roferon® (Hoffman-LaRoche) was approved by FDA in 1986 for the treatment of HCV chronic active hepatitis, RCC, HCL, cTCL, CML, and AIDS-related Kaposi sarcoma (KS). A pegylated form of IFN-α2a (Pegasys®, Hoffman-La Roche) was approved in 2002 for the treatment of HCV and HBV chronic active hepatitis.

IFN-α2b: as Intron A® (Shering-Plough) was approved in 1986 for the treatment of HCL, MM in 1995, FL, KS, for condiloma acuminata in 1988, HCV hepatitis in 1991, and HBV hepatitis in 1992. A pegylated form of IFN-α2b (Pegintron®, Shering) was approved in 2001 for HCV hepatitis, and another formulation of the same product (Sylatron®, Shering) was approved in the same year for the adjuvant treatment of malignant melanoma.

IFN alfacon-1: as Infergen® (Boerhinger) was approved in 1997 for the treatment of HCV hepatitis. IFN alfacon-1 is a synthetic Type I interferon derived by scanning the sequences of natural interferon alpha subtypes and by constructing a corresponding DNA sequence inserted in *Escherichia* coli.

IFN-β1b: as Betaseron® (Chiron, Bayer) was approved in 1993; Avonez® (Biogen) was approved in 1996, Rebif® (Ares Serono) was approved in 2002, and Extavia® (Novartis) was approved in 2009, all for the treatment of the relapsing forms of MS.

IFN-γ1b: as Actimmune® (InterMune) was approved in 1999 for the treatment of chronic granulomatous diseases. In 2004, the indication was extended to malignant osteoporosis [1–9].

EMEA approved Betaseron in 1995, Avonex in 1997, Rebif in 1998, and Extavia in 2008, all for the treatment of MS. Infergen was approved in 1999 for

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HCV hepatitis and withdrawn in 2006 upon request of the manufacturer. Intron A was approved in 2000 for the treatment of HBC and HBV chronic hepatitis, HCL, FL, CML, melanoma, carcinoid tumor, and multiple myeloma. Another recombinant formulation of IFN-α2b (Viraferon®, SP Labo N.V.) approved in 2000, was withdrawn in 2008 upon request of the manufacturer. A biosimilar formulation of IFN- β2a (Alpheon, Biopartners GmbH) was rejected in 2007.

A natural *human leukocyte-derived IFN-αN3*, as Alferon N injection® (Interferon Sciences) was approved by FDA in 1989 for intralesional treatment of refractory external condyloma acuminata, and in 2012 in Argentina under the name of Naturaferon®, Hemispherx Biopharma) for the same indication.

A natural *multi-subtype leukocyte-derived IFN-α* as Multiferon® (Sobi, Viragen) consisting of six mixed subtypes is used in a number of other countries (Sweden Chile, Philippines, Mexico, Bulgaria), for the treatment of selected viral infections and in adjuvant treatment of cancer, including melanoma. The product is recognized as orphan drug in some countries, and was investigated in one trial (NCT01171209) for the treatment of MS. At present is under investigation in two controlled trials (NCT01341158, NCT01387763) for the treatment of MM and CML, respectively. It is believed that natural interferons increase therapeutic response rates and result in a lower incidence and intensity of adverse events compared with recombinant interferons. However, this product is not approved in USA, Europe and Canada.

Additional formulations of recombinant IFNs have been approved in other Countries, such as the IFN-β1 biosimilar Cinnovex™ (CinnaGen) and Ziferon produced by the Center of Pharmaceutical Products in Iran, Reiferon® and Reiferon Retard® (Rhein-Minapharm) in Egypt, and Peg-IFN-α2b (Pegetron®, Shering) in Canada, for combined treatment of HCV hepatitis with ribavirin. New formulations of IFNs, such as pegylated and extended release products (Locteron®, OctoPlus), oral interferon, IFNω, IFNλ, are under investigation.

Overall, the safety profile for major IFN classes in therapy is based on over 20 years observations in controlled studies and postmarketing surveillance. Within this framework, variations in the AEs occurrence are present for each pharmaceutical product investigated with different protocols for specific underlying disorders. Therefore, a *standard IFN safety profile* based on pivotal clinical trials and on major postmarketing information will be depicted, and most relevant differences observed in specific investigations will be underlined.

The following general safety profiles are depicted on the basis of the most representative therapeutic formulations.

52.1 Alpha Interferons

In this class, two main IFNα products (Roferon®, Roche IFN-α2a; Intron® A, Merck IFN-α2b), and two pegylated forms (Pegasys®, Roche IFN-α2a; PEG-Intron/Sylatron, IFN-α2b) have been widely used. Standard dosages of the same IFN type differ in the various commercial products, according to their specific indications.

In particular, Roferon is indicated for HCV and HBV chronic active hepatitis, RCC, cTCL, FL, CML, and AIDS-related Kaposi sarcoma; Pegasys is indicated for HCV and HBV chronic hepatitis; Intron A is indicated for the treatment of HCL, MM, FL, Kaposi sarcoma, HCV and HBV chronic hepatitis, and condylomata acuminata; Peg-Intron is reserved for the treatment of HCV hepatitis.

Pivotal study on IFN-α2a (Roferon) was a Phase III Study NV14524 on 422 HCV patients treated with 6×10^6 units (MIU) for 3 months followed by 3 MIU for 3 or 9 months. Supportive studies were N3414 (B-154′511) and N3505 (B-154′512).

Pivotal studies for the pegylated IFN-α2a (Pegasys) were the Phase II NV15495 Study (271 patients) and two Phase III trials (NV15496, NV15497) for a total of 1,441 subjects with HCV chronic hepatitis treated for 48 weeks and followed for additional 24 weeks.

Pivotal study for IFN-α2b (Intron A) was the GELF trial on 273 FL patients treated either with CHVP chemotherapy (135), or with CHVP + 5 MIU for up to 18 months.

Pivotal study for Peg-IFN- α2b (PEG-Intron/Sylatron) was Phase III comparative study C/I97-010 on 1,224 patients with HCV chronic hepatitis treated with three different doses of pegylated (917) versus one dose (3MIU) of non-pegylated (307) IFN- α2b for 48 weeks and followed for additional 24 weeks.

The general safety profile of the IFN-α class includes, *neuropsychiatric disorders*, *hypersensitivity reactions, cardiovascular, cerebrovascular and ischemic disorders*, *gastrointestinal and hepatic disorders, infections/pyrexia, bone marrow toxicity*, *endocrine disorders, pulmonary disorders ophthalmic disorders, and pancreatitis.* In addition, a typical *flu-like-syndrome* (FLS) usually follows all types of IFN administration. Within this framework, variations in their occurrence or exacerbation of preexisting comorbidities are present.

Flu-like syndrome (FLS) is the most common encountered event in patients treated with IFN-α in controlled studies and in the postmarketing experience (see Chap. 3). It occurs within 2–4 h after IFN-α administration whatever the treatment indication, and usually lasts 4–8 h. The general profile, as obtained mostly from studies in HCV hepatitis patients includes fatigue (58 %), myalgia/arthralgia (51 %), pyrexia (28 %), chills (23 %), asthenia (6 %), sweating (5 %), leg cramps (3 %), and malaise (1 %).

FLS severity is dose-dependent, with pyrexia exceeding 40 °C and other systemic symptoms affecting over 60 % of patients treated with highest doses. Overall, no differences were noted depending on the type and formulation of marketed IFN-α.

In CML patients, signs appeared at higher rates as pyrexia (92 %), asthenia or fatigue (88 %), myalgia (68 %), chills (63 %), arthralgia/pain (47 %), and cephalea (44 %).

Neuropsychiatric disorders are the overall most relevant disorder. In HCV studies they occurred as depression (16 %), irritability (15 %), insomnia (14 %), anxiety (5 %), and behavior disturbances (3 %), including suicide and psychotic aggressive disorder.

Overall, the psychiatric events appeared related to IFN dose (9 % with 6 MIU; 6 % with 3 MIU), which influenced also the discontinuation rate (11 % with 6 MIU; 7 % with 3 MIU).

In HCL patients cephalea (64 %), dizziness (21 %), depression (16 %), sleep disturbance (10 %), decreased mental status (10 %), paresthesia (7 %), anxiety (6 %), lethargy (6 %), visual disturbance (6 %), and confusion (5 %) were observed. In the pivotal study with Peg-Intron one case of suicide was observed in the study group. Experience with Sylatron in similar studies showed depression (59 vs. 24 % in control), and severe depression (7 vs. < 1 %).

In CML patients the profile was similar and included cephalea (44 %), depression (28 %), decreased mental status (16 %), dizziness (11 %), sleep disturbances (11 %), paresthesia (8 %), involuntary movements (7 %), and visual disturbance (6 %). In experiences with the respective pegylated forms the overall profile did not show significant differences.

Similarly, in FL patients depression (9 %), suicide (2 vs. 0 % in controls), and paresthesia (13 %) occurred more frequently.

Gastrointestinal disorders were frequent as nausea/vomiting (33 %), diarrhea (20 %), anorexia (14 %), and abdominal pain (12 %), in HCV patient experience, and appeared increased in CML experience as anorexia (48 %), nausea/vomiting (37 %), and diarrhea (37 %). Signs of hepatotoxicity were usually transient and moderate. However, they could aggravate hepatic conditions already compromised, especially in patients with hepatitis and cirrhosis.

Hypersensitivity and skin reactions were observed as injection site reaction (29 %), partial alopecia (19 %), rash (8–18 %), sweating (15 %), xeroderma, or pruritus (7 %), with similar incidence in HVC and CML patients. Anaphylaxis is reported as a rare event.

Pulmonary and cardiovascular disorders were more frequent in HCL and CML than in HCV patients and included cough (19 %), dyspnea (8 %) arrhythmias (1–7 %), and rarely myocardial infarction (<1 %).

Hematological toxicity signs consist mainly in neutropenia, which varies from 10 % of cases in HCV treated patients to about 70 % in HCL, followed by thrombocytopenia (4–5 %–62 %, respectively), leukopenia (1.5–45 %), and anemia (0–31 %). Intermediate values were found in similar studies on CML patients.

Autoimmune events appear of particular interest, although not frequent, including a wide range of alterations, from the induction of asymptomatic and symptomatic autoantibodies, such as ANA and anti-DNA antibodies or antibodies directed to blood components (autoimmune hemolytic anemia and thrombocytopenia), to the induction of endocrinopathies (hyper- and hypothyroidism, diabetes), Myasthenia Gravis, GBC and related autoantibodies, and of systemic autoimmune diseases (SLE, Sjögren, exacerbation of RA, psoriasis). Among these, particular concern raised the induction of autoimmune hepatitis, which can be fatal. The overall incidence is low but uncertain. However, some of these events are also found in HVC hepatitis patients in the absence of such cytokine treatments [10].

Other *uncommon events* (<4 %) included constitutional signs, nervous and cognitive disorders, cardiomyopathy, UTI, viral infections, tonsillitis, otitis, bronchitis, pulmonary infections, and coagulative disorders.

As for major differences between *standard preparations of IFNα and pegylated forms* of the same cytokines, the overall spectrum of AEs profile resulted similar, but pegylated forms showed a tendency to produce a higher number of AEs, which seemed also dose-related. In particular, neutropenia and thrombocytopenia, with an indication for an associated risk of more infectious complications during and after treatment, was reported. The risk for increased bleeding was unclear. In addition, an unusual number of deaths in the peg group, was also observed.

Withdrawals and dose modifications induced by AEs were higher during treatment with pegylated products. However, no differences emerged among neuropsychiatric events, and a lower rate of anti-drug antibodies was detected, in the order of 10 versus 15 % in pegylated versus standard forms of IFNα, respectively. Neutralizing antibodies were in the range of 1 % [5, 6].

Overall discontinuation rates ranged 15–23 %, and were mostly determined by severe neutropenia (32 %) and psychiatric disorders (7–11 %). Interestingly, most of drug-related AEs tended to resolve spontaneously or after conventional therapy, and improved after drug discontinuation.

The trend of AEs for all IFNα tended to decrease over time and the general safety profile is well established after 26 years of therapy, and brought to the release of REMS requirements for some products, as approved from FDA for Pegasys on April 2011, and for Roferon on June 2011.

52.2 Beta Interferons

In this class there are two marketed products utilizing IFN-β1a (Avonex®, Biogen; Rebif®, EMD Serono), and two IFN-β1b formulations (Betaseron®, Chiron, Bayer; Extavia®, Novartis), all indicated for the treatment of relapsing forms of MS. Betaseron was the first biomedicine to be introduced for MS treatment.

The overall safety profile was based on the following pivotal controlled trial and on additional supportive studies. In particular,

Avonex: one Phase III pivotal 301 MS patients receiving 6 MIU weekly IM injections (158 treated), and followed up to 2 years. In particular, 182 patients completed one year study and 172 completed 2 years on study. Additional information resulted in 290 patients from a database on short and long term studies with IFN-β, in other disease indications. Updated information on 2012 prescribing information reports experience on 351 MS patients, of whom 319 were treated for 6 months and 288 for 1 year.

Betaseron: initial profile was based on one controlled trial on 338 MS patients, of whom 226 were treated with 9 MIU or 45 MIU, and 112 as placebo. Additional safety data from 1,440 patients treated with a various doses of Betaseron; 277 were

MS patients and 1,163 included HIV patients (464), subjects with solid tumors (587), hematologic malignancies (66), or had condyloma acuminata (46). However, part of these cohorts (877) was treated outside controlled studies. Updated information in the 2012 prescribing information refers to 1407 MS patients treated with Betaseron (0.25 mg every other day), of whom 1,261 patients were treated over 1 year.

Extavia: the safety profile of this kit for SC use refers to the same 1,407 MS patients treated with Betaseron (0.25 mg every other day), of whom 1,261 patients were treated over 1 year.

Rebif: one Phase III trial SC daily doses in MS patients (6 MIU in 189 subjects; 12 MIU in 184 subjects; 187 placebo) for 2 years. Supportive data came from 68 MS patients from a small open-label study, from a database including 565 relapsing-remitting MS patients, and from 126 subjects with other demyelinating disorders. Data reported in the last prescribing information dated December 2012 refer mainly to the previously reported pivotal Phase III study, to the open-label study on 565 relapsing-remitting MS, and to a randomized open-label comparator study in relapsing-remitting MS patients receiving SC (339 patients) or IM injections of Rebif for 48 weeks.

The general safety profile of IFN-β includes *psychiatric disorders* (depression, suicide), *hepatic injury* including autoimmune hepatitis, *injection site reactions*, and necrosis, *hypersensitivity* reactions including anaphylaxis, *FLS*, *congestive heart failure (CHF)*, *seizures*, *leukopenia*, and other *laboratory abnormalities*. Other *autoimmune disorders* were reported in the postmarketing experience.

Overall, the *most common* reported AEs, related to IFN administration in controlled studies include *FLS* (fever, chills, cephalea, fatigue, asthenia, myalgia, anorexia), *hematological abnormalities* (lymphopenia, neutropenia, thrombocytopenia, and anemia), and *hepatic toxicity* as expressed mainly by ALT/AST elevations.

Discontinuation rates ranged from 3 to 19 % according to the study and type of IFN used. They were usually more frequent among the study groups and tended to increase with dosage.

Psychiatric disorders were mainly occurring as depression, suicidal behavior, and psychotic disorders. Suicide cases were not detected in controlled studies up to 2 years treatment (Betaseron) and observation. Suicide attempts were 4 versus 1 % in respective controls, and depression was estimated as 20 % in the study group versus 13 % among controls. However, rates on a different database of 1,532 treated patients were 0.2 versus 0.1 % in controls, and suicide attempts were 0.5 versus 0.4 %.

In Rebif initial trials, there was one suicide among controls and one accidental death (fall) in the study group, which were considered not related to the trial medication. However, there were nine suicide ideation/attempts in the study groups and four cases in placebo.

Overall, no statistical significant increase in psychiatric disorders was observed (except for somnolence in some investigations) in these studies. Moreover, in the FAERS database on approximately 11,000 reports, nine cases of suicide (0.08 %), and 35 suicide attempts (0.3 %) were registered.

Hepatic toxicity is commonly evidenced by ALT/AST elevation observed in controlled studies as over fivefold baseline values (4–12 vs. 1–2 % in controls). These abnormalities produced discontinuations (1–2 %) or dose modifications during the study. However hepatic insufficiency, autoimmune hepatitis, and hepatic failures have been rarely experienced, and were observed mainly in the presence of comorbidities and/or in combined therapies.

In a comparative controlled study on 677 MS patients treated with Rebif or Avonex hepatic functional disorders were higher in the former (18 vs. 10 %). One case of fulminant necrotic hepatitis was observed in studies with Rebif. In the postmarketing settings most severe forms of hepatic injury and failure remain rare events. In pooled FAERS data referring to over 1,00,000 reports on three major IFN-β products (Avonex, Betaseron, Rebif) hepatic failures were 0.2 % (acute 0.04 %), and hepatic enzyme elevations were reported about 1 %.

Injection site reactions after SC administration of IFN-β are more frequent (78 %) than with other types of interferons, and showed the additional peculiarity to produce skin necrosis and deep cutaneous ulcers. This severe event was observed in 4 % of cases (0 % in controls), usually within the first 4 months therapy, with a tendency to decrease over time. In a comparative study, injection site reactions, including pain, were more common in the Rebif group as compared with Avonex, although most were mild to moderate in severity. One case of necrosis and abscess, and one associated to infective lymphadenopathy were observed after treatment with the former IFN formulation.

Hypersensitivity reactions are usually rare after IFN-β treatment, as compared also to IFN-α products. One case of severe anaphylaxis was reported after 6-month treatment with recombinant IFN-β1a in a young MS patient previously showing injection site reactions from long time [11]. One case had a positive intradermal test to IFN-β1b, but not to IFN-β1a or the diluents, suggesting a specific Type I IgE-mediated hypersensitivity reaction [12], and one case of eye contact dermatitis (IFN-β collyrium) were also reported [13]. Interestingly, one suspected case of anaphylactoid reaction encountered after 1-month therapy with Rebif revealed a sensitization to a component present also in the placebo formulation [14].

FLS is the most common event (43–51 %) related to IFN-β treatment, reported in clinical studies as a rapid insurgence of associated symptoms mostly represented by pyrexia (60–100 %), and by chills (3–6 %), cephalea (30–35 %), fatigue (16–74), asthenia, myalgia (10–42 %), and anorexia. In trials with Avonex, FLS was reported in 61 % of cases compared to 40 % in controls ($p = 0.001$), and was reported in 60–100 % of cases in other studies in HCV chronic hepatitis and MS. It must be noted that in long-term studies some confounding information may originate from seasonal illness as well. The incidence and severity of FLS associated with IFN-β does not seem to be dose-dependent. In one overdose exposure

due to a suicidal attempt with IFN-β1a prefilled syringes, only a modest increase in pyrexia was observed.

Although FLS is usually expressed as mild to moderate event, and can be controlled by conventional symptomatic therapy, it is among the frequent causes of therapy discontinuation, together with severe injection site reactions.

Overall, no marked differences were noticed between the two recombinant forms IFN-β-1a and IFN-β-1b.

Hematological signs of toxicity are mainly represented by neutropenia/leukopenia (8–40 % and 3–10 % respectively), with a trend to be dose-dependent. Severe neutropenia is in the range of 0.5–1 %. Occasional cases of severe lymphopenia were also observed. Similarly, cases of significant thrombocytopenia and anemia were observed in studies employing high doses of IFN-β. Cases of bleeding, clotting in VTE/ATE were observed in study group treated with Rebif.

Cardiovascular events, including infarction and CHF are not frequent. In the pivotal Avorex trial, one cardiac death occurred in one patient with history of pre-existing disorder.

Infections are frequent (11–50 %) but usually mild to moderate, and include rhinitis, sinusitis, pharyngitis, URTI, UTI, eresypela, and viral (herpetic) infections including pneumonia and varicella pneumonia. In Rebif studies, one case of abscess and one infectious lymphadenopathy occurred. However, the overall incidence of infections, including viral and serious infections was not significantly increased over controls, and they were not associated to neutropenia.

A few *nervous disorders*, included seizures, were observed (4 cases with Avonex, 3 serious). However, they occurred only in treated subjects and were generalized, while one case in controls was localized. Some patients showed also ataxia and migraine. In MS studies overall SAEs were approximately 20–25 %, but they were mostly represented by MS exacerbations.

Autoimmune disorders were mainly involving thyroid (5 %) as hypo/hyperthyroidism, usually mild and associated with TSH movement in a smaller portion of subjects.

Abnormal visual events were observed at high dose treatment. However, on a large database of patients treated with IFN-β, over 67 registered events, seven were considered as related to the drug in study (10.4 %), and one case in a healthy subject was suspect of optic neuritis, which indicated a possible drug-related risk in non MS patients.

Finally, in a comparative *head-to-head investigation* on 677 MS patients between Rebif and Avonex, both pertaining to IFN-β1a formulations, a number of interesting differences in AEs profile emerged. In particular, several rare, but important serious adverse events observed following treatment with Rebif, some of which were life-threatening and not encountered in other IFN-β, included anaphylaxis (2), fulminant hepatic failure, Stevens Johnson Syndrome, life-threatening cardiac arrhythmia, and erythema multiforme, all considered as drug-related. The case of fulminant hepatic failure (autoimmune hepatitis necrosis) was subsequently attributed to the associated nefaxodone therapy, as the responsible factor or cofactor. Overall, two cases of anaphylaxis were registered, one already

reported [11] and one observed in another supportive study (GF6789). A suspect case of abortion was also observed after Avonex treatment. FLS and depression appeared more frequently in Avonex, while "emotional lability", liver enzymes elevation, leukopenia, and injection site reactions were more common in the Rebif arm [14].

52.3 Gamma Interferon

Actimmune® (interferon gamma-1b, InterMune) is the major marketed recombinant product of this class, which is indicated for a different set of diseases, namely in chronic granulomatous disease (CGD) and malignant osteoporosis (MO). The product is not available in Canada and in EU. However, EMEA recognized IFN-γ an orphan designation in 2011 for Friedrich's ataxia, an inherited mitochondrial disease. Experiences in idiopathic pulmonary fibrosis in a controlled trial (INSPIRE) were unsuccessful and the study was stopped in 2007. Another formulation of the same IFN-γ1b (Imukin®, Boehringer) is available in about 20 countries for the treatment of CGD, including some European and South American States, Australia, and New Zealand [15, 16].

The mechanism of action in these diseases is unclear and may be related to macrophage activation and increase of superoxide production by granulocytes and monocytes. In the case of Friedrich's ataxia, an enhancing effect on mithocondrial frataxin production is presumed, with a relief effect on major symptoms.

Major warnings for Actimmune include *cardiovascular disorders, neurologic disorders, bone marrow toxicity,* and *hepatic toxicity*.

Pre-existing cardiac relevant conditions ischemia, CHF, arrhythmia) may be exacerbated by high doses.

Similarly, *psychiatric disturbances* and *seizures* are increased or exacerbated by high dose treatment.

Bone marrow toxicity is mainly expressed by neutropenia, and thrombocytopenia.

Hepatic toxicity is revealed by AST/ALT consistent elevations (up to 25-fold), with a trend to be more consistent in children <1 year of age.

The adverse effects related to FLS symptoms, as pyrexia, chills, dizziness, and cephalea, usually tend to decrease in severity over time.

Experience in CGD controlled study (63 treated, 65 controls) indicated an increase in study group versus controls of pyrexia (52 vs. 28 %), cephalea (33 vs. 9 %), rash (17 vs. 6 %), chills (14 vs. 0 %), injection site reactions (14 vs. 2 %), fatigue (14 vs. 11 %), vomiting/diarrhea (13–14 vs. 5–12 %), nausea (10 vs. 2 %), abdominal pain (8 vs. 3 %), myalgia (6 vs. 0 %), depression (3 vs. 0 %), arthralgia (2 vs. 0 %), and lumbalgia (2 vs. 0 %).

Similar safety data were observed in 24 MO patients.

A number of additional AEs were observed in studies with Actimmune generally administered at higher doses (>100 ug/m^2) in other, non indicated *off-label diseases*.

In particular, serious cardiac disorders (infarction, arrest, failure, tachyarrhythmia), nervous disorders (parkinsonian symptoms, TIA), gastrointestinal (hepatic insufficiency, gastrointestinal bleeding, pancreatitis-some fatal-), hematological (DVT and pulmonary embolism, neutropenia, thrombocytopenia), immunological (autoantibodies, LLS), respiratory (bronchospasm, interstitial pneumonitis), renal insufficiency, and exacerbation of dermatomyositis, were the most significant.

From these observations a potential role in inducing or exacerbating autoimmune disorders emerged. In fact, neutralizing anti-IFNγ antibodies could be raised and may be involved in cases of resistance to therapy [17], and in infection dissemination [18]. Moreover, a possible enhancing hemato-toxic effect of IFNγ on severe neutropenia and thrombocytopenia when associated to chemotherapy, was also observed [19].

Altogether, the long-term treatment of IFNγ in CGD confirmed a tolerable safety profile up to 9 years, with reasonable rates of serious infections (0.3–0.4 % P/Y) and mortality (1.5 % P/Y) which were lower than in placebo, and showing no abnormalities in normal growth and development of patients [20].

Recently, the association of IFN-γ with ribavirine and IFN-α, as combined therapy in 49 HBV chronic hepatitis patients previously resistant to IFN-α, allowed AEs evaluation before and after IFN-γ administration up to 48 weeks. Forty-one patients had mild/moderate AEs and one serious neutropenia in the first phase with IFN-α2a + ribavirine. However, two serious events (grade 4) were present among previous non-responders during triple therapy, and the number of AEs was slightly increased with a trend of a grading in severity. After therapy discontinuation the number of events was reduced to about 10 %. No new signals were observed [17].

52.4 Remarks

Overall, a drug class safety profile can be depicted for IFNs, with a number of minor differences in frequency rates among different interferon subtypes and product formulations. *FLS, psychiatric disorders, injection site reaction, hypersensitivity, bone marrow toxicity, infections, hepatic disorders, and autoimmune phenomena*, summarize the core of IFN-related AEs. More similarities exist within recombinant Type I (IFNα, IFNβ) dependent reactions, as compared to Type II (IFNγ), although no comparative studies were undertaken and differences in underlying diseases and age of treated populations may act as confounders. However, the recent limited experience of combined therapy with IFN-α and IFN-γ did not show significant synergism in AEs induction, as well as in efficacy.

While most common disorders (FLS, neutropenia, infections) are usually mild/moderate and manageable, more concern raises the less frequent but relevant depression, psychotic, and suicidal behavior, which appears as a common trait during IFN therapy. This is the case also for cardiovascular, hepatic, and hematologic abnormalities, which are rarely serious, but can be fatal. In all these

situations, an accurate evaluation of present comorbidities and their therapeutic control is essential to their prevention.

An intriguing AEs expression consisted in severe injection site reactions characterized by induration, indurated erythema, and *ulcerated cutaneous necrotic lesions* with some IFN formulations in a limited number of cases, and in particular with IFN-β2b. The mechanism of these rare events (about 30 cases in the literature) is unclear, but indicates the initiation of immune-mediated events leading to local thrombo-vascular inflammatory lesions, or perivascular dermatitis, or panniculitis [21]. The lesions usually appear after long-term treatment and heal after discontinuation or even after shifting administration at different sites. Some of these features remind a Type III hypersensitivity local reaction, such as Arthus reaction, although presumably mediated by local cytokine imbalance, more than by antigen–antibody complex inflammatory response. Interestingly, more rare cases of similar injection reactions have been described after IFN-α administration, where the thromboembolic pathogenesis appeared predominant [22, 23].

Information on the immunogenicity of recombinant IFNs is limited, although cases of *hypersensitivity, anaphylaxis, allergic contact dermatitis*, were reported, possibly at higher frequency after IFN-α administration.

Neutralizing antibodies were not frequently searched and reported, but in some experiences they were present as 12–38 % of patients treated for 2–3 year at a higher frequency with subcutaneous IFN-β1b compared with subcutaneous IFN-β-1a or intramuscular IFN-β1b. However, in other experiences they were found to cross react. In one patient, an intradermal test resulted positive to IFN-β-1b but not to IFN-β-1a or to the diluents, suggesting a specific Type I hypersensitivity reaction [24].

The overall relevance of neutralizing antibodies on efficacy is considered also limited, although in studies on MS they seemed to play a significant role in relation to disease activity and worsening.

The presence of *autoantibodies* has been observed after IFN-α and IFN-β treatment, apparently with a higher frequency in the former, although less investigated with the latter drug. *Autoimmune hepatitis* is the most serious encountered event, but *thyroid dysfunction* often associated with specific autoantibodies, was more frequently encountered, among other anecdotal reports on organ-specific autoimmune disorders. *Exacerbation of pre-existing autoimmune diseases*, such as psoriasis, sarcoidosis, vasculitis, and SLE, were also reported.

The experience with IFN-γ is more limited, but the safety profile is similar. In some off-label experiences, additional AEs were encountered with an overall accentuation of exacerbation of underlying autoimmune disorders, and in resistance to therapy associated to neutralizing antibodies, but in the absence of new AEs signals.

Finally, a particular role of AEs in IFNs history was related to approvals of various IFN subtypes as new drugs. According to FDA rules for orphan designation, the structural differences between the IFN-β1b products (Betaseron®, Avonex®, Rebi®) consisting only in minor amino acid sequences and glycosylation were not enough to consider them as different drugs, and therefore the Agency stated that

only clinical data would have to prove the diversity. Avonex treatment was found to have a significantly different and superior safety profile with regard to the incidence of injection site skin necrosis, and was thus concluded to be a different drug from Betaseron for orphan drug marketing exclusivity purposes [14].

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Hemopoietic Stimulatory Factors 53

Hemopoietic stimulatory growth factors encompass a series of soluble glycoproteins produced by cellular and stromal components of bone marrow constituting a sort of functional differentiation "niches" for all blood cell lines. Alternatively, some factors are produced outside bone marrow microenvironment, such as in kidney for erythropoietin. These substances are active at very low concentrations in inducing proliferation and maturation at all stages of cellular development, from uncommitted stem cells to final functional stages, either directly or by synergistic action and stimulation of additional growth factors. However, as long as the differentiation process irreversibly commits the precursors to any given cell lineage, the inducer factors become less redundant and linear specific. This complex network of cytokines tightly regulates the whole differentiation process with an extraordinary ability to rapidly increase the production of each hemopoietic cell line from a few pluripotent or precommitted stem cell stages up to several-fold the amount of differentiated cells with increased demand.

So far, clinical applications of these cytokines, experienced since 1989, have succeeded to support erythropoiesis, granulopoiesis, and megakaryocytopoiesis in various pathological conditions, while applications of factors acting on pluripotent stem cells are at early stages of development [1–3].

53.1 Erythropoietins and Epoetins

Erythropoietin (EPO) is the main differentiator factor of red blood cells. Its secretion is regulated by a renal feedback system, which senses the level of oxygen saturation in blood and consequently modulates erythropoietin production. The main molecular sensor, the hypoxia-inducible factor (HIF-1), is a heterodimeric

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transcription factor (HIF-1α, HIF-1β) of the HIF family that enhances the hypoxia inducible gene expression, such as vascular endothelial growth factor (VEGF) and EPO. The HIF-1β component is constitutively expressed, while HIF-1α under normal oxygen conditions is rapidly degraded by the von Hippel-Lindau ligase (VHL). During hypoxia HIF-1α is not degraded, thus allowing accumulation and heterodimerization into the active transcriptional factor.

EPO is a heavily glycosylated protein primarily expressed in renal tubular fibroblasts, and in liver, lung, spleen, brain, and testis, but their production is not sufficient to balance renal EPO insufficiency, as experienced in chronic kidney disease (CKD). The molecule consists of 165 aminoacids and four glycans expressed in different circulating isoforms. After cleavage of a small fragment during secretion, EPO binds to its receptor (EpoR), another homodimer member of the cytokine receptor family, situated on the surface of committed erythroid progenitors. Upon binding, EpoR changes its conformational state and, after internalization and degradation of the complex, activates the JAK2 and STATS5 downstream pathways, which inhibit the apoptosis of the erythroid precursor, thus allowing its maturation. EPO levels, which may increase of over 100-fold the baseline during anemia/hypoxia are controlled also by negative feedbacks mediated by phosphotyrosines associated with EpoR cytoplasmic domains, which prevent overactivation leading to erythrocytosis [4].

Among non-hematologic additional functions, erythropoietin elicits protective effects on various cell types through initiation of survival signals, including neurons and neural cells, via an attenuation effect on pro-inflammatory cytokines and by promoting repair by various mechanisms including angiogenesis, neurogenesis, and plasticity [5, 6]. In association with myeloid and stromal growth factors, GM-CSF and IL-3, EPO induces the formation of erythroid colonies in vitro, called burst forming units-erythroid (BFU-E), which differentiate into CSF-E, and eventually differentiate into mature erythrocytes showing a progressive increase of EPOR. The growth of BFU-E is promoted also by SCF, E-CSF, IL-5 stimulating stromal bone-marrow cells, IL-4, IL-11, and IL-9. By contrast, BFU-E formation is inhibited by several chemokines (MIP-1α, MIP2α, PF4, MCAF, IL-8, etc.).

Epoetins (rhEPOs) are recombinant molecules with the same aminoacid sequence and glycosylation sites of endogenous erythropoietin. Aminoacid changes are indicated by a prefix (*darb*-epoetin) and glycan diffrences by Greek letters (α,β, etc.). Therefore, the original recombinant Epoetin-α, and Epoetin-β show differences in their N- and O-glycans. These slight differences from the natural molecule do not have consequences on their function, but may affect the pharmacokinetics and/or immunogenicity. A genetically modified erythropoietin, called novel erythropoiesis-simulating protein (NESP), or darbepoetin-α (ARANESP), containing five mutated aminoacids to allow additional carbohydrate side chain addition, was developed to prolong the half-life (24–26 h) without functional modifications. Recently, a number of additional variations have been produced in the glycosylation of biosimilar epoetins, which have been approved by EMEA as epoetin equivalents.

Epoetin-α (Epogen®/Procrit®, Amgen, Ortho) obtained FDA approval in 1989 for the correction of anemia in adults with chronic renal failure (CRF). The indication was subsequently extended to treatment of anemia in patients with chronic kidney disease (CKD), in dialysis or not, in zidovudine treated HIV-infected patients, and in concomitance with myelosuppressive chemotherapy. Epoetin treatment was granted also to reduce the number of allogeneic RBC transfusions after non-cardiac, non-vascular surgery.

Additional studies (EPO-8702, 8905, 9002, 9118) for a total of 128 pediatric patients were submitted in 1997 for the extension to pediatric patients for correction of anemia and reduction of transfusion requirements in CRF children with the same adult indications. Both efficacy and safety profiles were very similar to that of adults.

The initial safety analysis was based on 335 CRF patients (200 treated) for the Procrit® formulation. The subsequent experience was essentially based on three large controlled trials including NHS (normal hematocrit study) trial on 1265 CKD patients on dialysis with documented CHF or ischemic heart disease, the CHOIR trial on 1,432 Epoin naive CKD patients with anemia not undergoing dialysis, and the TREAT study on 4,038 CKD anemic patients with Type 2 diabetes not on dialysis, treated with darbepoetin [7–11].

A BBW in the last prescribing information included the risk of *myocardial infarction*, *stroke, venous thromboembolism* (VTE), *thrombosis* of vascular access, *tumor progression or recurrence* in neoplastic patients, and *death*.

Additional relevant AEs included *hypertension*, *seizures*, *pure red cell aplasia* (PRCA), and *serious allergic reactions*.

This general safety profile varies, mainly in frequency, according to the underlying disease in study, and the most relevant differences are reported. Overall 148 patients were treated with Epoetin and 96 were treated with placebo.

The *safety experience on CKD* included 244 patients in dialysis, and 210 patients not in dialysis. The most common events included hypertension (28 % vs. 12 % in placebo), arthralgia (16 % vs. 3 %), muscle spasm (7 % vs. 6 %), pyrexia (10 % vs. 8 %), dizziness (9.5 % vs. 8 %), URTI (7 % vs. 5 %), and thrombosis (3 % vs. 1 %). Vascular access occlusion and clotting during dialysis occurred in about 8 % in the study group and 2–4 % in controls. Less frequent events were erythema and myocardial infarction (about 1 % each) occurring only in CKD patients not on dialysis.

Experience with Epoetin in *zidovudine-treated HIV patients* derived from 297 subjects (144 treated for 12 weeks). The most common events included pyrexia (42 % vs. 34 % in placebo), cough (26 % vs. 14 %), rash (19 % vs. 7 %), injection site reactions (7 % vs. 4 %), urticaria (3 % vs. 1 %), pulmonary embolism and respiratory tract congestion (1 % each in the study group).

The safety profile in *cancer patients undergoing chemotherapy* was based on 333 patients (168 treated for 16 weeks), and included vomiting/nausea (20–35 % vs. 16–30 % in placebo), myalgia/arthralgia (10 % vs. 5–6 %), stomatitis (10 % vs. 8 %), cough 9 % vs. 7 %), weight decrease (9 % vs. 5 %), leukopenia (8 % vs. 7 %), bone pain (7 % vs. 4 %), rash (7 % vs. 5 %), hypoglycemia (6 % vs. 4 %),

insomnia (6 % vs. 2 %), cephalea (5 % vs. 4 %), depression (5 % vs. 4 %), dysphagia (5 % vs. 2 %), thrombosis (5 % vs. 3 %), and hypokalemia (5 % vs. 3 %),

Finally a group of 461 patients (358 treated) *undergoing surgery* (orthopedic) in two studies received three different doses of Epoetin for 15 days or 4 weeks. The most common events included nausea (43–56 % vs. 45 % in placebo), vomiting (12–28 % vs. 14 %), pruritus (12–21 % vs. 14 %), cephalea (10–18 % vs. 9 %), injection site pain (9–13 % vs. 8 %), chills (0–7 % vs. 1 %), DVT (0–6 % vs. 3 %), cough (4–5 % vs. 0 %), hypertension (3-6 % vs. 5 %), rash (2–3 % vs. 1 %), and edema (1–3 % vs. 2 %).

Tumor progression, recurrence, and increase in related mortality (8.7 % vs. 3.4 %) were observed in advanced HNC in radiotherapy, breast cancer in chemotherapy, in lymphoid malignancies, and in other neoplasms including NSCLC not receiving such treatments. Notably, hypoxia represents a key tumor-promoting factor, and has been associated with tumor progression [12].

Additional information from postmarketing experience indicated the presence of additional AEs including seizures, PRCA, and serious allergic reactions, which were included in the BBW. Some PRCA cases were reported in association with anti-erythropoietin antibodies. Serious allergic reactions included anaphylactic reactions, angioedema, bronchospasm, skin rash, and urticaria.

Darbepoetin-α (ARANESP, Amgen) is a hyperglycosylated analog of Epoetin bearing 5 N-linked carbohydrate side chains (3 in the native molecule). FDA approval was granted in 2001 for the treatment of CKD patients (in dialysis or not), and in concomitance with myelosuppressive chemotherapy. The structural modification was considered potentially capable of inducing an immune response (anti-ARANESP antibodies) that could cross react with the endogenous erythropoietin. The issue was supported by rare documented cases of PRCA associated with cross-reacting anti-erythropoietin antibodies.

The general safety profile of darbepoietin is based on the same studies conducted for Epoetin-α, and some additional trials. In particular, experience in adult CKD patients included 766 patients treated with darbepoetin and 591 with epoetin. Pediatric experience was based on 81 CKD previously receiving epoetin. Experience during concomitant chemotherapy was based on 310 patients treated with darbepoetin and on 296 controls.

The most common AEs in 766 *adult CKD patients* treated with darbepoetin included hypertension (31 %), cough/dyspnea (12–17 %), peripheral edema (17 %), procedural hypotension (10 %), angina pectoris (8 %), vascular access complications (8 %), fluid overload (7 %), rash/erythema (5 %), and arteriovenous graft thrombosis (5 %).

The *pediatric safety profile in CKD* patients previously treated with epoetin followed by darbepoetin reported hypertension, injection site pain, and convulsions, being the former and the latter classified as serious events.

In *neoplastic patients* undergoing concomitant chemotherapy thromboembolic events were predominant (6–8 % vs. 4 % in placebo), and included ATE (1–3 % vs. 1 %), VTE (5 % vs. 3.5 %), myocardial infarction (1–2 % vs. 0 %), pulmonary embolism (2 % vs. 1 %), and cerebrovascular disorders(2–5 % vs. 2–3 %).

Overall, hypertension was the most serious event, together with seizures in children, which was reported increased with darbepoetin (40 %), as compared to the previous recombinant erythropoietin formulation (25–28 %).

The incidence of anti-erythropoietin antibodies with darbepoetin was of particular concern because of previous PRCA experiences. Sera of 1501 CKD patients and of 1159 cancer patients showed about 4 % of cases positive at baseline. One patient among the former group and 8 patients among cancer patients developed new anti-darbepoetin antibodies. However, no neutralizing antibodies directed to exogenous and endogenous erythropoietin were observed before and after treatment with the drug in study.

Epoetin-β (NeoRecormon®, Roche) was approved by EMEA in 1997 and the *pegylated form* (Mircera®, Roche) was approved by EMEA in July 2007 and by FDA in November 2007. The former was a successor of Recormon, under a new pharmaceutical form and strength, and with an extended indication for cancer patients treated with cisplatinum-based chemotherapy in addition to previous indications for CKD patients, whether or not in dialysis, to increase the yield of autologous blood, and anemia of prematurity. Additional pivotal studies were BP15984 in 54 healthy volunteers, and BA16108 on 844 patients including 228 patients with solid tumors (146 treated), and BO16196B on 1009 patients with lymphoid malignancies (MM, NHL, CLL). The overall safety analysis indicated most common events as: *cardiovascular* (mainly hypertension), *respiratory tract infections, injection site reactions*, and *laboratory abnormalities* (hyperkalemia, increase of liver enzymes). AEs of special interest were: *thromboembolic, hypertensive*, *allergic events*, and *neoplasms*. One of five serious thromboembolic events was considered as drug-related in the oncology group of patients. One case of angioedema and one urticaria were also detected. No new neoplasms occurred as related to the drug in study. Overall, the profile was reassuring and similar to the previous formulation [13].

Recently, a new class of drugs named CERA (continuous erythropoietin receptor activators) acts differently with respect to previous agents (ESA, NESP). They have an extended half-life (over 100 h) and a lower affinity (45 fold lower than epoetin-β) for EpoR so that they can dissociate and bind to another EpoR, thus expanding and prolonging their stimulatory effect [14].

Mircera is one representative of this class, showing a slower association and faster dissociation rates, an increased half-life (142h), a reduced activity in vitro and an enhanced activity in vivo. In particular, it shows a half-life 6 fold higher than darbepoetin-α, and 20 fold that of epoetin. The indication, approved by FDA in 2007, was for the treatment of CKD-associated anemia. Pivotal investigation were 6 Phase III studies including 2 correction studies in naive CDK patients in dialysis (BA16736) or not (BA16738), and 4 maintenance studies (BA16739, BA17238, BA17284) in patients on dialysis. All studies were open-label, and not blind. Comparator treatments were performed with epoetin-α, epoetin-β, and darbepoetin-α. Overall, 2,737 patients (1,789 exposed to drug in study, 948 to other ESA) were analyzed.

Altogether, safety evaluations were base on 28 clinical trials with SC and IV administrations. Pooled data were obtained from 2 Phase II studies and from the 6 pivotal trials. The BBW included *renal failure* and *cancer increased mortality*, and *tumor progression*. Additional warnings included *seizures* and *PRCA*, and *serious allergic reactions*. The most common AEs were *hypertension* (13 %), *diarrhea* (11 %), *nasopharyngitis* (11 %), *URTI* (9 %), *cephalea* (9 %), *hypotension* (5%; procedural hypotension 8 %), *muscle spasms* (8 %), *fluid overload* (7 %), *vomiting, cough, lumbalgia* (6 % each), *constipation, UTI pain in extremity*, and *arteriovenous thrombosis/fistula* (5 % each). DRAE were estimated in 7 % of cases versus 5 % in controls. SAEs were similar in patient receiving the drug in study compared to other ESA (38 vs 42 %), except for GI hemorrhage (1.2 vs 0.2 %). *Injection site reactions* were also higher in the study group (1.3 % vs. 0.4 %), together with *blood* (0.7 % vs. 0.3 %) and *skin disorders* (0.5 % vs. 0.1 %). However, 47 % of patients had at least one SAE in the study group and 54 % in controls. Discontinuation rates were 3 % versus 2 %. No ADA tested on 1,789 patients were found [15, 16].

It must be stressed that a number of biosimilar products are emerging, which will not be discussed here. One epoetin-α biosimilar (HX575) derives from the original epoetin-α previously registered as Eprex® (Janssen) in UK, and as Erypo® in Germany, now discontinued by the manufacturer (Janssen-Cilag). However, the commercial product (Abseamed®, Medice Arzeneimittel Pütter) was registered as epoetin-α although having a substantially different glycosylation with respect to the original molecule. Other glycosylated variants were identified as epoetin-τ (Biopoin®, CT Arzneimittel GmbH; Eporatio®, Ratiopharm GmbH), epoetin-z (SB309, Retacrit®, Hospira; Silapo®, Stada Arzneimittel AG). One biosimilar epoetin-α (Epostim ®, Reliance GeneMedix) was withdrawn by the manufacturer while under evaluation in 2011, and one formulation of epoetin-λ (Dynepo®, Shire Pharmaceuticals Ltd) approved by EMEA in 2002, was subsequently withdrawn in 2008. Glycosylated variants of epoetin-β (NeoRecormon Hexal®) are also available. These biosimilars were considered usually comparable in efficacy and safety to original molecules [17].

Peginesatide (Omontys®, Affymax), a new erythropoiesis stimulating agent (ESA) has been recently approved by FDA in 2012 for the treatment of CKD induced anemia in adult patients in dialysis [18, 19]. Peginesatide is a synthetic pegylated dimeric peptide consisting in two identical peptide chains covalently bound by an iminoacetic and β-alanine linker. The peptide sequence is not related to erythropoietin, but binds and activates its receptor with high specificity, and the consequent pharmacologic action is considered similar to epoetin-α and darbepoetin-α.

The BBW included *increased mortality, myocardial infarction, VTE, stroke, TVA,* and *tumor progression/recurrence*. The overall safety profile coincides, so far, with other experienced ESA, except for antibody-induced PRCA, which was not observed in controlled trials with peginesatide. Such profile was based on 1,066 CKD patients in dialysis and 542 placebo. Initial postmarketing records have registered serious allergic reactions. The immunogenicity testing on 2,357 patients showed peginesatide-specific antibodies in about 1 % of cases, with a higher

incidence after SC administration (2 %), with respect to IV injections. Pooled data from a recent experience in CKD patients not receiving dialysis (PEARL1, PEARL 2) showed an overall AEs incidence of about 94 % versus 91 % in controls treated with darbepoetin. No major differences emerged from the two groups, except for back pain (12 % vs. 7 % in controls). However, serious AEs were reported more frequently with the drug in study (48.5 % vs. 43 %), with a higher incidence of renal failure (8.5 % vs. 4 %), and anemia (3.5 % vs. 1.5 %). Interestingly, 10 patients treated with peginesatide raised specific antibodies, which were neutralizing in 8 of them [20]. On February 2013, the manufactured voluntarily recalled all lots of Omontys as a result of new postmarketing reports on serious hypersensitivity reactions, including life-threatening or fatal anaphylaxis. Fatalities were reported in about 0.02 % of patients following the first IV dose.

An intriguing off-label aspect of ESA therapy was based on to the mentioned "protective cytokine" of EPO, especially in brain and heart therapy. Although such treatment has shown to produce serious cardiac events, a number of studies were performed in patients with acute myocardial disorders. A recent meta-analysis on these studies analyzing 1,564 patients concluded that there was no clinical benefit for heart function, cardiovascular events, or mortality [21].

Finally, a number of preliminary studies analyzed the effect of ESAs on vascular, degenerative neurological diseases, and in some psychiatric disorders with alternate results. However, no new significant safety signals appeared to emerge [22].

53.2 Remarks

Epoetins have shown a potent anti-anemic effect in indicated therapies, although associated with a number of relevant AEs, including cardiovascular events, tumor progression, and PRCA. The first two events significantly increased the mortality rates. The CHOIR trial was terminated early because of serious AEs including major cardiovascular events (18 %). Nonetheless, a number of studies were performed in patients with acute myocardial disorders, because of the potential protective effect of erythropoietin and consequently of ESA, on various cell and tissues including myocardium. A recent meta-analysis on these studies analyzing 1564 patients concluded that there was no clinical benefit for heart function, cardiovascular events, or mortality [21].

The association of AEs in cancer patients is more complex. It involves stimulating effects on tumor cells and a number of additional risks, such as stroke and venous thromboembolism. As for the former activity, which seems related to the action of ESAs on triggering JAK/STATS and other downstream pathways in tumoral cells, recent data have shown contrasting activity of recombinant epoetins on breast cancer cells in vitro. In particular, short-term exposure reduced cell proliferation and chemotherapy-induced cytotoxicity, while long-term exposure increased both effects [23]. As for vascular adverse events, two meta-analyses reported a significant increase of thromboembolic events (6.1 % vs. 3.8 %), and venous thromboembolic events (7.5 % vs. 4.9 %).

In 2008, overall data on ESAs were considered sufficiently documented in SCLC with cisplatin-based chemotherapy (studies N93-004, 980297, and 2001-0145) to confirm no evidence of worsened survival or poorer tumor outcomes. In contrast, an increased risk of tumor promotion and increased mortality in HNC patients in radiotherapy (studies ENHANCE and DAHANCA), and increased mortality in patients with cancer not receiving chemotherapy (studies EPO-CAN-20 and 2001-0103) in relation to ESA administration were observed. In addition to tumor progression, these studies indicated an increase in thrombotic events, as well. On this basis ODAC considered the risks of ESA off label prescription as unacceptable [22].

These concerns induced to initiate a safety program for prescribers of epoetins in cancer patients (ESA APPRISE)[3].

PRCA remains an important, albeit infrequent event. Between 1993 and 1997 a number of cases in the literature were mostly associated with resistance to therapy, anaphylaxis, and elevated levels of specific antibodies, including patients with HIV-related anemia [10]. Up to 2002, 112 cases of antibody-mediated PRCA, related to one ESA product administered subcutaneously in CRF patients were reported by the manufacturer.

In the FAERS database, 812 cases of PRCA on 7,339 reports were registered after epoetin-α treatment, and 1,174 cases over 5,947 reports were filed after darbepoetin-α therapy.

Since, changes in glycosylation of recombinant epoetins are expected to modify the immunogenicity of these products, special attention should be given to recent biosimilar formulations. With this respect, the new asialo-carbomylated Epo (CEPO), and other synthetic non-glycosylated derivatives should be instructive both for the control of some AEs and application in non-hematologic disorders [24].

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Myelopoietic Stimulatory Factors

54

Myeloid stimulatory and growth factors are produced naturally by a number of different cell types, including macrophages, fibroblasts, osteoblasts, endothelial cells, and T cells. The bone marrow stromal cells play an important role in localizing (they express cell adhesion molecules) and conditioning in "niches" differentiating cells of all types [1]. As for erythropoietin, they are active at extremely low concentrations, bind to specific receptors, and activate the JAK/STAT signal transduction pathways. This group of cytokines are known also as colony stimulating factors (CSF) because of the initial finding that they could stimulate in vitro progenitor cells of different hemopoietic cell lineages into differentiated colonies of recognizable maturing cells with myeloid/monocyte morphology. They include the granulocyte CSF (G-CSF), the macrophage CSF (M-CSF), the granulocyte macrophage CSF (GM-CSF), the multi-CSF (CSF), and the stem cell factor (SCF). The acronyms denote the major function exploited by each factor, which is not only directed to initial precursors of one or more cell lineages, but involves maturational activities during the whole process of differentiation. Interleukin-3 (IL-3) stimulates also hemopoietic multipotent stem cells into myeloid, and erythroid (BFU-E) cells. However, in conjunction with IL-7 acts also on lymphoid precursors, and synergizes with other cytokines on further maturation steps of all myeloid lineages. These factors are also enhancers of cell migration, phagocytosis, and superoxide production.

Altogether, myeloid growth factors can induce pluripotent differentiation signals or more restricted maturation signals in specific cell lineages at different stages of commitment. Apparently, they exhibit a hierarchical and synergistic organization, with a considerable overlap in the targeted cell populations.

Early-acting growth factors include SCF, the Flt3 ligand (Flt3L), and IL-3 (or multi-CSF), and have some overlapping stimulating and regulatory functions on

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multipotent stem cells and on early stages (progenitor) of cell lineage commitments [2]. Ancestim (Stemgen®, SoBi), is a recombinant CSF approved for ex vivo stem cell expansion and in some countries, including Canada, Australia, and New Zealand, to induce in vivo stem and progenitor cell mobilization.

G-CSF is mainly restricted to neutrophils and stimulates proliferation, differentiation, and function of progenitor and maturing cells, enhancing phagocytic and cytotoxic activity. Direct influence on other cell lineages is limited, unless in synergy with other cytokines (IL-3, GM-CSF), but is a strong mobilizer of stem cell precursors (see Chap. 53). This cytokine is mainly produced by endothelial cells and macrophages. Filgrastim (Neupogen®, Amgen) is a recombinant human formulation (rhG-CSF) used to reduce the neutrophil recovery and the incidence of infections in cancer patients undergoing myelosuppressive/myeloablative chemotherapy. Pegfilgrastim (Neulasta®, Amgen) is a pegylated formulation of rhG-CSF developed for the same treatment indications.

GM-CSF stimulates committed progenitor cells to proliferate and differentiate into neutrophils, monocyte/macrophages, and myeloid-derived dendritic cells. This cytokine can also activate mature granulocytes and macrophages. Moreover, in association with other factors promotes the proliferation of megakaryocytes and erythroid progenitors. This cytokine shows also a positive chemotactic activity towards monocytes, and is secreted by macrophages, T cells, mast cells, NK cells endothelial cells, and fibroblasts. Sargramostim (Leukin®, Bayer) is a recombinant human GM-CSF used to reduce the neutrophil recovery and incidence of infections in AML patients undergoing myelosuppressive/myeloablative chemotherapy, and for myeloid reconstitution after bone marrow transplantation, transplantation failure, or engraftment delay. Recently (August 2012), FDA approved Tbo-filgrastim (Neutroval ®, Sicor) as a new rG-CSF, while EMEA considered this short-acting molecule as a biosimilar to Neupogen®, and marketed as Tevagastrim®, Teva.

The following safety profiles are depicted on the basis of the most representative therapeutic formulations of recombinant hemopoietic stimulatory factors.

54.1 Filgrastim, Pegfilgrastim, Sargramostim

Filgrastim (Neupogen®) was granted FDA approval in 1991 to decrease the incidence of infection in patients with non-myeloid malignancies undergoing myelosuppressive therapy causing severe febrile neutropenia. This short-lived molecule, with a half-life of about 3 h, is eliminated by glomerular filtration and by neutrophil-mediated clearance. Pivotal study for approval was a Phase III study on 210 patients (207 for safety evaluation) with small cell lung cancer. Filgrastim was administered SC for up to 14 days. Supportive data from Phase I-II studies derived from 40 patients with advanced urothelial cancer treated with escalated doses for 6–8 days. An additional Phase II study evaluated 18 patients undergoing high dose cytotoxic chemotherapy and filgrastim was administered by IV infusion at the end of each cycle up to 20 days. One pediatric study, 12 patients with

neuroblastoma received a similar treatment with the drug in study. Overall, efficacy and safety evaluation was based on about 350 treated patients [3, 4].

This indications in the prescribing information relate to four underlying disease conditions: patients receiving myelosuppressive chemotherapy; patients with AML under induction or consolidation chemotherapy; cancer patients receiving bone marrow transplant; mobilization of hematopoietic progenitor cells and collection by leukapheresis; patients with severe chronic neutropenia.

The overall safety profile, based on all adult patients receiving filgrastim in controlled studies, include *allergic reactions*, *splenic rupture*, *alveolar hemorrage/ hemophtysis*, *sickle cell disorders*, and *cutaneous vasculitis.*

In *pediatric patients* with congenital types of neutropenia (Kostmann's syndrome, congenital agranulocytsis, Schwachman-Diamond syndrome), *cytogenetic abnormalities*, and *transformation in MDS and AML* were also observed.

The resulting framework in 207 *SCLC patients* in myelosuppressive chemotherapy, exposure adjusted adverse events with higher frequency (≥1 %) than in placebo included skeletal pain (22 vs. 11 %), and pyrexia (12 vs. 11 %). No serious/life-threatening events or fatalities were observed. Transient hypotension not requiring therapy was observed in about 4 % of cases and cardiac events (infarction, arrhythmias) occurred in about 3 % of cases.

In *non-myeloablative chemotherapy* experience (350 patients), medullary bone pain was reported in 24 % of patients as the only consistent encountered AE, and infrequently was severe, with a dose–response trend.

In *AML* experience (259 patients) more frequent evens were petechiae (17 vs. 14 %) and epistaxis (9 vs. 5 %), The overall reported AEs were equally distributed (83 vs. 82 % in induction phase; 61 vs. 64 % in consolidation). Similarly, death rates (infection disiase progression, hemorrage) were equally distributed, except for cerebral hemorragic deaths (5 vs. 1 patient). Overall serious and fatal hemorrhagic events were higher in the study group (7 vs. 2 %).

In *bone marrow transplanted cancer patients* (167), nausea (10 vs. 4 %), vomiting (7 vs. 3 %), hypertension (4 vs. 0 %), rash (12 vs. 10 %), and peritonitis (2 vs. 0 %) occurred more frequently in the study groups, and were classified as non-related to filgrastim. One case of erythema nodosum was considered related to therapy in study. Additional serious events observed in non-randomized studies included 2 cases of renal insufficiency and one CLS.

Adverse events in 126 patients undergoing progenitor *cells mobilization and collection* were mild/moderate and included medullary bone pain (33 %) cephalea (7 %), and transient increase of ALP (21 %). However, anemia (66 %) and thrombocytopenia (97 %), occurring after leukapheresis, to which filgrastim may have contributed.

Finally, in *severe chronic neutropenia* bone pain (33 %) and musculoskeletal pain were more frequent in the study groups. Epistaxis (15 %) and thrombocytopenia (6 %) were related to therapy. Infrequent events were injection site reactions, rash, hepatomegaly, arthralgia, osteoporosis, cutaneous vasculitis, hematuria/proteinuria, alopecia, and exacerbation of skin disorders (psoriasis).

Cytogenetic abnormalities (mostly in chromosome 7) and *transformation in AML/MDS* appeared to be confined to congenital neutropenia in pediatric patients, with 531 patients recorded in the postmarketing setting and a potential cumulative risk of 16.5 % after 8 years of filgrastim treatment.

Additional data from the postmarketing surveillance include splenic rupture, ARDS, alveolar hemorrhage and hemoptysis, sickle cell crisis, cutaneous vasculitis, Sweet's syndrome, and osteoporosis in severe congenital neutropenia in chronic treatment with filgrastim.

Pegfilgrastim (Neulasta®) is the pegylated form of filgrastim, showing a prolonged half-life (15–80 h) with respect to the original recombinant molecule, due to a minimal elimination by renal clearance. The neutrophil-mediated elimination is nonlinear and is highly variable even in the same subject, being mainly related to the number and functionality of circulating neutrophils of each treated patient. FDA and EMEA approved pegfilgrastim in 2002 to decrease the incidence of infection in patients with non-myeloid malignancies undergoing myelosuppressive therapy causing severe febrile neutropenia, at a fixed SC dose of 6 mg per cycle of chemotherapy, as opposed to daily injections necessary with the unpegylated filgrastim [5, 6].

Pivotal studies consisted in two comparator-controlled trials (Study 980226 and 990749) in 467 patients with advanced metastatic breast cancer. In addition, a pediatric sarcoma investigation (Study 990130) and a retreatment experience (Study 990736) added supportive data. Previous dose-ranging Phase I-II studies were performed in healthy volunteers, and in patients with thoracic, breast cancer, and lymphoma (HL/MHL). Overall, 882 subject were enrolled, 540 received pegfilgrastim and 342 received filgrastim. The majority of treated subjects were breast cancer patients (823).

The *general safety profile* was mainly based on 461 patients treated with the drug in study and 467 placebo, and was largely similar in the two groups. The only AEs showing a higher incidence in patients treated with pegfilgrastim as compared to patients receiving filgrastim, were bone pain (31 vs. 26 %), and pain in extremities (9 vs. 4 %). Overall, all AEs were present in 100 % of patients in both groups. Severe/fatal events were 49 % in the study group and 50 % in the placebo treated group. In particular, SAEs (24 % in both groups), DRAEs (38 vs. 47 %), drug-related severe/fatal events (4 vs. 6 %), drug-related SAEs (0 vs. 1 %), and withdrawals (7 % in both), were similarly distributed. Warnings for serious potential events were indicated for splenic rupture, ARDS, serious allergic reactions, sickle cell crisis, and tumor progression, which were observed in the postmarketing setting.

As for *immunogenicity* of pegfilgrastim, preexisting antibodies were observed in 6 % of cases and nine subjects (0.8 %) developed new antidrug non-neutralizing antibodies.

Sargramostim (Leukine®) is a recombinant GM-CSF approved by FDA in 1991 to reduce the neutrophil recovery and incidence of infections in AML patients undergoing myelosuppressive/myeloablative chemotherapy, and for myeloid reconstitution after bone marrow transplantation, transplantation failure

or engraftment delay. The recombinant protein differs by only one aminoacid and possibly by the associated sugars with respect to the native cytokine [7].

Initial efficacy and safety experience was investigated in six underlying disease conditions: patients receiving myelosuppressive chemotherapy; Phase III study on 99 patients with AML under induction or consolidation chemotherapy [8]; cancer patients (NHL, ALL, HD) receiving autologous bone marrow transplant; two retrospective studies on 227 patients treated for mobilization of hematopoietic progenitor cells collection by leukapheresis and auto engraftment; three studies on 128 patients with lymphoid malignancies undergoing autologous bone marrow transplantation; one Phase II study on 109 patients with myeloid and lymphoid malignancies, HD, MM, and MDS, undergoing allogenic bone marrow transplantation; an analysis of case of bone marrow graft failure or engraftment delay in 140 patients with various hematologic and non-hematologic malignancies, MDS, and aplastic anemia [9, 10].

The overall safety profile warned for *fluid retention*, including *CLS*, *pleural and pericardial effusion, ARDS* due to sequestration of granulocytes in the pulmonary district, *arrhythmias*, *renal and hepatic dysfunction* in patients with precedent history of organ disease, and for *gasping syndrome* in neonates in consequence of a reaction to benzyl alcohol present in the liquid formulation of Leukine. The previous liquid formulation was withdrawn in 2008 because of serious AEs including syncope, with or without hypotension, correlated to the presence of EDTA in the solution.

Within this framework the selected experience in clinical trials on different underlying disease conditions reported variations in the respective safety profiles, mainly related to their frequency.

In 94 *AML patients* (47 treated) events occurring more frequently ($\geq$5 %) in the study group were non-infectious pyrexia (81 vs. 74 % in placebo), weight loss (37 vs. 28 %), vomiting (46 vs. 34 %), skin disorders (77 vs. 45 %), and metabolic disorders (58 vs. 49 %). Among these, skin disorders reached statistical difference over controls ($p = 0.002$). However, in a historically controlled analysis on additional 86 AML patients, weight gain, low serum proteins, and prolonged prothrombin time reached statistical significance.

In 156 pooled *autologous bone marrow transplanted pati*ents, any AEs were present in over 95 % of patients in study groups (79) and in controls (77). Among them, events occurring more frequently ($\geq$5 %) in the former included asthenia (66 vs. 51 %9, malaise (57 vs. 51 %), diarrhea (89 vs. 82 %), rash (44 vs. 38 %), and peripheral edema (11 vs. 7 %). Moreover, in some patients with preexisting renal or hepatic dysfunctions there was an increase of serum creatinine or bilirubin and hepatic enzymes, respectively.

Similarly, in 109 *allogenic transplanted patients* more frequent events were represented by abdominal pain (38 vs. 23 %), chills (15 vs. 9 %), diarrhea (81 vs. 66 %), vomiting (70 vs. 57 %), hematemesis (13 vs. 7 %), hemorrhage (11 vs. 5 %), pruritus (23 vs. 13 %), bone pain (21 vs. 5 %), arthralgia (11 vs. 4 %), eye hemorrhage (11 vs. 0 %), pharyngitis (23 vs. 13 %), anxiety (11 vs. 2 %), high

BUN (23 vs. 17 %), and high cholesterol (17 vs. 8 %). Moreover, in cases with graft failure increased frequencies of cephalea (26 %), pericardial effusions (25 %), arthralgia (21 %), and myalgia (18 %) were reported.

54.1 Remarks

Most of encountered AEs appear clearly related to the respective mechanisms of action of these myelopoietic stimulatory cytokines, and therefore they were expected. Differences among treated groups with distinct underlying diseases were mainly related to the frequency of AEs, more than to their typology.

Particular concern raised the transformation in MDS and AML in pediatric patients with congenital neutropenias, although they seemed restricted to this setting. Reports in the FAERS were limited to 19 AML cases and seven MDS on 1,112 consulted files for Filgrastim, and four AML and one MDS for Sargramostim.

CRL and CLS were also limited. In the same database there were four cases of CRS and five CLS in pooled reports of both therapeutic formulations.

Immunogenicity and allergic reactions can be considered low. Six cases of anaphylactic reactions were found in the same database for Filgratsim and none for Sargramostim.

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Thrombopoietic Stimulatory Factor 55

55.1 Interleukin-11

Interleukin-11 (IL-11) is a pleiotropic Type I cytokine pertaining to the functional superfamily of growth factors, and to the structural IL-6 family because of the sharing of gp130 protein in their receptors. IL-11 is produced by bone marrow stromal cells (fibroblasts, osteoblasts), but also by epithelial cells. IL-11 supports the proliferation of hemopoietic stem cells, and of megakaryocyte progenitor cells proliferation and differentiation. It shows synergism with IL-3, with the stem cell factor (SCF), and with the homologue Fms-related tyrosine kinase-3 ligand (FLT3LG) in producing its functions in vitro. IL-11 has also nonhemopoietic functions. In particular, this cytokine activates osteoclastogenesis and neurogenesis, enhances healing of epithelial intestinal lesions, inhibits adipogenesis, induces acute-phase proteins, stimulates tissue fibrosis, regulates chondrocytes and synoviocytes, stimulates T-cell dependent Ig-producing B cells, and controls the ovular implantation by supporting placentation and endometrium decidualization. IL-11 inhibits apoptosis/necrosis and the production of macrophage cytokine production, such as TNFα, IL-1 and IL-12.

In particular, primary osteoblasts and mature osteoclasts show IL-11 receptors and therefore are potential IL-11 targets.

The multimeric receptor (IL-11R) pertains to the Type I family and consists of a specific IL-11R α subunit combined with at least one promiscuous gp130 (IL6ST). The transmembrane downstream signaling is mediated by gp130 via the JAK tyrosine kinase pathway [1, 2].

Oprelvekin (Neumega®, Wyeth, Pfizer) is a nonglycosylated IL-11 lacking the N-terminal proline residue with respect to the natural cytokine. A recombinant human IL-11 is now manufactured in China (Kawin Technology).

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Oprelvekin was approved by FDA in 1997 for the prevention of chemotherapy-induced thrombocytopenia (CIT) and the reduction of the need for platelet transfusion in patients with nonmyeloid malignancies [3, 4].

Pivotal studies consisted in two Phase II (C9308, C9416) studies on 170 CIT patients. Supportive studies included 12 Phase II studies including healthy volunteers (4), in post-chemotherapy myelosuppressed patients with breast cancer (4), one trial in pediatric patients, and one Phase III ongoing study on CIT patients at time of evaluation. Overall, safety initial analysis was performed on over 300 patients, and 72 healthy volunteers. The experience covered cases of prior CIT, recovery of patients receiving dose-intensive chemotherapy or after myeloablative chemotherapy for breast cancer.

The emerging safety profile consisted in *allergic reactions* including *anaphylaxis*, for which a BBW was inserted in the prescribing information, *CLS*, *fluid retention* including *pulmonary edema*, *dilution anemia*, *cardiovascular events*, *cerebrovascular disorders, papilledema* and *renal failure*.

Overall, constitutional disorders and the general typology reported in detail in 224 patients were similar in study groups and placebo.

Most common events were edema (59 % vs. 15 % in placebo), dyspnea (48 % vs. 22 %), tachycardia (20 % vs. 3 %), conjunctival injection (19 % vs. 3 %), palpitations (14 % vs. 3 %), vasodilation (19 % vs. 9 %) and pleural effusions (10 % vs. 0 %). Most serious events included pyrexia (36 % vs. 28 %), neutropenic pyrexia (48 % vs. 42 %), syncope (13 % vs. 6 %), atrial fibrillation (12 % vs. 1 %), diarrhea (43 % vs. 33 %). Additional serious cases of papilledema, atrial arrhythmias (15 %, stroke and pneumonia occurred with unknown frequency. Two sudden deaths occurred during the study period and were considered drug-related.

Approximately 1 % (2 patients in study) developed *anti-oprelvekin antibodies*, and in one case they were neutralizing. Other laboratory abnormalities include a decrease in Hb levels, plasma protein concentration and hypocalcemia attributed to fluid retention, and consequent plasma volume expansion.

In the postmarketing experience cases of injection site reactions, CLS, renal failure anaphylaxis/anaphylactoid reactions, ventricular arrhythmia, papilledema and optic neuritis and vision abnormalities were reported. One case of possible interaction of oprelvekin with opioids producing somnolence, peripheral and pulmonary edema was also described [5]. No new signals were individuated.

Additional experiences derive from some *off-label treatments*. In a Phase II trial on nine patients with mild von Willebrand disease (a congenital bleeding disorders related to decrease of the carrier for FVIII), treated with oprelvekin for 8 weeks, AEs were all mild and included hypertension, fluid retention and hypokalemia. One case of anxiety and chest pain were observed in the same patient [6].

New formulations of IL-11 are emerging with the aim of reducing AEs and preserve or increase efficacy.

Recently, a *genetically modified IL-11*(*mIL-11*) was developed by deleting the first nine amino acids from the N-terminal and substitution of two amino acids at position 135. The molecule resulted more stable and therefore was used at lower dosages, with the attempt of reducing AEs.

The safety analysis on 153 CIT patients treated with mIL-11 (73 patients) or with oprelvekin (80), AEs were reported in about 85 % in the study group and 90 % in the controls. Drug-related events were 33 % in the study group and in over 51 % in the oprelvekin-treated controls. Most AEs with the genetically modified molecule were mild to moderate, while severe events were more frequent in the control group, including arrhythmia, dyspnea, myelosuppression and edema. Three patients discontinued treatment for drug-related AEs in controls and none in the study group. No antibodies were detected in both groups [7]. Another recent approach was directed to potentiate IL-11 functions by constructing fusion proteins containing only natural soluble IL-11Ra connected with IL-11 without insertion of artificial linkers (hyper IL-11). The preformed complex targets gp130 and appears to be more stable and effective at lower doses, and therefore are expected to produce less AEs [8]. The new formulations are now under investigation.

55.2 Remarks

IL-11 did not show relevant AEs and were all apparently related to its mechanism of action. In particular, CLS seems to characterize its safety profile with the known constitutional signs. No new signals emerged from this experience, so far.

The new attempts to modify IL-11, such as the hyper IL-11 fusion protein, to underline the possibility of splitting AEs inducing activity from the pharmacotherapeutic action of the cytokine, hopefully leading to better formulations.

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Pluripotent Growth Factors

56

In contrast with the mentioned hemopoietic stimulatory factors, mainly acting on precommitted stem cells and on later stages of hemopoietic cells maturation, *factors acting on uncommitted (pluripotent) stem cells* or immediate committing stages did not find, so far, large clinical application, except for some ex vivo agents employed as cell expanders [1].

Early acting growth factors include SCF, the Flt3 ligand (Flt3L), LIF, and IL-3 (or multi-CSF), and have some overlapping stimulating and regulatory functions on multipotent stem cells and on early stages (progenitor) of cell lineage commitments.

Although initial promising results stimulated a broad clinical investigation through the 1990s with IL-3, a typical stem cell stimulator, the experience in controlled studies on cancer patients, in MDS as monotherapy or in association with other myeloid growth factors, showed low efficacy and high rates of AEs. Synthetic IL-3R agonists, such as daniplestim, or hybrid IL-3/GM-CSF prototypes, such as pixykine, were evaluated as progenitor cells mobilizer or for bone marrow reconstitution after myelosuppression, without consistent results and were abandoned.

The Flt3 ligand showed relevant efficiency in expanding hemopoietic progenitors expressed by an increase of a number of hemopoietic cell lines, including B cells, NK cells, and dendritic cells in animal models. However, the expansion of the latter cells did not reach complete maturation. A recombinant human Flt3-ligand showed mobilizing and proliferative capacity on the same range of cells and was employed at clinical level in cancer patients and as a vaccine adjuvant, with alternate results. The factor does not stimulate proliferation of early hemopoietic cells, but synergizes with other CSFs and interleukins to induce growth and differentiation. This factor was also tested in association with filgrastim and sargramostim, and remains a potential multilineage stimulant to be further evaluated.

Similarly, a LIF human recombinant (emfilermin) gave modest results as thrombopoietic agent in Phase I studies, associated to dose-limiting toxicities (hypotension, rigors).

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SCF, as Flt3L, is a potent promoter of stem cell survival, but induces proliferation and differentiation only in association with other growth factors in vitro and in vivo. The association increases all bone marrow lineages, including mastocytes, and lymphocytes. The effects are slowly appearing in circulations, since their maturation and expansion needs about 2 weeks in animal models. Mastocytes are the most SCF-dependent (also called mast cell growth factor) lineage for survival, maturation, and degranulation.

In vitro SCF is a potent stem cells expander in association with other cytokines, such as IL-3, IL-6. IL.11, TPO, and GM-CSF. However, stimulating effects have been observed also on melanocytes, AML cells, and SCLC cells. Ex vivo expansion is used also for cord blood stem cell to reach a critical amount of cells for transplantation.

56.1 Ancestim

Ancestim (Stemgen®, Amgen), is a recombinant SCF approved for ex vivo stem cell expansion, and in Canada, Australia and New Zealand, to induce in vivo stem and progenitor cell mobilization since 1999. Initial clinical results were not particularly favorable in enhancing post-chemotherapy myelosuppression or as mobilizer in "poor donors" of peripheral blood precursor cells (PBPC). Nonetheless, its activity ex vivo is relevant [2, 3].

Ancestim is a human analogue of SCF, which induces proliferation of hemopoietic progenitors, but only in synergy with other growth factors, such as filgrastim, and is able to increase the number of circulating PBPC. The subcutaneous absorption half-life is 35–41 h and clearance (mostly renal) half-life is 2–5 h. Because of the strong activity on mastocytes, all treated patients require prophylactic administration of H1 and H2 antihistamines and a bronchodilator to mitigate systemic anaphylactoid reactions.

Pivotal experience for approval for human IV therapy was based on preliminary Phase I–II studies in 367 patients with breast cancer, NHL, and ovarian cancer, and in one Phase III trial on 205 breast cancer patients, in combination with filgrastim (Neupogen®). The in vivo treatment indication, in authorized countries, is for the setting of autologous PBPC transplantation for patients at risk for poor mobilization, to increase their yield in the apheresis harvest.

The safety profile includes warnings for *severe allergy and asthma* (due to mastocytes stimulation these patients were excluded from investigational trials), and for the *concomitant use with chemotherapy/radiotherapy* (due to potential toxic effects on proliferating myeloid cells, mastocytoma, and other neoplastic cells). Moreover, a potential risk of reinfusion on neoplastic cells after apheresis was postulated. The most common AEs reported in Phase III experience in 204 breast cancer patients treated with the ancestim-filgranstim association with higher frequency than in filgrastim-treated controls included injection site reactions (92 vs. 10 %), dizziness (16 vs. 6 %), tachycardia (8 vs. 0 %), respiratory disorders

(28 vs. 16 %), skin manifestations (21 vs. 7 %). Allergic *anaphylactoid reactions* appeared in three patients in study receiving antihistamines premedication. In an additional database of 434 patients treated with the same growth factors, 84 % of patients had injection site reactions, and diffuse skin reactions (rash, urticaria) in 16 % of cases (5 % in controls), usually as mild/moderate events. Interestingly, a recall reaction occurred in some cases where the reaction recurs at a distant point of inoculation. Allergic reactions were reported in 27 % of cases. Respiratory disorders (dyspnea, cough pharyngitis) also occurred (25 vs. 14 %).

Overall, the safety profile was in line with larger experiences with filgrastim, with a few increased events observed in the associated therapy, but in the absence of new relevant signals.

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Epidermal Growth Factors

57

The epidermal growth factor family includes a number of cytokines inducing the proliferation of epithelial cells. The founding member (EGF) is a small protein containing 53 amino acids and 3 disulfide bonds inducing the proliferation of cells sharing its receptor (EGFR), a member of the Erb family widely used as target of anti-tumoral biomedicines (cetuximab, nimotuzumab, panitumumab). Other members of the family include TGF-α, HB-EGF, amphiregulin, betacellulin, and epiregulin, all considered relevant regulators of epithelial function and regeneration. Members of this superfamily are characterized by the presence of EGF-like repeats, an evolutionary conserved protein domain, shared by many proteins involved in the regulation of cell cycle, proliferation, and developmental processes. However, being many cytokines pleiotropic, a series of other factor influence the growth of epithelial cells, such as fibroblast growth factors (FGF), IL-1, and the transforming growth factor-B (TGF-B). The FGF family includes the keratinocyte growth factor (KGF), binding to a specific cell receptor (KGFR), widely present in cutaneous and mucosal epithelia (oral and digestive tract) and in parenchymal organs (salivary glands, liver, lung, pancreas, kidney, urogenital tissues, and mammary gland). KGFR is present also on conjunctiva, cornea, and lens. It must be noted that the pituitary human growth hormone (HGH) is also active on hepatocytes, osteoblasts and fibroblasts, GM-CSF has some activity on fibroblasts, and the platelet-derived growth factor (PDGF) is active on fibroblasts and smooth muscle cells. Some of these factors stimulate also chemotaxis of keratinocytes (EPGF, FGF, GM-CSF), or of leukocytes (IL-1, GM-CSF). Most of these factors are of mesenchymal origin and are upregulated by epithelial tissue injury, thus acting in a mesenchymal-epithelial paracrine network.

EGF receptors are expressed in virtually all epithelia, and are often overexpressed in epithelial tumors. EGFR (ErB-1, HER1) is a transmembrane protein of a subgroup of Type 1 receptor tyrosine kinase (RTK), the ErbB family, which

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includes EGFR, HER2, HER3, and HER4. EGFR is constitutively expressed in many epithelial tissues, including skin and hair follicles as well as in epithelial cancer cells. There are 11 known natural ligands to these receptors, including TGFα, HB-EGF, EGF, epigen, betacellulin, AREG (amphiregulin), and EREG (epiregulin), which interact with EGFR. However, HB-EFG, betacellulin, and EREG interact also with HER4, but not with the other two ligands of the subgroup. Upon interaction, EGFR forms homo- or heterodimers with other ErbB receptors, a step related to activation of the receptor/ligand complex, via the intracellular tyrosine kinase pathway. The signaling produces essentially DNA synthesis, cell cycle progression, migration, adhesion, and proliferation of cells expressing EGFR. Therefore, this pathway is crucial for the homeostasis of epithelia, for innate immunity, and also as a downregulator of myelin regeneration. EGFR and HER2 are usually overexpressed on neoplastic cells of epithelial origin, and in particular on CRC, lung carcinoma, SCCHN, breast cancer, and on GBM, due to gene mutations/overactivity leading to uncontrolled cell division, angiogenesis, cell migration, and cellular invasion/metastasis. Therefore, anti-EGFR monoclonal antibodies have been raised to inhibit this activating pathway, e.g., cetuximab, panituzumab, nimotuzumab directed to EGFR; trastuzumab directed to HER2.

A number of EGFs have been experienced in various clinical conditions including, acute and chronic wound repair, diabetic ulcers, pressure ulcers, venous and arterial ulcers with alternate results. Their safety profile is depicted on the basis of clinical experience with two recombinat factors (rhEGF) approved for human therapy.

57.1 Palifermin and Becaplermin

Palifermin (Kepivance®, Amgen, Sobi), previously identified as rHuKGFδ23, is a purified recombinant truncated form of keratinocyte growth factor (rHuKGF). FDA approved the first formulation in 2004 to decrease the incidence and duration of severe oral mucositis in patients with hematological malignancies receiving myelotoxic therapy requiring hemopoietic stem cell support. The approval was based on one randomized, placebo-controlled investigation on 212 (107 treated) patients (Study 20000162) receiving high-dose cytotoxic therapy, followed by peripheral blood stem cell transplant (PBSCT) for the treatment of NHL, HD, AML, CML, MM, and CLL. Palifermin was administered IV (60 mg/kg) for three consecutive days prior to initiation of cytotoxic therapy and for 3 consecutive days after PBSCT. The second pivotal Phase II study (980231) was conducted on 169 patients (117 treated) receiving the same cytotoxic therapy and high-dose of CTX followed by PBSCT, treated for the same malignancies [1–4].

In animal models palifermin enhances epithelial proliferation, increase of tissue thickness, and improved survival after cytotoxic treatments. Similar results were obtained in vitro on human epithelial tumor cell lines and in xenograft models.

Therefore, a special warning for *potential stimulation of tumor growth* was included since the initial prescribing information.

The overall safety database consisted in 17 studies including 1168 (786 treated) patients.

The safety data profile form the primary pool was based on 409 treated patients and 241 controls observed prior to chemotherapy (PC), and 405 treated and 240 of them observed after chemotherapy (AC).

Any AEs were 84 vs. 79 % of controls in PC, and 100 % for both in PC. TEAEs were 38 % and 10 % in PC and 39 versus 22 % in PC, respectively.

Overall discontinuation rates related to drug in study were low (2 %).

The most common AEs were *skin toxicity*, as rash (62 % vs. 50 % in controls), erythema (35 % vs. 24 %), edema ((28 % vs. 21 %), pruritus (35 % vs. 24 %), *oral/mucosal toxicity* revealed by tongue discoloration/thickening (17 % vs. 8 %), dysesthesia/paresthesia (12 % vs. 7 %), and dysgeusia (16 % vs. 8 %). Other AEs were pain (16 % vs. 11 %), arthralgia (10 % vs. 5 %), and pyrexia (39 % vs. 34 %).

In a subset of patients (101) with MM the incidence of cataract or cataract progression was increased in the study group (46 % vs. 29 %).

Laboratory abnormalities included lipase (28 % vs. 23 %; severe 11 % vs. 5 %), and amylase (62 % vs. 54 %; severe 38 vs. 31 %) increase. Moreover, the presence of anti-palifermin antibodies accounted for 2 % of the 645 treated patients in controlled studies, as well as in the placebo group (321).

The postmarketing surveillance revealed additional cases of cataract, vaginal edema and erythema, and palmar-plantar erythrodysesthesia.

In long-term studies the disease progression rates were higher in the study group (27 % vs. 13 %) and in a group receiving fractionated radiochemotherapy (death rates 27 % vs. 24 %), while in multicycle therapy and in hematologic transplanted setting these rates were superimposable.

Becaplermin (Regranex®, Ortho, Janssen, Johnson) is a recombinant human PDGF consisting in two identical polypeptide chains covalently linked by disulfide bonds. It is prepared as a *gel for topical use*, and was granted FDA approval in December 1997. EMEA approval came in March 1999, but the marketing authorization was withdrawn in 2010 after a preceding restriction in use for cancer patients in areas close to the tumor, followed by the exclusion of treatment in all cancer patients. Meanwhile, FDA issued in 2008 a *BBW* on the increase of *cancer death* in patients who use three or more tubes of the product. On July 2011 the manufacturer informed that the production was discontinued for commercial reasons [5–9].

In the last available product information label, becaplermin is indicated for the treatment of lower extremity diabetic neuropathic ulcers extending in the subcutaneous tissue or beyond, and have an adequate supply of blood.

Pivotal studies were conducted on 922 patients with diabetic ulcers (475 treated) enrolled in 4 multicenter studies.

Malignancies distant from the site of application were reported in 3 % of cases of 291 subjects followed for about 20 months (1 % in the vehicle/standard of care group). The types of cancer varied and all were remote from the treatment site. In a short-term retrospective study the incidence rate for all cancer was 10.2/1.000P/Y and 9.1/1000P/Y in controls. Mortality rates were 1.6 and 0.9, respectively, which increased to 3.9 vs. 0.9 in patients using 3 or more tube of the product.

Minor site reactions were eventually expected in relation of the parabens or m-cresol excipients included in the gel.

It must be stressed that recombinant PDGF are used in human periodontal regeneration (Darby 2012) and in some bone grafts [10]. So far, no evidence emerged on chronic toxicity, carcinogenicity or tumor promotion in these studies. Minor reported AEs included local pain, swelling and pyrexia, soft tissue dehiscence, tooth abscess, sensitivity, and edema around the operative site. Mild laboratory abnormalities and no anti-drug antibodies were reported. However, investigators stressed data heterogeneity and recommended further studies.

Finally, an exogenous recombinant mouse PDGF-BB, together with other cytokines (TNFα, IFNγ, TGFa) able to stimulate endogenous PDGF, proved to accelerate prostate cancer growth by promoting the proliferation of mesenchymal stem cells [11].

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Part V
Overview

Biomedicines as Adverse Event Inducers

58

Biomedicines are new agents with old roots. Products of biological origin, such as vaccines, blood and serum components, human proteins, hormones, and immunoglobulins, are used from long time in human therapy.

After the discovery of a technique for producing monoclonal antibodies in 1975, and the first commercialization of muromonab in 1986 for the control of solid organ graft rejection, their expansion has been exponentially growing, leading to the development of new drug classes for the treatment of tumors, autoimmune diseases, and inflammatory diseases.

Their extraordinary efficacy, the parallel expansion of genetic engineering, and the increased knowledge on the physiopathology of the immune system soon stimulated the identification and production of other biologically active molecules, including fusion proteins, growth factors, hematopoietic stimulating factors, and other cytokines for therapeutic use such as enhancers, inhibitors, and antagonists of basic cell functions and of immune effector mechanisms.

The first cytokine-based therapy reached the market in 1986 with IFN-α2b and IFN-α2a. A recent business intelligence report retrieved 504 mAbs in clinical and market stages up to February 2013. By the end of 2011, about 270 new cytokine therapies, including cytokines, mimic-cytokines, cytokine inhibitors, and/or cytokine receptors were developed and investigated. Annual sales for cytokines, including IFN α/β, ESAs, and Hemopoietic Growth Factors, exceeded $1 billion in the same year. Annual sales of 30 monoclonal antibodies approved in US generated $ 44 billion in 2011. Adalimumab (Humira®), one of the top selling drugs worldwide in 2012, is expected to reach $13.7 billion in 2013.

Over a quarter of century of experience on efficacy and safety of most relevant new biomedicines has been so far accumulated.

Soon after the first clinical controlled experiences, it was clear that biomedicines could raise a number of adverse effects, sometimes impressive and life threatening. Muromonab showed extraordinary beneficial effects in the control of acute graft rejection, but they were associated to a heavy safety profile, including cardio-respiratory disorders, neuro-psychiatric events, serious infections, increase of malignancy rates, fatal anaphylaxis, and violent systemic reactions such as

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CRS, even during the first infusion. It was also evident that the increasing commercialization of new biomedicines and the expansion of indications of these products would have increased insurgence and incidence of new typologies of adverse events. Meanwhile, the growing availability of long-term clinical data and of more biomedicines with similar therapeutic indications, gradually offered the possibility of more solid and comprehensive evaluations on their safety, as single therapeutic agents or as drug classes sharing structural and/or functional properties.

On this basis, having initially examined the safety frameworks of the most relevant products of the area, some comparative analyses and common peculiarities in the generation of adverse events of some drug classes can be attempted.

In principle, AEs pathogenesis of biomedicines can be attributed to their mechanism of action and/or to their immunogenicity, i.e., to the consequences of targeting specific cell structures such as receptors or ligands, or to the specific structure of biodedicines, mostly consisting of glycoproteins containing animal (rodent) and/or human sequences. The first group of AEs can be considered consequent or associated to the pharmacological activity of the biomedicines, while the reactions caused by their immunogenicity are dependent mostly on the typical macromolecular, proteic structure of the agent, which acts as a strong foreign antigen promptly recognized by the recipient's immune system.

While AEs of the former group are frequently, but not always, linked to the therapeutic effect of the biomedicine, the latter reactions may not, and can not interfere with clinical effects by reducing drug availability. Therefore, during biomedicines' development it resulted urgent and more feasible to reduce their immunogenicity by progressive humanization of the molecules, up to fully human protein sequencing and glycosylation, than trying to dissect the efficiency from adverse reactivity, the "bonus" from "malus" activity at clinical level. Humanization procedures sharply reduced immunogenicity, although they were neither able to abolish AEs, nor to avoid their most severe and life threatening expressions [1, 2]. In fact, even fully humanization could not produce "stealth" molecules, since their structure can be still recognized as an allogenic "foreignness," yet able to induce sensitization of the recipient, and provoke hypersensitivity reactions of all types and severity.

However, surprising cases of tolerability were also experienced. For example, one patient previously showing a severe anaphylactic reaction to the chimeric murine basiliximab could receive the humanized daclizumab directed to the same IL-2R-α chain, without any adverse effects. Notably, the patient had a positive skin test to basiliximab and to horse and rabbit polyclonal anti-thymocyte antibody preparation, but not to daclizumab after prick and intradermal testing [3].

An alternative approach to reduce AEs among mAbs ($\simeq$147 kD) was the truncation of the Fc fragment, when the therapeutic effect was not critically linked to the expression of CDC and/or ADCC. In this case the shortage of the half-life of the remaining Fab portion was compensated by coupling the remaining Fab fragment with PEG, leading to products with reasonable durability and a lower AEs potentiality. For example, certolizumab is a pegylated recombinant

humanized Fab fragment (91 kD) composed of a single light and heavy chain derived from a murine IgG2a antibody, directed against soluble and transmembrane TNFα. The overall safety profile resulted more selective than other members of the same drug class. The absence of the Fc fragment avoided CDC and ADCC-dependent reactions. However, the incidence of infections and in particular of granulomatous infections, including new cases or reactivation of TB, were not reduced, thus indicating their strict relation to the Fab-mediated portion of the molecule and very likely to the expressed mechanism of action. Abciximab is a smaller fragment (47.6 Da) consisting in a disulfide-linked dimer of an Fd heavy chain fragment and an intact light chain. It is directed against the CD41 integrin and inhibits platelet aggregation. The safety profile consisted in hemorrhagic complications, strictly related to its mechanism of action, but also to its immunogenicity, which caused ITCP.

Pegylation has been used also for preparing therapeutic formulations of interferons (peg-IFNα-2a, and 2b), erythropoetins (peginesatide, peg-epoetinβ), of hemopoietic growth factors (pegfilgrastim), and mAbs (cetuximab) leading to improvement of their half-life and to mitigation of immunogenicity.

By contrast, in the case of fusion proteins, usually the addition of a human Fc fragment was necessary to express CDC/ADCC effector functions and increase their half-life, which inevitably carried some AEs enhancement as well.

Efforts to imbalance the risk/benefit ratio in favor of the latter were also attempted by increasing the affinity of the agent for its target. However, this was not always the case: motavizumab, for example, which was developed by affinity maturation from palivizumab, did not show a better efficiency, yet higher rates of AEs, SAEs, and death. Attempts to improve edrecolomab efficacy by increasing affinity up to 100 fold produced modest clinical results, but serious toxicities. By contrast, nimotuzumab—showing a lower affinity for EGFR, one log lower than cetuximab and 2 logs lower than panitumumab—apparently expressed a better safety profile in this drug class, without showing striking differences in terms of relative efficiency. In this case, the lower affinity seemed to better discriminate EGFR overexpressing neoplastic cells from normal epithelial cells, thus achieving a better risk/benefit balance. Notably, in these cases, as in others, the skin seemed to be a particularly sensitive target in evidencing, and discriminating among different safety profiles.

When immune-mediated effector functions were not needed in the mAb molecule, the IgG2 isotype was chosen, being an irrelevant inducer of CDC and ADCC activity, thus avoiding the related AEs events. This is the case of panitumumab, tositumomab, and daclizumab. Alternatively, the IgG4 backbone virtually not binding complement was preferred, such as for natalizumab, and gemtuzumab, or a hybrid IgG2/IgG4 combination as in eculizumab to take advantage of both properties.

Glycosylation was not immediately considered a crucial characteristic of biomedicines, but it became clear that the quality and quantity of glycosylation interfered with CDC and ADCC activity, as well as with immunogenicity, and therefore with the induction of AEs. For example daclizumab and the DAC HYP

analog have the same amino-acid sequence but a different glycosylation pattern affecting the binding of the latter molecule to the Fc receptors, resulting in decreased CDC and ADCC activity, expected to improve safety without altering efficiency.

Anomalous glycosylation patterns may provoke unexpected, unwanted immune reactions. In the case of cetuximab, its expansion in the murine Sp 2/0 cell line transferred galactose-α-1,3-galactose on the heavy chain of the Fab fragment, which at first infusion induced a severe IgE-mediated anaphylactic reaction, due to pre-existing antibodies in cetuximab recipients [4].

Glycosylation variability has also been of concern in the production of some biosimilar biomedicines, such as erythropoetins. In fact such variability, among others, can influence immunogenicity and has caused problems for the approval of some growth hormone biosimilars [5]. With this respect, the preparation of syalo-carbomylated and non-glycosylated erythropoietin recent formulations may help in better understanding their role in immunogenicity, and in AEs induction.

Taken together, it became evident that "biological" molecules fulfilled expectations more in terms of efficacy than in being "innocuous" or "invisible" to the immune system. Therefore, AEs will continue to be part of biomedicines' therapeutic scenery, although with milder characteristics when compared to chemotherapeutics and to other immunosuppressive drugs, but also with some additional peculiarities mostly related to their glycoproteic structure.

On this basis, in line with the general classification for all adverse drug-related events (see Chap. 2), AEs to biomedicines can be identified as:

(A) *AEs related to the mechanism of action*: They may derive from a direct and specific action (direct toxicity, induction of apoptosis), or as a consequence of the drug-target binding causing secondary toxicities (cytokine release, tumor lysis syndrome).

(B) *AEs related to the immunogenicity of the molecule*: They may occur as a consequence of hypersensitivity reactions triggered by the biomedicine recognition as a foreign complex of antigens, or by cross-link antigenicity with pre-existing antibodies or sensitized T cells.

However, some peculiarities need to be underlined. Predictability of DRAEs is mainly assigned to Type A reactions, while unpredictable immune-related Type B reactions are usually restricted to predisposed individuals (see Table 2.1). The overall frequency of ADEs was estimated to be over 80 % for Type A, and 10–20 % for Type B.

In the case of biomedicines, predictability is not so clear-cut between the two ADEs groups. One possible reason is the existence of multiple mechanisms, only partially known, involved in the pharmacological action of these agents. Moreover, being biomedicines proteic structures with a relatively high molecular weight they have high immunogenic potential; Type B reactions are expected to be more relevant than for small chemical therapeutic molecules. The different degree of "humanization" easily proved the possibility of reducing such immunogenic potential and the consequent capacity of inducing AEs, although leaving large margins of variability.

Therefore, a higher level of unpredictability in Type A and a higher frequency and variability in immune-related Type B than non-biological drugs are to be expected. The latter type of reactions in the case of biomedicines seems to be involved mainly in early events and in the reduction of pharmacological efficiency.

Finally, due to their relatively high immunogenic potential, ADEs induced by biomedicines (BAEs, Table 2.4) must be envisaged from a larger population of individuals than those usually identified as "predisposed," "genetically predisposed," or "atopic" subjects. However, these concepts better fit with specific hapten-directed immune events, more than with the more general reactivity to large multi-antigenic proteic structures.

In conclusion, ADEs in the treatment with biomedicines are an obligatory companion, which must be known, interpreted, prevented, and managed. Interestingly, the unwanted companion in some instances appears so strictly related to drug's efficiency to become a prognostic factor of clinical response, such as rash for cetuximab.

Two further approaches to reduce immune reactivity to biomedicines relate to procedures for *deimmunization* and *desensitization*. The former, in line with the mentioned more coarse techniques of mAb splitting and elimination of Fc fragment, is a new technology that allows to locate and selectively remove T cell epitopes responsible for the expression of immunogenicity within the variable region sequences of mAb, fusion proteins, or from any other proteic structure. Importantly, this technique influences the immunogenicity of the structural area involved in the mechanism of action of these biomedicines [6].

Desensitization is a known procedure widely used to mitigate allergic reactions to insect venoms and pollens. In this case the potential offending agent is administered in a stepwise, highly controlled regimen. Such procedure has been adopted, for example, to mitigate infusion reactions after rituximab, infliximab, cetuximab, and trastuzumab among others [7].

Both approaches deserve more attention from clinicians and biomedicines' manufacturers to mitigate and prevent the insurgence of undesired events.

Provided that the AEs expression variability is elevated among biomedicines and that experience is still limited with the most advanced formulations, it may be nonetheless useful for practical purposes to depict:

(1) The *general safety profile* of most relevant and frequent adverse events
(2) The *drug class safety profile*, at least for those categories represented by more than two therapeutic formulations.

In attempting to depict a *general safety profile* it is useful to group the analyzed biomedicines according to their common target, as reported in Table 58.1, which may help in better individuating shared AEs more strictly related to a similar mechanism of action. These agents can be also distinguished for having inhibitory effects (Class 1–10) or stimulatory effects (Class 11). In particular, among the inhibitory classes some are more strictly related to the targeted molecule, while other are more broadly grouped according to the targeted cell type/s. Typical target-specific groups are TNF inhibitors (Class1), anti-VEGF agents (Class 4), and anti-EGFR (Class 5). By contrast, Class 2 is characterized by the targeted

Table 58.1 Classes of biomedicines

Class	Target	Main expression	Biomedicines
		Inhibitory effect	
1	TNFα	Soluble and on T, M, Mθ, NK	Adalimumab
			Certolizumab
			Golimumab
			Infliximab
	TNFR	T, M, Mθ, NK	Etanercept
2	IL-1R	Ubiquitous	Anakinra
	CD25 (in IL-2R)	aT, aB, THY, MYpr, ODC	Basiliximab
			Daclizumab
	α-4β1, α-4β7(integrin)	T, B, M, Mθ, Bas, E	Natalizumab
	CD52	T,B, M/Mθ, NK(50 %)	Alemtuzumab
	IL6R (CD126/130)	Soluble and on T, B, G, F, Mθ	Tocilizumab
	CD11a (LFA-1)	T, B, Mθ, N	Efalimumab
	IL-2R	T, B, NK, M	Aldesleukin
			Denileukin-DT
	CD33	MY, M, ERpr	Gemtuzumab
	CD20	pre-B, B	Ibritumomab
			Ofatumumab
			Rituximab
			Tositumomab
	BLyS (TNF family)	Soluble	Belimumab
	CD80/CD86	T, DC	Abatacept
			Belatacept
	CD2	T	Alefacept
	CD3	T	Muronomab
	CD30 (TNF family)	Th2	Brentuximab
	CTLA-4 (CD152)	aT	Ipilimumab
3	IL-1β	Soluble	Canakinumab
	IL-1α, IL-1β	Soluble	Rilonacept
	IL-12/IL-23	Soluble	Ustekinumab
4	VEGF	Ep, E, R, F, M, Mθ, NEU	Aflibercept
			Bevacizumab
			Ranibizumab

(continued)

Table 58.1 (continued)

Class	Target	Main expression	Biomedicines
		Inhibitory effect	
5	EGFR	Epithelia	Cetuximab
			Nimotuzumab
			Panitumumab
	EpCAM	Epithelia	Catumaxomab
			Edrecolomab
	HER-2 (CD340)	Epithelia	Pertuzumab
			Trastuzumab
	EpGFR (epidermal)	Epithelia, Keratinocytes	Palifermin
			Becaplermin
6	RANKL	OB, OC, BMSC, other	Denosumab
7	IFNAR	Epithelia, Virus infected cells	rHuIFN-α, -β
	IFNGR		rHuIFN-γ
8	RSV	Respiratory Syncytial Virus	Palivizumab
9	CD41	Thrombocytes	Abciximab
10	C5	Soluble	Eculizumab
	IgE	Soluble	Omalizumab
		Stimulatory effect	
11	IL-11R	Blood cell precursors	Oprelvekin
	TPOR	Thrombocytes	Romiplostim
	EPOR	ERpr	rHuEPO-α, -β
			Darbepoetin–α
	GFR	G, M	Filgrastim
			Sargramostim
	SCR	BMSC, PBPC	Ancestim

aN activated neutrophils; aT, aB activated lymphocytes; Bas basophils; BMSC bone marrow stem cells; DC dendritic cells; E eosinophils; E/Ep endothelia/precursors; ERpr erythroid precursors; F fibroblasts; G granylocytes; M monocytes; Mpr myeloid precursors; MY/MYpr myeloid cell lineage/precursors; Mθ macrophages; N neutrophils; NEU neurons; NK natural killer cells; OB, OC osteoblasts, osteoclasts; PBPC peripheral blood presursor cells; R renal cells; T,B lymphocytes; Th2 T-helper cells; THY Thymocytes. See also list of acronyms.

cells, mostly represented by mAbs directed to a variety of molecules expressed on WBC, either widely shared or specifically restricted to a cell type (T, B) or even to a subgroup of them (Th, aT). Clearly, whenever inhibitory effects are directed against downregulators of the immune response (CD8+T cells, Treg), overstimulation, and autoimmune reactions can be expected as outwardly paradoxical

Table 58.2 Classes of biomedicines and their safety profiles

Class	Inhibitory effect		Safety profile	
	Target	Biomedicine	BBW	Main additional group features
1	TNFα	Adalimumab	SI, TB, M	OI, TB
		Certolizumab	SI, TB, M	H/A
		Golimumab	SI, TB, M	M: L/LK, HSTCL, TCL,NMSC, Solid tumors
		Infliximab	SI, TB, M	HBV, DD (MS, GBS, PNP, etc.): exacerbation and new
	TNFR	Etanercept	SI, TB, M	HF: LLS; CP
2	IL-1R	Anakinra	–	SI, H/A, IR, M, NP, ISR (TNF inhibitors increase infections)
	CD25 (in IL-2R)	Basiliximab	–	I, IR, H/A, HYP, PY
		Daclizumab	–	CT, H/A, HYP, HYG, PY, GI,WH, Edema, Tachycardia, Bleeding Thrombosis
	α-4β1, α-4β7(integrin)	Natalizumab	PML	H/A, HT, SI, IR, IRIS, WBC and nucleated RBC increase
	CD52	Alemtuzumab	CT, SI, IR	A,OI (CMV), IR
	IL6R (CD126/130)	Tocilizumab	SI	A, CT, DD, GIP, HT, ILD, IR, M, MAS, NP, OI,TCP, TB, WH Dyslipidemia
	CD11a (LFA-1)	Efalizumab	PML, SI	OI (CMV),DD (GBS, PNP), IHA, M, NF, ITCP, DW
	IL-2R	Aldesleukin	CLS, DI, CT	PY, TCP, HT, NPD, AKF, Chemotaxis impairment
		Denileukin-DT	CLS, IR, V	HT, Hypoalbuminemia, Visual and color acuity disorders
	CD33	Gemtuzumab	H/A, IR, HT	Severe pulmonary events during IR, TLS
	CD20	Ibritumomab	MCR, IR, CP	MDS/AML, FT, ST (SJS, exfoliative dernatitis, etc.)
		Ofatumumab	–	IR, CP (NP), SI (OI), PML, HBV, IO
		Rituximab	IR, TLS, MCR, PML	SI, HBV, CT, GIP, RT, CP, Hypo-Ig
		Tositumomab	H/A, CP, RE	M (MDS/AML, solid tumors), Hypothyroidism, FT
	BLyS (TNF family)	Belimumab-fh-IV	–	SI, H/A, Depression, Increased mortality
	CD80/CD86	Abatacept	–	H/A, SI,TB, M, IR, (TNF inhibitors increase infections; COPD increase respiratory AEs)
		Belatacept	SI, M(PTLD)	PML, OI (CMV), TB, PVN, Solid tumors, NMSC, HYP, Dyslipidemia
	CD2	Alefacept	–	SI, M (NMSC, HL, NHL), H/A, HT, LP
	CD3	Muromonab		
	CD30°	Brentuximab - ch-IV	PML	PNP (mostly sensory), IR, NP, TLS, PML, SJS,
	CTLA-4 (CD152)	Ipilimumab	IMAE	IMAE: hepatitis, endocrinopathies, SJS, TEN, Enterocolitis, GBS, PNP

(continued)

Table 58.2 (continued)

Class	Inhibitory effect			Safety profile
	Target	Biomedicine	BBW	Main additional group features
3	IL-1β	Canakinumab	–	SI (URTI, some OI), H/A, ISR, (TNF inhibitors increase infections)
	IL-1α, IL-1β	Rilonacept	–	SI (URTI, bacterial meningitis), H/A, ISR, Dyslipidemia (TNF inhibitors increase infections)
	IL-12/IL-23	Ustekinumab	–	SI (Mycobacteria. BGC, Salmonella), M (solid tumors), H/A, RPLS
4	VEGF	Bevacizumab	HD, GIP, WH	Hemorrhage, non-GIP, ATE, HYP, RPLS, Proteinuria, IR, ovarian failure
		Aflibercept (zaltrap)	HD, GIP, WH	Hemorrhage, non-GIP, ATE, HYP, RPLS, Proteinuria, IR,NP, Diarrhea
		Aflibercept (eylea)	–	SI (endophthalmitis), Retinal detachment, IOP, ATE
		Ranibizumab	–	SI (endophthalmitis), Retinal detachment, IOP, ATE, D (DME)
5	EGFR	Cetuximab	IR, CT	Cardiopulmonry arrest, PT (ILD), ST (acneiform rash), Hypomagnesemia
		Nimotuzumab	–	IR, HYP, ST (mild), PY, Hypomagnesemia
	EpCAM	Catumaxomab	–	CRS, SIRS, GI disorders, HYP, LP, SI, Rash
		Edrecolomab		GI disorders (diarrhea), H/A
	HER-2 (CD340)	Pertuzumab	FT	LVEF dysfunction, IR, H/A
		Trastuzumab	IR, CT, PT, FT	LVEF dysfunction, ILD, NP, Anemia, SI, RT, TE, Diarrhea
	EpGFR (epidermal)	Palifermin	–	M (epithhelial), Rash, Tongue/taste altered, Dysesthesia, Lipase/amylase increase
		Becaplermin	M	M (local and distant; increased mortality)
6	RANKL	Denosumab	–	Hypocalcemia/phosphatemia, ONJ, FT
7	IFNAR	rHuIFN-α, rHuIFN-β	–	NPD, HT, H/A, CHF, LKP, AID (ITCP, AIH, THY), Seizures
	IFN-alfacon-1	synthetic IFN-α	D	D:(in NPD, AID, SI, CVD). FT, PT, HT, RF, H/A, OD, AID, PNP, Colitis, Pancreatitis
	IFNGR	rHuIFN-γ	–	CT, CRS/FLS, HT, NPD, ISR
8	RSV	Palivizumab	–	H/A, PY, TCP, ISR, Rash
9	CD41	Abciximab		TCP, Bradycardia, H/A, ARDS, Hemorrhage
10	C5	Eculizumab	SI	SI (meningo, strepto, haemophilus), IR. URTI, Tachycardia
	IgE	Omalizumab	–	H/A, TCP, ISR

(continued)

Table 58.2 (continued)

Class	Inhibitory effect		Safety profile	
	Target	Biomedicine	BBW	Main additional group features
11	IL-11R	Oprelvekin	H/A	CLS, Edema (facial, pulmonary), Papilledema, Anemia (dilutional), CT, RF
	TPOR	Romiplostim	–	M (MDS/AML progression), TE, TCP, BMRF, Erythromelalgia
	EPOR	rHuEPO-α, rHuEPO-β	M, CT	D (in CKD), M (progress/recurr; solid/lymphoid), H/A, HYP, Seizures, PRCA, Stroke
		Darbepoetin-α	M, CT, TE, D	D (in CKD), M (progress/recurr; solid/lymphoid), H/A, HYP, Seizures, PRCA, Stroke
	GFR	Filgrastim/ pegfilgrastim	–	Splenic rupture, Bone pain, ARDS, H/A, Sickle cell crisis, M (MDS/AML), ISR
		Sargramostim	–	CLS, Edema, CT, RF
	SCR	Ancestim	–	H/A, M (SCLC, MCL, MM), Leukocytosis, ISR (distant recall)

A anaphylaxis; AID autoimmune disorders; AIH autoimmune hepatitis; AKF acute kidney failure; ANAs anti-nuclear antibodies, all types; ARDS acute respiratory distress syndrome; ATE artero-thrombotic event; BMRF bone marrow reticulin formation; CHF congestive heart failure; CKD chronic kidney disease; CLS capillaty leak syndrome; CMV cytomegalovirus; COPD chronic obstructive pulmonary disease; CP cytopenia; CRS/FLS cytokine release syndrome/ flu-like syndrome; CT cardiotoxicity; CVD cerebrovascular disorders (stroke, etc.); exacerbation and new; D death (increased mortality); DD demyelinating disorders; DME diabetic macular edema; DW disease worsening (in treatment); FT fetal toxicity; GBS Guillain Barré syndrome; GI gastrointestinal disorders; GIP gastrointestinal perforation; H, H/A hypersensitivity, and including anaphylaxis; HBVr hepatitis B virus reactivation; HD hemorrhagic disorders; HF heart failure, all type; HL Hodgkin lymphoma; HSTCL hepato-splenic Tcell lymphoma; HT hematotoxicity/bone marrow toxicity; HYG hyperglycemia; HYP hypertension; IHA immune hemolytic anemia; ILD interstitial lung disease; IMAE immune-mediated adverse events (Tcell activation); IO intestinal obstruction; L/LK lymphoma/ Leukemia; IOP intraocular ocular pressure (increased); IR infusion reaction; IRIS immune restoration inflammatory syndrome; ISR injection site reaction; ITCP immune thrombocytopenia; LKP leukopenia; LLS lupus-like syndrome; LP lymphopenia; LVEF left ventricular ejection fraction; M malignancy; MAS macrophage activating syndrome; MCL mastcell leukemia; MCR muco-cutaneous reaction; MDS/AML myelodisplastic syndrome/acute myeloid leukemia; MM malignant melanoma; MS multiple Sclerosis; NF necrotizing fascitis; NHL non-Hodgkin lymphoma; NMSC non melanoma skin cancer; NP neutropenia; NPD neuro-psychiatric disorders; OD ocular disorders; OI oppertunistic infections, all type; ONJ osteonecrosis of the jaw; PML progressive multifocal leucoenchephalopathy; PNP peripheral Neuropathy (polyneuropathy); PRCA pure red cell aplasia; PSD psychiatric disorders; PT pulmonary toxicity; PTLD post-transplant lymphoproliferative disorder; PRCA pure red cell aplasia; PSD psychiatric disorders; PT pulmonary toxicity; PTLD post-transplant lymphoproliferative disorder; PVN polyoma virus nephropathy; PY pyrexia (relevant); RE radiation exposure; RPLS reversible posterior leukoencephalitis syndrome; RT renal toxicity; SCLC small cell lung cancer; SI serious infections; SIRS systemic inflammatory response syndrome; ST skin toxicity; TB tuberculosis (reactivation and new); TCL T cell lymphoma; TCP thrombocytopenia; TE thromboembolism; TEN toxic epidermal necrolysis; THY thyroiditis (autoimmune); URTI upper respiratory tract infections; WH wound healing retardation

See also list of acronyms

effects. Finally, some agents directed to specific targets act as carriers of toxins (denileukin-diftitox) or radionuclides (ibritumumab-tiuxetan-Yttrium, tositumomab-iodine), thus combining therapeutic actions and adverse reactions as well. They have a limited use and cannot be assimilated into a specific drug class.

On this basis, a specific *drug class safety profile* can be attempted, as summarized in the following Table 58.2.

58.1 General Safety Profile

58.1.1 Infusion Reactions and Injection Site Reactions

Possibly the most common and typical early event following biomedicines administrations, infusion reactions, usually occur during the first or second exposure. They are generally well tolerated, manageable, and in part prevented or mitigated by prophylactic therapy, but can be severe and sometimes fatal. Their incidence can be observed well over 50 % of recipients after mAbs administration such as, alemtuzumab, gemtuzumab, or rituximab, and at lower frequency with fully human products, such as panitumumab (about 5 %). This kind of reactions is generally non dose-dependent, and can be partially masked/mitigated by premedication.

Seven biomedicines (alemtuzumab, gemtuzumab, ibritumomab, rituximab, cetuximab, trastuzumab, and denileukin) have a BBW on infusion reactions, indicating their potential severity in their expression. They are not directed to the same targets, but five of them are mainly expressed on leukocytes and two are directed to epithelial surface molecules. Similarly, they do not pertaining to the same structural class and include mAbs, fusion proteins, cytokines, and cytokine receptor analogues. However, 12 additional agents can induce infusion reactions without having a special warning for them. Overall, 13/19 involved biomedicines are directed to cell surface structures expressed by leukocytes (mainly on T cells, B cells, and monocytes), 4 were directed to epithelial cells, and 2 against VEGF molecules (Table 58.1).

Importantly, infusion reactions tend to decrease over time at subsequent administrations. This phenomenon has been attributed to a hypothetical “acquired tolerance”, yet to be ascertained.

Infusion reactions have been also attributed to the presence of pre-existing antibodies against murine or human antigens in normal subjects, cross-linking with the respective analogs inserted in the mAb structure. More frequently they appear induced by direct action on immune-related receptors and ligands, inhibited or stimulated by a number of biomedicines, mimicking such events, and even producing impressive systemic reactions such as CRS. TLS, and SIRS (see Chap. 3). The role of glycosylation in modulating these responses has been previously mentioned.

Fusion proteins indicated for intravenous administration appeared to elicit a lower number of reactions, such as belatacept (5–25 %), abatacept (6 %), and denileukin diftitox (8 %) underlining the crucial role of the Fc fragment, which was truncated in these formulations.

Altogether, these events are difficult to distinguish from concurrent classical hypersensitivity Type I (IgE-mediated) and Type II cytotoxic (IgG/IgM- mediated) reactions in response to their immunogenicity.

It must be stressed that the existence of drug-induced allergic responses was already known for low molecular weight conventional drugs, which can trigger

immune reactivity either acting as haptens conjugated to endogenous proteins after administration, or by direct interaction with immune receptors [8], even after non-covalent binding to MHC and TCR molecules [9].

Injection site reactions with biomedicines, anyway injected, are frequent, but usually not worrisome. Etanercept can induce reactions in over 40 % of patients, but have the tendency to decrease with prolonged use, a trend observed also with other biomedicines. Histologically, they showed CD8+ T lymphocyte and eosinophil infiltration, with an increased expression of HLA-DR on keratinocytes [10]. Occasional severe ulcerated necrotic reactions were observed with IFN formulations, and in particular with IFN-β2b [12]. Noteworthy, most systemic treatments with conventional drugs, especially directed against cancer, are associated with similar reactions during which it is difficult to distinguish hypersensitivity phenomena from direct toxicities induced by the various agents often administered in complex combinations. However, reactions caused by biomedicines tend to appear earlier and at the very first administration. For all of them accurate prevention, proper administration, and symptomatic therapy are crucial to significantly mitigate their effect [10–12].

58.1.2 Infections

Infectious complications are common events during treatment with biomedicines inducing direct or indirect immunosuppression, thus causing a transient secondary immunodeficiency that can be profound and prolonged.

All TNF inhibitors are relevant inducers of infections. They differ both in typology (e.g., TB and other opportunistic infections, mainly Pneuocystis Histoplasmosis) and frequency (higher with infliximab than with etanercept, etc.), although rarely reaching statistical significance in comparative analyses.

Ten biomedicines have a BBW for serious infections. They can be all included in Type A reactions, and pertain mainly to Classes 1, 2, and 10 (with a BBW warning), and to Classes 3, 4, and 7 (without a BBW). Indeed, in these groups infections are particularly severe. They include fungal, viral, TB reactivation or new onsets, and other opportunistic infections with a trend to be disseminated.

In the case of local (intravitreal) administrations, aflibercept, ranibizumab, and bevacizumab (in off-label administration) cause endophthalmitis, which is infrequent albeit serious.

From this overall experience some relevant proofs of concept have emerged in relation to the crucial role of distinct receptors and ligands, blocked by biomedicines, in immune defense from specific infectious agents.

Eculizumab, blocking the C5 factor and the consequent activation of terminal complement cascade, showed its fundamental role in the protection against Neisseria infections, thus mimicking the rare cases of C5 complement congenital deficiency observed in humans.

The reactivation or new insurgence of TB during anti TNF-α therapy indicated the key role of this cytokine pathway in organizing the defense against mycobacterial infection and in the modulation of inflammatory granuloma formation. Moreover, the experience of various biomedicines available in the anti-TNF drug class revealed the existence of a hierarchy among inhibiting signals expressed by a different incidence and gravity of emerging infections, which were also influenced by the underlying disease under treatment [see also certolizumab, Chap. 14].

The reactivation of viruses, such as HBV, EBV, and JC virus had been observed in a number of clinical conditions during treatment. In particular, cases of HBV reactivation and/or possible new infections were observed with certolizumab, efalizumab, etanercept, golimumab, infliximab, muromonab, ofatumumab, rituximab, tocilizumab, and ustekinumab. Reactivation of EBV was observed with alemtuzumab, belatacept, brentuximab, canakinumab, daclizumab, and muromonab. Finally, the most intriguing JC virus reactivation was detected after belatacept, efalizumab, natalizumab, rituximab, and tocilizumab. The wider spectrum of pathways intercepted by biomedicines indicated that virus replication and diffusion are under the control of many immune mechanisms, although the TNF pathway appeared particularly important.

JC virus reactivation was particularly concerning because of the rapid induction of PML. In particular, the insurgence after natalizumab treatment clearly indicated the role of integrins, which are involved both in the T cell trafficking and cell adhesion. Moreover, natalizumab mobilizes CD34+ hemopoietic cells—which are considered a reservoir of JCV—thus contributing to virus diffusion to CNS, being such transfer through BBB possibly facilitated by the anti-integrin effect of the monoclonal. These recent data may help in designing more selective biomedicines, with the aim of improving the risk/benefit balance.

The virus activation observed with efalizumab was also instructive. In fact, this mAb was particularly active in inducing viral and mycobacterial infections, including PML and TB infections, indicating the crucial role of another integrin (LFA-1R) in these processes. Efalizumab was withdrawn from market in 2009.

The overall incidence of infections is increased by all immunosuppressive biomedicines, and when particularly effective they cause also opportunistic infections whit a tendency to be disseminated. Comparative data on 8 mAbs, 3 fusion proteins, and one IL-1R antagonist (anakinra) indicated a higher risk of serious infections with certolizumab, infliximab, and tocilizumab; thus indicating possible differences related to the respective mechanisms of action, as repeatedly reported in this volume. In contrast, the risk of TB appeared increased (OR: 4.68, 95 % CI 1.18 to 18.60) for the whole group of examined biomedicines [13].

Importantly, patient's accurate selection, antibiotic prophylaxis, and close monitoring are crucial for their control.

58.1.3 Hematological Events

Hematotoxicity is common among biomedicines and for some of them this event was expected, being strictly related to their mechanism of action. This is the case of abciximab, an anti-GPIIB/IIIa receptor specifically blocking platelet aggregation causing hemorrhage. However, in the case of alemtuzumab, an anti-CD52 protein expressed on virtually all immune cells but not on megakaryocytes and platelets, a less expected diffuse hematotoxicity included severe (up to 50 % of cases in some studies) and fatal cases of TCP, which only in a minority of cases were found to be immune-mediated (ITCP). Similarly, mild to severe unexpected events were also observed after the administration of agents not specifically directed to bone marrow and blood cell components. For example, TCP was observed after efalizumab (anti-integrin), infliximab (anti-TNF), and rituximab (anti-CD20, exclusive of B cells). In the case of efalizumab, an immune-mediated thrombocytopenic activity was detected in some cases.

Among anti TNF-α agents, thrombocytopenia, as well as neutropenia, hypercoagulability, pancytopenia, and aplastic anemia are uncommon, but can be fatal. Interestingly, it seems that in vitro TNF-α can elicit both stimulatory and inhibitory effects on hemopoietic progenitors, which would indicate that under certain conditions anti-TNF therapy may also induce inhibiting effects on hemopoietic stem cells differentiation [14]. In the case of rituximab, an anti-CD20 transmembrane differentiating agent virtually expressed only on B cells, thrombocytopenia was observed in about 11 % of cases and was serious in over 4 %. Notably, rituximab was effective in restoring platelet levels in ITCP, yet for unexplained reasons, since levels of anti-platelet antibodies remained unchanged in these patients, while the platelet counts increased [see rituximab, Chap. 35]. Hematotoxicity signs are also reported for gemtuzumab (conjugated with the cytotoxic antibiotic ozogamicin), pertuzumab, ofatumumab, tocilizumab, trastuzumab, aldesleukin, denileukin (conjugated with diphteria toxin), and IFNs.

Overall, the pathogenetic mechanisms of a number of drug-related blood disorders remain substantially unknown, yet they are often included into the wide and vague category of "bone-marrow toxicities." Therefore, several aspects of hematotoxicity not directly related to the therapeutic mechanisms of action still need to be investigated, in order to better understand their pathogenesis, and hopefully develop agents in which secondary mechanisms of toxicity could be split off.

Meanwhile, accurate pre-clinical investigation, patient's selection and supportive therapy, also with the powerful bone marrow stimulating factors, are crucial for the control and mitigation of such events.

58.1.4 Anti-Drug Antibody Response

The induction of various types of antibody response is a frequent event with biomedicines for reasons repeatedly mentioned. Anti-drug antibodies may be

developed against mAbs and fusion proteins, either murine (HAMA), chimeric (HACA) or human (HAHA), mainly as IgG, but also as IgM, IgA, and IgE in more limited occasions [15]. Less frequently, these antibodies are neutralizing, and consistently interact with pharmacokinetics of the injected drug [16]. In fact, they impact on safety and efficacy of biomedicines, through altered biodistribution and clearance of the product.

Although mitigated by a number of procedures [2], they remain a major concern, and therefore specific guidelines for their assessment during development of biotechnology-derived therapeutic proteins were issued by some control Agencies [17]. The incidence of such antibodies ranges from about 5 to 65 % according to—yet not strictly dependent on—their level of humanization. The major consequences are immediate adverse reactions and reduction of drug efficiency due to the presence of neutralizing antibodies [16, 18]. However, their presence and role not always appears sufficiently investigated, such as with respect to the Ig subclass role on specific AE outcomes. In some instances, it is surprising that their presence was reported not to interfere with clinical efficiency with respect to observed clearance of the drug in study. Quite rare are specific investigations on IgE presence during Type I hypersensitivity reactions.

58.1.5 Autoimmune Events

Agents interfering with the regulation of the immune system, through immunosuppressive or immunostimulating actions are expected to imbalance the endogenous immunosurveillance, thus enhancing the possibility for autoreactive cell clones to sneak through. Autoimmune phenomena, such as the production of autoantibodies, exacerbation of pre-existing autoimmune diseases or insurgence of new immune disorders, have all been observed during and after the aministration of a number of biomedicines. Overall, they tend to be expressed more frequently in patients with existing immune dysregulations or overt autoimmune disease. For example, exacerbation and new cases of rheumatic disorders were observed with abatacept (Ps), adalimumab (demyelinating disorders), anakinra (RA), certolizumab (RA, CD, Ps), efalizumab (Ps), etanercept (demyelinating disorders), golimumab and infliximab (palmar pustular psoriasis), natalizumab (CD), ustekinumab (Ps), and rituximab (Ps). Aldesleukin showed a complex multi organ safety profile including new onset and exacerbation of autoimmune disorders. It must be stressed that these complications are quite distinct from rebounding of autoimmune disorders undergoing treatment after therapy interruption or discontinuation.

Among autoimmune conditions particularly evidenced during such treatments there are the lupus-like syndrome (LLS), autoimmune thyroiditis, and autoimmune colitis.

LLS was infrequently observed with natalizumab, rituximab, infliximab, etanercept, certolizumab, alemtuzumab, and adalimumab. The syndrome is associated

with the presence of autoantibodies (ANA, dsDNA), but has not been observed in all antibody-positive patients. Interestingly, LLS tended to subside after therapy discontinuation.

Autoimmune thyroiditis was frequently observed after off-label alemtuzumab administration reaching 25 % of treated MS patients. However, thyroid dysfunction is a common event during treatment with IFNs, IL-2, TYK inhibitors, ipilimumab, tositumomab, daclizumab, abatacept, denileukin-diftitox, and with non-biological agents. In particular, primary hypothyroidism is the most common occurring event, but cases of hyperthyroidism and thyrotoxicosis have been also described. The overall incidence ranges from 20 to 50 % of treated cases, but possibly the amount of the drug-induced dysfunction has been underestimated because of the existence of a number of subclinical forms, often confounded by underlying disease symptoms [19].

Autoimmune enterocolitis/colitis and hepatitis have been observed after ipilimumab and tremelimumab (now in Phase III advanced evaluation with unsatisfactory results) administration, both acting as inhibitors of CTLA-4, a member of the Ig superfamily expressed on T cells including Treg lymphocytes. CTLA-4 generates inhibiting signals on T cells and APC cells. Notably, complete knockout of CTLA-4 signals is lethal in animal models and induce massive infiltration of T cells into parenchymal tissues, leading to organ destruction.

Cases of autoimmune hepatitis were also observed after etanercept, infliximab daclizumab, tocilizumab, and after IL-2 (aldesleukin, denileukin) treatment. Noteworthy, fatal cases of autoimmune hepatitis were also observed with IFNs (α, β, and γ).

Some of these disorders are partially reversible after therapy discontinuation. Unfortunately, their prevention is unsatisfactory or not possible. Administration of oral iodine is usually performed before and during treatment for prevention of hypothyroidism. However, cases of hypothyroidism related to the administration of saturated solutions of potassium iodine have also been reported.

58.1.6 Cutaneous Reactions

Skin is a highly sensitive monitor of ADEs, either immune-mediated or not. It has been calculated that cutaneous eruptions are related to drugs in 1–8 % of cases, but these figures appear clearly underestimated when referred to biomedicines. Acute and chronic reactions may involve epithelial, dermal, and vascular skin components with various clinical expressions, from mild to life-threatening syndromes. Generally, mild cutaneous BAEs include rash, maculopapular eruptions, fixed drug eruptions, urticaria, purpura, and vasculitis as the major representative clinical expressions. Severe, life-threatening conditions are mainly represented by Stevens-Johnson syndrome, toxic epidermal necrolysis, acute generalized exanthematous pustulosis, and a more complex and generalized pathology recently called drug-induced hypersensitivity syndrome (DIHS) or drug reaction with

eosinophilia and systemic symptoms (DRESS), characterized by variable skin eruptions, pyrexia and multi-organ involvement associated to signs of lymphocyte activation (lymphadenopathy, lymphocytosis, atypical circulating lymphocytes) eosinophilia, and to frequent endogenous virus reactivation.

Biomedicines with immunosuppressive activity, mainly targeting T cells (muromonab, efalizumab, alefacept, abatacept), anti-TNF agents (adalimumab, infliximab, etanercept), or consisting in IL-2 formulations and in EGF topical and systemic formulations can promote serious cutaneous events (SJS, exfoliative dermatitis, acneiform dermatitis, palmar-plantar erythrodysesthesia) including the insurgence of cutaneous tumors and other distant epithelial malignancies. Signs of severe skin toxicity have been observed with ibritumomab, bevacizumab, cetuximab, nimotuzumab, panitumumab, and trastuzumab.

It must be noted that the skin microenvironment shows some immune autoregulatory peculiarities, which may explain its exquisite local reactivity to allergens, drugs, and some paradoxical events observed during biomedicines' administration. For example, adalimumab showed to increase the number of Langerhans cells in healing psoriasic plaques, thus suggesting that these specialized cutaneous dendritic cells were somehow involved in an anti-inflammatory process induced by the mAb with favorable consequences for the psoriasic disorder [20].

Estimation of the real incidence and prevalence of cutaneous ADEs are difficult, because of the lack of dedicated studies with observational controlled data collection. Some available estimated rates range from 1.8 to 7 cases per 1,000 hospitalized patients. This type of data collection clearly indicates that milder ADEs not requiring hospitalizations were not considered, and therefore figures are likely underestimated being referred only to most serious events.

Systematic overall estimations of cutaneous BAEs are lacking. A network meta-analysis and Cochrane overview performed in 2011 limited the investigation to 9 biomedicines for TB reactivation, serious infections, and lymphoma indicating higher rates in treated groups, but no data were evaluated at cutaneous level. Other studies limited the safety evaluations to specific drug classes, such as anti-TNF agents, and to serious events. Moreover, some biomedicines used in cutaneous pathologies mimic cutaneous ADEs, or induce exacerbation of pre-existing disease, or add new cutaneous events to pre-existing events, thus increasing difficulties in diagnostic interpretation and etiological assessment.

Recently, a number of cutaneous reactions associated with the use of some biomedicines (mainly, TNF inhibitors) were indicated as mimicking skin diseases, and included: psoriasiform eruptions associated with both anti-TNF agents and with rHuGM-CSF; lichenoid eruptions, vasculitis, LLS, linear IgA eruptions associated to rHuGM-CSF administration; acneiform eruptions mainly associated with anti-EGFR agents (cetuximab, panitumumab, nimotuzumab); interstitial granulomatous dermatitis, alopecia, hirsutism, and other hair disorders [21].

The case of anti-EGFR biomedicines (cetuximab, panitumumab) is instructive, since the epidermis is an ineludible co-target of these mAbs directed to epithelial tumors originating in other organs and tissues. Monoclonals induce acute rash and

acneiform dermatitis distinct from acne vulgaris and resistant, when not worsened, by topical therapy for acne. This ADE is so strictly linked to the mechanism of action of the anti-EGFR agents that eruptions not only correlate with their administration, but they are considered a positive prognostic sign.

Surprisingly, the third anti-EGFR mAb, nimotuzumab, showed a lack of severe skin reactions; rash was practically absent and tolerability was considered excellent also for extra cutaneous AEs (see nimotuzumab, Chap. 28).

Finally, a relevant confounding factor in assessing cutaneous ADEs derives from their clinical evaluation, usually not performed by dermatologists in this kind of safety observations.

58.1.7 Cardiotoxicity

Mild to moderate signs of cardiotoxicity are experienced during therapy with biomedicines, mainly in patients with a pre-existing history of cardiovascular disorders. In fact, macromolecules, such as mAbs and FPs do not have substantial access to ion channels in the myocardium, and therefore they are not expected to affect ion currents or channel selectivity as usually occurring with small molecule drugs. Nonetheless, higher rates and more serious events were observed with adalimumab, aflibercept, bevacizumab, etanercept infliximab, pertuzumab, tocilizumab, trastuzumab, and ustekinumab, inducing a number of CHF, LVEF decrease, myocardial infarction, and other functional disorders. Moreover, because of elevated TNF-α in advanced heart failure, their antagonists were proposed and experienced for therapy with lack of efficacy and increase in mortality.

The case of trastuzumab is instructive, since cardiotoxicity seems related to its mechanism of action inducing HER2 blockade. HER2 is overexpressed in epithelial breast cancer cells, but is crucial in MOMP mitochondrial functioning. In fact, cardiotoxicity seems related to the blocking of downstream HER2 signaling causing membrane permeabilization of myocytes, cytochrome-c release, caspase activation resulting in apoptosis, impaired contractility, and LVEF decrease. Furthermore, trastuzumab inhibits neuregulin1 (NRG1), a protein acting on EGFR, which is essential for heart functioning (see Chap. 38).

It must be noted that some of cardiotoxic effects are reversible, but may also be aggravated by therapeutic associations, such as with anthracyclines [22]. Efforts are being made to separate anti-tumoral from cardiotoxic effects, and to individuate preventive screenings for cardiotoxicity during pre-clinical development [23].

58.1.8 Systemic Syndromes

A number of systemic syndromes mostly related to massive cytokine release and/or other bioactive cellular components have been described in Chap. 3, and Table 3.1. They include CRS, CLS, TLS, IRIS, SIRS, and MAC expressing a

variety of symptoms, from mild flu-like signs to life-threatening impressive reactions. PML and RPLS are considered localized forms of IRIS and CLS, respectively. Table 3.2 reports biomedicines more frequently capable of their induction. These syndromes remain mostly uncommon/rare and moderate events, and are preventable and manageable, but in a minority of cases they can be deleterious.

A recent and intriguing new phenomenon is related to the induction of immune-related (mediated) adverse events (IrAEs or IMAEs) as a consequence of therapy with biomedicines exerting an enhanced activity of immune aggression, such as after ipilimumab administration. In this case the inhibition of a natural inhibiting signal mediated by CTLA-4, triggers a number of multiorgan inflammatory processes driven by the massive activation oT cells. IrAEs are highly concerning, yet to be fully investigated and understood (see Chap. 25)

58.1.9 Malignancies

A number of biomedicines express immunosuppressive actions, and therefore they are all considered therapies at risk of malignancy, whether or not an effective increase in tumor incidence was observed during controlled studies. The unwanted effect is considered not linked to direct oncogenic properties of these agents, but to a lowering of immunosurveillance on abnormal proliferating cell clones escaping destruction by cytotoxic effector immune mechanisms.

Most of these agents are used in autoimmune and inflammatory diseases, which already have higher rates of malignancies with respect to the background of the healthy population. Therefore, in most cases, data on the ADE-related increased risk of malignancies are controversial. Anti-TNF agents, such as adalimumab, certolizumab, daclizumab, etanercept, golimumab, and infliximab, as well as biomedicines directed against T, B, other leukocytes, and accessory immune cells are reported as potential inducers of malignancies, with variable and controversial frequencies. Anti-TNF agents are considered at higher risk mainly of lymphoma and leukemia, especially in children and adolescents. However, it must be noted that the area of therapeutic intervention consists of populations per se at higher risk of malignancy, such as rheumatic diseases. Skin cancer, and in particular NMSC, is among the most represented epithelial induced neoplasm, followed by a number of other solid tumors. In some instances, peculiar types of neoplasms were apparently increased after treatment with specific biomedicines. For example, the risk for hepatosplenic T cell lymphoma was increased in IBD young patients treated with infliximab. Malignancies were also expected and observed after radiolabeled mAbs (Ibritumomab-tiuxetan-^{90}Yttrium; Iodine^{131} tositumomab) treatment, including MDS, AML and a number of solid tumors, although rates were not particularly increased in long-term observations. Epoetins iincrease tumor progression and recurrence. EGF, such as becaplermin and palifermin, respectively used for treatment of severe oral mucositis and for diabetic ulcers at

lower extremities, show a consistent stimulation of tumor growth, with increased related mortality, and insurgence mainly of solid tumors in various districts distant from the site of application. Finally, a higher risk for malignancy was theoretically anticipated for ustekinumab, because of potential oncogenic activities of both IL-12 and IL-23 combined with the immunosuppressive effects of this mAb. In fact, epithelial tumors and melanoma in situ were observed, although significantly increased values were confirmed only for NMSC.

Taken together, the risk of malignancy is apparently real in these treatments, but is difficult to estimate in relation to the respective diseased population, while comparison with rates in the normal populations are questionable because of the lack of data on fairly matched groups. An additional confounder consists in the frequent association with immunosuppressive chemotherapy, known to exert further oncogenic effects.

58.1.10 Other AEs Typologies

Constitutional signs and gastrointestinal signs, which represent common reactions to many drugs, rarely show peculiarities during treatments with biomedicines, compared to standard chemotherapy, or other immunosuppressive interventions, which are usually more serious and frequent.

Agents targeting VEFG, such as bevacizumab and aflibercept are particularly aggressive at gastrointestinal level, causing also perforations. Similarly, although to a minor level, cetuximab (anti EGFR), ipilimumab (anti-integrin), and tocilizumab (anti IL-6R) expressed intestinal toxicity and cases of perforation, which mainly are related to underlying pathological conditions (e.g. diverticulitis).

Neuropsychiatric events, as vascular accidents, demyelinating disorders, or infectious complications and cognitive disorders do not show distinctive features or particular associations with specific biomedicines. Neuropathies are also expressed with some frequency during treatment with a number of biomedicines, without showing a peculiar relation with their mechanisms of action or structure. IFNs is associated with an increased trend for psychotic and suicidal disorders. PML and RPLS are specific syndromes observed during treatment with mAbs such as natalizumab, rituximab, brentuximab, ustekinumab, and others [Table 3.2].

At respiratory level, most complications related to infections, which are particularly frequent as nasopharyngitis, URTI, and pneumonia. Interstitial lung disease (ILD) is considered among signs of pulmonary toxicity, and was observed after cetuximab, rituximab, panitumumab, trastuzumab, and etanercept, while COPD was observed after infliximab, rituximab, and etanercept, mainly as exacerbations of previous underlying pathology.

Interestingly, the endocrine system, except for the mentioned autoimmune thyroiditis (see 58.1.5) is not particularly involved. Rare cases of hypophysitis caused by ipilimumab, and more rare cases of diabetes (etanercept) appeared to be rather protected from biomedicines' complications.

58.2 Drug Class Analysis

Having considered individual safety profiles of biomedicines, and most relevant typologies of related AEs, attempts to consider their distribution according to the major drug classes of biomedicines can be instructive.

In Table 58.2 the biomedicines in study are grouped according to the previously described targeted classes. For each product a synthetic safety profile consisting in BBW specifically issued so far, and a number of additional warnings considered more relevant and typical, is reported. Their allocation in the table, allows also the identification of the overall characteristics within each group, as well as the relevant differences in safety profiles among classes and individual agents.

58.2.1 TNF Inhibitors

The essential safety triad expressed by TNF inhibitors includes serious infections, TB reactivation and new, and malignancies reported in BBW of all formulations.

Most members of this class are used for the treatment of rheumatic disorders, Crohn's disease and psoriasis with remarkable results in some of them, although not long-lasting and therefore requiring continuous treatment. Notably, not all diseases in which a relevant pathogenetic role had been attributed to TNF cytokines responded to specific TNF blockade (Sjögren syndrome, vasculitis, and Wegener granulomatosis). Moreover, the responsive diseases, such as RA, JIA, Ps, and CD did not equally respond to treatment, or to any agent of this class.

Although TNF cytokines were shown to play a role in a number of different disorders, such as those involving the cardiac function, CHF resisted or worsened after anti-TNF treatment. Notably, some unwanted effects could be bypassed by shifting to another member of the same drug class.

These differences within the same drug class were reflected also in the expression of other AEs.

Both Type A and Type B DRAEs were observed in this class, the most concerning categories being infections and malignancies consequent to the immunosuppressive activity of all class members. However, their expression, together with other relevant AEs, such as TB reactivation, hepatotoxicity, and induction of anti-drug antibodies varied in frequency and severity according to the agent used. In particular, TB cases appeared more frequently with mAb than with fusion proteins of the same drug class. In addition to the raise of anti-drug antibodies, formation of autoantibodies (ANA, anti-dsDNA) was also observed during anti-TNF treatment, which appears unexpected in the presence of the consistent immunosuppressive activity of this therapy. The concomitant reduction of Treg lymphocytes and consequent decrease of endogenous immunosurveillance have been evoked as a potential pathogenetic mechanisms of antibody response. Interestingly, the presence or entity of autoantibodies does not seem to correlate with increased clinical

signs of disease, and only a portion of positive patients showed associated syndromes, such as LLS.

Negative synergic effects were also observed when employing biomedicines combinations, such as TNF-inhibitors and anakinra (IL-1Ra antagonist), which brought to recommend avoidance of such association. However, the convenience of administering combined therapies for blocking two targets remains a debated issue. For example, it is not clear if the double action by two different biomedicines individually targeting VEGF, for inhibition of tumor vasculature, combined to those killing specific tumor cells, significantly increases efficacy or the insurgence of ADEs. Since the APRIL-dependent pathway is considered important for lupus nephritis, attempts to double block BLys and APRIL though the association of belimumab with atacicept, have been performed. Unfortunately, such attempts have produced a remarkable increase of serious infections, leading to an anticipated termination of the study (see belimumab, Chap. 9).

Although the effective increase of malignancies deserves conclusive data, the overall trend of this class is in favor of the existence of such risk, although not particularly related to length of treatment.

The effect of anti-TNF therapy on MS or other demyelinating disorders is controversial, given the alternate responses to therapy. Furthermore, insurgence of new demyelinating disorders, including MS, during therapy with anti-TNF inhibitors for rheumatic diseases (RA) was also observed.

The introduction of pegylated, Fc deprived mAbs, such as certolizumab, has contributed in understanding the typology of AEs derived from Fc immunogenicity and from its capacity to activate CDC and ADCC immune effector functions, which apparently are not crucial for therapeutic efficacy [24–26].

Overall, differences in the TNF inhibitors' capacity to induce adverse events and their relation to molecular structure or binding affinity, still need to be clarified, and will eventually contribute to future formulations of agents with better risk/benefit balance.

58.2.2 T Lymphocyte Inhibitors

T cell blockade was first attempted with polyclonal anti-lymphocyte and anti thymocyte sera to control rejection of solid organ transplants, leading to the development of the first monoclonal antibody licensed for human therapy, muromonab. This anti-CD3 agent produced a potent inhibition of the whole T cell compartment expressed by a profound immunosuppression, which successfully controlled allograft rejection, but generated an entire set of serious AEs as a consequence of immunosuppression and mainly of the strong immunogenicity of this fully murine mAb. The important learned lesson from muromonab was that monoclonal antibodies could be very effective but dangerous, and indicated the main road for future development: individuate more selective targets and cut down immunogenicity.

The subsequent products, such as basiliximab, daclizumab, and the fusion proteins abatacept, alefacept, and ustekinumab followed such strategy. Daclizumab and basiliximab were directed at CD25, a basic component of IL-2R, inhibiting the immune response and thus allowing the control of graft rejection. The spectrum of AEs was reduced, possibly because the CD25 target is structurally incapable of transmembrane signaling, behaving as an inert surface component after the specific mAb binding.

Infections appeared as more localized to the urinary and respiratory tract, especially in patients with a COPD history, and opportunistic infections were virtually absent. Abatacept induced a slight increase of infections and a lighter overall safety profile. By contrast, alefacept, binding to the CD2 component of LFA-3, interfered with T cell activation causing profound and persistent lymphopenia, serious infections, and malignancies in over 1 % of cases within the first 24 weeks of observation. This framework was associated with a rather low response to treatment (30 %), indicating the relevant role of LFA-3 pathway inhibition in the induction of adverse events. Alefacept was discontinued in 2011, and a supportive program was provided up to March 2012.

Ustekinumab expressed a general immunosuppressive activity, blocking IL-12 and IL-23 shared by activated T cells, NK cells, and other immune accessory cells. This caused an increase in the risk of infections and malignancies, although sparing some immune cells (naive T cells, Th1, Treg) and cytokine production from memory CD4+ cells, thus indicating the existence of different roles of cell subsets in tumorigenesis and/or the presence of alternative pathways yet to be identified. Nonetheless, a better dissection between inhibited and spared immune functions was more evident, and produced encouraging and protracted results, yet showing a considerable induction of AEs.

The long-lasting depleting effect on T cell produced by some of these biomedicines remains to be explained. In a study on RA patients CD4+ T cells and NK cells were still below normal levels after 12 years from treatment. This phenomenon, together with an unbalanced reconstitution of lymphocytes subsets after treatment with some mAbs, possibly leading to autoimmunes disorders, eems to be peculiar of these biomedicines (see for example alemtuzumab, Chap. 7).

Overall, inhibition of T cell functions greatly improved the control of allograft rejection, and showed considerable effects in some rheumatic diseases, but indicated their essential role in immune defense. When comparing the safety profiles of biomedicines affecting more than one immune cell lineage (alemtuzumab, tocilizumab, natalizumab), with more selective agents targeting a single cell lineage (rituximab, belimumab, alefacept, muromonab) or even a cell subset (brentuximab), some improvement in the safety profile could be noticed, although not much influencing the quality of BBW issued within the whole group, and confirming the pivotal role of T cell inhibition in the generation of most serious AEs. Nonetheless, it also showed, yet with uncertain results, the possibility of dissecting the specific T-dependent immune reactions to be inhibited. This could represent an intriguing strategy for future developments [27, 28].

58.2.3 B Lymphocyte Inhibitors

B cell inhibition and elimination are considered crucial for antibody-based autoimmune disorders, and for B cell leukemia and lymphoma. It is expected that such selective interventions expose to less risks than using anti T lymphocytes, since antibodies are only one terminal arm of the complex immune defense. In fact, primary selective immunodeficiencies have shown that the impairment of antibody production is less crucial than T-cell depletion, since most regulatory and effector functions of the immune system are based on T lymphocytes efficiency.

The major class of B cell inhibitors is directed to CD20, a virtually exclusive antigen at B lymphocyte cell surface. Rituximab, ibritumomab, ofatumumab, and tositumomab are all directed to this antigen and therefore they represent, together with anti TNF-inhibitors, the most furnished drug class of biomedicines.

As expected, infections (15–37 %) were common as mild to moderate event (about 80 %), with a relatively low rate (5–10 %) of serious and opportunistic forms, despite the prolonged depletion of B cells. Infections were mostly extracellular bacterial infections, since antibodies have a particular efficiency against them, while T lymphocytes are essential for intracellular infections of bacterial, viral, and fungal origin. Interestingly, the level of circulating immunoglobulins was moderately reduced but remained stable during treatment. Notably, mature plasma cells do not exhibit CD20 on their surface, although this condition does not fully explain the Ig production duration in long-term treatments, nor can be totally reassuring about late AEs, including the risk of insurgence of malignancy, for which longer observations are still needed. However, some concerning signs of an inefficient antibody protection emerged, such as virus reactivations including HBV and JC virus, the latter leading to insurgence of PML. Despite specific antibody suppression, hypersensitivity reactions were observed, particularly at first infusions, with possible multifactorial immune and non-immune mechanisms taking place in concomitance. The response to some no-live vaccines was reduced.

A more selective inhibition was obtained with omalizumab directed exclusively to IgE. This monoclonal acted also as proof of concept on the role of IgE in severe asthma, in a portion of chronic urticaria, and parasitic infections. In the latter case, no dedicated studies were available, but in particular geographic areas (Brazil) over 50 % of treated patients showed at least one helminth infestation. Despite humanization of this IgG1k mAb, hypersensitivity reactions including anaphylaxis, as early or late event, were observed. Malignancies (mainly solid, including parotid tumors), serious systemic eosinophilia, and serum sickness (presumably generated by IgE/omalizumab complexes) were also observed, once again confirming the crucial role of IgE in their control. Interestingly, two unexpected events were also observed during omalizumab therapy which both raising concerns and possibly indicating additional functional roles of IgE: elevated levels of myeloid cell counts after 29 month treatment, being normal before therapy and recovering after discontinuation; a cluster of constitutional new signs in an off-label treatment, including sleep disturbance, vertigo, exercise intolerance, myalgia,

joint pain without effusion, crippling fatigue, and feebleness, all gradually disappearing after omalizumab discontinuation [29].

Overall, the B-dependent safety profile, appeared more selective than the T-dependent profile, but revealed as much serious expressions, mainly when targeting CD20 molecules.

58.2.4 VEGF Inhibitors

In this class there are two monoclonal antibodies, bevacizumab and ranibizumab, and one fusion protein, aflibercept, which are used in oncology (bevacizumab, aflibercept/Zaltrap), and in the treatment of AMD (ranibizumab, aflibercept/Eylea, and bevacizumab as off-label intraocular administration).

The anti-angiogenic effect of these biomedicines used systemically (IV) or locally (IVI) produced significant general and local AEs, mainly as Type A reactions related to the expected toxicity at endothelial level, and mostly represented by bleeding disorders at both levels. Serious and sometimes fatal hemorrhages were observed mainly at gastrointestinal level with aflibercept/Zaltrap and bevacizumab, followed by ATE/VTE in various districts, including CNS.

However, some unexpected events—apparently related to vascular toxicity—also occurred, such as RPLS with aflibercept and bevacizumab, now considered a local form of CLS, or ONJ with bevacizumab (and aflibercept in the postmarketing setting). In the latter case, the damage at vascular level was questioned as pathogenetic, while the delay in wound healing appeared more in line with the anti-angiogenic effect of these biomedicines and with the presence of VEGF on fibroblasts.

Neutropenia and infections were less expected as drug-related AEs due to anti-angiogenic effect, although VEGF was observed on macrophages.

IVI administrations produced local hemorrhagic events, together with endophthalmitis, retinal detachment, ATE, increased intraocular pressure and local injection-related events. However, systemic complications in addition to non-ocular hemorrhage, such as sepsis, pneumonia, and gastrointestinal disorder were also observed. Notably, systemic AEs occurred also after IVI administration mimicking IV administrations, although to a lesser extent [30–32; see also aflibercept Chap. 42].

58.2.5 Cytokines

As previously mentioned, cytokines are a complex of heterogeneous factors both for structure and function, and therefore they cannot be considered as a unique drug class when considering their capacity of inducing AEs. As for their therapeutic use and related consequences, their functional classification (Chap. 48) seems more appropriate, although some structural peculiarities are relevant for the

understanding of their potential immunogenicity. The overall scenario of AEs is complex as well, but not surprising, since a number of them exert pleiotropic functions, and may behave differently according to their dose and their reciprocal systemic interactivity. The overall more peculiar expression of their action may be summarized in the induction of systemic syndromes, as described in Chap. 3, and their functionally related consequences.

After initial attempts with pro-inflammatory interleukins in cancer therapy, IL-1, and IL-2 studies were discontinued for their heavy safety profile. Two subsequently developed recombinant IL-2 (aldesleukin, denileukin-diftitox) are currently available, yet not extensively used.

IL-1 was associated with a modest antitumoral activity, and a concerning stimulatory effect on the hemopoietic stem cell compartment. IL-2, the first recombinant cytokine introduced in human therapy, was shown to exert a potent stimulatory effect on CD8+ lymphocytes and on NK cells. Due to the insurgence of relevant systemic AEs—mostly represented by CRS, CLS, and related complications—that rapidly limited the use in vivo, these interleukins resulted more successful as ex vivo expanders of hemopoietic stem cells, and for the production of autologous LAK cells, in association with other interleukins and growth factors.

These studies were also instructive for the understanding of pyrexia and of an entire cohort of symptoms caused by IL1 administration, such as arthralgia, myalgia, and hypotension resistant to indomethacin. The safety profiles of IL-1α and IL-1β were substantially similar.

IL-2 related AEs were dose-dependent and long-term treatment showed additional signs such as diffuse edema, thyroid dysfunction, and musculoskeletal algia.

Aldesleukin, a recombinant IL-2 approved by FDA (orphan drug designation for EMEA) for the treatment of metastatic renal carcinoma and melanoma, has a paradigmatic and heavy multi-organ safety profile including a series of exacerbations and new onset of autoimmune disorders. Notably, immunogenicity as revealed by the raise of non-neutralizing antibodies was frequent (70 %).

Denileukin-diftitox, a recombinant IL-2 fused to DT has a complex safety profile in which the toxic actions of distinct components are difficult to evaluate.

For the purpose of the present work, the safety profile of a non-glycosylated form of IL-11 has been considered within the group of hemopoietic stimulatory factor, because of its specific activity on megakaryocytes.

Interestingly, a new glycosylated formulation of IL-7 (CYT017), recently designated as an orphan drug for the treatment of PML, has shown to promote T cell expansion preferably of effector memory cells, without effects on other T cells, B cells, and NK cells, with consequent immune recovery without significant toxicity [33, 34].

58.2.6 Interferons

Alpha and beta IFNs are widely used in human therapy, and their safety profile includes neuropsychiatric disorders hypersensitivity reactions, cardiac and cerebrovascular disorders, multiorgan, and bone marrow toxicities. A common trait of this drug class is FLS of different severity (see CLS, Chap. 3), which appears to be dose-dependent. Autoimmune disorders appear also of particular interest, not because of their frequency but for their wide typology, including hematologic disorders (AIHA, ITCP) endocrinopathies (hyper- hypothyroidism, diabetes), MG, GBS, and systemic autoimmune disorders (SLE, RA, and hepatitis). The spectrum of safety was similar in standard and pegylated form of IFN, with a trend to produce a higher incidence of AEs and related discontinuations in the latter, but with no difference in neuropsychiatric events. Notably, their frequency tended to decrease over time.

When observed in detail, some differences appeared among various preparations of IFN. For example, in a large one head-to-head investigation comparing two IFNβ formulations, Rebif® and Avonex®, only the former induced rare cases of anaphylaxis, fulminant autoimmune hepatitis, Stevens-Johnson syndrome, erythema multiforme and cardiac disorders all considered as drug-related, while FLS and depression appeared more frequently with the latter.

Gamma IFN, or immune interferon, is a different molecule, although the safety profile was similar to other IFNs.

The peculiar necrotic skin reactions after subcutaneous IFN administration have been previously mentioned (see Chap. 52, p 555, and ref 21–23).

58.2.7 Hemopoietic Stimulatory Factors

Erythropoietic factors (epoetins) and myelopoietic stimulatory factors, which are usually considered as separate drug classes, have different safety profiles.

Epoetins increase the risk for multiorgan thrombotic events, tumor progression or recurrence, and death. Additional relevant AEs include hypertension, seizures, PRCA, and serious allergic reactions. Overall, hypertension, thrombotic events, and seizures in children were the most frequent occurrences together with allergic reactions. Anti-erythropoietin antibodies were also observed, but they were not neutralizing against endogenous and exogenous factors. PRCA was of special concern although appearing rarely, and was correlated with resistance to therapy and with the presence of specific antibodies.

Pegylated forms of epoetins, such as the synthetic peginesatide showed a similar safety profile, although with a trend to induce more renal failures, and anemia, but with a lower tendency to raise anti-erythropoietin antibodies with respect to recombinant formulations.

Particular concern in the treatment of cancer patients raised the observation that epoetins have stimulating effects on neoplastic cells, possibly related to their

activity on the JAK/STATs downstream pathways, although with contrasting time-related effects (see erythropoietins, Chap. 53). These concerns led to launch of a safety program on the use of these products in cancer patients (ESA APPRISE).

Myelopoietic stimulatory factors include a series of recombinant molecules exerting powerful stimulatory activity on stem cells (CSF, SCF) and on granulocyte/monocyte cells in various stages of maturation (G-CSF, GM-CSF). Their overall safety profile includes allergic reactions, splenic rupture, alveolar hemorrhage/hemoptysis, sickle cell disorders, and vasculitis as the more representative events. Moreover, cytogenetic abnormalities and transformation in MDS and AML were observed in pediatric patients with congenital neutropenia, deeply influencing the safety profiles. For example, ARDS due to sequestration of granulocytes in the pulmonary district, and CLS with related fluid retention after sargramostim administration were preferably observed in hematological malignancies, while renal and hepatic dysfunctions were more frequent in patients with precedent history of organ disease. Skin disorders were particularly elevated in AML patients compared to controls, with a statistically significant difference. However, overall variations in the AEs profiles rather concerned their frequency than their typology.

A distinct position is reserved to the thrombopoietic stimulatory factor, oprelvekin, a non-glycosylated form of IL-11, and possibly to a less known recombinant IL-11 manufactured in China. Oprelvekin safety profile consists of allergic reactions including anaphylaxis, CLS and related fluid retention including pulmonary edema, dilution anemia, cardiovascular and cerebrovascular events, papilledema, and renal failure. Among serious events there are pyrexia and neutropenic pyrexia, syncope, atrial fibrillation and diarrhea, all consistently higher than in controls. New formulations of IL-11 are in progress with the aim of reducing AEs and preserve therapeutic efficacy. Among these, a genetically modified formulation showed in fact a lower incidence and a milder profile of undesirable events than the reference oprelvekin preparation. Recently, a potentiated IL-11 fusion protein (hyper IL-11) was developed, and showed to be more stable and effective at lower doses, thus promising to have a better risk/benefit balance.

Finally, a potent stem cell stimulatory factor, ancestim, acting in association with other hemopoietic growth factors, is used in vivo only in some Countries, while it has a larger use for ex vivo stem cell expansion. The limited experience in vivo showed severe allergy and asthma in cancer patients including frequent (92 %) injection site reactions, cardio-respiratory disorders. Overall, the safety profile was similar to that of filgrastim.

58.2.8 Epidermal Growth Factors

The major concern with EGFs is the relevant potential stimulation of tumor growth experienced with the two available formulations, palifermin for IV administration, and becaplermin for topical use, which is an important limitation for their use in

oral mucositis induced by myelotoxic chemotherapy, and for lower extremities diabetic ulcers, respectively. Palifermin, employed as systemic treatment, induces also frequent signs of skin and mucosal toxicity. Becaplermin, although used topically, is able to increase the incidence of solid tumors (even distant from the site of application) and to raise the cancer death rate in patients using more than three tubes of the gel formulation.

In conclusion, the methodological methodological approach proposed in this chapter is more meant to suggest the need of building up a framework useful to untangle the complex panorama of adverse events to biomedicines, more than attempting a systematic organization of this recent intricate, and galloping area of medicine.

Some drug groups already have a few products to justify a comprehensive class analysis, but many of them only include one or two, that are on the market from too short a time to even start drawing conclusions. Nonetheless, being aware of such limitations, the proposed attempt may be of some help for a better understanding of the accumulated experience on AEs to biomedicines, while waiting for more solid information to come.

The major difficulty in evaluating the safety profile of a biomedicine relates to their frequent use in combination with other therapeutic agents, often composed of multiple associations of drugs sometimes capable of inducing heavier AEs.

The major difficulty in evaluating AEs within each drug class of biomedicines is the substantial lack of head-to-head studies. In a recent (March 2012) updated Drug Class Review from the Health and Science University of Oregon evaluating efficacy and safety of mAbs and fusion proteins in RA, only 18 direct comparative studies, almost exclusively observations studies, provided direct evidence of the AEs association with such treatments. On the other hand, over two hundred randomized controlled trials provided indirect comparative data. Moreover, the mentioned report stressed the particularly limited experience in pediatric patients, and the consequent lack of adequate data. These features are paradigmatic for the whole class of biomedicines [13].

Finally, an important approach for practical purposes consists in assessing safety profiles of biomedicines for the treatment of a single pathology. As an example, a recent attempt has considered the safety profiles of TNF inhibitors—anakinra, tocilizumab, abatacept, and rituximab in patients with RA.

All these agents gave considerable results in this disease, but showed a number of safety concerns that make difficult to evaluate the risk/benefit balance when deciding the strategy to be adopted in each patient. However, they showed that some of them could be avoided/mitigated by changing drug class or even substituting agents of the same class.

One crucial aspect relates to evaluation of short versus long-term safety issues in determining the appropriate therapy, and consequent strategies to be adopted for prevention and monitoring AEs during the course of therapy with biomedicines. From this kind of analyses, two sets of recommendations have been produced. In particular, one relates to prevention and diagnosis of infections, and one

specifically addresses TB infections, before, during, and after therapy in RA patients [35]. A similar procedure is advisable for other pathologies where a sufficient number of biomedicines are already available.

The drug class approach has relevant bias because of the experienced unpredictability of AEs expression among biomedicines. Nonetheless, when approaching new-marketed products—with a limited experience accumulated on a few trials on highly selected patients—drug class comparisons become essential and represent a unique support for such narrow experience to define better strategies for prevention, monitoring, and management of the expected "stone guest."

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Conclusions and Perspectives

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Safety of biomedicines is an important limitation to their expansion. Therefore, the analysis and knowledge of adverse events are crucial for improving strategies to lower their burden, both at bench and at bedside.

However, AEs evaluation in controlled studies is not an easy task for a number of reasons: the profound differences in the investigational protocols; their privileging the analysis of efficiency parameters more than the emerging of adverse conditions; the consistent lack of comparative studies; the frequent lack of preliminary evaluations to achieve proper statistical dimensioning of trials at predetermined endpoints. Systems investigating and reporting safety data are particularly heterogeneous. Methodologies for collecting data are highly variable, as well as the selection of events to be followed. Long-term comparative studies are lacking or limited. Short-term treatment and observations, mostly performed in the range of 24–48 weeks, usually have adequate controls within that range, but long-term evaluations usually do not, for a number of understandable reasons, which cannot compensate for their absence, nor justifies extrapolations from short- to long-term risk evaluation.

Going through a number of meta-analyses, such as those of the Cochrane organization (www.thecochranelibrary.com), it becomes immediately apparent that the majority of studies are not properly designed to reach their goals [1, 2].

Nonetheless, an accurate analysis of AEs is of outmost importance for their control and even more for the future development of better medicines.

Therefore, instead of avoiding to face these problems, studies should be focused on how to achieve an accurate safety data collection, to predispose guidelines for the prevention and treatment of AEs, and to organize a rapid and correct diffusion of official information. These aspects are essential for controlling AEs and for a comprehensive understanding of risk and benefits at patient's level.

In fact, public perception has changed, and ought to be followed [3, 4]. Media have become the primary source of medical information and when information—or prompt information—from the competent sources is lacking, media can influence the decision making process and the public opinion, sometimes with sensational or distorted breaking news, even producing inappropriate official issues [5].

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An additional and intriguing problem relates to research financing and personal support of pharmaceutical companies to prominent scientists for preapproval clinical studies. To quote one elegant example, a recent meta-analysis conducted at the Mayo Clinic and recently reported by a widely diffused and qualified science magazine, evidenced that most favorable opinions expressed on one anti-diabetic drug were released by scientists with a conflict of interest [6]. In fact, the disclaimer listing is particularly rich after drug reports and controlled trials, which are pivotal for the subsequent evaluation for approvals.

Another intriguing aspect of safety evaluation is related to the different rules and policies adopted for drug approvals, even when the methodological approach of committed Agencies appears to be similar.

Interestingly, even under the same legislative framework, it can happen that a BBW is issued only for some products of the same drug class [7]. For instance, abatacept and belatacept are both directed to CD80/CD86 targets, but only the latter has a BBW. Among four mAbs targeting CD20, ofatumumab, approved in October 2009, is the only monoclonal not carrying a BBW, up to the last label revision (September 2011). Noteworthy, the pivotal study on this mAb was organized as single arm, open label trial, and subsequent studies have reported AEs at higher frequency, such as neutropenia and related consequences.

Postmarketing surveillance is active and valuable, yet accessibility and operating rules of databases are different. In a recent report on the topic, the editor of the Canadian Medical Association Journal judged the FDA database (FAERS) "*not searchable and… often incomprehensible to consumers*" and held that Health Canada's Med-effect database, which was made public in 2005, was "*not in a form that many consumers or health researchers say is necessarily useful*" [8].

The postmarketing European Eudravigilance (EUV) has been opened to public and to professionals only since June 2012.

The information collected in these databases does not meet the hard scientific and statistical criteria, and therefore are considered of limited scientific use. According to the same eminent Canadian source, these datasets would be ameliorated if data reporting were mandatory and committed to remunerated physicians, who are thus expected to provide more solid information in their reports [9]. This may not be the right solution, but the problem is real and needs to be solved.

It must be also considered that postmarketing databases may be partially overlapping; the same case report is usually sent to different Agencies and to the manufacturer, which often subsequently forward the same data to Agencies under a different coding procedure. Nonetheless, the role of postmarketing surveillance remains crucial for monitoring the long-term evolution of AEs, especially for those occurring rarely and/or far from the completion of therapy, and therefore it should be ameliorated, rather than being considered of inferior utility.

Recently, some efforts have been made for establishing European Registries for biomedicines and providing harmonized policies for the AEs assessment [10], but this trend does not seem to be followed at larger scale.

In licensing products for pediatric use, the age range may differ from one Agency to another, even when such decisions are taken on the basis of the same

clinical investigations submitted with the respective applications (see for example basiliximab, etanercept).

An additional concern relates to the progressive expansion of off-label drug use (either unlicensed or prescribed outside the terms of license), especially in the pediatric age, clearly associated with an increased risk of ADEs, reported from quite some time [11, 12]. It has been recently calculated (AAP National Conference, October 2012) that 96 % of 492 drugs was prescribed as off-label treatment in hospitalized pediatric patients. However, major concerns come from clinical practice, where decisions and controls may be less stringent. In this case, family pediatricians may play a relevant role in mitigating expectancies and in contributing to keep AEs well monitored and reported [13].

The off-label use in adult patients raises concern as well, due to its impressive growing expansion on a wider range of diseases.

It has been estimated that 50–75 % of drugs or biologics for cancer therapy are used as off-label in US; this fact has led to the release of recommendations on how to use them at least within the frame of controlled clinical trials.

Interestingly, such approach with biomedicines has been attributed to physicians influencing each other through congress participations and specialist societies, rather than to pharmaceutical companies [14–16].

Approval or rejection may be decided on the basis of the same studies, due to a different overall evaluation of safety signals, which is quite addling both for physicians and patients. In some instances, a product is accepted or rejected by an Agency and designated as orphan drug by another, which again sounds puzzling and creates ambiguity [17].

Nowadays, being the nearly instantaneous sharing of information the usual setting, such decisions are immediately available worldwide to professionals and non-professionals, thus creating doubts or undue expectancies among patients.

Frequently, official reports submitted for applications by manufacturers and subsequent Agencies' evaluations are not at the best intelligibility, not as much for data typology or completeness, but rather for the way they are exposed and tabulated. Recently, FDA refused to file an application requesting a Priority Review designation for Lemtrada, on the grounds that the provided data needed to be reorganized in the proposal, thus raising complains of the manufacturer (Sanofi press release, August 27, 2012). Rejection may not be the solution, but the problem is real and requires a more comprehensive solution.

The role of differences in AEs terminology as a source of further confusion has been stressed since the introductory remarks of this volume.

Another aspect worth mentioning is related to some typology and modality of expression of AEs to biomedicines that are different from the conventional ones, with consequent difficulties in their timely assessment.

A JAMA report evidenced that since 1995 one out of four biomedicines approved by FDA or EMEA had at least one subsequent safety-related regulatory issued for them, and 11 % received a BBW, some after considerable time. In the case of rituximab, it took over 8 years for the first BBW designation for infusion reactions, mucocutaneous toxicity, and TLS, and over 9 years for the PML

additional update warning. Efalizumab, licensed in 2003, was given a BBW for PML after 2008.

Apparently, most intriguing signals were identified at postapproval stages from the postmarketing settings but it takes several years of debate before they can be taken in proper consideration.

The growing number of approved biomedicines has produced postcommunications to health care professionals on about 24 % of them, and 82 safety-related regulatory actions, clearly indicating that these drugs were more susceptible to raise AEs not intercepted before approval.

This implies that preapproval clinical studies do not adequately characterize AEs and that rules currently in force do not adequately control biomedicines before their licensing, thus exposing the recipients to a higher relative risk [17–19].

By contrast, manufacturers claim that Agencies' testing rules and requests are overwhelming, cause drug cost increase and delays in having superior drugs available on the market, and even frustrate the development of potential new drugs because of these stumbling conditions. Although proposals of market liberalization are certainly of much higher concern, a more streamlined methodology, without harming safety and effectiveness, is advisable.

Overall, safety of medicines and biomedicines is satisfactorily controlled, but it can be improved in the interest of all.

The comprehensive overview emerging from these considerations may be overestimating the negative impact on professional and on the public opinion, but certainly something must be done to ameliorating both the investigation of drug AEs and the related information provided to the public.

To remain in the field of biomedicines, safety-related regulatory actions are different and follow autonomous policies. Reassuring actions of major Agencies specifically intended for biomedicines have been increasing in the last years. For example, EMEA and FDA recently issued new guidelines for the immunogenicity assessment of proteic medicines, and for the labeling of biomedicines [20–22]. However, their actions, together with those of other Agencies, should be more collaborative and coordinated to better fit with a globalized society and market.

After reaching different opinions in full autonomy, Agencies should convene to confront their dissenting evaluations and to establish common basic aspects, such as on specific indications and exclusions, or on the age range of applicability. Most of all, attempts should be made in depicting more harmonized safety profiles and recommendations, for issuing more homogeneous BBW or similar major warnings.

As it happened in the case of bevacizumab, the criteria used by each Agency may lead to different results with regard to the same biomedicine, despite the fact that decisions are taken on the basis of the same reported data. An harmonization of these criteria would certainly provide a more authentic and reliable profile of each biomedicine, which will greatly improve the impact on recipients, and help clinicians and health care professional in choosing strategies and in monitoring of AEs development.

Particular efforts should be given to protocol standardization for safety and efficacy evaluation, to be used in clinical trials aimed at drug approvals.

On the other hand, evaluation procedures of Agencies should become more expedite, obviously with the highest level of accuracy, in order to timely make decisions of great relevance for manufacturers.

Accelerated approvals for cancer drugs, although valid and understandable, have produced drawbacks [23–25]. Up to 2004, 18 drugs for 22 different cancer treatments were made available through this procedure, which allows, among other facilitations, the use of single-arm studies. For example, the accelerated approval for bevacizumab in mBC granted in 2008 was withdrawn in 2011, both for safety and efficacy concerns. Therefore, it is expected that if the current average time for standard evaluation procedures were adequately shortened, accelerated procedures would be limited.

Due to their unique characteristics with respect to other drugs, biomedicines should have specific safety protocols, AEs of special interest to be followed, and peculiar follow-up procedures. Head-to-head investigations should be encouraged, if not made mandatory, at this stage of development.

Long-term observations are even more crucial with these agents, because of the potential delayed expression of AEs, mainly as malignancies and autoimmune phenomena. With this respect, the comparison with background morbidities of selected populations, sharing ethnicity, and environmental conditions with the subjects in study, is essential. Quite often, multicenter controlled trials performed worldwide refer to background level of a single area e.g., US. Experiences of lupus nephropathy among Afro-Americans and of drug-related pneumonia in Japan are instructive to evaluate the impact that such differences may have in the expression of AEs and in efficacy outcomes. [26, 27].

Further support may come from specialized Registries for diseases or drug typology for long-term comparative analysis, especially on rare disorders. In this case, efforts should be made to pool data of vast and rather homogeneous areas, such as EU and US.

Another relevant issue is related to extrapolation of safety data from controlled trials to clinical practice, which raises concerns since the selected population in controlled studies is far from everyday reality of patients receiving the same treatment. Postmarketing studies on real-world medical settings should be encouraged and may be less expensive, although more complex to analyze [28].

Although a number of recent initiatives are clearly aimed at solving some of the problems posed by biomedicines, yet they appear fragmentary.

The overall complexity most likely requires a global revisiting of the whole process of development, approval procedures, from clinical trials to postmarketing surveillance, allowing a fine tuning of the entire procedure better fitting with the new biomolecules.

Postmarketing observation for oncology drugs, and in particular for biomedicines, should be differently planned. In fact, AEs may occur more than three decades after administration, and a particular awareness is demanded to professionals for monitoring patients at such long distance [29].

More stringent rules should be adopted for off-label uses of medicines and biomedicines. Often, these attempts are anecdotal, uncontrolled, and unjustified. Moreover, existing rules seem not to be strictly observed, as revealed by the number of legal controversies on their breaking in promoting off-label uses by a number of manufacturers.

Antibody engineering will continue to be a powerful tool to implement future therapeutic monoclonals and similar agents, but more efforts should be dedicated to AEs mitigation rather than to over-increasing their affinity [30]. In fact, past experience has shown that affinity enhancement did not always improve efficacy, but often increased adverse reactivity. Improvements may be obtained by enhancing complementarity, thus achieving a tighter binding and better specificity without increasing affinity, although this approach seems to be less considered by investigators (see basiliximab, palivizumab, Chap. 8, and palivizumab vs motavizumab, Chap. 31). High-affinity antibodies have shown to poorly penetrate in tumor tissues, while a lower affinity seems to favor in-deep access [31]. Moreover, the frequent use of biomedicines in association with other therapeutic agents, have shown to improve efficacy, but the summing up of AEs is still the major limiting factor. In these circumstances it appears that mAbs with lower affinity may be the right answer for balancing risks and benefits of multidrug therapy in oncology and autoimmunity, especially when the target is overexpressed, as observed in a number of neoplastic cells, such as for HER2, EGFR, ICAM, and CD30. Interestingly, in the mentioned case of the very similar fusion proteins, abatacept and belatacept, the latter has a higher affinity and received a BBW for serious infections and malignancies (including PTLD), and has a warning for PML, while abatacept did not. A different model may come from the recent CERA agents, which seem to exert a prolonged action due to a lower affinity that allows to expand their action to a larger number of targets, by continuously associating and dissociating their bindings (see Chap. 53).

This seems to be also the case of nimotuzumab, directed to EGFR, that has an optimized intermediate affinity for the target compared to high affinity agents of the same class (cetuximab, panitumumab), and shows a milder safety profile, although with cumbersome efficacy. This may prove to offer better chances in combined therapies, to improve efficacy without increasing AEs. and a low safety profile.

In pursuing future strategies, the safety profiles become more appropriate and crucial for guiding the development of new biomedicines.

New expanding areas for biomedicines are at hand, such as the treatment of infectious diseases and asthma (so far represented by only one product, palivizumab and omalizumab, respectively), hypercholesterolemia, Alzheimer disease, and others [32].

It is reasonable to expect different safety profiles emerging from the experience of these products in quite different pathological situations [33].

Another frontier is represented by the need to overcome resistance to biomedicines through a more personalized therapy. With this respect, individuals that have the best balance between risks and benefits using these agents will be selected

on the basis of their AEs expression and of the selective reactivity of their immune functions [34].

Further efforts should be concentrated on the predictability of AEs in preclinical stages of investigation on the basis of the accumulated clinical experience with available products, and on new in vitro testing models.

In particular, immunotoxicity of biomedicines with potential immunomodulatory effects should be specifically investigated during safety preclinical testing, with a more precise strategy and better timing in the drug development process [35, 36].

By combining pharmacological data with clinical observations in a systemic computation model, the predictability of AEs can be improved [37]. However, the existing instruments need to be further refined to achieve more reliable analyses. Their development should be encouraged, also in the light of the use in specific drug classes [38].

More attention should be devoted to diversities of reactions observed with biomedicines to better guide strategies for their prevention and treatment [39].

Finally, the problematic conflict of interest in clinical evaluations should find a better solution than declaring disclosures, which render their existence explicit, but cannot be considered problem solving [40].

Indirect supporting through a common funding system controlled by Authorities and/or a "peer review" system of independent evaluation in preapproval stages may help in reassuring on such fundamental activity that is entrusted to autonomous experts.

AEs expression analysis is fundamental for developing better drugs. The evolution of mAbs and fusion proteins engineering, and the glycosylation and de-glycosylation strategies applied to them and to epoetins are clear signs of the role played by immune reactivity through AEs against these molecules, that were successfully mitigated by molecular humanization and proper glycosylation. The experience of highly efficient biomedicines withdrawn due to safety issues can be highly instructive as well, and indicate the need of a more realistic confrontation with AEs.

Adverse events are ineludible companions of therapy and their understanding remains the main strategy to prevent, mitigate, or reduce their negative impact on patients and drug manufacturers. A late assessment of safety risks, other than producing negative consequences in subsequent therapy diffusion and compliance, may also cause significant financial drawbacks. Most of all, it fosters negative psychological attitudes toward new therapeutic approaches, even when extraordinary for their efficiency and safety compared to previous treatments, as in the case of many biomedicines.

Learning to accept the risk of adverse reactions, as the counterweight of the extraordinary benefits of modern therapies, would be greatly facilitated if all possible efforts to increase benefits in the balance were clearly transmitted to patients and health operators, showing with transparency and continuity both progress and difficulties during development, experimentation, and everyday practice with each therapeutic agent. On November 15, 2013 executive members of EMA argued on individual risks vs. public health advantages from new drugs, and suggest additional criteria for a better evaluation of risk/benefit ratios during

licensing procedures, including the opinion of patients on acceptable risks, in the interest of public health [41].

An open ans easy access to such information at all levels and a constant feedback to health operators will increase confidence and improve their involvement in proper reporting. The common perception that such high responsibility is felt and shared among all partners of this extraordinary enterprise would be the most reassuring message for all.

It is in this spirit that this monograph has been designed.

In Seneca's *Epistulae morales ad Lucilium, Liber XI-XIII,* a collection of moral epistles to Lucilius, the then procurator of Sicily, it is stated: "…*Ergo bona nasci ex malo non possunt*". Most of the times, this is not far from the truth. But in the case of adverse events to old and new medicines I believe this can happen: these events were generated in the attempt of producing a good therapeutic result—*ex bonum malo* –, and in turn their occurrence can generate further improvements for human therapy—*ex malo bonum*.

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