

Chapter 2

Cancer: Clinical Background and Key Challenges

Antonio Llombart-Bosch, Ulrik Ringborg, Sergio Rutella and Julio E. Celis

Abstract This chapter is aimed at a wide audience ranging from biologists to medical students and cancer specialists. It provides a comprehensive overview of systems approaches to the pathology and treatment of cancer. In particular, it addresses diagnosis and therapy by interconnecting various aspects of cancer at both the molecular and clinical level, and contrasts the unifying features of malignancies with the daunting diversity of cancer types, stages, and evolutionary processes during treatment. The importance is emphasized of both prevention and innovative treatments in reducing the cancer burden, and of early detection as the link between these two major areas. It sets the stage for analysis of cancer by means of systems biology, bioinformatics, and systems medicine. These methods involve the processing of cytological, histological, and imaging data, combined with genetic and expression profiling. The application of systems approaches to cancer-related clinical practice and research is discussed. The necessity is demonstrated for signalling pathways analysis to be fully integrated into grading and clinical staging of cancers, as well as into the process of discovering novel targets and biomarkers for diagnosis and prognosis. Key challenges and limitations are outlined for systems approaches to cancer, and areas are indicated where research needs to be focused in the future. Finally, pointers are provided to the paths that must be followed in order to move from a carefully controlled biological investigation, to approaches and technologies that will eventually accelerate the translation of new discoveries into prevention and clinical applications.

2.1 Introduction

Cancer is one of the major health issues affecting our society, and is forecast to worsen globally as the population ages. The WHO statistics from 2008 (Boyle and Levin 2008) estimate an increase of new cancer cases from 12.4 million to 20 million world-wide by 2030. Over the same period, the number of deaths from cancer will increase from 8.3 million to 12.9 million, and the number of patients living with cancer from 28 to 82 million, with similar trends in Europe (Ferlay et al. 2010a). In 2008, there were 1.7 million deaths due to cancer, a number that corresponds

A. Llombart-Bosch (✉)

Department of Pathology, School of Medicine, University of Valencia, Valencia, Spain
e-mail: antonio.llombart@uv.es

to more than three deaths every minute; while the number of new cases was 3.2 million. As the population ages, the burden is expected to increase even further. Moreover, as a result of more effective treatments for cancer, its prevalence as a chronic disease will sharply increase, particularly in countries such as the EU where life expectancy is already high. Cancer is one of the main chronic diseases, a fact that translates into a substantial extra demand on health care systems, because of the required surveillance and recurrent treatment of both the disease and observed side-effects.

Worldwide, lung cancer is the most frequent form of the disease and causes the majority of deaths. Among men, the commonest types of cancer are lung, prostate, stomach and colorectal; among women, breast, cervix, uteri, and colorectal. The principal cause of death is cancer of the lung, followed by stomach and liver (Ferlay et al. 2010b). There are many differences in occurrence between the more developed countries as compared to the less developed ones; for example, breast, prostate, lung, and colorectal cancer are the most frequent malignancies in developed countries, while in less developed ones liver, cervical, and oesophageal cancers are more prevalent (Kamangar et al. 2006; Boyle and Levin 2008).

A number of factors affect incidence rates in different countries, the most important of which are demographic aspects, external environment, lifestyle, and economic status. Differences in genetic risk factors can also contribute to dissimilarity and disparity in the results of diagnostic activities, and variations in the recording of cancer statistics make comparison between countries very difficult (Parkin 2004).

The cure rate of some cancers has increased significantly (for a review, see DeVita et al. 2008). For example, patients with some forms of leukaemias, lymphomas, and paediatric tumours have been treated successfully with combinations of antitumoural agents, while the increased cure rate for patients with cutaneous malignant melanoma is mainly owing to early detection (Cohn-Cedermark et al. 2000; Balch et al. 2003; Aitken et al. 2010). Early detection has improved the cure rate for breast cancer, with additional curative effects from both radiation therapy and antitumoural agents (Fletcher and Elmore 2003; Berry et al. 2005; Clarke et al. 2005, 2008; Cuppone et al. 2008; Madarnas et al. 2008; Dowsett et al. 2010; Richards 2009). The average mortality reduction for all forms of cancer is, however, modest. In the follow-up of the European Code against Cancer, a 9% reduction was achieved over a period of 15 years (Boyle et al. 2003b).

Significant relief of the cancer problem can only be achieved by concerted actions aimed at improving prevention and therapeutic strategies. Moreover, early detection is of fundamental importance both for prevention and improving cancer treatments. Prevention initiatives might focus on either high-risk individuals or total populations. Thanks to molecular genetics and epidemiological studies, a number of risk factors, both inherited and lifestyle-acquired, have been identified. Advances in understanding the molecular pathways involved in tumour initiation and progression have provided unique opportunities for research on the correlation between molecular biomarkers and risk factors. Approximately one-third of cancers are considered to be preventable. Successful prevention, however, largely depends on change in lifestyles, a challenging problem for behavioural sciences. Examples

of risk factors are tobacco smoking (Doll et al. 2004; Pleasance et al. 2010); Human papilloma virus (HPV) infections (Cogliano et al. 2005); alcohol abuse (Boffetta and Hashibe 2006); UV radiation, obesity and insufficient physical activity (Boyle et al. 2003a; Boyle and Levin 2008).

Early detection is often used synonymously with secondary prevention. For breast, cervical, and colorectal cancer population screening is considered to be a satisfactorily evidence-based method (Boyle and Levin 2008). An important area of modern cancer research is the identification of premalignant (precursor) lesions likely to progress to invasive cancers; this is crucial to the development of innovative approaches to prevention. For prediction, appropriate molecular markers are required, since premalignant lesions represent a heterogeneous group intermingled with benign lesions unlikely to develop malignant behaviour.

Today's treatment strategies aim to develop personalized cancer medicine based on the understanding of the molecular mechanisms underlying the disease. To achieve this goal, predictive biomarkers of primary and metastatic disease need to be identified, and sensitive and accurate methods have to be developed to monitor response to treatment and the occurrence of side-effects. Moreover, knowledge of the mechanisms underlying sensitivity and resistance to anticancer agents will constitute an important aspect of the endeavour, given that treatment failures are often the result of existing or acquired drug resistance. A close coordination of preclinical with clinical research is required in order to gain insight into which of the molecular pathways are disturbed and drive tumour growth. Novel targets for therapy will thereby be generated, which should foster the development of new anticancer agents. We expect, however, that major advances can only be made in the short term by choosing the appropriate combination of already existing drugs based on extensive diagnostic information.

The purpose of this chapter is to provide a pathological and clinical overview of cancer, as well as to pinpoint major challenges that need to be addressed if we are to fulfil the goal of bringing individualized cancer care closer to reality. Initially, we cover tumour nomenclature, grading and staging, and various technological approaches to categorizing morphology. The various methods and strategies for treatment are then outlined. With this background, several major cancers are then treated in detail, including various further classifications and modifications which have been developed for each individual type, leading to implications for treatment. Finally, these descriptions are linked to a systems biology and medicine context, demonstrating both the need for and the advantages of these approaches.

2.2 Pathology Integration in Cancer Biology Systems

For many years, the study of morphology, as applied to biology and medicine, has played a major role in basic and clinical science, helping to establish and confirm the diagnosis and prognosis of disease. Histopathology, with the aid of old and new ancillary techniques, remains a key protagonist in medicine today. Nevertheless,

new complementary classifications based on gene expression profiles enhance or more accurately predict survival in the case of certain neoplasms (Gravendeel et al. 2009; Aparicio and Huntsman 2010).

Over 150 years have elapsed since Rudolf Virchow postulated the key approach *‘on the structural basis of diseases and their anatomical location as a consequence of an altered response to any external or internal injury produced to the cell or the tissue.’* Even though the biological concept of disease has changed to a great extent over the years, the anatomo-clinical approach for diagnosing diseases remains the basis of medicine (Llombart-Bosch 2001; Costa 2009).

This concept is particularly valid in oncology, where the notion of malignancy is associated with a loss of control in normal cell growth, tissue and cell differentiation. Malignancy is a biological concept, according to which the anarchic proliferation rate of the newly transformed tissue overgrows the normal, and extends into distant areas of the organism (metastasis), often producing the death of the patient in what is known as the ‘natural history of cancer’. In this situation, the morphological (i.e. an image) diagnosis, provides objective support to the clinical diagnosis.

Histopathology and cytology, based upon the microscopic examination of tissues and cells, continue to play a seminal role, and are reliable methods for diagnosing patients with cancer. The hematoxylin-eosin (H&E) staining of paraffin-embedded tissues is considered the gold standard for the histological diagnosis of cancer (Rosai 2007). In addition, a number of new technological approaches based both on the microscopical analysis of cells and tissues, such as electron microscopy, enzyme histochemistry, immunohistochemistry (IHC), fluorescence in situ hybridizations (FISH) etc., yield enhanced information on the initiation, promotion, and progression of cancer cells, both in human and experimental models. These technologies are described in detail in Sect. 2.3.

The findings obtained with these methods have been supplemented by the application of molecular biology approaches to the study of cells in normal and pathological conditions. Thus, molecular pathology, in the process of complementing conventional histopathology, raises new challenges for considering cancer not only as a disease of the organ-tissue-cell system, but also as a consequence of genetic disease involving several complex biological systems (Hahn and Weinberg 2002; Celis 2008; Abelloff et al. 2008).

Nevertheless, concepts such as benign or malignant, as well as tumour typing, continue to be connected to the conventional notion of pathology, as defined by use of a microscope. Thus, morphology continues to be a reliable tool for the diagnosis of a neoplasm, as well as for controlling tissue quality in molecular biological and oncological research (Rosai 2001; Llombart-Bosch 2001; Beckman 2006).

2.2.1 Definition of a Neoplasm

A neoplasm consists of a new growth of cells, initiated within a tissue by substituting for normal cells and causing the emergence of a mass (tumour) in which the

cells exhibit high growth rate, loss of differentiation, self-autocrine feedback, loss of cell death self-control, ability for angiogenic modulation, and metastatic capacity. The degree of failure in these biological controls, which conditions the benign or malignant nature of the neoplasm, threatens the death of the host. Evolution towards malignancy may be rendered irreversible by genomic instability and the acquisition of new biological properties, as a result of continuous genetic and epigenetic remodelling (Hanahan and Weinberg 2000; Weinberg 2007).

2.2.2 Tumour Nomenclature

The nomenclature of tumours is based either on cell origin (histogenesis) or on microscopical similarity with normal tissue (histology); both categorizations may sometimes overlap.

Two types of tissue compose solid tumours: neoformed tissue (*parenchyma*), whether *carcinoma* in epithelial context (tissue composed of cells that line the cavities and surfaces of the body) or *sarcoma* in mesenchymal (connective tissue, bone, cartilage, and circulatory and lymphatic); and the *stroma* component, which provides the support cells and the vessels. Haematological neoplasia (*leukaemia*) lacks stroma while growing within the bone marrow and the blood compartment. The stroma component is an active partner in tumour behaviour and seems to be induced by the malignant cells in tumours, providing a decisive role in invasion and metastasis. Interplay between both components varies from case to case, giving a fleshy or dense consistency. The amount of the collagenous involvement causes increased density or *desmoplasia*.

Tumour nomenclature is based upon the type of parenchymal cells. Two principal types of tumours occur, benign and malignant, although a number of intermediate clinical possibilities must also be considered, such as semi-malignant, pseudo-malignant, and of questionable malignancy. The most important features used to characterize a tumour are its predictable clinical behaviour and its microscopical appearance. Benign neoplasms are harmless and slow-growing, whereas malignant lesions exhibit rapid proliferation, invade adjacent tissue, and metastasize.

In general, the suffix *-oma* follows the originating cell type, independently of grade (benign or malignant). Thus, the name for an epithelial benign tumour is *adenoma* when originating in glandular tissue (gastro-intestinal tract, breast, kidney or liver, etc.), and *papilloma* if the cells arise from the non-secretory epithelial surfaces (skin, respiratory mucosa, lower urinary tract). An additional number of terms complement this generic histological terminology, based on the presence of cysts, micropapillae, or mixed components. The terms for benign mesenchymal tumours are based on the cell type: *fibroma* is used when fibroblasts constitute the tumour, *lipoma* in the case of adipocytes, and *osteoma* for osteoblasts. Tumours may present a variable extent of interstitial components such as myxoid, angiomatous, collagen, or elastic fibres. Mixed epithelial-mesenchymal tumours are named fibropapilloma, adenofibroma and so on. A *polyp* is a composite tumour containing both the

neoformed epithelial parenchyma and the stroma; it may be pediculated or sessile. The term polyp has no implication for clinical outcome.

The generic name for a malignant epithelial neoplasm is *carcinoma*. When a dual component is present, either glandular or ductal, the term is *adenocarcinoma*. A tumour originating in a specific cell type, such as terminal lobules of the breast, is named *lobular carcinoma*. When derived from squamous or keratin producing epithelia, they are called *squamous* or *epidermoid carcinomas*. Neoplasms with hybrid features of glandular and squamous participation are known as *adenosquamous carcinoma*. Histological varieties of glandular differentiation such as cords, trabecula, tubes, papillae, or cysts, provide a variety of histological subtypes. Neoplasms may also be named by the organ from which they originate: *hepatocellular carcinoma* is a generic name for a malignant tumour originating from hepatocytes, and *mesothelioma* for tumours of mesothelial origin.

The size of the cell is also used in some poorly differentiated neoplasms such as *small-cell* or *large-cell carcinoma* of the lung. Cell secretory activity provides additional varieties with names such as *mucinous*, *colloid*, *serous*, *apocrine*, or *neuroendocrine* for carcinomas in diverse anatomical locations such as breast, ovary, or GI tract. Malignant epithelial cells may suffer metaplasia, conditioning the so-called *metaplastic carcinoma*, in which the carcinomatous and sarcomatous components appear together.

Malignant tumours of mesenchymal origin are designated sarcomas; several varieties of sarcomas are defined based upon their histological resemblance to the normal cell counterpart: *liposarcoma* resembles adipocytes, *fibrosarcoma* mimics fibroblasts, *leiomyosarcoma* resembles smooth muscle, *angiosarcoma* is similar to blood vessels.

The cell line of differentiation is not sufficient in several malignant mesenchymal tumours to define their true nature, thus in these cases the terminology *pleomorphic sarcoma*, *myxoid sarcoma*, *small round cell sarcoma*, or *spindle cell sarcoma* is employed, based exclusively on the cell configuration, cell size, and type of stroma. Composite tumours may be found within the same neoplasms (*dedifferentiated sarcomas*). Epithelial-like components occur in some sarcomas (*synovial sarcoma*, *epithelioid sarcoma*, *alveolar sarcoma*) making the differential diagnosis with carcinomas difficult.

The nomenclature for embryonal (occasionally ectopic) neoplasms (germ-cell tumours) of the testis or ovary is mainly dependent on tissue similarity to the cell of origin, while exhibiting malignant behaviour, and includes *seminoma*, *embryonal carcinoma*, *yolk sac tumour*, *granulosa cell tumour*. *Teratoma*, *hamartoma*, and *choristoma* are terms associated with embryonal cell growth, showing one, two, or all three germ-cell layers (ectoderm, endoderm, and mesoderm) in a variable degree of differentiation or state of maturation. No particular clinical outcome is implied by these terms for the patient. For example, teratoma may be malignant or benign depending on the cells present and their level of maturity.

Tumours of haematopoietic tissue are named either *leukaemia* or *lymphoma*. *Lymphoma* is frequently associated with haematogenous extension (leukemic). *Leukaemia* acquires a tumour component when seeding into a solid organ. Many

types are classified according to their cell of origin: cancer stem cell (lymphocytes, myelocytes, granulocytes, monocytes, erythrocytes) and their degree of maturation. For further details, see the WHO tumour classification (Chan 2001).

Tumours of the nervous system are often named from their similarity to the cells of origin. Other terms focus on histological similarities, as is the case for *glioma*, when initiated from the glial cells, *medulloblastoma* and *neuroblastoma* for primitive stem neuroblasts or embryonal neuroblasts. A tumour derived from Schwann cell is called *schwannoma* when benign, and *malignant peripheral nerve sheath tumour* when malignant. As with haematological tumours, a large number of terms are employed for the several varieties existing within these tumours.

The term *blastoma* is used for tumours composed of germ cells of a highly malignant behaviour. The term is preceded by the name of the potential cancer stem cell that may be the origin of the neoplasm: *nephroblastoma* for embryonal tumour of the kidney (also called Wilm's tumour); *hepatoblastoma* for a similar embryonal neoplasm of the liver. Malignantly transformed germinal cells of the ovary are referred to by the term *disgerminoma*. Highly malignant neoplasms such as grade III (see below) gliomas are denominated *glioblastoma*.

2.2.3 Tumour Grading

Nomenclature provides only a partial view of the biology and clinical outcome of neoplasms. Additional information is therefore necessary for a clear characterization of each tumour type. To this end, several grading systems have been proposed (Rosai and Ackerman 1996). Tumour grading, from **benign** to **malignant**, is based primarily on: degree of differentiation of the tumour cells, nuclear features, rate of growth (mitotic activity), stromal response (angiogenesis, inflammatory reaction). The grading criteria vary greatly for different neoplasms and the correlation between histology and clinical behaviour is in some cases imperfect. Accuracy in grading is difficult because of the heterogeneity existing in some neoplasms as well as being dependent on site-related events.

2.2.3.1 Benign Tumours

These lesions are made up of mature, differentiated cells mimicking the tissue from which they originate and resembling the normal tissue, but showing a lower grade of architectural organization: stroma is poor, and vessels scarce. Nuclear pleomorphism is almost absent or very rare, while mitosis may be limited, and there is a lack of chromosomal abnormalities. In most cases the nuclei have a diploid DNA content. The tumour is well delimited and is frequently encapsulated or polypoid. Such lesions are occasionally multiple and synchronous (uterine *leiomyomas*, breast *fibroadenomas*, GI *polyps*). Some reach large sizes (*fibromas*, *leiomyomas*, *ovarian enteric cysts*, *prostatic adenomas*). Exceptionally, they are functional and hormone

producing (*pancreatic insular adenomas*); nevertheless, they may cause severe clinical problems and even a fatal outcome. Basically, a benign tumour is not a metastasizing neoplasm, but benign metastasizing tumours have been described in solid or in endocrine organs (thyroid gland, kidney, ovary, testes) and bone (giant cell tumour). In order to distinguish a benign from a malignant well-differentiated neoplasm, meticulous attention must be paid to the presence of any capsular or vascular infiltration which would indicate the capacity to metastasize. Tumours with potential metastatic capacity have also been defined as neoplasms with uncertain biological potential.

2.2.3.2 Malignant Neoplasms

These lesions are characterized by a lesser degree of structural and architectural organization, providing a poor histological configuration when compared to their normal counterpart. Nevertheless, the degree of differentiation may vary from well to poor, from undifferentiated to dedifferentiated neoplasia. The latter two terms are used indiscriminately, even though they represent two diverse biological situations: the first means a lack of differentiation, while the second expresses a loss. Lack of differentiation is one of the major structural patterns of malignancy, since the tissue mimics its embryonal counterpart. The existence of ‘cancer committed stem cells’ as progenitors for tumours indicates that the arrest of tumour maturation in a given stage is probably the cause of loss of architectural organization. Additionally, the stroma plays a role in the loss of differentiation (Kalluri and Zeisberg 2006).

Neoplastic cells induce angiogenesis by secreting angiogenic factors, and also induce fibroblastic proliferation, interfering with the normal reparatory process associated with tumour growth (Bergers and Benjamin 2003). An inflammatory reaction may participate in the process, imparting additional complexity to the tumour phenotype.

The cellular and nuclear pattern is the second attribute that characterizes a malignancy. The cytological criteria of a malignant tumour are well known and used for diagnosis, either by tumour scraping (cervical cytology) or by aspiration (fine needle aspiration cytology). Histology also provides adequate structural criteria. The loss of cytoplasm and nuclear structures varies greatly from tumour to tumour. Cells may be larger or smaller than normal cells, or they may resemble embryonic cells (loss of the nuclear to cytoplasmic ratio) with large nuclei and nucleoli, occasionally multiple and with an irregular contour. The distribution and configuration of the chromatin varies widely from cell to cell (hyperchromatism). Total loss of cell configuration is described as *anaplasia*: the neoplasm lacks any structure that resembles its normal histological counterpart. Anaplasia is associated with higher malignancy, but may also be caused by therapy (chemo- or radiotherapy). In addition to undifferentiation and pleomorphism, some tumours present abundant cellularity with dominance of the parenchyma over the stroma. Cell rich neoplasms, mainly in sarcomas, are associated with a poor prognosis.

Invasive growth is another characteristic of malignant neoplasms. In carcinomas, the normal surrounding tissue is infiltrated by neoplastic cells which disrupt the

basal membranes and extend into the neighbouring stroma, producing new structural patterns such as cords, trabecula, tubules or glands. The malignant cells may appear isolated within the neoformed stroma or produce tiny nests. Infiltration is a microscopic event and conditions the capacity of some tumours to local relapse. The lack of defined spatial limits is associated with the absence of a capsule as happens in benign neoplasms. Some slow-growing carcinomas may induce a pseudo-capsular structure mimicking a benign tumour. Particular attention has to be paid to these tumours (*follicular carcinoma of the thyroid* is an example) because they may be confused with an adenoma. Thus, the limits of invasion have to be confirmed in order to assure tumour-free margins for treatment. To this end, multiple histological sections of the neoplasia are necessary, and are made possible by colouring the excision margins with Indian ink (which resists discoloration when treated with alcohol-xylene dehydration), embedding in paraffin, and staining with H&E.

Tumour invasion may affect the neighbouring tissues and vessels, thus originating local relapses. The invasion of the vessels involves regional (lymph nodes) or distant metastasis. A particular type of invasion is the so-called *skip metastasis*, corresponding to tumour implants in the skin adjacent to a primary neoplasm due to their local extension. This type of metastasis occurs mainly in melanomas or sarcomas.

Sarcomas possess highly invasive growth potential and imprecise margins, lacking anatomical boundaries that necessitate large compartmental resections to avoid local relapses. The absence of invasion of the margins is a mandatory requisite, to assure the absence of relapse in the preserved limb. New therapeutic methodologies for malignant tumours have created the possibility of preserving organs or limbs, avoiding previously necessary organ resections.

Necrosis is another feature present mainly in malignant tumours. Tumour progression is dependent on a balance between cell proliferation, differentiation, and death. Necrosis is the usual end for many tumour cells, independently of *apoptosis* (programmed cell death). This variety of non-programmed destruction of cells is expressed by a loss of the architectural and cytological (*pyknosis*, *karyorrhexis*, *karyolysis*) structures in a focal or large area within the tumour.

Most of these necrotic foci adopt a geographical distribution depending on the amount of neovascularisation. Only cells located at less than 200 μm from a vessel receive sufficient oxygen and nutrients to stay alive. Fast-growing carcinomas and sarcomas present an imbalance between angiogenesis and cell proliferation, and therefore suffer extensive areas of necrosis. In contrast, slow-growing epithelial or mesenchymal tumours maintain a good angiogenic capacity and so the degree of ischemia is more limited. This is also the case for benign tumours, which rarely suffer necrosis.

2.2.3.3 Miscellaneous and Borderline Lesions

In addition to benign and malignant neoplasms, a number of biological possibilities arise out of the clinical behaviour of some neoformations. *Pseudomalignant*

tumours are neoformations that express many of the attributes of malignancy such as cell pleomorphism, loss of architectural patterns, and mitosis. Nevertheless, their clinical behaviour is benign. Endocrine tumours such as *pheochromocytoma* or *paraganglioma* may present these peculiarities. The *pleomorphic adenoma* of the salivary glands also belongs to this category. In the skin, a *keratoacanthoma* may resemble a squamous epidermoid carcinoma, but with benign behaviour and may even regress spontaneously. Other examples are pigmented tumours in the skin such as *cellular blue nevi* or *Spitz nevi*. Some soft tissue neoplasms display this peculiarity (*pseudosarcomatous fasciitis*).

Semimalignant tumours are neoplasms with a peculiar biology. They express local aggressivity like malignant neoplasms and have cell atypia and mitosis, infiltrating neighbouring tissues; nevertheless, they lack metastatic capacity, even if they may relapse locally. *Basal cell carcinoma* of the skin is a good example. *Dermatofibrosarcoma protuberans* can also be included in this category, as can *phylloides tumours of the breast*, both of which rarely metastasize.

Currently there is a tendency to consider these neoplasms as *low-grade tumours* (tumours with questionable malignancy), meaning proliferations with scant aggressivity, but which may occasionally relapse or even metastasize. Examples of these are atypical lipomatous tumour or the *angiectatic pleomorphic tumour* in soft tissue. They should not be confused with another category of neoplasia: *borderline lesions*, a term denoting the inability of the pathologist to define the possible clinical outcome of neoplasms. The number of lesions classified within the context of *borderline* is progressively increasing, and represents a substantial grey area between benign and malignant processes. Examples include borderline pigmented lesions of the skin, or borderline lesions in mucinous and serous tumours of the ovary, as well as lesions present in the breast or in the prostate.

2.2.4 Growth Rate of a Tumour

Growth rate constitutes one of the main biological features marking the distinction between benign and malignant tumours. Within the latter category, slow-growing tumours are distinguished from those that possess a rapid rate of proliferation and therefore a higher biologically aggressive behaviour.

The study of tumour cell kinetics has clarified the mechanisms by which a tumour proliferates. This rate is also dependent on the degree of differentiation (cell maturation) and programmed cell death (cell loss). Thus, it would be helpful to know not only how many cells replicate and go into mitosis, but also how many are within the cell cycle by maturation or remain outside the cycle (cancer progenitor stem cells). In addition, the growth rate is also dependent on other factors such as cell doubling time or the amount of the replicative pool. Finally, tumour growth is balanced between the predominance of cell proliferation over cell loss. Thus, in tumours with a relatively high growth fraction, the disparity is large; resulting in more rapid growth than in tumours in which cell production exceeds cell loss by only a small margin.

The number of cells in mitosis or in cell cycle provides two ways to measure the growth rate within a neoplasm. Histologically, both features are easily and objectively assessable. Mitotic counts have been performed for many years as the most reliable way to measure the proliferation activity of a given neoplasm. It is well known that benign tumours lack or have few mitoses, while carcinomas and sarcomas display high mitotic count together with abnormal mitosis, aberrant chromosomes and loss of ploidy (aneuploidy). There is a good correlation between the mitotic cell count in a tumour and prognosis. Breast carcinoma is a good example of this correlation with the so called Bloom index (Nottingham index) (Elston and Ellis 1998). In soft tissue sarcomas, all grading systems (American system, French Federation system) include the mitotic count as a reliable parameter to measure the histological grade of malignancy.

A second method is to evaluate the number of cycling cells. Several immuno-histochemical techniques offer reliable ways to measure their number and establish correlations with the mitotic count and prognosis. Today, the most popular antibody used in histology is Ki67 (MIB1) for assessing the proliferative rate of a tumour. Numerous publications confirm its value (Ueda et al. 1989; Hoos et al. 2001; Meara et al. 2007; Lopez-Guerrero et al. 2011).

2.2.5 *Dysplasia and Carcinoma in situ*

The process of cancer promotion and progression involves a number of genetic and molecular events that are variably expressed with a number of structural changes in the phenotype of the cell and tissue (Ponten 2001). The loss of cytological and architectural organization is consistent with the term *dysplasia* (altered form) and can be considered a step in the transition from normal to cancer cells. The presence of dysplasia in a tissue does not necessarily mean a precancerous lesion or *in situ* cancer. Many of the dysplastic alterations may reverse or are secondary to an adapted cell response to a metabolic injury, inflammation, or tissue repair. Thus, the distinction between what represents a harmless dysplasia and a precancerous lesion exceeds the limits of histology and occupies an imprecise grey area. German pathologists of the early twentieth century used to consider this the realm of ‘*Persönlich einstellung*’ (personal appreciation), because at that time histological diagnosis was based exclusively on the experience of the expert. One of the principal advances in modern genetics and molecular biology is to have provided objectivity in differentiating these types of lesion, in which histopathology has a limited capacity for definition.

Most lesions presenting dysplasia occur in the epithelia, either stratified or glandular, and are associated with *metaplasia* (transformation of a tissue originating in a given germ layer to another of the same origin) or *hyperplasia* (increase in the number of cell layers within an epithelia, but preserving their normal phenotype). Hyperplasia, metaplasia, and dysplasia are biological events that merge in both early precancerous lesions and carcinoma *in situ* or invasive cancer. Examples of these lesions can often be seen in resected specimens of the bronchial mucosa as-

sociated with carcinoma of the lung (Franklin et al. 2004). Similarly, the presence of intestinal metaplasia of the gastric mucosa is consistent with a precancerous lesion (Whitehead 1994), while the processes of hyperplasia-metaplasia and dysplasia characterize GI polyps and are also related to the successive genetic rearrangements that precede carcinoma (Shia et al. 2003).

In situ carcinoma occurs in numerous stratified glandular epithelial mucosa and in the skin. Several examples have been described that characterize this entity. The most frequently quoted models are uterine cervix for squamous epithelia, and the breast for glandular epithelia, perhaps because they are commonly occurring lesions of a type easily defined by cytology or histology. This form of carcinoma depends primarily on two components that preserve the capacity of the transformed cells to maintain the organization and integrity of the epithelia, namely the continuity of the basal membrane that isolates the epithelia from the supporting connective tissue, and maintenance of cell to cell attachment, owing to the activation of several cytoplasmic and membranous adhesion molecules. The loss of these functions leads to early invasion and infiltration (microinvasion).

Additionally, the tumour induces angiogenesis which provides a seminal capacity for growth, invasion, and metastasis. The tumour vessel formation mimics vascular embryogenesis, promoting several processes. These mechanisms are very complex and may occur together inside the same tumour and their surrounding stroma. A number of possible processes have been recognized:

- *Neo-angiogenesis*: new vessels grow by branching from pre-existing vessels (mainly capillaries). The process occurs through *vascular sprouting* or by *intussusceptions* in which interstitial columns of tissue are incorporated into the lumen of newly formed or pre-existing vessels.
- *Vasculogenesis*: *de novo* production of vessels originating from undifferentiated precursors (mesenchymal pluripotent cells with angioblastic capacity) forming an initial tubular network. At this stage the endothelial cells mature and integrate closely with smooth muscle cells, pericytes, and the surrounding matrix.
- *Angiogenic remodelling*: the initial network is modified by pruning and vessel enlargement to form interconnecting branching figures, characteristic of a more mature vasculature.
- *Lymphangiogenesis*: the endothelial vessels proliferate, producing new lymph vessels, either by angiogenesis or a vasculogenesis-like mechanism, induced by lymphatic endothelial growth factors and their receptors.
- *Vascular co-option*: groups of avascular tumour cells co-opt with pre-existing host vessels and initiate as well-vascularised small tumours.
- *Mosaic vessels*: the tumour cells come in contact with a lumen, together with neoformed endothelial cells, producing an interface or mosaic on the surface of the intratumoural capillaries.
- *Vascular mimicry*: the tumour cells transform themselves into a pseudoendothelium, mimicking new vessels that are incorporated into the vascular network.
- *Capillary drop-out*: regression induced in recently developed microvessels by anti-angiogenic drugs, such as Avastin (bevacizumab).

2.2.6 Metastasis

Metastasis is an especially complex process which occurs through a series of sequential steps in which tumour cells first migrate from the primary tumour, penetrate blood vessels, circulate within the bloodstream, and after migration, finally colonize distant sites, reproducing the disease. Metastases are tumour implants discontinuous with the primary tumour. New data indicates that the mechanisms controlling metastasis are regulated independently of the primary tumour growth and are due to a sequence of events mediated by different classes of metastatic genes (Meyer and Hart 1998; Chiang and Massague 2008; Nguyen et al. 2009). Approximately 30% of cancer patients harbouring solid tumours present metastases at diagnosis, this being the most common cause of death, even if the patients respond transiently to conventional therapy.

The dissemination of cancers may occur through several pathways: by direct extension and seeding into neighbouring organs, surfaces, or body cavities, or by either lymphatic or haematogenous vascular spread. Transplacental tumour extension has also been reported, even though it may be very unusual. Direct implants of tumour cells caused by surgery may rarely occur.

The term *local metastasis* refers to lymphatic invasion or direct spread of tumour cells into the surrounding interstitial tissue and/or space, as in the case of the peritoneum, pleurae, SNC liquoral ventricles, etc. In the breast, local invasion of the subcutaneous tissue and dermis produces a skin retraction (*peau d'orange*). *Metastasis in continuity* affects mainly the natural ducts such as the bronchial tree for lung cancer, or urethra in carcinoma of the bladder, due to the seeding of neoplastic cells in proximity of the original neoplasia. Children's medulloblastoma in the Central Nervous System (CNS) may extend directly into the lateral and medial ventricles, while high-grade glioblastoma produces massive local invasive growth, but does not usually metastasize outside the CNS. A particular type of local invasion is *Paget's disease of the nipple*, in which an intraductal breast carcinoma originating in the main collector ducts of the gland progressively substitutes, as isolated or small cell nests, for the normal superficial stratified cutaneous epithelia.

Tumour extension into the natural cavities affecting the serosa is frequent in the abdominal cavity and pleura, secondary to serous or mucinous carcinoma of the ovary or GI tract, lung carcinoma, and malignant mesothelioma. Tumour implants may be microscopical in size or form large nodules (*carcinomatosis peritonei/pleurae*). *Primary peritoneal carcinomatosis (pseudomyxoma peritonei)* is almost always secondary to a mucinous carcinoma of the appendix or the ovary, while in the pleural spaces, the origin of focal or diffuse *pleuritis carcinomatosa* is mainly secondary to carcinomas of the lung or the breast.

Peritoneal implants caused by borderline mucinous carcinoma of the ovary are not necessarily cases of true metastasis, in that the tumour deposits only superficially and does not invade the serosa, prognosis being favourable in this case. Detection of pleural or peritoneal tumour extension is, however, an indication of poor clinical outcome.

Lymphatic nodes may be colonized by tumour cells (mainly carcinomas and melanomas, more rarely sarcomas), a fact that signifies the regional extension of the tumour. The early detection of nodal metastasis is an important aim in cancer assessment. Contrast radiological lymphography has been used for years to detect the presence of metastasis in nodes. Other imaging technologies such as Positron Image Tomography—PET and Computed Tomography—CT/PET, have greatly improved the detection of small metastasis in nodes, since they are able to detect and depict with high precision areas of relatively intense metabolism as actively replicating tumour cells form in their growth process. Even so, the histological study of the resected lymph nodes is still required for the purpose of precisely determining the “pathological staging” of the tumour (pTNM, see Sect. 2.2.7). Small deposits of tumour cells have been called ‘clandestine metastasis’ and their prognostic and therapeutic significance is today of high oncological value. The presence of isolated tumour cells, freely located in the cortical sinus of a node, has no clinical significance, unless the tumour increases in size to 2 mm or more, in which case it is considered as a positive metastasis node.

The *sentinel lymph node* is the node anatomically located in closest proximity to the cancer, and provides excellent information on the extension of the neoplasia into the regional territory. This is of great value in breast carcinoma, sparing unnecessary surgery on the residual axillary nodes while the sentinel nodes continue to present negative. Other tumours may benefit from this approach (melanoma, gastric and colon cancer, prostate). In addition, the capsular invasion by tumour cells and their extra nodal extension are associated with a negative prognosis. Furthermore, histological study of all nodes remaining after surgery offers the possibility of gaining additional information about the presence or absence of metastasis, which will condition both therapy and clinical outcome.

2.2.7 Tumour Staging

Several types of local, regional, and distant metastasis are characterized by *tumour staging*, which is based upon the presence or absence of any various types of tumour extensions together with the size of the neoplasm. Staging of the tumour is an important procedure before deciding on treatment. The widely used classification and staging system is the Tumour Node Metastasis (TNM). Data for the vast majority of solid neoplasms is integrated in the framework of the TNM system. The T, N, and M parameters and their combination define the stage of disease and represent a powerful criterion in the therapeutic decision making process. The presence of distant metastasis puts the tumour at the most advanced stage, irrespective of the T and N status.

Two versions of TNM exist: the one developed by UICC, the International Union Against Cancer—<http://www.uicc.org>—(Sobin et al. 2009) and the other by AJCC, the American Joint Committee on Cancer—<http://www.cancerstaging.org>—(Edge et al. 2010).

- **T** indicates the size of the primary tumour and its behaviour towards surrounding structures (adjacent/in contact versus infiltrating).
- **N** represents the involvement of regional lymph nodes.
- **M** the presence of metastases (loco-regional and/or distant).

The clinical staging (producing the cTNM status and the cStage), performed at the moment of the initial diagnosis, depends essentially on imaging techniques (Computed Tomography (CT- and PET-CT); Nuclear Magnetic Resonance (NMR), Ultrasound (US), radionuclide scan, etc.) and outlines, defines the presence and characteristics of the local and loco-regional disease (including the T and N status), and the presence and characteristics of loco-regional and distant metastases. Over time, the process of revision of the TNM staging system is a forum of continuous discussion and validation. The pathological staging (producing the pTNM status and the pStage), assessed if surgery is performed, is essentially based on the final pathology. The T and N statuses are unequivocally characterised by histology. The M status can be confirmed cytologically or histologically if metastases are simply biopsied or surgically removed. The M status is recorded at the time of clinical staging as well as pathological staging, even if a final pathology is not obtained on neoplastic tissue originating from the detected metastases, because of the fact that these are not surgically removed (in general terms, in fact there is no surgical indication for the removal of local and distant metastase, except in selected cases).

An unusual situation arises where a tumour has been judged to be resectable owing to its locally advanced stage, as opposed to a cancer judged to be inoperable because of distant spread. Whereas chemotherapy and/or radiation is typically administered as ‘adjuvant’ primary treatment for unresectable tumours. In such cases, after primary therapy has been administered, the tumour status is re-assessed in what is known as “clinical re-staging” and a yTNM plus yStage is produced. This provides a basis for clinicians to evaluate whether the situation is better (down-staged), the same (unchanged), or worse (progression) and to make decisions as to further treatment options. If surgery is indicated, a pTNM and pStage will be determined.

Since staging is fundamental for the choice of local, loco-regional, or systemic treatment, it is important to improve staging methods. In the future, by using molecular biomarkers, it will be possible to predict local and metastatic disease with much higher precision. It may even be possible to predict different metastatic phenotypes of the primary tumour, such as regional and systemic metastases. Promising developments of both molecular pathology/cytology and imaging, which will incorporate molecular biomarker research outcomes into clinical technologies, should soon have an important impact on clinical staging procedures.

The presence of distant metastasis is associated with vascular spread and represents Stage IV of the disease (the most advanced). Patient survival at this stage is generally poor, with therapy being based on a palliative approach. Haematogenous spread occurs when the tumour cells irrupt into the vessel, circulate within the blood, adhere to the endothelia, transverse the wall vessel, and invade, colonizing a distant tissue. The site of the metastasis depends on the anatomical configuration

and the local circulatory network in the distant organ, into which the tumour grafts and reproduces a new growth with some similarities to the original. Here again, the cancer cells induce their own new vessels by secreting vascular growth factors (Kerbel 2008). There are two main theories postulating the mechanism for metastatic organ distribution. The ‘mechanical theory’ maintains that the spreading of metastasis depends primarily on the number of vessels present in the tissue: tissues with dense vascularisation are better predisposed to receive metastasis. This is the case for the liver, lung, CNS, or bone marrow, but not in others such as the spleen. There is evidence for a direct relationship between venous blood drainage and the anatomical location of the metastasis. Basically, some neoplasms show a tendency to venous invasion; an example is the paravertebral plexus in the prostate or renal veins in kidney carcinoma. Colon cancer extends not only to the local regional lymph-nodes, but can also affect the liver as the most frequent first distant location. This is also the case in gastric cancer. Nguyen et al. 2009, and others before them (Mehlen and Puisieux 2006), have argued for another possible mechanism to explain the metastatic process known as the ‘root and seed’ process. This implies the existence of tumour cell specificity for dissemination and organ-specific colonization. The organ specificity of the metastatic cells is determined by a particular infiltrative and colonizing capacity, gained after its dissemination from a primary tumour. For a given type of cancer, these events occur within particular temporal kinetics and in a unique organ site. The varied latency period for metastasis occurring in certain tumours suggests a need for a specific tumour progression allowing the cells to adapt to the microenvironments of the particular organ. The acquisition of specific pro-metastatic functions, earlier during primary tumour promotion, might enable distinct cancer types to express different timings to relapse.

Mehlen and Puisieux 2006 have provided additional evidence that metastatic potential is associated with an increased resistance to apoptosis. They postulate the concepts of *anoikis* and *amorphosis* as barriers to metastasis. *Anoikis* should be considered as cell death induced by disruption of the cell attachment and interactions between the cell-matrix complexes, whereas *amorphosis* appears to depend on tumour cell death stimulated by the loss of cytoskeletal architecture. Both processes interfere with metastatic spread.

Sanchez-Garcia 2009 in a review on ‘the crossroads of oncogenesis and metastasis’ analysed two known possibilities of the metastatic cascade in cancer. In the classic model of human cancer progression, the metastatic process occurs in the advanced clinical stage. However, recent studies support a second possibility that could be modulated by activation of protein expression that mediates the epithelial-to-mesenchymal transition. This would concomitantly activate both the malignant conversion and the metastatic dissemination occurring in early tumour stages. According to this model, dissemination of the initiated malignant cells could happen at any time during cancer promotion or progression and not only in the advanced stage of the disease. If this theory were accepted, cancer could be considered as a systemic disease from its early phase (*ab initio*) and should therefore be treated as such. Studies of certain ovarian and breast carcinomas (Naora and Montell 2005; Weigelt et al. 2005) support this possibility.

In conclusion, evidence suggests that some tumours bear a genetically controlled metastatic phenotype, and are prone to dissemination from the beginning of their growth, even where the tumour size is so small that it cannot be clinically detected (generalized metastasis with unknown primary). A number of metastatic genes have been postulated to be involved in this process. The detection of what could be a metastatic phenotype, using biomarkers to identify distant metastases, would aid in the selection of patients to be treated with targeted therapy.

2.2.8 Cytology and Diagnosis

Clinical cytology, used in conjunction with histology, is the most reliable tool available for the microscopical diagnosis of cancer for precancerous lesions and other pathological conditions such as atypical reactive proliferations or inflammatory processes. It has also been used for hormonal checks in women and in addition serves to orient a rapid diagnosis of internal lesions that are radiologically detectable as lumps or scars. Image diagnosis is complemented with a quite simple procedure using fine-needle aspiration (FNA) of the suspected process (Arisio et al. 1998). This is an economical and very reliable way to assess pathology diagnosis of cancer in neoplasms located in deep organs which are not easily accessible and require surgery.

The application in population-based screening programs of systematic cytology examinations using cervical exfoliation or endometrial aspiration has provided one of the more valuable methods to detect early-stage cancer or precancerous lesions. The pioneering work of George Papanicolaou deserves honourable mention; in 1941 he proposed exfoliation of cells of the cervix as a reliable method for the diagnosis of cervical cancer. This simple and quite inexpensive test has probably saved more women's lives than any other diagnostic procedure in clinical practice. Cervical screening with the 'Pap-test' has continued to be an almost indispensable system for controlling cervical cancer and following up the response to therapy or the hormonal status. The application of this test has been extended to other mucosa (oral mucosae, gastric, bronchial tree, and urinary tract) and corporeal fluids (urine, sputum, liquids in pleural or peritoneal cavities).

The FNA technology was first developed at the Radiumhemmet, the Karolinska Hospital in Sweden in the early 1950 by the group of cytologists and pathologists lead by Sixten Franzén and Jozef Zajicek and extended rapidly to the USA, becoming nowadays a very popular technique in pathology laboratories all over the world. Nonetheless, it must be taken into account that exfoliative or FNA cytology shares the limitations inherent in any clinical tests: the balance between sensitivity and specificity. Generally, few cases of neoplasia are missed when diagnosed by an expert cytopathologist, which means a higher sensitivity of the technique, nevertheless a number of patients without neoplastic disease will need additional follow-up testing which means a lower specificity. The balance between sensitivity and specificity varies greatly from organ to organ, and is also dependent on the good quality

of the material obtained by the technical extraction procedure of the cells (scraping or aspiration) and on the quality of smearing cells onto the slide together with good fixation and staining. Quality control within cytological laboratories and training of personnel is a major problem in developing countries, contributing to failures that may discredit this kind of test.

Cytology material has been successfully used in numerous conventional and molecular techniques, such as immunohistochemistry (IHC), morphometry, electron microscopy and in recent years in molecular biology DNA and RNA extraction for blotting, PCR, or microarray gene cluster analysis.

2.3 Technological Approaches to Morphology and Pathology

2.3.1 Hematoxylin-Eosin (H&E) Staining in Histological Diagnosis

The rising use and value of histopathology in cancer diagnosis has for many years been based mainly upon a relatively simple and economical technology that has stood the test of time and should continue to do so in the future, provided that it remains in the hands of adequately trained pathologists. Microscopical observation of tumour slides with hematoxylin-eosin (H&E) staining offers an especially selective and sensitive means of approaching cancer diagnosis. Occasionally, however, H&E staining of paraffin-embedded tissue sections is insufficient to confirm a diagnosis of malignancy: Immunohistochemistry (IHC) staining should additionally be performed to acquire sufficient information for a final diagnosis. There is no problem with IHC staining of formalin-fixed paraffin-embedded tissue, and it does not require the extra time or major expense of more sophisticated diagnostic procedures such as electron microscopy (EM) or FISH. However, in view of the fact that some special stains have specific requirements for uncommon tissue fixation and processing, the need for special stains must be anticipated to ensure that adequate tissue samples are appropriately processed, e.g. fixation for electron microscopy or preservation of fresh tissue for cell cultures, xenografting or RNA extraction. Tissue banking, not only with paraffin blocks, but also with fresh normal and tumour tissue, is becoming mandatory in all modern, well-equipped laboratories. The EM procedure is quite expensive and very time consuming when compared to IHC, so its use is therefore restricted to specific tumour types or the search for a particular inclusion. In fact, a large number of laboratories have abandoned this diagnostic procedure, limiting it to a very few research indications.

2.3.2 Immunohistochemistry

Immunohistochemistry (IHC) is an important ancillary tool that has provided great support to the diagnosis and prognosis of tumours. IHC is an old technique, still used

in fluorescence microscopy, deriving from the pioneering work of Albert Coons in 1941, who labelled antibodies with fluorescein isocyanate. The data it yields must be considered together with other information available, such as histology, molecular findings, and clinical records. It should not constitute the sole decision criterion for a final diagnosis, but must be integrated into the decision-making process of the pathologist (Taylor and Cote 1997; Natkunam and Mason 2006).

However, transmission light microscopy offers greater advantages in evaluating morphology over fluorescence, because cells and tissues are more clearly recognized and tumour architecture is well preserved, allowing better histological correlations. This makes enzyme IHC (peroxidase, alkaline phosphatase, biotin-avidin) more useful for routine diagnosis. The sensitivity of fluorescence is higher, but is mostly limited to FISH (Fluorescence In Situ Hybridization) for procedures such as detection of chromosomal segments or gene probes (Jin and Lloyd 1997). The enzymatic procedure involves three steps in which the selected antibody is first bound to an unlabeled antibody and posteriorly to a second antibody having generic specificity for the first, and is then conjugated with biotin, providing a bridge for the subsequent binding of an avidin-biotin horseradish peroxidase complex that completes the immunochemical assembly. Nuclei are counterstained with hematoxylin.

Usually the antigenic determinants that are targets of the IHC stains are not totally tumour-specific. Therefore, it is necessary to employ more than one antibody in order to elaborate sets of primary antibodies for the identification of the subtype of a given tumour, thereby constructing an 'antigenic fingerprint' that will allow final interpretation. On this basis a number of algorithms have been constructed to facilitate the classification or differential diagnosis of histologically similar neoplasms.

Several examples of these antigenic fingerprints are discussed in Sect. 2.5, related to colorectal, breast and bronchial carcinoma. Differential diagnosis of 'small round cell tumours' including lymphomas located in soft tissue or in bone, provides a good model of integrating histology with a panel of IHC markers in molecular genetically similar neoplasms.

2.3.3 *Electron Microscopy*

Electron microscopy (EM) can be especially helpful when other specialized techniques do not provide a definitive diagnosis. It should not be expected to be able to differentiate benign from malignant cells, as they may not display specific patterns (Dardick and Herrera 1998; Ordonez and Mackay 1998). Samples have to be prepared with extreme care; small pieces of tissue are processed in a special fixative, embedded in epoxy resin, and thinly sectioned (0.1 mm thick), after which they are impregnated with osmium. This technique, by enabling a differential absorption of the focused electron beam traversing through the specimen, can produce an image that is directly observed on a fluorescent screen, stored on photographic film, or digitized. The image should be evaluated by a trained electron microscopist, analysing specific, but previously focused images at low magnification. Study of the cell configuration, membrane preservation, and intracellular content has to be progressively

reviewed with higher magnifications to look for specific structural organelles and other features; the type of malignant cell may be characterized, as well as cytoplasmic contents such as filaments, neurosecretory granules, secretory material, lipid droplets, or inclusions such as viruses located either in the cytoplasm or nucleus. All this information has to be comprehensively analysed, by comparing conventional histological slides of the tumour and the semi-thin slides coloured with Alcian blue.

2.3.4 Tissue Microarray (TMA)

Tissue microarray (TMA) technology is based upon the concept of obtaining high-throughput phenotyping profiles of intact tissues. The conventional investigation of fresh frozen/paraffin embedded tissues is too expensive and time consuming for analysis of hundreds and thousands of genes associated with tumours. Therefore, TMA is based on the idea of miniaturization and a high-throughput rapid and cost effective approach to the validation of molecular targets in a large number of tissue specimens at DNA, RNA, or mainly protein level with IHC (Shergill et al. 2004).

Donor blocks must be at least 1 mm thick, but an optimal thickness of 3–4 mm is recommended for better results. Recipient array blocks are prepared by pouring paraffin into moulds of about 5–10 mm thickness. The block surface is made flat and parallel to the underside of the plastic cassette by trimming off in a microtome. The TMA technique has the advantage of permitting rapid analysis of genomic alterations in a large number of specimens, and of achieving an unprecedented level of standardization with minimal destruction of original tissue blocks. A uniform staining quality is also achieved with internal positive and negative controls, and efficiency is high, saving reagents, time, and money.

Nevertheless, the technique presents a number of limitations, such as possible loss on the glass slide of samples floated off during sectioning or unmasking procedure, and the tendency of shallower samples to be used up more quickly. On some occasions inadequate tumour tissue is present in the sample, which may therefore fail to be representative of the entire lesion, owing to the tumour heterogeneity (cells may be intermingled with areas of stroma and necrosis). Even so, several studies have confirmed that two cores from each tumour give 95% accuracy (Kallioniemi et al. 2001).

The applications of TMA are numerous. Some of the possibilities include routine protocols for IHC, *in situ* RNA hybridization, or interphase FISH. Another possible application is the analysis for prevalence of genetic alteration in one or more tumour samples lacking clinicopathological information. In addition, TMA can be used to analyse molecular markers in relation to various stages of the tumour, or for prognosis studies linking molecular findings and clinical outcome.

The TMA technique, as distinct from gene microarrays, covers many samples for one antibody or *in situ* hybridization product, whereas with gene microarrays, one tissue sample analyses several probes delivering a gene expression profile of amplifications/deletions.

Frozen tumour TMA technology (Schoenberg Fejzo and Slamon 2001) for the analysis of tumour RNA, DNA, and proteins may be useful in some circumstances. It is known that paraffin embedding incurs a problem of fixation, which could partially mask some antigenic targets. This can introduce chemical modification of RNA, resulting in less optimal conditions for *in situ* analysis of DNA, RNA, or proteins. Frozen TMA for IHC and *in situ* RNA hybridization with FISH have been developed to overcome the fixation problem.

A matter of debate is whether TMA automation is necessary for routine pathology. There are arguments in favour of automatic screening, since a pathologist may need to review up to 1000 samples in 1–2 h. An argument against automation is that the procedure is based on signal intensity/spot and lack of distinction between different cell types, such as epithelial and stromal. Automation may also underestimate staining in samples with few cancer cells.

Bioinformatics-type analyses in TMA ‘virtual cores’ with software tools have been developed for archival IHC data in TMA (Liu et al. 2002). Analysis and storage of the large amount of data generated by the staining results is accomplished by recording directly into Microsoft Excel work sheets. Data is subsequently reorganised by a program (‘TMA -DECONVOLUTER’) into a format suitable for hierarchical cluster analysis. The immunoprofile of a case can be retrieved and reviewed by using the ‘STAINFINDER’ software. Digital images are available at <<http://genome-http://www.stanford.edu/TMA/explore.shtml>>.

The National Human Genome Research Institute (NHGRI) and National Cancer Institute (NCI) have created the Tissue Array Research Program (TARP) (http://ccr.cancer.gov/tech_initiatives/tarp/) of the National Cancer Institute (NCI) with the goal of promoting TMA research and development and providing assistance in arraying unique tissue materials such as those collected from clinical trials. The TARP programme also provides training and arranges workshops and protocols concerning the TMA technology.

2.4 Treatments

In Western countries about half of cancer patients have advanced cancer at the time of diagnosis, with either advanced visible tumours or concurrent micrometastases. It therefore follows logically that treatment is mostly multimodal in nature, involving combinations of surgery, radiation therapy, and medical treatment. For example, in a Swedish investigation 84% of those patients treated by radiation therapy were also treated with surgery and/or antitumour agents (Ringborg et al. 2003). Multidisciplinarity, as reflected by diagnostic activities, is essential, as well as psychosocial oncology, rehabilitation, high-quality supportive and palliative care. The cure rate of patients in European cancer registries was 21–47% in men and 38–59% in women (Francisci et al. 2009). In general, about 1/3 of patients are cured by surgery alone. The addition of radiation therapy increases the cure rate to about 50%, while further medical treatment increases the cure rate to about 60% in countries with well-developed cancer care. These, of course, are approximate.

2.4.1 *Surgical Treatment*

The main aim of surgical treatment is to remove the primary tumour and loco-regional metastases. Over the years a number of surgical methods have been tested, all too often with mutilation as a consequence. During the last decades, however, our enhanced understanding of the biology underlying the various cancer types has resulted in a reduction of the invasiveness of surgical interventions (the “organ sparing” philosophy). Malignant melanoma is a good example of this trend, as the availability of better parameters for predicting recurrent disease has considerably reduced the size of resection margins (Cohn-Cedermark et al. 2000; Balch et al. 2003). Similarly, combining surgery with radiation therapy in breast cancer has enabled strategies to be changed from mastectomies to breast-conserving treatments. The outcome is the same, but the quality of life of the patients is improved (Clarke et al. 2005). For other tumour types like rectal cancer, improved surgical techniques and combined chemo-radiation plus surgery approaches have reduced the local recurrence rate and the need for extensive *demolitive surgery* (Simunovic et al. 2009). The same is true in head and neck cancer, where combined chemo-radiation therapy has replaced extensive surgery (Lango 2009).

2.4.2 *Radiation Therapy*

Innovations in radiation physics have significantly improved the quality of radiation therapy (Levitt et al. 2006, 2008; Verellen et al. 2007). Modern imaging technologies now permit the volume of the tumour to be defined and delineated in a more precise way that facilitates image-guided radiotherapy. The availability of high-energy, well-collimated radiation facilities, together with effective dose planning, makes it possible to save more normal tissue, while at the same time increasing the dose delivered to the tumour. Moreover, with intensity-modulated radiation therapy, the dose can be shaped to fit almost any irregular tumour mass in the body. Methods have also been developed for breathing-synchronized irradiation.

Stereotactic radiation therapy (radiosurgery) can effectively kill primary tumours and metastases and can, for selected patients, replace surgery (Baumann et al. 2009). Some cancer centres are currently evaluating treatments with light ions having different radiobiological effects as compared to x-ray photons, the source of energy most widely used (Jakel et al. 2008).

The main role of radiation therapy is to eliminate loco-regional tumour disease. This is done by irradiating the total tissue volume around the primary tumour as well as the anatomical region where *in-transit* and lymph-node metastases may grow. Total systems irradiation may be indicated in cases of sensitive disseminated disease. Radiation therapy also has an important role to play in the palliative treatment of patients. Prediction of regional metastases, *in-transit*, and/or lymph-node metastases is particularly important when they are microscopic. If these can be detected using biomarkers, molecular imaging may be used to determine the target volume for radiation.

Table 2.1 Effects of cyto-static/cytotoxic treatment

<i>Treatment may cure</i>
Acute leukaemia, above all in children
Choriocarcinoma
Malignant lymphoma
Testicular carcinoma
Wilm’s tumour
<i>Treatment may prolong survival and be of palliative value</i>
Breast cancer
Colorectal cancer
Chronic leukaemia
Myeloma
Ovarian carcinoma
Sarcoma
Small-cell lung cancer
Urinary bladder cancer
Cancer of corpus uteri
<i>Treatment has modest effects</i>
Gastrointestinal (except colorectal) cancer
Malignant glioma
Malignant melanoma
Prostate cancer

An important area of research is the investigation of molecular mechanisms underlying sensitivity and resistance to radiation therapy, since this has a bearing both for tumours and normal tissues. The optimal dose and optimal fractionation of the radiation are crucial questions. Here a number of radiobiological factors, including molecular factors linked to DNA damage response, are of strategic importance (Sarkaria and Bristow 2008). Understanding the molecular radiobiology of ionizing radiation is expected to lead to new prediction methods regarding radiosensitivity and resistance. Currently, there is a trend to combine radiation therapy with antitumour agents, with the aim of increasing the combined effect.

2.4.3 Systemic Treatment

Medical treatment of malignancies is the youngest of the treatment modalities currently available. Even if surgery and radiation therapy jointly cure the majority of potentially curable patients, medical treatment is effective in several forms of the disease. Its role has increased considerably over time, as the main challenge is to solve the problem posed by disseminated disease, which currently have different levels of response to treatment (Table 2.1). A large number of antitumoural agents are in clinical use, with different modes of action (for a review, see Chabner and Longo 2006). Examples of *cytostatic/cytotoxic agents* are presented in Table 2.2.

Table 2.2 Examples of cytostatic/cytotoxic drugs in clinical use

<i>Alkylating agents and platinum compounds</i>	<i>Topoisomerases inhibitors</i>
Busulfan	Amsacrine
Cisplatin	Daunomycin
Carboplatin	Doxorubicin
Cyclophosphamide	Epirubicin
Chlorambucil	Etoposide
Dacarbazine	Idarubicin
Ifosfamide	Irinotecan
Lomustine	Mitoxantron
Melphalan	Topotecan
Oxaliplatin	
Temozolomide	
<i>Antimetabolites</i>	<i>Mitotic inhibitors</i>
Azathioprine	Docetaxel
Capecitabine	Estramustin
Chlorodeoxyadenosine	Paclitaxel
Cytarabine	Vinblastine
Fludarabine	Vincristine
Gemcitabine	Vinorelbine
Mercaptopurine	
Methotrexate	
Thioguanine	
<i>Other drugs</i>	
Bleomycin	
Actinomycin D	
Mitomycin C	

2.4.3.1 Cytostatic/Cytotoxic Agents

Alkylating agents bind preferentially to the N7-position of guanine in DNA and are effective in the resting phase of the cell cycle. Some are monofunctional alkylating agents, but the majority of the agents are bifunctional and form intra- and interstrand crosslinks in DNA. There are two main groups of alkylating agents. Nitrogen mustard gas derivatives, which include melphalan, chlorambucil, cyclophosphamide, and ifosfamide, are the most frequently used agents and are clinically active in the treatment of both haematological malignancies and solid tumours. The other group includes the nitrosoureas which are represented by the lipid soluble chloroethylnitrosourea compounds carmustine (BCNU), lomustine (CCNU), and methyl-CCNU. Following intracellular degradation, the active part of the chloroethylnitrosourea binds to the O⁶-position of guanine before crosslinking the DNA. The O⁶ of guanine is the target for the DNA repair protein O⁶ –methylguanine-DNA methyltransferase, which can modify the cytotoxic effect of chloroethylnitrosoureas (Bobola et al. 2005; Hansen et al. 2007). Antitumour effects are

observed in several types of malignancies such as lymphomas, small-cell lung cancer, melanoma, and brain tumours. Streptozotocin and chlorozotocin are variants of nitrosureas; they are monofunctional alkylating agents that are active in the treatment of endocrine pancreatic cancer. Busulfan, an alkyl alkan sulfonate, causes bifunctional DNA crosslinks and its antitumour activity has been shown in CML (chronic myelogenous leukaemia). Procarbazine, a monofunctional alkylating agent, is used in combination chemotherapy for malignant lymphoma, while dacarbazine (DTIC) is active in malignant melanoma. Temozolomide has a similar mode of action and has shown positive clinical effects in brain tumours and malignant melanoma.

Platinum compounds react with DNA, noting that the most frequent DNA lesions are intrastrand crosslinks. Cisplatin is active in several types of solid tumours, particularly in testicular carcinoma. Combination of chemotherapy with cisplatin cures 70% of patients with disseminated testicular cancer (Ehrlich et al. 2010). Carboplatin, a second-generation platinum agent, has similar antitumour effects, while oxaliplatin is a more complex molecule active in the treatment of colorectal cancer.

Topoisomerase inhibitors interfere with the rejoining of DNA strands after topoisomerase action (topoisomerase I and II). Antitumour antibiotics like the anthracyclines daunorubin and doxorubicin are specific for the action of topoisomerase II. Daunorubicin has a role in treatment of leukaemias, while doxorubicin is active in the treatment of several solid tumours. Epirubicin is a derivative of the anthracycline molecule with less cardiac toxicity as compared to doxorubicin. Idarubicin is another anthracycline with activity in CML. Etoposide and teniposide are podophyllotoxin derivatives that interact with topoisomerase II. Etoposide has a role in the treatment of lung cancer, leukaemia, lymphomas, and testicular carcinoma. Derivatives of camptotecin, such as topotecan and irinotecan, are inhibitors of topoisomerase I that have antitumour effects for several solid tumours. Mitoxantrone is used in the treatment of breast cancer and haematologic malignancies and amasacrine in the treatment of acute leukaemia.

Antimetabolites are substances that simulate normal precursors of DNA and RNA and are cell-cycle specific. Methotrexate is a folic acid analogue that inhibits dihydrofolate reductase. It is active in treatment of leukaemias and lymphomas, but also in the treatment of several solid tumours. Mercaptopurine, thioguanine, and azathioprine (Imuran, an immuno-suppressant), are purine analogues which are phosphorylated to triphosphates with inhibitory effect on DNA synthesis. They are used in the treatment of haematologic diseases. The purine nucleoside analogues chlorodeoxyadenosine and fludarabine are mainly active in myeloproliferative diseases. Cytarabine is a pyrimidine analogue active in treatment of leukaemia. 5-Fluorouracil is a fluoropyrimidine with antitumour activity in treatment of colorectal and breast cancer. Capecitabine is a pro-drug which is metabolized to 5-fluorouracil in the tumour. Gemcitabine, a cytidine analogue, inhibits ribonucleotide reductase and can also be incorporated into DNA. Gemcitabine is active in the treatment of several solid tumours, such as pancreatic, non-small-cell lung, and head-neck cancer.

Mitotic inhibitors are represented by vinca alkaloids (vinblastine, vincristine, vindesine, and vinorelbine), compounds that act by binding to tubulin. Neurotox-

icity is a common side-effect particularly after treatment with vincristine, which is active in leukaemia, lymphoma, and testicular carcinoma. Vinblastine has fewer neurotoxic side-effects and is used in combination therapy for testicular and ovarian carcinoma as well as lymphoma. Vindesine is used in the treatment of ALL (acute lymphatic leukaemia) in children, malignant melanoma, and squamous cell carcinoma. Vinorelbin is used in non-small-cell lung and breast cancer and has limited toxicity to normal tissues. Taxanes (paclitaxel and docetaxel) are mitotic inhibitors that interfere with microtubule function. Anticancer activity has been demonstrated for non-small-cell, ovarian, and breast cancer. Estramustine, composed of estradiol and non-nitrogen mustard, is cytotoxic mainly by binding to tubulin and is used in the treatment of hormone refractory prostate cancer.

Bleomycin is a mixture of glycopeptides. Its cytotoxic effects are most probably caused by strand brakes in DNA. Bleomycin is used in the treatment of malignant lymphoma, testicular cancer, and squamous cell carcinoma of the head and neck and in combination with radiation therapy for penis and anal cancer. Actinomycin D, an inhibitor of RNA synthesis, is active in colon carcinoma, Wilm's tumour, neuroblastoma, embryonic rhabdomyosarcoma, and Ewing sarcoma. Mitomycin D is a DNA crosslinker with antitumour effect in several solid tumours.

2.4.3.2 Endocrine Treatment

A special area of medical oncology is endocrine treatment of malignant tumours, as several tumour diseases are dependent on both steroid and peptide hormones. Most endocrine therapies aim at decreasing the stimulating effects of steroid hormones in malignant cells. Examples are anti-oestrogen and aromatase inhibition of breast cancer (Dowsett et al. 2010) and LHRH agonists for treatment of cancer of the breast (Sharma et al. 2008) and prostate (Albertsen 2009). Glycocorticoids may be effective in the treatment of lymphomas, and gestagenes in the treatment of breast cancer and corpus carcinoma. Tyrosine is used for treatment of thyroid carcinoma with the aim of decreasing the stimulatory effect of thyreotropin on the disease.

2.4.3.3 Targeted Treatment

During recent decades, targeted therapies have been developed on the basis of enhanced knowledge of the molecular mechanisms underlying cancer (Chabner and Longo 2006; Baselga 2006; Baselga and Swain 2009; Yamanaka and Saya 2009, and references therein). The main molecular targets used to develop anticancer drugs are cell surface receptors, signal transduction pathways, gene transcription targets, ubiquitin-proteasome/heat shock proteins, and tumour microenvironment constituents (Fig. 2.1). Examples of targeted drugs are given in Tables 2.3 and 2.4 and Fig. 2.1. Currently, there are widespread efforts to identify new targets and develop new compounds, and it is expected that about 10 new antitumoural agents

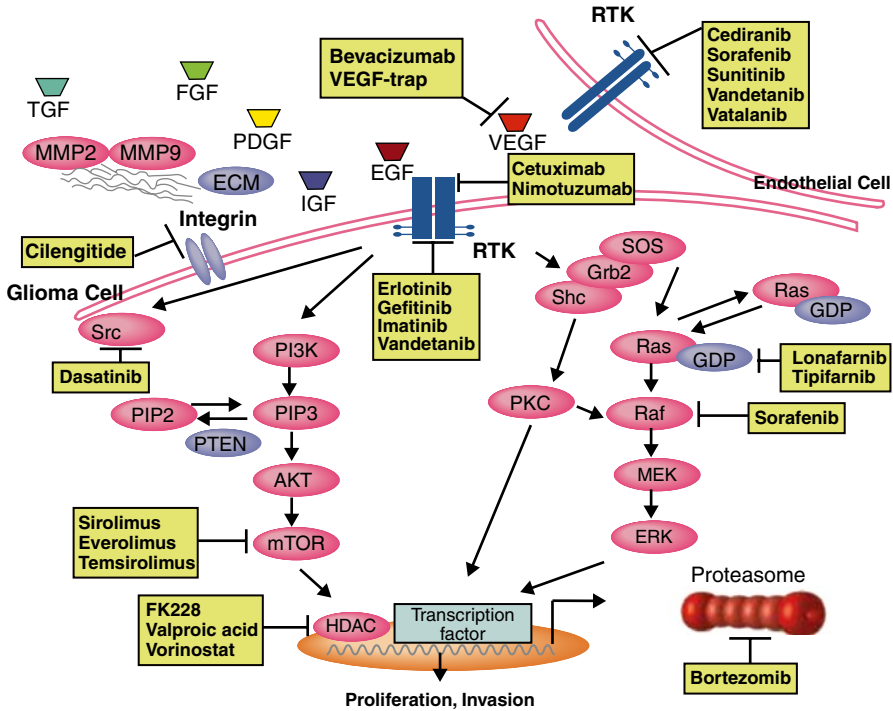


Fig. 2.1 Molecular targeted therapy for glioma. A representation of signalling pathways and therapeutic molecular targets in glioma cells. ECM=extracellular matrix; EGF=epidermal growth factor; ERK=extracellular regulated kinase; FGF=fibroblast growth factor; GDP=guanosine diphosphate; Grb2=growth factor receptor-bound protein; GTP=guanosine triphosphate; HDAC=histone deacetylase; IGF=insulin-like growth factor; MEK=mitogen-activated protein extracellular regulated kinase; MMP=metalloproteinase; mTOR=mammalian target of rapamycin; PDGF=platelet-derived growth factor; PI3K=phosphatidylinositol-3-kinase; PIP2=phosphatidylinositol (4,5) biphosphate; PIP3=phosphatidylinositol (3,4,5) triphosphate; PKC=protein kinase C; PTEN=phosphatase and tensin homolog; RTK=receptor tyrosin kinase; Shc=Src homology 2 domain containing transforming protein; SOS=son of sevenless homolog; Src=Schmidt-Ruppin A2 viral oncogene homolog; TGF=transforming growth factor; VEGF=vascular endothelial growth factor. From Yamanaka and Saya (2009)

will be registered per year in the near future. Estimates indicate that there are 800–1000 new compounds in the pipeline for anticancer drug development.

Today, most of the targeted therapies are aimed at growth factor signalling pathways and tyrosine-kinase receptors. The main group of trans-membrane tyrosine kinase receptors correspond to the ErbB family that includes EGFR (epidermal growth factor receptor, HER1), ERBB2 (HER2), ERBB3 (HER3) and ERBB4 (HER4). The receptors have an extra-cellular domain which is a target for the ligand, a trans-membrane segment, and an intracellular tyrosine kinase domain. Binding of the ligand to the receptor causes its dimerization and this in turn leads to receptor autophosphorylation and to pathway activation and a cascade of downstream events.

Table 2.3 Examples of protein kinase inhibitors

	Target	Clinical effects in
Imatinib	BCR-ABL	CML
	c-Kit	GIST
	PDGFRA	Chronic myelomonocytic leukaemia
Gefinitib	EGFR	Small-cell lung cancer
Erlotinib	EGFR	Non-small-cell lung cancer Pancreatic cancer
Sunitinib	VEGFR	GIST
	PDGFR	Renal cell carcinoma
	c-Kit	
Sorafenib	VEGFR	Liver carcinoma
	PDGFR	Renal cell carcinoma
Dasatinib	SRC-ABL	CML
		ALL
Lapatinib	EGFR HER2	Breast cancer
Nilotinib	BCR-ABL	CML
Temozolimus	mTOR	Renal cell carcinoma
		Mantle cell lymphoma

Table 2.4 Examples of monoclonal antibodies

	Target	Clinical effects in
Rituximab	CD20	Follicular non-Hodgkin's lymphoma
		B-cell lymphoma
		CLL
Trastuzumab	HER2	Breast cancer
Alemtuzumab	CD52	CLL
		Sézary's syndrome
		Cutaneous T-cell lymphoma
Cetuximab	EGFR	Colorectal cancer
		Head-neck carcinoma
Bevacizumab	VEGF	Colorectal cancer
		Breast cancer
		Non-small-cell lung cancer
Panitumumab	EGFR	Colorectal cancer

In many malignancies, overexpression and mutations of receptor tyrosine kinases cause pathologic molecular signalling which leads to uncontrolled cell proliferation and invasion. So far, drugs interfering with EGFR and HER2 have shown the most significant clinical effects. Targeting aberrant tyrosine kinase activities has opened new possibilities for therapies having more specific antitumour activity, and also fewer side-effects.

Imatinib, a small molecule inhibitor that binds to the site of tyrosine kinase activity, is a potent BCR-ABL inhibitor and the standard of care for first-line treatment of chronic myeloid leukaemia (CML) (Druker et al. 2001; O'Brien et al. 2003; Bacca-

rani et al. 2009). Imatinib also blocks other tyrosine kinases like c-Kit and PDGF receptors which are aberrantly expressed in GISTs (gastrointestinal stromal tumours) (van Oosterom et al. 2001; Heinrich et al. 2003; Heinrich and Corless 2004). Owing to other PDGF alterations, the antitumour activity of imatinib has also been demonstrated for chronic myelomonocytic leukaemia (Baselga and Arribas 2004).

Trastuzumab is a monoclonal antibody that binds to the extra cellular domain of HER2. Amplification of the HER2 gene occurs in about 25% of invasive primary breast cancers (Slamon et al. 1987). Treatment of HER2-positive early breast cancer patients with trastuzumab has shown significant reduction of recurrent disease (Baselga 2006; Smith et al. 2007).

Cetuximab is an anti-EGFR monoclonal antibody with clinical effect in colorectal and head and neck cancer. Gefinitib and erlotinib are small molecules that inhibit the tyrosine kinases associated with EGFR, effective against lung and pancreatic cancer. Dasatinib, a second generation of small molecules targeting tyrosine kinases, is clinically active in patients with imatinib-resistant CML. Sunitinib, which targets different tyrosine kinases associated with EGFR, PDGFR, and c-Kit, has been shown to have anti tumour activity in imatinib resistant GISTs and renal cell carcinoma. Lapatinib, on the other hand, is a second generation of tyrosine kinase inhibitors associated with EGFR and HER2 that exhibits antitumoural effect in trastuzumab-resistant breast cancer. Sorafinib targets VEGFR and PDGF and is effective for the treatment of liver cancer and renal cell carcinoma. Bortezomib is a proteasome inhibitor which is used in the treatment of multiple myeloma. Retuximab binds to CD20 with antitumour activity on follicular non-Hodgkin's lymphoma, diffuse large-cell B-cell lymphoma, and CLL (chronic lymphatic leukaemia). Alemtuzumab is another antibody that targets CD52 with clinical effects in treatment of CLL, Sézary's syndrome, and cutaneous T-cell lymphoma. Bevacizumab targets VEGF and interferes with the angiogenesis regulatory process. The antibody has been shown to have clinical effect for the treatment of colorectal carcinoma.

2.4.3.4 Other Treatment Modalities

Biological treatment includes a group of agents with natural functions in the body. Some of them have antitumour characteristics, as is the case for interferons and interleukins. Throughout the years, different types of immunotherapies have been explored, based on antibodies and cellular immunity (for a review, see Chabner and Longo 2006). From the increased knowledge about molecular defects causing tumour development, different possibilities to correct these defects have been identified in experimental systems, but so far gene therapy remains an experimental approach.

2.4.3.5 Drug Resistance

Drug resistance is becoming an increasingly important clinical problem. Some tumours like melanoma, renal cell carcinoma, and non small-cell lung cancer are

often primarily resistant, while others like myeloma and breast cancer may recur after a remission. Recurrent disease may depend on the heterogeneity of the tumour cell population with a minority of resistant cells surviving the primary treatment. Several types of resistance mechanisms have been described. Some anti-tumour agents are dependent on active transport through the cell membrane, and decreased uptake of the drug may lead to resistance. One special resistance mechanism is linked to the action of the 170 kDa membrane protein from the MDR-1 (multidrug resistance) gene which acts to transport molecules outside the cell, some cytostatics included (Marie et al. 1991). To be active, a number of anticancer agents require metabolization by specific enzymes. On the other hand, cytostatics may be inactivated by glutathione conjugation. Thus, changes in activation as well as inactivation may lead to drug resistance. Genetic instability of tumour cells may represent one of the main causes of acquired resistance. Gene amplification and mutations may cause alterations in the amount of target molecules, or cause qualitative changes of these molecules with decreased efficacy of the anticancer agent. Drugs causing DNA damage will be more or less effective depending upon DNA repair mechanisms. Examples are activities of 06-methylguanine-DNA-methyl-transferase and nucleotide excision repair. Several antitumour agents induce apoptosis, and changes in apoptosis-regulating molecules may simultaneously confer drug resistance. This can be seen as a system-level phenomenon (see Chap. 12 and 17).

2.4.3.6 Side-Effects

An important aspect of treatment with anticancer agents is side-effects. Acute side-effects like nausea, vomiting, and fatigue occur frequently. Depending on their mode of action, different anticancer agents cause organ related side-effects. A large number of anticancer agents produce alopecia, and neurotoxicity is common following treatment with vincristine and cisplatin. A majority of antitumour agents cause bone marrow toxicity, and gastrointestinal side-effects are common after treatment with antimetabolites. Anthracyclines can induce specific cardiotoxicity. Cisplatin and methotrexate (Lederetrexate, an antineoplastic antimetabolite with immunosuppressant properties) are examples of drugs causing nephrotoxicity. A long-term side-effect may be secondary tumours caused mainly by alkylating agents.

2.4.4 Treatment Strategies

Combining antitumour agents with different modes of action has been successful in the treatment of lymphomas and leukaemias (DeVita et al. 2008), with strong posi-

tive effects being demonstrated in paediatric oncology (Pinkerton et al. 2007; Trigg et al. 2008). With some exceptions, e.g. in testicular carcinoma (Ehrlich et al. 2010), treatment of solid tumours poses a more difficult problem. An important concept is applying adjuvant systemic treatment at an early stage of the metastatic disease. For example, it has been shown that for breast cancer, being subject to most oncologic activities, adjuvant systemic treatment after primary surgery and radiation therapy in high-risk individuals, significantly improves survival (Clarke et al. 2005, 2008; Cuppone et al. 2008; Madarnas et al. 2008). For treatment of patients with solid tumours, the general trend is towards systemic treatment after primary surgery/radiation therapy for high-risk individuals, regarding the presence of micro-metastases. Another application of chemotherapy for solid tumours is in preoperative treatment in order to reduce the tumour volume before surgery and to treat potential metastatic disease as early as possible.

There are a large number of anticancer agents with positive clinical effects on only a fraction of patients and often for only a limited time. To improve cure rates, treatments tailored to the individual patient are required, taking into account the specific phenotypic and molecular characteristics of the tumour and the patient. This includes treatment of the cancers at an early stage. Early detection should therefore include prediction or detection of micrometastatic disease. Systemic treatment is indicated in such cases, as it will increase the probability of cure.

Personalized cancer medicine has as its goal delivery of ‘the right treatment to the right patient at the earliest possible time’. The time is now ripe to identify and validate prognostic biomarkers anticipating the risk that the patient will develop progressive disease and predictive biomarkers that point to the likely response of the tumour to a particular intervention as well as side-effects. There are three steps to biomarker discovery: identification, retrospective validation in archival material, and prospective validation in clinical trials. For this purpose we need molecular pathway-driven and adaptive clinical trials, before the potential benefit of predictive biomarkers used for stratification of patients will be evaluated in randomized clinical trials. Since one expects a high correlation between the biomarker profile and the response to a particular treatment, this approach requires trials with only a small number of patients in a specific treatment area. This strategy, along with the implementation of systems biology and systems medicine approaches, will likely help in solving the dilemma of the present approach of evidence-based cancer medicine, where large numbers of patients are needed for time-consuming trials which involve identifying small differences between treatment groups. This should lead to a much faster development and implementation of new therapies. Molecular pathology/cytology will be fundamental in predicting prognosis and treatment outcomes. The development of imaging technologies, already spectacular, will benefit in the future from molecular pathology discoveries, making the biological analyses of tumours possible with fewer surgical intervention (Fass 2008).

2.5 Major Cancers, Diagnosis, Disease-specific Supplementary Classifications, and Treatment Implications

In light of the evidence discussed so far we will now discuss the features and treatment of some of the ‘big killers’ at a level of detail that illustrates the complexity which needs to be ultimately addressed by systems approaches in conjunction with existing classification methods, to produce improved outcomes for patients.

2.5.1 Colorectal Cancer

Colon and rectal cancer (CRC) is a major cause of death in Western countries and the incidence is increasing rapidly worldwide due to the adoption of Western lifestyles and increased ageing of the population. About 1 out of 20 people will be affected by this disease, and in the European Community alone around 200,000 new cases per year are diagnosed. Nevertheless, in recent years, understanding of the initiation and progression of the adenoma-to-carcinoma sequence, and the genes involved in these processes, has increased enormously, opening up new preventive and therapeutic approaches. Survival has increased thanks to better surgical treatments, especially for rectal carcinoma, with a reduction in local recurrences, and to new targeted therapies with monoclonal antibodies, together with neoadjuvant radiotherapy and improved conventional chemotherapy.

In all these steps, accurate pathological reporting of the specimens plays an increasing role in two situations: the diagnostic and the post-surgical phases (Bosman 1995). The role of histopathology consists both in confirming the presence of clinically suspected intestinal adenoma or atypical adenoma to *in situ* or invasive carcinoma sequence, and is furnishing a precise description of the surgically resected specimen, including the resection margins and the nodes. This double role will influence the therapeutic approach and clinical management, and predict the survival of the patient.

The modified, original Duke’s prognostic classification (Hutter and Sobin 1986; Whitehead 1994; Fenoglio-Preiser et al. 1999) is based upon pathological examination of the surgically resected specimen and the assessment of the tumour extension in the depth of the intestinal wall affecting or not the neighbouring tissues together with nodal invasion or the presence of distal metastasis. This classification has been further improved with the addition of TNM staging (Edge et al. 2010). T describes the size of the tumour and whether it has invaded nearby tissue, N describes regional lymph nodes that are involved, M describes distant metastasis (spread of cancer from one body part to another).

Duke’s classification is based upon the depth of tumour invasion:

A: limited to the mucosa;

B1: limited to the *muscularis propria*, but with negative nodes;

- B2: penetrates the *muscularis propria*, with negative nodes;
- C1: limited to wall, but with positive nodes;
- C2: extends through the wall and nodes are positive;
- D: any grade, but with the presence of distal metastases.

Colorectal cancer–TNM classification (Sobin et al. 2009) includes four stages:

- Stage I: Tumour invades muscularis propria, but has not spread to nearby lymph nodes.
- Stage II: Tumour spreads into the subserosa and/or perirectal tissues with up to three regional lymph nodes, or directly invades adjacent tissues without lymph node involvement.
- Stage III: Any depth of tumour invasion, with four or more positive lymph nodes, but without distant metastases.
- Stage IV: Any depth of tumour involvement, any number of involved lymph nodes, with distant metastases.

Staging at diagnosis has been recognized as the most powerful indicator of clinical outcomes (e.g. survival rates) in patients with colorectal malignancies, which varies from 3% to 100%, depending upon the grade and stage of the tumour. An especially important aspect of staging is the determination of the N status. The introduction of sentinel-node detection has proved to be of value, similar to that in breast carcinoma. In the majority of patients, the sentinel node procedure is successful, and almost one-quarter of the clinically suspected node-negative patients have microscopic disease, which has profound implications for the outcome of the disease. However, approximately one-third of node-negative patients have recurrent disease. Another important parameter affecting prognosis and guidelines is accurate pathological staging after neoadjuvant radio- and chemotherapy prior to surgery. An added problem is the morphological prediction of response to therapy, but several approaches have been proposed with promising results (Dworak et al. 1997; Saad et al. 2006).

Histological typing of the tumour follows the accepted international histological classification of CRC proposed by the WHO (Hamilton and Aaltonen 2000). Adenocarcinoma is the basic epithelial neoplasm displaying several histological varieties (mucinous-colloid, signet-ring cell, medullary, small-cell, squamous cell, adenosquamous, and undifferentiated). Several grading systems have been proposed, but a common accepted standard is lacking. The College of American Pathologists proposed a system based upon the proportion of gland formation, with an average of more than, or less than 50%, and also 0%, thus distinguishing between well-differentiated and undifferentiated adenocarcinoma. The proportion of glands present in the tumour allows a diagnosis of well/moderate, low grade (grades 1 and 2) to poorly/undifferentiated, high grade carcinomas (grades 3 and 4). Some types are directly categorized as high grade: signet ring, small-cell, and undifferentiated carcinomas, while medullary carcinoma has a better prognosis. Neuroendocrine carcinomas, which may present a small-cell pattern or be undifferentiated with large cells, display a worse prognosis. The presence of isolated neuroendocrine cells in

a conventional adenocarcinoma does not, however, imply an adverse prognosis. In fact, patients with the worse prognosis tend to be at a more advanced stage of the disease, according to pTNM classification and pStage.

In colo-rectal cancer, completeness of the mesorectum removal during surgery allows a good assessment of the adequacy of excision and the regularity of the circumferential resection margin, while the evaluation of mesorectal completeness provides significant information on the prognosis. Patients with an incomplete mesorectum have a higher risk of recurrence and the circumferential margin involvement is one of the most powerful predictors of local recurrence. Although there has been discussion about the definition of positive margins, Nagtegaal and van Krieken (2002) have shown that in order to predict local recurrence, margins smaller than or equal to 2 mm should be regarded as involving an increased risk of local recurrence, while margins of 1 mm or less are predictive of an increased risk of developing distant metastases and therefore of shorter survival times.

A decisive factor in prognosis is the presence of nodal metastases at the time of surgical treatment. The TNM guidelines recommend examining at least 12 lymph nodes; they must be tumour-free before a case can be classified as N0. In daily practice this number is hard to attain, and the number may vary from one patient to another; in elderly patients retrieved lymph-nodes are less numerous. In addition, pre-operative neoadjuvant treatment (normally chemo-radiotherapy) influences the number and status of lymph nodes that are resected and examined. Detailed macroscopical examination of the resected specimen is an important element in determining the number of lymph nodes as well; there are in fact considerable differences in the numbers of lymph nodes retrieved by different pathologists in different institutions or even within the same laboratory (Ingoldsby and Callagy 2009).

However, data obtained from colon or colorectal cancer patients may not be applicable to rectal cancer patients. It is clear that the mean number of examined nodes in the ascending and descending colon may be almost twice as high as the number of nodes in the rectum. Very small lymph nodes that are missed using routine examination can be detected using fat clearance techniques. However, these techniques are time-consuming and expensive and might interfere with determination of the circumferential resection margin. Immunohistochemical and molecular support provide promising results, but their relevance is still not clear.

Several linear analyses (Jass 2007; Markowitz and Bertagnolli 2009) are consistent with the subdivision of all CRC developed in the general population into five major classes: Hereditary nonpolyposis colorectal cancer (HNPCC), suspected HNPCC, juvenile cases, familial tumours, and apparently sporadic cases. All these cases evolve through several genetic pathways defined by specific molecular expressions: (1) DNA microsatellite instability (MSI) status being sub-stratified as MSI-high (MSI-H), MSI-low (MSI-L) and MS stable (MSS), and (2) CpG island methylated phenotype (CIMP) divided as CIMP-high, CIMP-low and CIMP-negative (CIMP-neg). Jass (2007) proposed the presence of a morphological correlation in the five molecular subtypes:

Type 1 (CIMP-high/MSI-H/BRAF mutation),

Type 2 (CIMP-high/MSI-L or MSS/BRAF mutation),

Type 3 (CIMP-low/MSS or MSI-L/KRAS mutation),
Type 4 (CIMP-neg/MSS) and
Type 5 or Lynch syndrome (CIMP-neg/MSI-H).

These molecular states can be detected at an early evolutionary stage and are present in polyps with precancerous lesions. For instance, serrated polyps are the precursors of Types 1 and 2 of CRC, whereas Types 4 and 5 evolve through the steps of conventional adenomatous polyp, *in situ* carcinoma, and invasive carcinoma sequence. Type 3 CRC may arise within either type of polyp.

In addition, a better understanding of the molecular pathways that characterize CRC cell growth, cell cycle, apoptosis, angiogenesis, and invasion has led to the identification of novel targets for cancer therapy. Duff et al. (2006) have given an interesting overview on proteins that play a role in CRC, grouped according to their location in the cell: membrane receptor targets (epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), insulin-like growth factor receptor (IGFR), platelet-derived growth factor receptor (PDGFR), tumour necrosis factor-related apoptosis-inducing ligand receptor (TRAIL-R), and c-Met, intracellular signalling targets (Ras/Raf/MAPK pathway, phosphatidylinositol-3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR), src kinase, and p53/Hdm2, as well as other protein kinases that control cell division.

These molecular approaches give further understanding to the sequence of already well known morphological events. This evolution is a consequence of an aberrant gain in cell proliferation that starts with somatic or germinal mutations and leads to the gene instability that typifies the progression of CRC. Thanks to these findings, medicine today has a better opportunity to prevent and clinically manage the polyp-adenoma sequence before malignant transformation (Fearon and Vogelstein 1990; Lynch and de la Chapelle 2003).

2.5.2 Breast Carcinoma

Breast neoplasms comprise a heterogeneous group of proliferative processes including distinct entities, many of which present a malignant behaviour. Breast carcinoma is the primary cause of cancer morbidity in women in developed countries, and its incidence is increasing worldwide, resulting in more than 500,000 deaths annually according to the WHO.

Pathology participates decisively in combating this process through microscopical diagnosis, which joins other image techniques such as routine mammographic screening, echography, and MRI. Needle-core biopsy (NCB) is widely used, being a well validated technique that reduces the need for diagnostic breast surgery and almost totally excludes fine needle aspiration cytology. The current increase in cure rates is based on early and generalized tumour screening, improved surgery, better radiation therapy, and more effective chemotherapy regimens. Nevertheless, breast cancer mortality remains high, not only when the diagnosis is performed at advanced stages (Stages III and IV), but also in subgroups of patients affected with

small tumours in early stages (stage I and stage II) prior to any evidence of distant metastasis.

The advances attained on knowledge of the disease have been spectacular in recent decades; mainly thanks to the fields of genetics and molecular biology. New biological modulators and monoclonal antibodies targeted to specific cell pathways disturbed in cancer, have contributed to identifying particular types of tumours that benefit from a more effective therapy. Nevertheless, progress remains limited and many questions these new bio-technological approaches have raised are still largely a matter of laboratory and clinical investigation. Closer integration by systems approaches of diverse fields of science is a pressing and necessary desideration for conquering the scourge of cancer.

For many years the grading and the staging of breast tumours (Tavassoli and Devilee 2003) has constituted the basis for therapy, follow-up, and prognosis. In this context, determining the clinical status of the patient and the pathology of the tumour (TNM) is mandatory before starting any type of treatment. These include a number of clinical parameters such as the tumour size, the nodal status, the presence or absence of distant metastasis, and the histological type and grade. These have been historically complemented with the determination of the status of oestrogen (ER) and progesterone (PgR) receptors and more recently of the epidermal growth factor receptor 2 (HER2/neu) (Payne et al. 2008; Faratian and Bartlett 2008).

Malignant progression to carcinoma of the breast follows a successive number of steps similar to what happens in other glandular epithelia. In this process, a ductal-lobular unit initiates a hyperplastic focus proceeding to a usual dysplasia, which leads to an atypical ductal or lobular dysplasia (atypical intraductal or lobular proliferation), and eventually into an *in situ carcinoma* (ductal or lobular) which may progress to invasion and metastasis into axillary nodes or distant organs. Other changes include columnar cell changes, complex sclerosing lesions, and papillary proliferations. Obviously, not all breast carcinomas necessarily follow these successive steps, and a few probably become malignant *ab initio* (from the matter) without manifesting the precancerous stages. Fortunately, a large number of women suffer a dysplasia that will never progress into carcinoma.

The NHS proposed in 2001 (Hayes and Quinn 2009) a coding system designed to simplify the screening programs as guidelines for classifying the lesions obtained with NCB:

- normal non-malignant breast (B1),
- benign (B2),
- uncertain malignant potential (B3),
- suspicious of malignancy (B4), and
- malignant (B5).

Why there is so much heterogeneity in the biological and pathological behaviour of the mammary gland is still poorly understood. This diversity affects not only the histology of the dysplastic lesions, but also the varieties of *in situ carcinoma* including their transition to infiltrating carcinoma. Below we highlight some particular breast cancer aspects of more general interest.

2.5.2.1 Histological Types: WHO Classification and Nottingham Grading

Three major histological varieties (Tavassoli and Devilee 2003) of invasive carcinomas can be distinguished: ductal carcinoma (IDC), some of which may lack a specific organization (not otherwise specified, NOS) 70%, lobular carcinoma (ILC) 8%, and combined infiltrating ductal lobular carcinoma (IDLC) 12%. In addition, there are around 10% of cases with miscellaneous phenotypes (colloid, mucinous, secretory, medullary, papillary, micropapillary, anaplastic, etc.).

The most frequent form of breast carcinoma is IDC. This category mainly comprises those easily identifiable cases with enlarged ducts filled with more or less pleomorphic cells and necrosis (comedo-necrosis is not always seen). They present a varied diversity of stroma infiltrations such as ducts, cords, papillae, or solid nests that extend into the fat tissue and invade local structures, large ducts, deep muscle, or superficial dermis. Most of the NOS varieties initiate as an IDC. The limits of the neoplasm are usually better defined than in ILC and contain grouped microcalcifications which help the mammographic detection in the early invasion stage or even as carcinoma *in situ*. Desmoplasia varies and the elastosis produces a central sclerotic core that mimics a benign radial sclerosis or sclerosing complex, lesions being visible senographically as a dense stellate lump. Their size varies and is a prognostic factor together with the presence or not of axillary nodal metastasis. Some histological varieties display better prognosis than others; tubular, papillary carcinomas are low-grade tumours, while solid invasive comedocarcinoma, micropapillary, or colloid IDC are high-grade. IDC varies widely in the expression of ER, PgR, HER2/neu, cytokeratins, EGFR, or proliferative markers such as MIB1. In addition, most cases express the E-Cadherin adhesion molecule, which helps differentiate them from the ILC negativity. New molecular microarray genetic expression analysis has led to a more advanced molecular classification with the support of IHC in paraffin slides, complementing present histological types and better adapted to the available targeted therapies (Reis-Filho et al. 2006; Badve and Nakshatri 2009).

ILC shows peculiar clinical characteristics in its lack of microcalcifications, the absence of distinct borders, and the difficulty in visualizing it with conventional mammography. Histological analysis may reveal remaining *in situ* areas with dilated lobules filled with small cells of bland nuclear appearance, associated with infiltration in files of single cells (Indian files) caused by the loss of expression of certain adhesion molecules such as E-Cadherin. There are subtypes that display major nuclear polymorphism and solid, alveolar, or mixed architecture with desmoplasia. Hormone receptors (ER and PgR) are positive in 90% of cases, while HER/neu2-positive cases are low (about 8%) and mostly correspond to the pleomorphic subtype.

In terms of prognosis, ILC presents a better clinical outcome compared to IDC in early years, but this trend reverses later on, 6 years or more after diagnosis, achieving IDC mortality rates. Local recurrences and contralateral association are higher when compared to IDC, but total mastectomy is not justified, and lumpectomy is the choice for small tumours.

Clinical and histological data may be used to stratify the carcinoma in order to determine its prognosis and therapy. The pTNM and WHO Stage classifications are based upon tumour size, axillary nodes status, and the presence of distal metastasis, in conjunction with histologic type and grade (Bloom and Richardson 1957 modified by Elston and Ellis 1998), known as the Nottingham grade. Computation of all this information stratifies carcinomas into low, intermediate, and high-grade, with an apparent different clinical outcome (relapse-free disease, disease-free survival, and overall survival) dependent on the response to therapy which combine neoadjuvant and/or adjuvant chemotherapy, hormonal inhibitors, surgery, and high-energy beam radiotherapy, in regimens, depending on the patient's age, the disease stage, and hormonal status (Eden et al. 2004).

There are, however, shortcomings in the results; they are not always as clear-cut as might be expected. Divergences between the clinical behaviour of tumours considered as low grade, but which relapse early or present shortened overall survival, are in contrast with high-grade neoplasms that display an unexpected favourable clinical outcome. Additionally, carcinomas with analogous grade and stage may respond differently to a similar therapeutical protocol. Mortality from breast cancer has tended to decline; an increase in survival of over 10% since the late nineties is attributable in part to early detection by population screening and the implementation of hormonal and adjuvant chemotherapy. However, the disease still remains a major cause of morbidity and mortality, and global survival rates are not satisfactory. Further improvements are expected, given newly available diagnostics and targeted therapeutical agents.

2.5.2.2 Immunohistochemistry and a New Molecular Classification

In recent years, microarray gene expression measurements on fresh tissue RNA, analysed by hierarchical clustering, have offered new possibilities for genetic profiling of breast carcinomas (Perou et al. 2000; O'Shaughnessy 2006; Reis-Filho et al. 2006). This technology has provided new sub-classifications, dividing breast carcinomas into groups that facilitate more precise prognostic and therapeutical approaches. Microarray technology is, however, impractical or difficult to implement in daily routine, partly because of current high costs (Desmedt et al. 2008; Correa Geyer and Reis-Filho 2009).

Nevertheless, these advances have provided seminal information leading to more accessible and cheaper methods, such as IHC in paraffin embedded tissues combined with tissue microarray technology. Using these two techniques, large series of tumours can be tested in single slides with a particular antibody, not only retrospectively, but also in prospective studies. Carcinomas of the breast have been reclassified not only according to their histology, but also by the positive or negative expression of a number of marker proteins that possess clinical, prognostic, or therapeutic relevance. This classification has now been validated by retrospective clinical analysis, and new studies, currently underway, will combine microarray gene expression analysis with the methodology, hope-

fully resulting in a better and more comprehensive view of breast cancer biology (Tang et al. 2009).

Four major types of breast carcinoma are recognized to date (McCafferty et al. 2009). These types are known as: *Luminal A*, *Luminal B*, *Basal-like*, and *HER2/neu*; all identified using a four-marker immunopanel based on hormone receptor status: oestrogen receptor (ER) status, progesterone receptor (PR) status, HER2/neu, and Ki-67 proliferation index. At least two to four more subtypes have been proposed. The proposed addition of CK 5/6 and EGFR allows the sub-classification of the Basal-like subtypes in a triple negative and in core basal phenotypes. Moreover, an Apocrine phenotype (based upon AR status and GCDP-15), which is close to the HER2/neu and 'Claudin 1 low' (stem-cell like) phenotypes (Pinero-Madrona et al. 2008), has been proposed. Although this immunohistochemical-molecular classification has attracted wide interest, its clinical validation is still in progress.

The *Luminal A subtype*, which expresses ER and PR positivity, is HER2/neu negative, and displays a low proliferative index (KI-67). This is the most common breast tumour mimicking normal luminal cells (positivity for luminal low-molecular-weight cytokeratins 8/18) involving genes associated with an active ER pathway. It corresponds histologically to low grade carcinomas, mainly ductal, tubular, cribriform, and lobular, following the WHO classification, and therefore presents low clinical stages and favourable prognosis.

The *Luminal B subtype* is the second most frequent breast tumour. They express ER but the PR status is low or negative. The lesions are HER2/neu negative and show a high Ki-67 proliferative index. They consist of derivatives of luminal cells (positivity for low molecular-weight cytokeratins 8/18), with activated ER gene pathways and p53 mutations. The histological counterparts are mainly high grade ductal, NOS, and micropapillary carcinomas. Clinical outcome and prognosis is worse than for tumours of luminal type A, but the B subtype responds well to chemotherapy with Taxotere Adriamycin Cyclophosphamide (TAC) or Fluorouracil Adriamycin Cyclophosphamide (FAC), and to hormonal control. The clinical stages may be more advanced (stages II and III).

Basal-like subtypes comprise a low number of tumours (around 15% of breast carcinomas correspond to this category), but they can be subdivided in groups that have prognostic implications (Dent et al. 2007; Cheang et al. 2008). All basal-like carcinomas have the characteristics of positivity for basal high-molecular-weight cytokeratins and specific myoepithelial cell markers (CK5/6, CK17, Caveolin1, Calponin1, P63), as well as lack of ER, PR, and HER2/neu expression (triple negative). The lesions exhibit a high Ki-67 proliferation index and harbour both p53 mutations and DNA repair defects. At present, there is controversy regarding these groups of tumours, because not all triple-negative types are genetically basal-like and not all basal-like genetically confirmed tumours display triple-negative features (Rakha et al. 2006). Moreover, a subgroup of basal-like carcinomas expresses EGFR and C-KIT positivity. The latter have a worse prognosis when compared to the already known unfavourable clinical outcome and poor response to therapy of the subgroup. The basal-like category also covers medullary, adenoid cystic, and metaplastic carcinoma, and includes a small subgroup of high grade NOS in the

WHO classification. The category as a whole has more numerous BRCA1 germ line mutation carriers. Some authors prefer to consider as ‘unclassified’ those tumours with the triple negative features with negativity for CK5/6 and EGFR should be considered as unclassified tumours (Tang et al. 2009).

The *HER2/neu subtype* comprises carcinomas with definite positivity for immunostaining with this antibody (clone DAKO, 3+) and confirmation by FISH or CRIST analysis. These tumours may belong to the luminal B type, but the majority correspond to the category of ER and PR negative tumours with a high Ki-67 positivity and occasional low CK 5/6 expression. They are very aggressive high-grade ductal NOS carcinomas; but respond well to HER2 tyrosine kinase inhibitors (trastuzumab).

The *Apocrine type* is very infrequently diagnosed. Focal apocrine features are found in the majority of ductal not-otherwise-specified (NOS) carcinomas; the term is limited to exceptionally pure histological apocrine carcinomas. Apocrine metaplasia is very usual in benign ductal dysplasia and less frequent in *in-situ* carcinoma. Clinically, apocrine carcinomas correspond to high grade tumours, are negative for ER and PR, and present AR positivity together with intense, but focal GCDP-15 and occasionally HER2/neu 3+. Their genetic profile has recently been partially identified (Celis et al. 2007, 2008). However, it is not clear if this group, as is also the case for ‘*Claudin1 low stem cell like carcinoma*’ (Pinero-Madrona et al. 2008), constitute particular clinical entities or should be included within any of the above indicated categories.

Normal cell breast-like type carcinoma has been considered by some authors as another specific entity (Sorlie et al. 2006). However, the characteristics of this tumour, detected via unsupervised hierarchical clustering analysis by the Stanford group (Perou et al. 2000), are unclear because it mimics normal epithelial cells; its histological and clinical significance has still to be determined (Brenton et al. 2005; Tang et al. 2009).

Breast cancer control has progressed dramatically in the past few years, mainly because detection of clinical stage I disease has been improved by modern imaging and screening campaigns, thus reducing cases of more advanced phases involving regional tumour spread and distant metastasis. Simultaneously, a better understanding of the morphological and molecular mechanisms underlying the disease has resulted in additional diagnostic tools and better tailored therapy approaches. Incorporation of this knowledge into systems biology is an example of the new modern approaches to enhancing prevention, early diagnosis, and cure of this disease.

2.5.2.3 Tumorectomy vs. Mastectomy: Free Margins and Sentinel Lymph Nodes

Breast cancer is now considered as a systemic disease and not merely a localized problem. Hence, the approach to its control has changed, with surgery limited to lumpectomies with tumour excisions instead of large amputations (radical mastectomy with complete dissection of axillary lymph nodes), and increased use of

adjuvant chemotherapy and targeted drugs including hormonal inhibitors. This new trend allows surgery to be geared to preserving the mammary gland in the case of small localized tumours. The axillary nodes too may be preserved if the sentinel lymph-node is free of metastasis.

The pathologist plays a seminal role in these new therapeutic directions. Keeping margins free from carcinoma invasion is essential, if local tumour relapse is to be avoided. To this end, it is necessary to excise at least 10 mm of tumour-free margins containing normal tissue surrounding the neoplasm (Lopez-Guerrero et al. 2006). Several procedures have been proposed for determining the extent of the tumour-free margins in the resected surgical specimen; the usual procedure involves colouring the resected specimen with permanent ink that resists embedding in paraffin and does not discolour in the histological section. The practice of free-margin tumorectomies does not preclude the need to complement the area around the excised tumour with local irradiation therapy: the incidence of tumour relapse decreases from 10% to 3–4% in irradiated patients (Lopez-Guerrero et al. 2006).

Sentinel-node biopsy is a standard procedure in operable breast cancer for clinically negative axillae, replacing axillary node dissection in the staging of breast carcinoma. Its value has been confirmed by numerous randomized studies demonstrating improved quality of life and reduced morbidity in patients that undergo this procedure.

Nevertheless, several problems have been raised regarding this new procedure, both of a technical and oncological nature. A technical problem is whether the pathologist should perform a diagnosis of frozen samples, with or without the support of IHC. Another is the variability of histological techniques employed, leading to diversity of results emanating from the different laboratories. Although the American College of Pathology guidelines do not recommend routine IHC as mandatory for diagnosis, many pathologists use it (pan-cytokeratins cover over 90% of epithelial cells, but also non epithelial cells such as the normal reticular cell) and consequently not only metastasis (>2 mm in size) but also micrometastases are detected (0.2–2 mm or isolated or grouped free tumour cells located in the cortical sinus). An oncological problem concerns the determination of micrometastases.

Another controversy concerns the substitution of conventional microscopy diagnosis traditionally performed by the pathologist, with molecular assaying techniques (RT-PCR) based on GeneSearch and OSNA by Sysmex, both using cytokeratin 19. This molecular procedure requires lyzing the node and therefore causes loss of tissue. Nevertheless, preliminary results indicate that the diagnostic capacity of this assay is at least similar or superior to intra-operative imprint cytology or frozen sections, even with the help of IHC.

2.5.3 Lung Cancer

Bronchial carcinoma (lung cancer) is today the most common neoplasia in the world (12.6% of all new cancers) with a male-female gender ratio of 2.7:1 affecting

populations of both developed and developing countries. While the incidence in the developed countries remains stable or even decreases, in developing countries it is increasing. The primary cause of lung cancer in 90% of patients is smoking, and it is estimated that carcinoma will develop in 10–15% of all smokers. Environmental factors may play a role, as well as genetic predisposition in a multistep carcinogenic process, but stopping cigarette smoking is the most effective and least expensive means of reducing the risk.

Lung cancer is the deadliest of human neoplasiae, and yet no early diagnosis is currently available. Staging is still mainly based on histopathological and clinical criteria, which have a limited capacity to predict relapses and survival. In recent years, a major effort to improve the control of lung cancer has been carried out by introducing molecular profiling to typify different groups of bronchial carcinomas, and to provide more accurate predictions of the outcome after treatment, particularly with new targeted therapies. A good example is the EGFR mutations and amplifications that identify patients with non-small-cell lung cancer who may respond well to EGFR tyrosine kinase inhibitors.

Four major histological groups have to be considered, aside from a small number of rarer tumours that display unusual behaviour. The main groups are: *squamous carcinoma*; *adenocarcinoma*; *large-cell carcinoma*; *small-cell carcinoma*. In addition, combined types exist such as adeno-squamous, neuroendocrine (carcinoids), sarcomatoid, and some other more infrequent carcinomas. Mesenchymal and lymphoid neoplasms, together with dysgenetic pulmonary blastoma, complete the picture of this family of malignant lung tumours, excluding pleural mesothelioma (Travis et al. 2004).

For the processes of clinical staging and therapeutic indications, tumours are divided in two major categories based upon cell size. ‘Small-cell Lung Cancer’ (SCLC) and ‘Large-cell Lung Cancer’ (LCLG) or ‘Non Small-cell lung Cancer’ (NSCLC). The last comprising squamous carcinoma, adenocarcinoma, large-cell carcinoma, and adeno-squamous carcinoma, each with different clinic outcomes and distinct available therapeutic approaches.

Small-cell lung cancer (SCLC) was previously known as ‘oat cell carcinoma’. It corresponds to an anaplastic epithelial neoplasm, microscopically composed of small cells of round and/or spindle contour, containing one nucleus with dense chromatin and scanty poorly-defined cytoplasm. It is not infrequently combined with composite structures (about 10% of the tumour extent is necessary for it to be considered a mixed type) such as spindle-shaped, squamous, adenomatous, large, or even giant cells. Mitotic activity is high and necrosis may be massive, producing a diagnostic dilemma when the sample is small. IHC support is necessary to distinguish SCLC from lymphomas or metastatic small-round-cell-tumours (SRCT) (see Sect. 2.5.4), found in other anatomical locations. The SCLC tumour expresses neuroendocrine antigens (chromogranin and synaptophysin) and TTF-1, containing neurosecretory granules which are detectable with EM. The p53 gene is very frequently mutated, with a type of mutation related to cigarette smoking, mainly in women. A large number of genetic rearrangements have been described, that not only differentiates from classic neuroendocrine carcinoma, but also from NSCLC.

There is no detectable *in-situ* phase. Histogenesis involves a pluripotential stem cell of the bronchial tree with neuroectodermal differentiation and neuroendocrine expression. The tumour is not however considered as a true member of the family of neuroendocrine carcinomas.

Due to its high malignancy and generally adverse but unpredictable clinical outcome, the neoplasia is not graded following the TNM system, but is clinically considered as being either at a limited or an advanced stage of the disease. The advanced stage is associated with the presence of distant metastasis. Clinical symptom diagnosis of SCLC are generally reflected in disseminated disease with metastasis in liver, brain, or bone marrow. Original neoplasms located in the hilar or parahilar area may be asymptomatic and remain occult, even where the mediastinal node is involved, or where distant metastasis exists.

Non small-cell lung cancer (NSCLC) comprises a large number of histological varieties of bronchial carcinomas. For clinical purposes, three major histologic subtypes are considered: squamous-cell carcinoma, adenocarcinoma, and large-cell carcinoma. Smoking causes all types of lung cancer, but is more strongly linked with squamous-cell carcinoma, while adenocarcinoma is more frequent in patients who have not smoked. Staging of NSCLC is based on the TNM system. Before treatment, the tumour size, lymph-node status, and the possible presence of metastases must be determined. Lung cancer often spreads to the nodes in the hilum and mediastinum. The combination of PET and X-ray CT scans appears to have great sensitivity and specificity, and the use of both is recommended as part of the clinical evaluation before making any therapeutical decision.

We now focus on the histology of the three major carcinomas and on recent advances in the molecular study of the origin and biology of squamous-cell carcinoma and adenocarcinoma (Pass et al. 2000).

Squamous cell carcinoma is more frequent in men (44%) than in women (25%). Microscopical investigation shows it to possess large cells with diverse amounts of keratinization and pearl formation. The relative amount of squamous maturation serves to stratify well-differentiated tumours (with abundant keratinization) and undifferentiated tumours (with intercellular bridges and focal cytoplasmic keratin only in occasional large cells). Cytologic atypia with highly hyperchromatic nuclei, mitosis, and necrosis are hallmarks of this type of tumour. Several subtypes are considered: clear-cell, small-cell, basaloid, and alveolar carcinomas, as well as the adenocarcinoma mixed phenotype. IHC supports the diagnosis with positivity for low- and high-weight molecular keratins. In addition, EMA (epithelial membrane antigen) and CEA (carcinoembryonic antigen) stain focally isolated cells. TTF1 (thyroid transcriptions factor1) is positive only in some tumours.

Adenocarcinoma, which is becoming more frequent and surpassing the incidence of squamous carcinoma, occurs currently at a rate of almost 80% of NSCLC (Thun et al. 2006), a fact that is explicable due to changes in smoking behaviour. Its anatomical location varies within the lung, most usually as a peripheral tumour close to the pleura or with mesothelioma-like pleural extension, followed by central bronchial and endobronchial siting. The tumour sometimes adopts a pneumonia-like infiltration with nodular foci in the basal lobes owing to a bronchioloalveolar

extension. Scar carcinoma with desmoplasia is quite rare. In histology, glandular differentiation is the dominant pattern, with or without mucin secretion and acinar, papillary, or bronchioloalveolar associated phenotypes. Some adenocarcinomas adopt a solid configuration in which mucin production is lost or limited to isolated fine droplets within the cell. During histological grading, in cases of mixed histology, the least differentiated grade has to be measured. The bronchioloalveolar pattern is consistent with a grade 1, while solid adenocarcinoma is grade 3.

For the differential diagnosis with a metastatic carcinoma in the lung, IHC may be of interest. CAM 5.2, EMA, CEA, and CK7 are very frequently positive, but provide little additional value for the differential diagnosis of a metastasis. TTF1 positivity (75% of cases) gives support to the bronchial origin because other adenocarcinomas, excluding thyroid, are negative.

Large-cell carcinoma accounts for 9% of all lung cancers. This carcinoma combines old terminology: large cell anaplastic carcinoma and large-cell undifferentiated carcinoma, to which should be added the large-cell neuroendocrine carcinoma (LCNEC) and some rare combined forms (lymphoepithelioma-like, basaloid, and rhabdoid phenotype). The stage at diagnosis is similar to other NSCLC. Anatomically, they present large nodules generally located at the periphery of the lung, infiltrating pleura and even the chest wall or large bronchi.

Their histology encompasses a variety of microscopical patterns that contain large undifferentiated cells with large polygonal cytoplasm and prominent nuclei exhibiting numerous mitosis, but lacking squamous, adenoid, or microcellular differentiation. Large-cell neuroendocrine carcinomas (LCNEC) may present alone or be combined with other microscopical components such as adenocarcinoma, squamous, or even giant cells. They stain for chromogranin and synaptophysin, but also for TTF-1. Histological prognostic criteria for LCNEC are controversial, with apparently better outcome than with conventional large-cell carcinomas. Depending upon the staging, the 5-year survival of localized tumours with <3 cm resected NSCLC reaches almost 100% (T1). The local IIA decreases to 55%, while locally advanced (IIB, IIIA, IIIB) shows 39%, 23% and 3%, while IIIB is quite similar to advanced stages (stage IV) (Spira and Ettinger 2004).

Several alterations have been found in histologically apparently normal specimens of bronchial epithelium from smokers. Changes such as hyperplasia and metaplasia have been considered as a slightly abnormal epithelium and are regarded as early changes. The lesion affects normal bronchial mucosa composed of stratified cylindrical epithelia with cilia, and leads to a hyperplasia with or without metaplastic changes of squamous type. These modifications are not necessarily cancer precursors and may regress spontaneously. Nevertheless, in smokers the epithelial metaplasia may progress into dysplasia and subsequently to *in situ* squamous carcinoma. These microscopic lesions are usually multicentric in the bronchial tree and are frequently found in resected lungs of smokers, cohabiting with invasive squamous carcinoma. The molecular variations detected in dysplasia are regarded as occurring at an intermediate stage, whereas those found *in situ* or in invasive carcinoma are consistent with late changes. This multiple-step genetic rearrangement present in most lung cancers varies in its progression during the preneoplastic process.

In fact, the origin of lung cancer depends on a number of interactions between the environment and host genetic susceptibility, including changes in deregulated signalling pathways, which are potential targets for new therapeutical approaches. New techniques for genomic, transcriptomics, epigenetic, and proteomic profiling (Patz et al. 2007; Esteller 2008; Herbst et al. 2008) have improved the clinical approach to several histological varieties of NSCLC, identifying particular molecular markers of individual sensitivity, prognosis, and response to treatment. Good examples are the EGFR and VEGF inhibitors (erlotinib and bevacizumab), which have improved the clinical outcome in these patients (Shepherd et al. 2005; Sandler et al. 2006).

2.5.4 Small Round Cell Tumours (SRCT)

This category comprises a number of malignancies consisting predominantly of small round cells or round-spindle cells, independently of their origin or anatomical setting. Their histological patterns are very similar, thus a differential diagnosis becomes necessary in order to provide clinical information for therapy and prognosis. Among these, the most frequent is Ewing's Sarcoma (ES) and its family of tumours (ESFT) (including peripheral neuroectodermal tumour—PNET). Other frequent SRCT malignancies are neuroblastoma, rhabdomyosarcoma, and lymphoma (non-Hodgkin). A more extensive categorization might include tumours such as microcellular anaplastic osteosarcoma, myxoid chondrosarcoma, small-cell carcinoma of the lung, and small-cell neuroendocrine carcinoma (e.g. Merkel's tumour of the skin). Clinically, SRCT occurs in varied anatomical locations: not only bone and soft tissue, but also solid organs (ovary, testes, kidney, lung, meninges) and skin. There are no gender differences and it may occur at any age, although children and young adults are affected more frequently. Recently developed ancillary techniques such as cytogenetics, molecular biology, FISH, DNA or RNA microarray, and TMA, are offering new diagnostic and prognostic possibilities. Nevertheless, immunohistochemistry and electron microscopy continue to play an important role in the characterization of SRCTs.

Histologically, several variants of ESFT have been described, combining images of conventional or classical ES with other varieties such as atypical ES with large cells, ES with neuroectodermal pattern, and ES with endothelial features (Llombart-Bosch et al. 1996, 2009). Moreover, peripheral neuroepithelioma of soft tissue belongs to this group of neoplasms and may mimic a conventional undifferentiated neuroblastoma. Although neuroblastoma in adults is a rare event, it may still occur (Hasegawa et al. 2001); the differential diagnosis is mainly based upon immunohistochemical and molecular genetic findings. Adamantinoma-like and desmoplastic ES are other unusual variants of this family (Folpe et al. 2005). Genetic confirmation is essential for their identification.

Several immunohistochemical techniques are necessary for diagnosis. Antibody HNK 1 (CD 57), neuron-specific enolase, S-100, NF-70, synaptophysin, chromo-

Table 2.5 Chromosomal and genetic rearrangements in ES/pPNET tumours

Tumour	Translocation	Fusion gene	Frequency (%)
ES/pPNET	t(11;22)(q24;q12)	EWS/FLI-1	85
ES/pPNET	t(21;22)(q22;q12)	EWS/ERG	10
ES/pPNET	t(7;22)(p22;q12)	EWS/ETV1	<1
ES/pPNET	t(17;22)(q12;q12)	EWS/EAF	<1
ES/pPNET	t(2;22)(q33;q12)	EWS/FEV	<1

granin and PG-9.5, lend support to a neuroectodermal lineage (Navarro et al. 2007). Cytokeratin positivity (AE1/3) has been seen in several cases. The cell surface protein product p30/32 MIC-2 (Fellinger et al. 1991) CD99 is expressed in nearly 99% of cells in these tumours, independent of the histological subtype, but is absent in neuroblastoma, while positive in other SRCT unrelated to ESFT, such as B-lymphomas, rhabdomyosarcomas, synovial sarcomas (Weidner and Tjoe 1994; Llombart-Bosch et al. 2009). Caveolin 1 has also been confirmed as an excellent marker for this family of tumours (Llombart-Bosch et al. 2009).

Moreover, it has been demonstrated (Nilsson et al. 1999) that the 68 kDa fusion protein derived from the EWS/FLI1 hybrid gene can be specifically detected by Western blotting using a polyclonal antibody to the C-terminal of FLI1 on fresh tissue as well as paraffin-embedded ES. Eighty percent of the tumours exhibited a positive reaction for the FLI1 antibody, mainly with a nuclear location, but negative in neuroblastoma (Llombart-Bosch and Navarro 2001; Folpe et al. 2005).

Combining histology with immunohistochemistry confirms the structural heterogeneity of ESFT, which varies from conventional ES to atypical ES (including the large-cell variants) to PNET, with numerous Homer-Wright rosettes. Intermediate types may be found within a single tumour, reinforcing this heterogeneous microscopic pattern, such as the presence of vascular lakes with endothelial-like cells. The combination of staining with four antibodies, CD99, HNK1, FLI1, and Caveolin1, provides 100% of positivities in genetically confirmed tumours (Llombart-Bosch et al. 2009) aside from their histology.

Electron microscopy provides further support to the diagnosis. The cells are characterized according to their homogeneity. Large amounts of well-preserved glycogen are seen, and cell contacts show desmosomes. The detection of neurosecretion does not alone exclude ESFT; exclusion is confirmed by other cytoplasmic inclusions such as myofilaments (rhabdomyosarcoma) or interstitial deposits of osteoid material (small-cell osteosarcoma) (Llombart-Bosch et al. 1996).

Accuracy in diagnosis additionally requires confirmation by cytogenetic and molecular biology, which should indicate the chromosomal and genetic rearrangements detected in this group of tumours (Table 2.5). Moreover, the EWS gene presents fusion products with transcription factors: ATF (clear cell sarcoma of soft tissue), WT1 (desmoplastic small round cell tumour), and TEC (myxoid chondrosarcoma).

The detection of a balanced translocation in the ES/pPNET tumours, t(11;22)(q24;q12) shown in Table 2.5, turned out to be a formidable diagnostic marker pro-

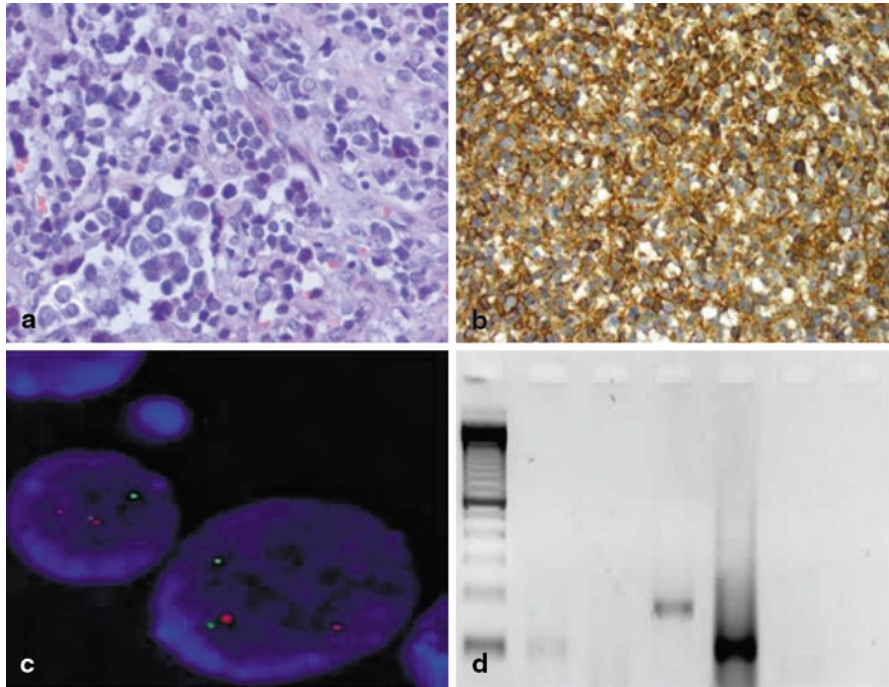


Fig. 2.2 **a** Small round cell tumour Ewing sarcoma type H/E 40X; **b** Membranous CD99 positivity +++ 40X; **c** FISH EWSR1 positive translocation (split signal) 100X; **d** RT-PCR positive (EWS/FLI1) gene fusion

viding a new phenotypic trait in this group of SRCT. More recently, new chromosomal translocations have been observed, independent of the morphological subtype of tumour. These, in decreasing frequency, are as follows: $t(21;22)(q22;q12)$ in approximately 10% of cases; $t(7;22)(q22;q12)$ in approximately 1% of tumours; $t(17;22)(q12;q12)$ also in less than 1% of tumours, and finally a very rare translocation $t(2;22)(q33;q12)$ described only in three ES/pPNET tumours. Thus, this family of tumours is characterized by at least five variants of translocations, in which the locus of the chromosome 22q12 is affected (Delattre 2008).

Nonetheless, approximately 5% of ES/PNET tumours with histological consistency and clinical evidence, using RT-PCR, remain negative for such types of transcript, with the result that a small number of SRCTs still remain outside any genetical phenotyping. New tools are evolving to facilitate the discrimination of most of these breakpoints. For example, the complementary technique of FISH employs specific probes flanking the ES breakpoint regions, not just metaphase chromosomes, but interphase nuclei as well (Bridge et al. 2006; Machado et al. 2009) (see Fig. 2.2a–d).

Gene expression profiling using cDNA microarrays, which allows simultaneous analysis of multiple markers, has been used to categorize different types of SRCT, especially when complemented with artificial neural networks (ANNs) (Khan et al.

2001; Kauer et al. 2009; Zambelli et al. 2010). Gene-expression signatures associated with specific variants of these tumours have thereby been identified: ESFT versus neuroblastoma, rhabdomyosarcoma, and Burkitt lymphoma.

These findings confirm the number of additional mutations that have occurred in ES, which add further insights into the malignant transformation of the initiating cell. These new mutations are not necessarily tumour specific, but may account for the clinical and histological variability of these neoplasms and even show prognostic value. Among them, trisomy 8 appears in 50% of ES and trisomy 12 appears in 20% of tumours. Moreover, an unbalanced translocation $t(1;16)$ has been described in several tumours with ES phenotype. Furthermore, several oncogenes, such as *ras*, *CMYC*, *MDM2* and tumour suppressor genes (*p53*, *p16*, *pRb*) have been analysed in ES tumours. Alterations expressed in oncogenes are not representative, while suppressor genes may play a major role. The frequency of *p53* mutation is low (approximately 10% of ES) and *pRb* is not inactivated (Parham et al. 1999). In contrast, *p16* homozygous deletions have been described in one third of ES without chromosomal aberrations of the 9q21 locus for the *p16* gene (Kovar et al. 1997). In addition, our group has performed a molecular analysis of the 9p21 locus and *p53* genes in this family of tumours using cell lines and original neoplasms (Lopez-Guerrero et al. 2001). Hence, the molecular alteration in either or both the *pRb* and *p53* pathways seems to constitute a multi-step process with equivalent cellular effects, in which the EWS-ETS gene fusion seems to be the initiating mechanism.

A search for prognostic factors is necessary, since only one third of children with non-metastatic disease and 10% of metastatic patients survive the neoplasm. Several multivariate analyses show that the clinical prognosis provides the most valid criteria at present: those criteria are male sex, age greater than 12, fever at presentation, anaemia, high lactate dehydrogenase, axial location, and older chemotherapy regimes associated with adverse outcome (Cotterill et al. 2000). The determination of neuroectodermal expression in ES provides no differences in overall survival or disease-free survival, but histological atypical variants show a worse clinical outcome when compared to classical ES (Terrier et al. 1995; Parham et al. 1999; Llombart-Bosch et al. 2009).

Several assays have been undertaken to ascertain the molecular changes that could indicate further clinical prognostic markers in ES. In the analysis of the EWS/ETS gene fusion types, it was found that the most common fusion type is EWS/FLI-1 exon 7/6 (type 1), whereas in a third of cases the FLI-1 exon 5 is included in the transcript joined to the EWS exon 7 (type 2 fusion seen in 30% of cases). More occasionally, the fusions result in inclusion of the EWS exon 9 or 10 (10%) with FLI-1 exon 4 or 6 (10%) (Ladanyi 1995). Other studies (Zoubek et al. 1996) suggest a better clinical outcome for patients with localized ES, carrying mutation type 1. Thus, the EWS/ETS gene fusion type could serve as a prognostic indicator in both localized and metastatic disease (de Alava et al. 1998). Other molecular factors could also play an important role from the prognostic point of view. These genes include the cell cycle regulators *p53* and *p16* and the recently demonstrated Ki67 in localized disease, whereby those cases expressing Ki67 in more than 5% of nuclei of tumour cells had the worst behaviour independently of the type of treat-

ment (Lopez-Guerrero 2010). In addition, Zambelli et al. (2010) have proposed that lectin galactoside-binding soluble 3 binding protein (LGALS3BP) is a novel and reliable prognostic indicator for ES/PNET patients showing associated high mRNA expression levels of HINT1, STOML2 and c.MYC.

2.5.5 Leukaemias and Lymphomas

Molecular genetics has been at the forefront of research into cancer pathogenesis. The identification of recurrent chromosomal translocations has provided insights into the molecular events leading to leukaemic transformation. The classification of leukaemias has been, until recently, based on morphology and immunophenotype. The recognition of the correlation between distinctive morphologies and specific translocations mainly occurring in *de novo* leukaemias has had an indisputable impact on leukaemia classification, leading to the introduction of a subset of “acute myeloid leukaemias (AML) with recurrent genetic abnormalities” in the new WHO classification of acute leukaemias. The majority of the translocation events produce a fusion gene that encodes an aberrant protein, in which the ‘5 end of one translocation partner encodes the N-terminal protein sequence of the fusion protein, and the 3’ end of the other translocation partner encodes the C-terminal protein sequence of the fusion protein. The fusion genes produced as a result of translocation events or the mutated genes are transcriptional regulatory proteins with altered properties of transcriptional activation or repression. It is now realized that many leukaemia cases that appear to be cytogenetically normal have point mutations or deletions in genes encoding key regulatory proteins, such as the *fms*-like tyrosine kinase-3 (FLT3) or the CCAAT/enhancer binding protein- α (C/EBP α).

More than one genetic hit is usually necessary for the development of leukaemia. The concept that multiple genetic defects are involved in leukaemogenesis is supported by the discovery of frequent FLT3 mutations in leukaemias with recurrent translocations. The breakthroughs in understanding the molecular genetics of leukaemia have had a direct impact on clinical treatment. In this respect, chronic myeloid leukaemia (CML) has been the paradigm for the translation of basic research to clinical treatment. CML was the first leukaemia to be associated with a recurrent translocation, t(9;22)(q34;q11) (the Philadelphia chromosome) and the first leukaemia for which the product of the translocation, BCR-ABL, was characterized. In addition, a specific molecular inhibitor, imatinib (Gleevec), was designed for CML and was successfully used for patient treatment. The Philadelphia (Ph) chromosome is also the most frequent recurring translocation in adult acute lymphoblastic leukaemia (ALL) occurring in 15–30% of patients, and is also present in 5% of paediatric B-cell ALL. In both clinical scenarios, it is an adverse prognostic factor. The most common breakpoint region within the BCR gene, the major breakpoint cluster region (M-bcr), results in a fusion protein of 210 kD, referred to as p210^{bcr-abl}. A minor breakpoint, the m-bcr, results in a truncated fusion protein of 190 kD (p190^{bcr-abl}). Importantly, p210^{bcr-abl} is much more common in CML, whereas

p190^{bc_r-abl} is present in 80–90% of paediatric Ph+ ALL and 50% of adult Ph+ ALL. BCR-ABL has leukaemogenic properties as a constitutive tyrosine kinase that activates multiple downstream signal transduction intermediates, including ras, PLC γ and PI3 kinase, leading to proliferation and resistance to apoptosis.

Conceivably, similar mechanisms are operative in Ph+ ALL, a disease whose treatment is highly problematic. Remissions tend to be short-lived and stem cell transplantation is the most effective way of attaining durable control of the disease. The 2-phenylaminopyrimidine derivative, imatinib, is an ABL-specific tyrosine kinase inhibitor that constrains the proliferation of CML cell lines by inhibiting BCR-ABL kinase activity. The drug is administered orally and is generally well tolerated. In a multicenter phase II trial, imatinib at 400 mg/d induced a complete haematological response in 95% of patients and a major cytogenetic response in 60% (Kantarjian et al. 2002). After a median follow-up of 18 months, 95% of the patients were alive and CML had progressed to accelerated or blast crisis in 11% of patients. This has represented a dramatic breakthrough in the treatment of CML, a disease context where initial therapies were aimed at controlling the elevated white blood cell count, reducing the symptoms of concomitant splenomegaly, and treating metabolic complications arising from profound marrow proliferation, such as hyperuricaemia and gout.

Unfortunately, the issue of resistance to imatinib is beginning to emerge. Of patients who start imatinib in the early chronic, late chronic, and accelerated phases of CML, 12%, 32%, and 62% respectively develop resistance mutations within two years of commencing treatment, these being attributable to either a single amino acid substitution in the ATP-binding region of BCR-ABL or, occasionally, to progressive BCR-ABL gene amplification. New BCR-ABL tyrosine kinase inhibitors are currently being evaluated in clinical trials: the improved-potency, selective Abl inhibitor nilotinib, and the highly potent dual Src/Abl inhibitor dasatinib. Despite their greater activity, there is some concern arising from *in vitro* studies of dasatinib that these drugs too will turn out to be unable to clear the leukaemic stem cells (Copland et al. 2006). Clinical trials will show whether these second-generation tyrosine kinase inhibitors should be used alone or in combination as first-line therapy for newly diagnosed CML.

Another elegant example of the interaction between molecular advances and clinical treatment is the case of acute promyelocytic leukaemia (APL). The t(15;17)(q22;q21) translocation in APL is associated with the characteristic morphology of hypergranular blast cells with frequent Auer rods or the microgranular variant. An initial report from China (Huang et al. 1988) indicated that APL could be treated successfully with all-trans retinoic acid (ATRA). This observation preceded the discovery that the t(15;17) translocation involved the retinoic acid- α gene on chromosome 17. In the t(15;17)(q22;q21), the most common translocation associated with APL, the 5' portion of the fusion protein is encoded by the PML (promyelocytic leukaemia) gene from 15q22, and the 3' portion is encoded by the RAR α gene from 17q21. The wild-type RAR α is a nuclear receptor acting as a transcription factor and binding to retinoic acid response elements (RARE) in the promoter of many genes, including those implicated in myeloid differentiation (granulocyte col-

ony-stimulating factor [G-CSF], cell-surface adhesion molecules [CD18, CD11b], regulators of apoptosis [Bcl-2], and several transcription factors). In the absence of retinoic acid, the wild-type RAR α binds to corepressor proteins and histone deacetylases, resulting in transcriptional repression. Wild-type PML protein is localized in subnuclear oncogenic domains, called nuclear bodies, and may act as a tumour-suppressor protein, although it does not bind DNA directly. In APL, the aberrant fusion protein PML-RAR α is delocalized from the nuclear bodies to a microspeckled nuclear pattern and acts in a dominant negative manner, competing with wild-type RAR α for binding to the RAREs in the absence of ligand. However, pharmacological concentrations of retinoic acid are required to convert PML-RAR α into a transcriptional activator. These observations have provided the rational basis for the efficacy of ATRA treatment in patients with APL to induce the differentiation of leukemic promyelocytes.

The t(8;21) translocation is present in approximately 15% of patients with AML and involves the RUNX1 (AML1) gene which is located on chromosome 21q22.3. The fusion partner of RUNX1 in t(8;21) is named eight-twenty-one (ETO) and is a transcriptional regulator. The murine counterpart of RUNX1 was first described as part of the core binding factors, which are essential for haematopoietic development, as indicated by gene deletion experiments in mice. RUNX1 is a transcriptional activator regulating lymphoid genes such as B-cell tyrosine kinase, T-cell receptor α and β , interleukin (IL)-3, and granulocyte proteins. The RUNX1-ETO fusion protein binds to the same DNA-binding site as RUNX1 and acts as a dominant negative inhibitor of wild-type RUNX1 but also as an active transcriptional repressor. Targets of RUNX1-ETO repression are presumed to be genes important for granulocytic differentiation and tumour suppressors such as p14ARF and NF1.

More recently, development of the technology of microarray analysis has led to a better understanding of the global changes in gene expression that occur as a result of leukaemic transformation and has allowed the subtyping of acute leukaemias. When patients with AML are grouped on the basis of gene expression signatures, most clusters correspond to the common recurrent translocations or known gene mutations. Valk et al. 2004 identified 16 groups when analysing blood or bone marrow from 285 patients with AML. Patients with recurrent translocations (t(8;21), t(15;17) and inv(16)), and thus with cytogenetically defined disease subsets, formed clear clusters, thus validating the significance of the gene expression patterns and suggesting that microarray analysis may allow subclassification of leukaemias into meaningful groups with unique prognosis and pathogenesis.

Lymphomas are a heterogeneous group of malignant diseases of the lymphoid system, whose classification remains a confusing and controversial issue. They comprise the *non-Hodgkin lymphomas* (NHL) and *Hodgkin lymphoma* (HL). In 1994, the Revised European American Lymphoma (REAL) classification was proposed and listed “real disease entities recognized and diagnosed in daily practice”. More recently, the WHO classification has provided a comprehensive definition of lymphomas by morphology, immunophenotype, genetics, and clinical information. The NHL are a diverse collection of lymphoid neoplasms with varied pathology, cell of origin, natural history, and response to treatment. The histological diagno-

sis of NHL is among the most difficult tasks that surgical pathologists are asked to undertake. The molecular genetic lesions of pathogenic importance in selected forms of NHL have recently been unravelled via the molecular analysis of structural chromosomal abnormalities that alter critical genes regulating growth and/or differentiation.

There is a worldwide epidemic of NHL and its rise has been faster than that of all other malignancies except lung cancer in women, melanoma, and prostate cancer. More than 60,000 new cases per year will be diagnosed in the United States in the 2000s. The majority of patients with NHL presents with painless lymphadenopathy, more commonly in the cervical or supraclavicular regions, but extranodal disease, mainly of the gastrointestinal tract, can be detected at presentation in up to 40% of patients. Systemic symptoms are associated with advanced stages of disease and portend a poor prognosis. Actually, there is no substitute for tissue diagnosis, although PET scanning using ^{18}F -fluorodeoxyglucose has been used as a diagnostic technique for disseminated disease and to assess treatment response (Haioun et al. 2005). Disease prognosis is highly variable, as might be expected from the broad spectrum of NHL subtypes, and biological differences have been described between young and old patients, translating into a greater mortality in some series of elderly patients compared with younger cohorts. The type of therapy is generally based on pathology and its intensity is based on both pathology and stage of disease. Independent prognostic determinants include advanced stage, tumour bulk as reflected by size and LDH, and number of extranodal sites of involvement.

Recent advances in clinical scoring systems and in molecular and phenotypic markers have improved our ability to predict therapeutic responses. In this respect, immunotherapy approaches are rapidly advancing and include non-specific immunostimulation with interferons, passive therapy with anti-lymphoid antibodies such as rituximab, radioimmunotherapy, patient-specific autologous anti-idiotypic vaccines, and novel cellular immunotherapy modalities. Rituximab is a chimeric IgG anti-CD20 monoclonal antibody composed by fusing a light and heavy chain-variable domain of a murine IgG with a human IgG light and heavy chain-constant region. Rituximab has rapidly become an effective component as a single agent or in combination with chemotherapy in the treatment of all types of NHL, producing an undisputed survival advantage over chemotherapy alone. New monoclonal antibodies directed against CD20 and other B-cell antigens are under investigation, including epratuzumab, a chimeric anti-CD22 antibody.

Multiple myeloma (MM) is a highly treatable but incurable neoplastic plasma-cell dyscrasia characterized by the clinical pentad of anaemia, a monoclonal protein in the serum and/or urine, abnormal radiographs and bone pain, hyperuricaemia, and renal insufficiency or failure. Until recently, the higher response rates seen with regimens that combine multiple agents as initial therapy (alkylators, anthracyclines, corticosteroids, and interferon) had not resulted in improved survival rates. Over the last five years, we have witnessed enormous progress in fundamental and therapeutic research in MM. The current preferred therapies are all in the “novel” category. In particular, a new class of immune modulatory drugs has dramatically changed the previous scenario. The recognition in 1999 of the activity of thalidomide against

MM and the subsequent development of lenalinomide and bortezomib, has made MM treatment more promising and rewarding. Thalidomide is believed to inhibit angiogenesis in MM but also to target the surrounding stroma and cytokines and to affect NK cells. When used as a single agent, thalidomide induces response rates of 25% in previously untreated patients and is now considered a standard therapy for MM. Bortezomib is the first drug in its class of proteasome inhibitors. Bortezomib selectively and reversibly inhibits the proteasome, an intracellular complex that degrades ubiquitinated proteins and plays a key role in cell cycle regulation, protein degradation, and gene expression. Single-agent response rates in relapsed/refractory MM range from 28% to 38% and the median duration of response is 8 months (Weber et al. 2003). The long-term outcome of treatment with novel drugs is presently not known, because of the short duration of follow-up. The protagonists of the current treatment armamentarium embrace a holistic total-therapy approach and combine multiple agents. In the near future, we may be able to ascertain biological differences among disease subsets and direct specific forms of therapies to their biology. Cytogenetic testing is an integral element to establish prognosis and a treatment plan for newly diagnosed MM. Nearly all MM patients have cytogenetic abnormalities diagnosed by FISH, but abnormal karyotypes are seen in only 18–30% of cases. Molecular classification systems have been proposed based on gene expression profiling but these are deemed not to be ready for general clinical application, as distinct from cytogenetic classification systems that are easily applied to the clinic at present. Nearly 85% of newly diagnosed MM patients have gene expression-defined good risk features and fare so well that the prospect of cure has become a reality. By using high-density oligonucleotide microarrays and hierarchical clustering analysis, four distinct subgroups of MM (MM1, MM2, MM3 and MM4) have been identified (Zhan et al. 2003). Of interest, clinical variables associated with poor prognosis, including abnormal karyotype and high serum β 2-microglobulin levels, were most prevalent in MM4. Also, over-expression of genes involved in DNA metabolism and cell cycle control was primarily observed in MM4. Whether this information may be incorporated into novel prognostic algorithms and be used to tailor current treatment strategies, remains to be addressed.

2.6 Systems Biology of Cancer: Key Challenges for the Future

As mentioned earlier, recent advances in molecular and cellular cancer biology, as well as the explosion of novel technologies within genomics, transcriptomics, proteomics, and functional genomics, promise to have a major impact on clinical practice. These developments are likely to change the way in which diseases will be diagnosed, treated, and monitored in the future (Celis et al. 2005). Major areas of research that will benefit from these developments include the identification of molecular biomarkers for non-invasive early diagnosis, subclassification based on clinical outcome, prediction of prognosis and response to treatment, as well as

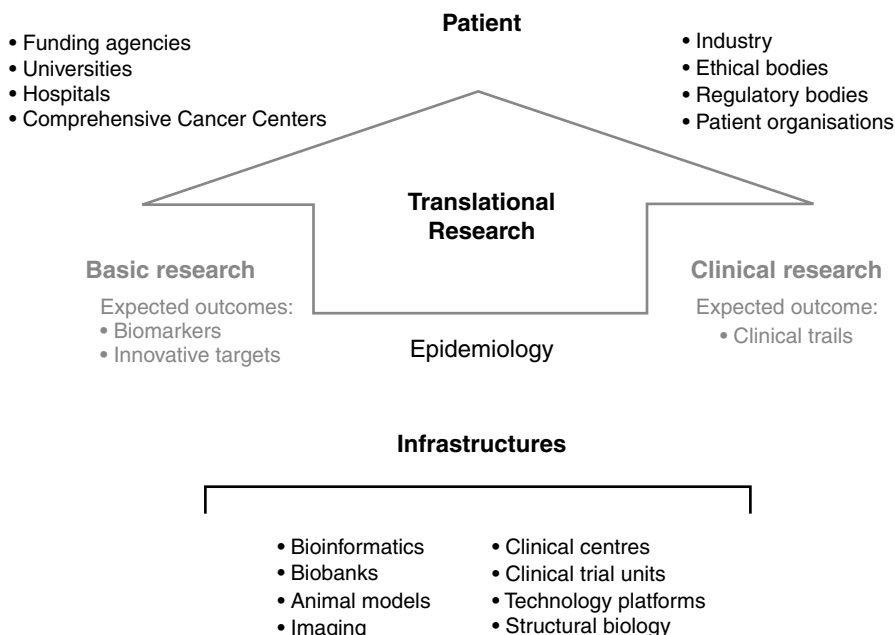
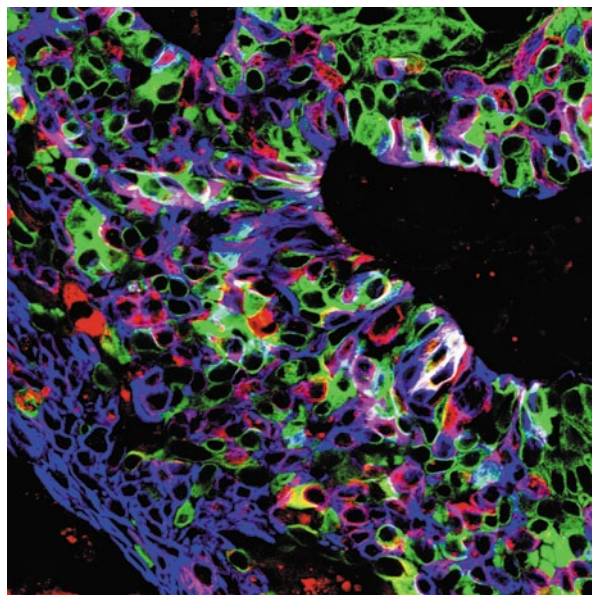


Fig. 2.3 Graphical illustration of stakeholders and support infrastructures in discovery-driven translational cancer research

determination of drug efficacy and toxicity (Zhang et al. 2007; Ransohoff 2009; Alymani et al. 2010 and references therein). Also, the identification of novel therapeutic targets—identified on the basis of systems biology approaches to the analysis of pathways that are affected in cancer cells (Aebbersold et al. 2009; Laubenbacher et al. 2009; Kreeger and Lauffenburger 2010)—will be another area that will greatly benefit from high-throughput ‘omic’ technologies (Sara et al. 2010).

Research efforts in these areas, however, must be supported by the development of bioinformatics and modelling tools for integrating and mining data, as well as by proper technological and clinical infrastructures. In the long run, this integrated approach should lead to a better understanding of the biology underlying cancer cells, which in turn will lead to a more effective translation of basic discoveries into new diagnostics and therapies (Fig. 2.3). The daunting heterogeneity of human cancer, in terms of cellular phenotypes, genetic make-up, molecular profiling, and clinical behaviour is however posing major challenges that must be addressed if we are to fulfil the dream of bringing personalized medicine and individualized cancer care closer to reality. Tumours usually contain malignant cells showing different degrees of differentiation (Celis et al. 2003; Celis et al. 2007) as well as other cell types, which together compose the ‘tumour microenvironment’ (Celis et al. 2004; Tlsty and Coussens 2006; Witz 2009). The heterogeneity-related problems in characterization and treatment have been partially addressed using techniques that allow the dissection of a defined set of purified cell populations (Espina et al. 2007), but these

Fig. 2.4 Triple IHC stained of a breast carcinoma *in situ* reacted with antibodies against CK's 8, 15, and 19. Photograph kindly provided by Jose Moreira



technologies cannot solve the problem altogether, as heterogeneity can be observed even in a small number of cells within a given lesion, as illustrated in Fig. 2.4. In this particular case a breast carcinoma *in situ* has been stained with antibodies against cytokeratins 8, 15, and 19. As seen in Fig. 2.4, the heterogeneity of the epithelial cells in terms of phenotype is such that it would be very difficult to interpret expression data generated from it, unless one had access to speedy procedures for validation at the cellular level; for example, using a large battery of specific antibodies (Tlsty and Coussens 2006). The fact that only a few cells in this pre-cancerous lesion may harbour the malignant phenotype underlines the complexity of the problem and emphasizes the need to develop strategies to identify biomarkers that specifically predict the prognosis of each cell type expressing a given phenotype. In addition, we must increase our efforts to identify cancer stem cells (which generate cellular heterogeneity), as these will be the focus for developing new targeted therapies (Nirmalanandhan and Sittampalam 2009; Watt and Driskell 2010).

It is also urgent to address the problem of clinical relevance when selecting the source of samples used to derive new biomarkers and targets (Celis et al. 2005). The use of well-annotated and accessible clinically relevant samples to generate new data is important, as the final outcome will very much depend on the quality and relevance of the data. Accordingly, we are increasingly moving from the study of cultured cells to the analysis of freshly collected cells, tissue samples, and bio-fluids, but one of the main challenges one faces is how best to apply the powerful 'omics' technologies to the study of clinically relevant samples in a well-defined clinical and pathological framework (Celis et al. 2003). There is still a significant gap between mechanistic research based on cellular model systems, and their potential in clinical applications.

Finally, we must also acknowledge the value of long-term research and provide the appropriate legal and ethical framework to encourage collaboration among all the stakeholders in the cancer continuum. Bridging the gap between basic and clinical research, facilitating the engagement of industry, establishing new infrastructures, as well creating innovative clinical trials, are among the items that require urgent action (Fig. 2.3). The aim of cancer research is to improve the life expectancy and quality of life of patients and we must make every effort to coordinate current activities in order to achieve this goal.

Acknowledgements We are indebted to Laila Fischer for expert secretarial assistance. This work was supported by the IVO Cancer Institute and the EuroBoNeT consortium, a network of excellence granted by the European Commission for studying the pathology and genetics of bone tumours (to ALLB), the Stockholm Cancer Society (to UR), and the Danish Cancer Society, the Danish Medical Research Council, and the John and Birthe Meyer Foundation (to JEC).

References

- Abeloff MD, Armitage JO, Niederhuber JE, Kastan MB, Gillies McKenna W (2008) *Abeloff's clinical oncology*, 4th edn. Churchill Livingstone, UK
- Aebbersold R, Auffray C, Baney E, Barillot E, Brazma A, Brett C, Brunak S, Butte A, Califano A, Celis J, Cufer T, Ferrell J, Galas D, Gallahan D, Gatenby R, Goldbeter A, Hace N, Henney A, Hood L, Iyengar R, Jackson V, Kallioniemi O, Klingmuller U, Kolar P, Kolch W, Kyriakopoulou C, Laplace F, Lehrach H, Marcus F, Matrisian L, Nolan G, Pelkmans L, Potti A, Sander C, Seljak M, Singer D, Sorger P, Stunnenberg H, Superti-Furga G, Uhlen M, Vidal M, Weinstein J, Wigle D, Williams M, Wolkenhauer O, Zhivotovsky B, Zinovyev A, Zupan B (2009) Report on EU-USA workshop: how systems biology can advance cancer research (27 October 2008). *Mol Oncol* 3(1):9–17
- Aitken JF, Elwood M, Baade PD, Youl P, English D (2010) Clinical whole-body skin examination reduces the incidence of thick melanomas. *Int J Cancer* 126(2):450–458
- Albertsen P (2009) Androgen deprivation in prostate cancer—step by step. *N Engl J Med* 360(24):2572–2574
- Alymani NA, Smith MD, Williams DJ, Petty RD (2010) Predictive biomarkers for personalised anti-cancer drug use: discovery to clinical implementation. *Eur J Cancer* 46(5):869–879
- Aparicio SA, Huntsman DG (2010) Does massively parallel DNA resequencing signify the end of histopathology as we know it? *J Pathol* 220(2):307–315
- Arisio R, Cuccorese C, Accinelli G, Mano MP, Bordon R, Fessia L (1998) Role of fine-needle aspiration biopsy in breast lesions: analysis of a series of 4110 cases. *Diagn Cytopathol* 18(6):462–467
- Baccarani M, Cortes J, Pane F, Niederwieser D, Saglio G, Apperley J, Cervantes F, Deininger M, Gratwohl A, Guilhot F, Hochhaus A, Horowitz M, Hughes T, Kantarjian H, Larson R, Radich J, Simonsson B, Silver RT, Goldman J, Hehlmann R (2009) Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet. *J Clin Oncol* 27(35):6041–6051
- Badve S, Nakshatri H (2009) Oestrogen-receptor-positive breast cancer: towards bridging histopathological and molecular classifications. *J Clin Pathol* 62(1):6–12
- Balch C, Houghton A, Sober A, Soong S (2003) *Cutaneous melanoma*, 4th edn. Quality Medical, St Louis
- Baselga J (2006) Targeting tyrosine kinases in cancer: the second wave. *Science* 312(5777):1175–1178

- Baselga J, Arribas J (2004) Treating cancer's kinase 'addiction'. *Nat Med* 10(8):786–787
- Baselga J, Swain SM (2009) Novel anticancer targets: revisiting ERBB2 and discovering ERBB3. *Nat Rev Cancer* 9(7):463–475
- Baumann P, Nyman J, Hoyer M, Wennberg B, Gagliardi G, Lax I, Drugge N, Ekberg L, Friesland S, Johansson KA, Lund JA, Morhed E, Nilsson K, Levin N, Paludan M, Sederholm C, Traberg A, Wittgren L, Lewensohn R (2009) Outcome in a prospective phase II trial of medically inoperable stage I non-small-cell lung cancer patients treated with stereotactic body radiotherapy. *J Clin Oncol* 27(20):3290–3296
- Beckman M (2006) Tumor complexity prompts caution about sequencing. *J Natl Cancer Inst* 98(24):1758–1759
- Bergers G, Benjamin LE (2003) Tumorigenesis and the angiogenic switch. *Nat Rev Cancer* 3(6):401–410
- Berry DA, Cronin KA, Plevritis SK, Fryback DG, Clarke L, Zelen M, Mandelblatt JS, Yakovlev AY, Habbema JD, Feuer EJ (2005) Effect of screening and adjuvant therapy on mortality from breast cancer. *N Engl J Med* 353(17):1784–1792
- Bloom HJ, Richardson WW (1957) Histological grading and prognosis in breast cancer; a study of 1409 cases of which 359 have been followed for 15 years. *Br J Cancer* 11(3):359–377
- Bobola MS, Silber JR, Ellenbogen RG, Geyer JR, Blank A, Goff RD (2005) O6-methylguanine-DNA methyltransferase, O6-benzylguanine, and resistance to clinical alkylators in pediatric primary brain tumor cell lines. *Clin Cancer Res* 11(7):2747–2755
- Boffetta P, Hashibe M (2006) Alcohol and cancer. *Lancet Oncol* 7(2):149–156
- Bosman FT (1995) Prognostic value of pathological characteristics of colorectal cancer. *Eur J Cancer* 31A(7–8):1216–1221
- Boyle P, Autier P, Bartelink H, Baselga J, Boffetta P, Burn J, Burns HJ, Christensen L, Denis L, Dicato M, Diehl V, Doll R, Franceschi S, Gillis CR, Gray N, Gričute L, Hackshaw A, Kasler M, Kogevinas M, Kvinnslund S, La VC, Levi F, McVie JG, Maisonneuve P, Martin-Moreno JM, Bishop JN, Oleari F, Perrin P, Quinn M, Richards M, Ringborg U, Scully C, Siracka E, Storm H, Tubiana M, Tursz T, Veronesi U, Wald N, Weber W, Zaridze DG, Zatonski W, Zur HH (2003a) European code against cancer and scientific justification: third version. *Ann Oncol* 14(7):973–1005
- Boyle P, d'Onofrio A, Maisonneuve P, Severi G, Robertson C, Tubiana M, Veronesi U (2003b) Measuring progress against cancer in Europe: has the 15% decline targeted for 2000 come about? *Ann Oncol* 14(8):1312–1325
- Boyle P, Levin B (2008) World cancer report. <http://www.iarc.fr/en/publications/pdfs-online/wcr/2008/index.php>
- Brenton JD, Carey LA, Ahmed AA, Caldas C (2005) Molecular classification and molecular forecasting of breast cancer: ready for clinical application? *J Clin Oncol* 23(29):7350–7360
- Bridge RS, Rajaram V, Dehner LP, Pfeifer JD, Perry A (2006) Molecular diagnosis of Ewing sarcoma/primitive neuroectodermal tumor in routinely processed tissue: a comparison of two FISH strategies and RT-PCR in malignant round cell tumors. *Mod Pathol* 19(1):1–8
- Celis JE (2008) Editorial. *Mol Oncol* 2(1):1–1
- Celis JE, Gromov P (2003) Proteomics in translational cancer research: toward an integrated approach. *Cancer Cell* 3(1):9–15
- Celis JE, Gromov P, Cabezon T, Moreira JM, Ambartsumian N, Sandelin K, Rank F, Gromova I (2004) Proteomic characterization of the interstitial fluid perfusing the breast tumor microenvironment: a novel resource for biomarker and therapeutic target discovery. *Mol Cell Proteomics* 3(4):327–344
- Celis JE, Gromov P, Cabezon T, Moreira JM, Friis E, Jirstrom K, Llombart-Bosch A, Timmermans-Wielenga V, Rank F, Gromova I (2008) 15-prostaglandin dehydrogenase expression alone or in combination with ACSM1 defines a subgroup of the apocrine molecular subtype of breast carcinoma. *Mol Cell Proteomics* 7(10):1795–1809
- Celis JE, Gromov P, Gromova I, Moreira JM, Cabezon T, Ambartsumian N, Grigorian M, Lukaniadin E, thor Straten P, Guldberg P, Bartkova J, Bartek J, Lukas J, Lukas C, Lykkesfeldt A, Jaatela M, Roepstorff P, Bolund L, Orntoft T, Brunner N, Overgaard J, Sandelin K, Blichert-Toft

- M, Mouridsen H, Rank FE (2003) Integrating proteomic and functional genomic technologies in discovery-driven translational breast cancer research. *Mol Cell Proteomics* 2(6):369–377
- Celis JE, Gromova I, Cabezon T, Gromov P, Shen T, Timmermans-Wielenga V, Rank F, Moreira JM (2007) Identification of a subset of breast carcinomas characterized by expression of cytokeratin 15: relationship between CK15+ progenitor/amplified cells and pre-malignant lesions and invasive disease. *Mol Oncol* 1(3):321–349
- Celis JE, Moreira JM, Gromova I, Cabezon T, Ralfkiaer U, Guldberg P, Straten PT, Mouridsen H, Friis E, Holm D, Rank F, Gromov P (2005) Towards discovery-driven translational research in breast cancer. *FEBS J* 272(1):2–15
- Chabner BA, Longo DL (2006) Cancer chemotherapy and biotherapy. Principles and practice, 4th edn. Lippincott Williams & Wilkins, Baltimore
- Chan JK (2001) The new world health organization classification of lymphomas: the past, the present and the future. *Hematol Oncol* 19(4):129–150
- Cheang MC, Voduc D, Bajdik C, Leung S, McKinney S, Chia SK, Perou CM, Nielsen TO (2008) Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. *Clin Cancer Res* 14(5):1368–1376
- Chiang AC, Massague J (2008) Molecular basis of metastasis. *N Engl J Med* 359(26):2814–2823
- Clarke M, Coates AS, Darby SC, Davies C, Gelber RD, Godwin J, Goldhirsch A, Gray R, Peto R, Pritchard KI, Wood WC (2008) Adjuvant chemotherapy in oestrogen-receptor-poor breast cancer: patient-level meta-analysis of randomised trials. *Lancet* 371(9606):29–40
- Clarke M, Collins R, Darby S, Davies C, Elphinstone P, Evans E, Godwin J, Gray R, Hicks C, James S, MacKinnon E, McGale P, McHugh T, Peto R, Taylor C, Wang Y (2005) Effects of radiotherapy and of differences in the extent of surgery for early breast cancer on local recurrence and 15-year survival: an overview of the randomised trials. *Lancet* 366(9503):2087–2106
- Cogliano V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F (2005) Carcinogenicity of human papillomaviruses. *Lancet Oncol* 6(4):204
- Cohn-Cedermark G, Rutqvist LE, Andersson R, Breivald M, Ingvar C, Johansson H, Jonsson PE, Krysaner L, Lindholm C, Ringborg U (2000) Long term results of a randomized study by the Swedish Melanoma Study Group on 2-cm versus 5-cm resection margins for patients with cutaneous melanoma with a tumor thickness of 0.8–2.0 mm. *Cancer* 89(7):1495–1501
- Copland M, Hamilton A, Elrick LJ, Baird JW, Allan EK, Jordanides N, Barow M, Mountford JC, Holyoake TL (2006) Dasatinib (BMS-354825) targets an earlier progenitor population than imatinib in primary CML but does not eliminate the quiescent fraction. *Blood* 107(11):4532–4539
- Correa Geyer F, Reis-Filho JS (2009) Microarray-based gene expression profiling as a clinical tool for breast cancer management: are we there yet? *Int J Surg Pathol* 17(4):285–302
- Costa J (2009) Systems approach to the practice of pathology: a new role for the pathologist. *Arch Pathol Lab Med* 133(4):524–526
- Cotterill SJ, Ahrens S, Paulussen M, Jurgens HF, Voute PA, Gadner H, Craft AW (2000) Prognostic factors in Ewing's tumor of bone: analysis of 975 patients from the European Intergroup Cooperative Ewing's Sarcoma Study Group. *J Clin Oncol* 18(17):3108–3114
- Cuppone F, Bria E, Carlini P, Milella M, Felici A, Sperduti I, Nistico C, Terzoli E, Cognetti F, Giannarelli D (2008) Taxanes as primary chemotherapy for early breast cancer: meta-analysis of randomized trials. *Cancer* 113(2):238–246
- Dardick I, Herrera GA (1998) Diagnostic electron microscopy of neoplasms. *Hum Pathol* 29(12):1335–1338
- Alava E de, Kawai A, Healey JH, Fligman I, Meyers PA, Huvos AG, Gerald WL, Jhanwar SC, Argani P, Antonescu CR, Pardo-Mindan FJ, Ginsberg J, Womer R, Lawlor ER, Wunder J, Andrulis I, Sorensen PH, Barr FG, Ladanyi M (1998) EWS-FLI1 fusion transcript structure is an independent determinant of prognosis in Ewing's sarcoma. *J Clin Oncol* 16(4):1248–1255
- Delattre O (2008) Ewing's tumours, genetic and cellular aspects. *Pathol Biol (Paris)* 56(5):257–259
- Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, Lickley LA, Rawlinson E, Sun P, Narod SA (2007) Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res* 13(15):4429–4434

- Desmedt C, Ruiz-Garcia E, Andre F (2008) Gene expression predictors in breast cancer: current status, limitations and perspectives. *Eur J Cancer* 44(18):2714–2720
- DeVita VT Jr, Hellman S, Rosenberg SA (2008) Cancer: principles and practice of oncology. Lippincott Williams and Wilkins, Baltimore
- Doll R, Peto R, Boreham J, Sutherland I (2004) Mortality in relation to smoking: 50 years' observations on male British doctors. *BMJ* 328(7455):1519
- Dowsett M, Cuzick J, Ingle J, Coates A, Forbes J, Bliss J, Buyse M, Baum M, Buzdar A, Colleoni M, Coombes C, Snowdon C, Gnant M, Jakesz R, Kaufmann M, Boccardo F, Godwin J, Davies C, Peto R (2010) Meta-analysis of breast cancer outcomes in adjuvant trials of aromatase inhibitors versus tamoxifen. *J Clin Oncol* 28(3):509–518
- Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-Jones S, Sawyers CL (2001) Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* 344(14):1031–1037
- Duff SE, Jeziorska M, Rosa DD, Kumar S, Haboubi N, Sherlock D, O'Dwyer ST, Jayson GC (2006) Vascular endothelial growth factors and receptors in colorectal cancer: implications for anti-angiogenic therapy. *Eur J Cancer* 42(1):112–117
- Dworak O, Keilholz L, Hoffmann A (1997) Pathological features of rectal cancer after preoperative radiochemotherapy. *Int J Colorectal Dis* 12(1):19–23
- Eden P, Ritz C, Rose C, Ferno M, Peterson C (2004) “Good Old” clinical markers have similar power in breast cancer prognosis as microarray gene expression profilers. *Eur J Cancer* 40(12):1837–1841
- Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A (2010) AJCC cancer staging manual, 7th edn. Springer, Berlin
- Ehrlich Y, Brames MJ, Beck SD, Foster RS, Einhorn LH (2010) Long-term follow-up of Cisplatin combination chemotherapy in patients with disseminated nonseminomatous germ cell tumors: is a postchemotherapy retroperitoneal lymph node dissection needed after complete remission? *J Clin Oncol* 28(4):531–536
- Elston C, Ellis I (1998) Systemic pathology: the breast. Elsevier, Churchill Livingstone, London
- Espina V, Heiby M, Pierobon M, Liotta LA (2007) Laser capture microdissection technology. *Expert Rev Mol Diagn* 7(5):647–657
- Esteller M (2008) Epigenetics in cancer. *N Engl J Med* 358(11):1148–1159
- Faratian D, Bartlett J (2008) Predictive markers in breast cancer—the future. *Histopathology* 52(1):91–98
- Fass L (2008) Imaging and cancer: a review. *Mol Oncol* 2(2):115–152
- Fearon ER, Vogelstein B (1990) A genetic model for colorectal tumorigenesis. *Cell* 61(5):759–767
- Fellinger EJ, Garin-Chesa P, Su SL, DeAngelis P, Lane JM, Rettig WJ (1991) Biochemical and genetic characterization of the HBA71 Ewing's sarcoma cell surface antigen. *Cancer Res* 51(1):336–340
- Fenoglio-Preiser CM, Noffsinger AE, Stemmermann GN (1999) Gastrointestinal pathology: an atlas and text. Lippincott Williams & Wilkins, Baltimore
- Ferlay J, Parkin DM, Steliarova-Foucher E (2010a) Estimates of cancer incidence and mortality in Europe in 2008. *Eur J Cancer* 46(4):765–781
- Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM (2010b) Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 127(12):2893–2917
- Fletcher SW, Elmore JG (2003) Clinical practice. Mammographic screening for breast cancer. *N Engl J Med* 348(17):1672–1680
- Folpe AL, Goldblum JR, Rubin BP, Shehata BM, Liu W, Dei Tos AP, Weiss SW (2005) Morphologic and immunophenotypic diversity in Ewing family tumors: a study of 66 genetically confirmed cases. *Am J Surg Pathol* 29(8):1025–1033
- Francisci S, Capocaccia R, Grande E, Santaquilani M, Simonetti A, Allemani C, Gatta G, Sant M, Zigon G, Bray F, Janssen-Heijnen M (2009) The cure of cancer: a European perspective. *Eur J Cancer* 45(6):1067–1079

- Franklin W, Muller KM, Wistuba II, Sozzi G, Geisinger K, Brambilla E, Lam S, Gazdar A, Hirsch FR (2004) Squamous dysplasia and carcinoma in situ. Pathology and genetics of tumours of the lung, pleura, thymus and heart. WHO—IARC press, Lyon
- Gravendeel LA, Kouwenhoven MC, Gevaert O, Rooi JJ de, Stubbs AP, Duijm JE, Daemen A, Bleeker FE, Bralten LB, Kloosterhof NK, De MB, Eilers PH, Spek PJ van der, Kros JM, Sillevius Smitt PA, Bent MJ van den, French PJ (2009) Intrinsic gene expression profiles of gliomas are a better predictor of survival than histology. *Cancer Res* 69(23):9065–9072
- Hahn WC, Weinberg RA (2002) Modelling the molecular circuitry of cancer. *Nat Rev Cancer* 2(5):331–341
- Haïoun C, Itti E, Rahmouni A, Brice P, Rain JD, Belhadj K, Gaulard P, Garderet L, Lepage E, Reyes F, Meignan M (2005) [18F]fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) in aggressive lymphoma: an early prognostic tool for predicting patient outcome. *Blood* 106(4):1376–1381
- Hamilton SR, Aaltonen LA (2000) Pathology & genetics of tumours of the digestive system. WHO—IARCPress, Lyon
- Hanahan D, Weinberg RA (2000) The hallmarks of cancer. *Cell* 100(1):57–70
- Hansen RJ, Nagasubramanian R, Delaney SM, Samson LD, Dolan ME (2007) Role of O6-methylguanine-DNA methyltransferase in protecting from alkylating agent-induced toxicity and mutations in mice. *Carcinogenesis* 28(5):1111–1116
- Hasegawa T, Hirose T, Ayala AG, Ito S, Tomaru U, Matsuno Y, Shimoda T, Hirohashi S (2001) Adult neuroblastoma of the retroperitoneum and abdomen: clinicopathologic distinction from primitive neuroectodermal tumor. *Am J Surg Pathol* 25(7):918–924
- Hayes BD, Quinn CM (2009) Pathology of B3 lesions of the breast. *Diagnostic Histopathol* 15(10):459–469
- Heinrich MC, Corless CL (2004) Targeting mutant kinases in gastrointestinal stromal tumors: a paradigm for molecular therapy of other sarcomas. *Cancer Treat Res* 120:129–150
- Heinrich MC, Corless CL, Demetri GD, Blanke CD, Mehren von M, Joensuu H, McGreevey LS, Chen CJ, Van den Abbeele AD, Druker BJ, Kiese B, Eisenberg B, Roberts PJ, Singer S, Fletcher CD, Silberman S, Dimitrijevic S, Fletcher JA (2003) Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J Clin Oncol* 21(23):4342–4349
- Herbst RS, Heymach JV, Lippman SM (2008) Lung cancer. *N Engl J Med* 359(13):1367–1380
- Hoos A, Stojadinovic A, Mastorides S, Urist MJ, Polsky D, Di Como CJ, Brennan MF, Cordon-Cardo C (2001) High Ki-67 proliferative index predicts disease specific survival in patients with high-risk soft tissue sarcomas. *Cancer* 92(4):869–874
- Huang ME, Ye YC, Chen SR, Chai JR, Lu JX, Zhao L, Gu LJ, Wang ZY (1988) Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. *Blood* 72(2):567–572
- Hutter RV, Sobin LH (1986) A universal staging system for cancer of the colon and rectum. Let there be light. *Arch Pathol Lab Med* 110(5):367–368
- Ingoldsby H, Callagy G (2009) Pathology of minimal metastatic disease in sentinel lymph nodes in breast cancer. *Diagn Histopathol* 15(10):470–477
- Jakel O, Karger CP, Debus J (2008) The future of heavy ion radiotherapy. *Med Phys* 35(12):5653–5663
- Jass JR (2007) Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. *Histopathology* 50(1):113–130
- Jin L, Lloyd RV (1997) In situ hybridization: methods and applications. *J Clin Lab Anal* 11(1):2–9
- Kallioniemi OP, Wagner U, Kononen J, Sauter G (2001) Tissue microarray technology for high-throughput molecular profiling of cancer. *Hum Mol Genet* 10(7):657–662
- Kalluri R, Zeisberg M (2006) Fibroblasts in cancer. *Nat Rev Cancer* 6(5):392–401
- Kamangar F, Dores GM, Anderson WF (2006) Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. *J Clin Oncol* 24(14):2137–2150
- Kantarjian H, Sawyers C, Hochhaus A, Guilhot F, Schiffer C, Gambacorti-Passerini C, Niederwieser D, Resta D, Capdeville R, Zoellner U, Talpaz M, Druker B, Goldman J, O'Brien SG, Russell N, Fischer T, Ottmann O, Cony-Makhoul P, Facon T, Stone R, Miller C, Tallman M,

- Brown R, Schuster M, Loughran T, Gratwohl A, Mandelli F, Saglio G, Lazzarino M, Russo D, Baccarani M, Morra E (2002) Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N Engl J Med* 346(9):645–652
- Kauer M, Ban J, Kofler R, Walker B, Davis S, Meltzer P, Kovar H (2009) A molecular function map of Ewing's sarcoma. *PLoS One* 4(4):e5415
- Kerbel RS (2008) Tumor angiogenesis. *N Engl J Med* 358(19):2039–2049
- Khan J, Wei JS, Ringner M, Saal LH, Ladanyi M, Westermann F, Berthold F, Schwab M, Antonescu CR, Peterson C, Meltzer PS (2001) Classification and diagnostic prediction of cancers using gene expression profiling and artificial neural networks. *Nat Med* 7(6):673–679
- Kovar H, Jug G, Aryee DN, Zoubek A, Ambros P, Gruber B, Windhager R, Gadner H (1997) Among genes involved in the RB dependent cell cycle regulatory cascade, the p16 tumor suppressor gene is frequently lost in the Ewing family of tumors. *Oncogene* 15(18):2225–2232
- Kreeger PK, Lauffenburger DA (2010) Cancer systems biology: a network modeling perspective. *Carcinogenesis* 31(1):2–8
- Ladanyi M (1995) The emerging molecular genetics of sarcoma translocations. *Diagn Mol Pathol* 4(3):162–173
- Lango MN (2009) Multimodal treatment for head and neck cancer. *Surg Clin North Am* 89(1):43–52
- Laubenbacher R, Hower V, Jarrah A, Torti SV, Shulaev V, Mendes P, Torti FM, Akman S (2009) A systems biology view of cancer. *Biochim Biophys Acta* 1796(2):129–139
- Levitt SH, Perez CA, Hui S, Purdy JA (2008) Evolution of computerized radiotherapy in radiation oncology: potential problems and solutions. *Int J Radiat Oncol Biol Phys* 70(4):978–986
- Levitt SH, Purdy JA, Perez CA, Vijayakumar S (2006) Technical basis of radiation therapy. Practical clinical applications, 4th edn. Lippincott Williams & Wilkins, Baltimore
- Liu CL, Prapong W, Natkunam Y, Alizadeh A, Montgomery K, Gilks CB, Rijn M van de (2002) Software tools for high-throughput analysis and archiving of immunohistochemistry staining data obtained with tissue microarrays. *Am J Pathol* 161(5):1557–1565
- Llombart-Bosch A (2001) De la anatomia patologica estructural a la patologia molecular. Discurso de Recepcion. Real Academia de Medicina de la Comunidad Valenciana. RAMCV Press, Valencia, Spain
- Llombart-Bosch A, Contesso G, Peydro-Olaya A (1996) Histology, immunohistochemistry, and electron microscopy of small round cell tumors of bone. *Semin Diagn Pathol* 13(3):153–170
- Llombart-Bosch A, Machado I, Navarro S, Bertoni F, Bacchini P, Alberghini M, Karzeladze A, Savelov N, Petrov S, varado-Cabrero I, Mihaila D, Terrier P, Lopez-Guerrero JA, Picci P (2009) Histological heterogeneity of Ewing's sarcoma/PNET: an immunohistochemical analysis of 415 genetically confirmed cases with clinical support. *Virchows Arch* 455(5):397–411
- Llombart-Bosch A, Navarro S (2001) Immunohistochemical detection of EWS and FLI-1 proteins in Ewing sarcoma and primitive neuroectodermal tumors: comparative analysis with CD99 (MIC-2) expression. *Appl Immunohistochem Mol Morphol* 9(3):255–260
- Lopez-Guerrero JA, Machado I, Scotlandi K, Noguera R, Pellin A, Navarro S, Serra M, Calabuig-Farinas S, Picci P, Llombart-Bosch A (2011) Clinicopathological significance of cell cycle regulation markers in a large series of genetically confirmed Ewing's sarcoma family of tumors. *Int J Cancer* 128(5):1139–1150
- Lopez-Guerrero JA, Llombart-Cussac A, Noguera R, Navarro S, Pellin A, Almenar S, Vazquez-Alvadalejo C, Llombart-Bosch A (2006) HER2 amplification in recurrent breast cancer following breast-conserving therapy correlates with distant metastasis and poor survival. *Int J Cancer* 118(7):1743–1749
- Lopez-Guerrero JA, Pellin A, Noguera R, Carda C, Llombart-Bosch A (2001) Molecular analysis of the 9p21 locus and p53 genes in Ewing family tumors. *Lab Invest* 81(6):803–814
- Lynch HT, la Chapelle A de (2003) Hereditary colorectal cancer. *N Engl J Med* 348(10):919–932
- Machado I, Noguera R, Pellin A, Lopez-Guerrero JA, Piqueras M, Navarro S, Llombart-Bosch A (2009) Molecular diagnosis of Ewing sarcoma family of tumors: a comparative analysis of 560 cases with FISH and RT-PCR. *Diagn Mol Pathol* 18(4):189–199

- Madarnas Y, Trudeau M, Franek JA, McCready D, Pritchard KI, Messersmith H (2008) Adjuvant/neoadjuvant trastuzumab therapy in women with HER-2/neu-overexpressing breast cancer: a systematic review. *Cancer Treat Rev* 34(6):539–557
- Marie JP, Zittoun R, Sikic BI (1991) Multidrug resistance (*mdr1*) gene expression in adult acute leukemias: correlations with treatment outcome and in vitro drug sensitivity. *Blood* 78(3):586–592
- Markowitz SD, Bertagnolli MM (2009) Molecular origins of cancer: molecular basis of colorectal cancer. *N Engl J Med* 361(25):2449–2460
- McCafferty MPJ, Healy NA, Kerin MJ (2009) Breast cancer subtypes and molecular biomarkers. *Diagn Histopathol* 15(10):485–489
- Meara RS, Cangiarella J, Simsir A, Horton D, Eltoum I, Chhieng DC (2007) Prediction of aggressiveness of gastrointestinal stromal tumours based on immunostaining with bcl-2, Ki-67 and p53. *Cytopathology* 18(5):283–289
- Mehlen P, Puisieux A (2006) Metastasis: a question of life or death. *Nat Rev Cancer* 6(6):449–458
- Meyer T, Hart IR (1998) Mechanisms of tumour metastasis. *Eur J Cancer* 34(2):214–221
- Nagtegaal ID, Krieken JH van (2002) The role of pathologists in the quality control of diagnosis and treatment of rectal cancer—an overview. *Eur J Cancer* 38(7):964–972
- Naora H, Montell DJ (2005) Ovarian cancer metastasis: integrating insights from disparate model organisms. *Nat Rev Cancer* 5(5):355–366
- Natkunam Y, Mason DY (2006) Prognostic immunohistologic markers in human tumors: why are so few used in clinical practice? *Lab Invest* 86(8):742–747
- Navarro S, Giraudo P, Karseladze AI, Smirnov A, Petrovichev N, Savelov N, Varado-Cabrero I, Llombart-Bosch A (2007) Immunophenotypic profile of biomarkers related to anti-apoptotic and neural development pathways in the Ewing's family of tumors (EFT) and their therapeutic implications. *Anticancer Res* 27(4B):2457–2463
- Nguyen DX, Bos PD, Massague J (2009) Metastasis: from dissemination to organ-specific colonization. *Nat Rev Cancer* 9(4):274–284
- Nilsson G, Wang M, Wejde J, Kreicbergs A, Larsson O (1999) Detection of EWS/FLI-1 by immunostaining. An adjunctive tool in diagnosis of Ewing's sarcoma and primitive neuroectodermal tumour on cytological samples and paraffin-embedded archival material. *Sarcoma* 3(1):25–32
- Nirmalanandhan VS, Sittampalam GS (2009) Stem cells in drug discovery, tissue engineering, and regenerative medicine: emerging opportunities and challenges. *J Biomol Screen* 14(7):755–768
- O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, Cornelissen JJ, Fischer T, Hochhaus A, Hughes T, Lechner K, Nielsen JL, Rousselot P, Reiffers J, Saglio G, Shepherd J, Simonsson B, Gratwohl A, Goldman JM, Kantarjian H, Taylor K, Verhoef G, Bolton AE, Capdeville R, Druker BJ (2003) Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med* 348(11):994–1004
- O'Shaughnessy JA (2006) Molecular signatures predict outcomes of breast cancer. *N Engl J Med* 355(6):615–617
- Ordenez NG, Mackay B (1998) Electron microscopy in tumor diagnosis: indications for its use in the immunohistochemical era. *Hum Pathol* 29(12):1403–1411
- Parham DM, Hijazi Y, Steinberg SM, Meyer WH, Horowitz M, Tzen CY, Wexler LH, Tsokos M (1999) Neuroectodermal differentiation in Ewing's sarcoma family of tumors does not predict tumor behavior. *Hum Pathol* 30(8):911–918
- Parkin DM (2004) International variation. *Oncogene* 23(38):6329–6340
- Pass HI, Mitchell JB, Johnson DH, Turrise AT, Minna JD (2000) Lung cancer: principles and practice, 2nd edn. Lippincott Williams & Wilkins, Baltimore
- Patz EF Jr, Campa MJ, Gottlin EB, Kusmartseva I, Guan XR, Herndon JE (2007) Panel of serum biomarkers for the diagnosis of lung cancer. *J Clin Oncol* 25(35):5578–5583
- Payne SJ, Bowen RL, Jones JL, Wells CA (2008) Predictive markers in breast cancer—the present. *Histopathology* 52(1):82–90
- Perou CM, Sorlie T, Eisen MB, Rijn M van de, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lonning PE, Borresen-Dale AL, Brown PO, Botstein D (2000) Molecular portraits of human breast tumours. *Nature* 406(6797):747–752

- Pinero-Madrona A, Polo-Garcia L, Onso-Romero JL, Salinas-Ramos J, Canteras-Jordana M, Sola-Perez J, Galindo-Fernandez PJ, Illana-Moreno J, Bermejo-Lopez J, Navarrete-Montoya A, Parrilla-Paricio P (2008) Immunohistochemical characterisation of breast cancer: towards a new clasification? *Cir Esp* 84(3):138–145
- Pinkerton R, Matthay K, Shankar AG (2007) Evidence-based pediatric oncology, 2nd edn. Blackwell, Oxford
- Pleasantance ED, Stephens PJ, O'Meara S, McBride DJ, Meynert A, Jones D, Lin ML, Beare D, Lau KW, Greenman C, Varela I, Nik-Zainal S, Davies HR, Ordóñez GR, Mudie LJ, Latimer C, Edkins S, Stebbings L, Chen L, Jia M, Leroy C, Marshall J, Menzies A, Butler A, Teague JW, Mangion J, Sun YA, McLaughlin SF, Peckham HE, Tsung EF, Costa GL, Lee CC, Minna JD, Gazdar A, Birney E, Rhodes MD, McKernan KJ, Stratton MR, Futreal PA, Campbell PJ (2010) A small-cell lung cancer genome with complex signatures of tobacco exposure. *Nature* 463(7278):184–190
- Ponten J (2001) Cell biology of precancer. *Eur J Cancer* 37(Suppl 8):97–113
- Rakha EA, Putti TC, bd El-Rehim DM, Paish C, Green AR, Powe DG, Lee AH, Robertson JF, Ellis IO (2006) Morphological and immunophenotypic analysis of breast carcinomas with basal and myoepithelial differentiation. *J Pathol* 208(4):495–506
- Ransohoff DF (2009) Promises and limitations of biomarkers. *Recent Results Cancer Res* 181:55–59
- Reis-Filho JS, Westbury C, Pierga JY (2006) The impact of expression profiling on prognostic and predictive testing in breast cancer. *J Clin Pathol* 59(3):225–231
- Richards MA (2009) The size of the prize for earlier diagnosis of cancer in England. *Br J Cancer* 101(Suppl 2):S125–S129
- Ringborg U, Bergqvist D, Brorsson B, Cavallin-Stahl E, Ceberg J, Einhorn N, Frodin JE, Jarhult J, Lamnevik G, Lindholm C, Littbrand B, Norlund A, Nylen U, Rosen M, Svensson H, Moller TR (2003) The Swedish Council on Technology Assessment in Health Care (SBU) systematic overview of radiotherapy for cancer including a prospective survey of radiotherapy practice in Sweden 2001—summary and conclusions. *Acta Oncol* 42(5–6):357–365
- Rosai J (2001) The continuing role of morphology in the molecular age. *Mod Pathol* 14(3):258–260
- Rosai J (2007) Why microscopy will remain a cornerstone of surgical pathology. *Lab Invest* 87(5):403–408
- Rosai J, Ackerman LV (1996) Ackerman's surgical pathology. CRC Press, Boca Raton
- Saad RS, Kordunsky L, Liu YL, Denning KL, Kandil HA, Silverman JF (2006) Lymphatic microvessel density as prognostic marker in colorectal cancer. *Mod Pathol* 19(10):1317–1323
- Sanchez-Garcia I (2009) The crossroads of oncogenesis and metastasis. *N Engl J Med* 360(3):297–299
- Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, Lilienbaum R, Johnson DH (2006) Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med* 355(24):2542–2550
- Sara H, Kallioniemi O, Nees M (2010) A decade of cancer gene profiling: from molecular portraits to molecular function. *Methods Mol Biol* 576:61–87
- Sarkaria JN, Bristow RG (2008) Overview of cancer molecular radiobiology. *Cancer Treat Res* 139:117–133
- Schoenberg Fejzo M, Slamon DJ (2001) Frozen tumor tissue microarray technology for analysis of tumor RNA, DNA, and proteins. *Am J Pathol* 159(5):1645–1650
- Sharma R, Hamilton A, Beith J (2008) LHRH agonists for adjuvant therapy of early breast cancer in premenopausal women. *Cochrane Database Syst Rev* (4):CD004562
- Shepherd FA, Rodrigues PJ, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, Campos D, Maoleekoonpiroj S, Smylie M, Martins R, Kooten M van, Dediu M, Findlay B, Tu D, Johnston D, Bezjak A, Clark G, Santabarbara P, Seymour L (2005) Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* 353(2):123–132
- Shergill IS, Shergill NK, Arya M, Patel HR (2004) Tissue microarrays: a current medical research tool. *Curr Med Res Opin* 20(5):707–712

- Shia J, Ellis NA, Paty PB, Nash GM, Qin J, Offit K, Zhang XM, Markowitz AJ, Nafa K, Guillem JG, Wong WD, Gerald WL, Klimstra DS (2003) Value of histopathology in predicting microsatellite instability in hereditary nonpolyposis colorectal cancer and sporadic colorectal cancer. *Am J Surg Pathol* 27(11):1407–1417
- Simunovic M, Smith AJ, Heald RJ (2009) Rectal cancer surgery and regional lymph nodes. *J Surg Oncol* 99(4):256–259
- Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 235(4785):177–182
- Smith I, Procter M, Gelber RD, Guillaume S, Feyereislova A, Dowsett M, Goldhirsch A, Untch M, Mariani G, Baselga J, Kaufmann M, Cameron D, Bell R, Bergh J, Coleman R, Wardley A, Harbeck N, Lopez RI, Mallmann P, Gelmon K, Wilcken N, Wist E, Sanchez RP, Piccart-Gebhart MJ (2007) 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial. *Lancet* 369(9555):29–36
- Sobin LH, Gospodarowicz MK, Wittekind C (2009) TNM classification of malignant tumours, 7th edn. Wiley-Blackwell, Oxford
- Sorlie T, Perou CM, Fan C, Geisler S, Aas T, Nobel A, Anker G, Akslen LA, Botstein D, Borresen-Dale AL, Lonning PE (2006) Gene expression profiles do not consistently predict the clinical treatment response in locally advanced breast cancer. *Mol Cancer Ther* 5(11):2914–2918
- Spira A, Ettinger DS (2004) Multidisciplinary management of lung cancer. *N Engl J Med* 350(4):379–392
- Tang P, Skinner KA, Hicks DG (2009) Molecular classification of breast carcinomas by immunohistochemical analysis: are we ready? *Diagn Mol Pathol* 18(3):125–132
- Tavassoli FA, Devilee P (2003) Pathology & genetics: tumours of the breast and female genital organs. IARC press—WHO, Lyon
- Taylor CR, Cote RJ (1997) Immunohistochemical markers of prognostic value in surgical pathology. *Histol Histopathol* 12(4):1039–1055
- Terrier P, Henry-Amar M, Triche TJ, Horowitz ME, Terrier-Lacombe MJ, Miser JS, Kinsella TJ, Contesso G, Llombart-Bosch A (1995) Is neuro-ectodermal differentiation of Ewing's sarcoma of bone associated with an unfavourable prognosis? *Eur J Cancer* 31A(3):307–314
- Thun MJ, Henley SJ, Burns D, Jemal A, Shanks TG, Calle EE (2006) Lung cancer death rates in lifelong nonsmokers. *J Natl Cancer Inst* 98(10):691–699
- Tlsty TD, Coussens LM (2006) Tumor stroma and regulation of cancer development. *Annu Rev Pathol* 1:119–150
- Travis WD, Brambilla E, Muller-Hermelink HK, Harris CC (2004) Pathology & genetics of tumours of the lung, pleura, thymus and heart. IARC press—WHO, Lyon
- Trigg ME, Sather HN, Reaman GH, Tubergen DG, Steinherz PG, Gaynon PS, Uckun FM, Hammond GD (2008) Ten-year survival of children with acute lymphoblastic leukemia: a report from the Children's Oncology Group. *Leuk Lymphoma* 49(6):1142–1154
- Ueda T, Aozasa K, Tsujimoto M, Ohsawa M, Uchida A, Aoki Y, Ono K, Matsumoto K (1989) Prognostic significance of Ki-67 reactivity in soft tissue sarcomas. *Cancer* 63(8):1607–1611
- Valk PJ, Verhaak RG, Beijen MA, Erpelinck CA, Barjesteh van Waalwijk van Doorn-Khosrovani, Boer JM, Beverloo HB, Moorhouse MJ, Spek PJ van der, Lowenberg B, Delwel R (2004) Prognostically useful gene-expression profiles in acute myeloid leukemia. *N Engl J Med* 350(16):1617–1628
- Oosterom AT van, Judson I, Verweij J, Stroobants S, di Donato PE, Dimitrijevic S, Martens M, Webb A, Sciort R, Van GM, Silberman S, Nielsen OS (2001) Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. *Lancet* 358(9291):1421–1423
- Verellen D, Ridder MD, Linthout N, Tournel K, Soete G, Storme G (2007) Innovations in image-guided radiotherapy. *Nat Rev Cancer* 7(12):949–960
- Watt FM, Driskell RR (2010) The therapeutic potential of stem cells. *Philos Trans R Soc Lond B Biol Sci* 365(1537):155–163
- Weber D, Rankin K, Gavino M, Delasalle K, Alexanian R (2003) Thalidomide alone or with dexamethasone for previously untreated multiple myeloma. *J Clin Oncol* 21(1):16–19

- Weidner N, Tjoe J (1994) Immunohistochemical profile of monoclonal antibody O13: antibody that recognizes glycoprotein p30/32MIC2 and is useful in diagnosing Ewing's sarcoma and peripheral neuroepithelioma. *Am J Surg Pathol* 18(5):486–494
- Weigelt B, Peterse JL, van't Veer LJ (2005) Breast cancer metastasis: markers and models. *Nat Rev Cancer* 5(8):591–602
- Weinberg RA (2007) *Biology of cancer*. Garland Science, London
- Whitehead R (1994) *Gastrointestinal and oesophageal pathology*. Churchill Livingstone, London
- Witz IP (2009) The tumor microenvironment: the making of a paradigm. *Cancer Microenviron* 2(Suppl 1):9–17
- Yamanaka R, Saya H (2009) Molecularly targeted therapies for glioma. *Ann Neurol* 66(6):717–729
- Zambelli D, Zuntini M, Nardi F, Manara MC, Serra M, Landuzzi L, Lollini PL, Ferrari S, Alberghini M, Lombart-Bosch A, Piccolo E, Iacobelli S, Picci P, Scotlandi K (2010) Biological indicators of prognosis in Ewing's sarcoma: an emerging role for lectin galactoside-binding soluble 3 binding protein (LGALS3BP). *Int J Cancer* 126(1):41–52
- Zhan F, Tian E, Bumm K, Smith R, Barlogie B, Shaughnessy J Jr (2003) Gene expression profiling of human plasma cell differentiation and classification of multiple myeloma based on similarities to distinct stages of late-stage B-cell development. *Blood* 101(3):1128–1140
- Zhang X, Li L, Wei D, Yap Y, Chen F (2007) Moving cancer diagnostics from bench to bedside. *Trends Biotechnol* 25(4):166–173
- Zoubek A, Dockhorn-Dworniczak B, Delattre O, Christiansen H, Niggli F, Gatterer-Menz I, Smith TL, Jurgens H, Gadner H, Kovar H (1996) Does expression of different EWS chimeric transcripts define clinically distinct risk groups of Ewing tumor patients? *J Clin Oncol* 14(4):1245–1251

Cancer Systems Biology, Bioinformatics and Medicine
Research and Clinical Applications

Cesario, A.; Marcus, F. (Eds.)

2011, XXVIII, 484 p., Hardcover

ISBN: 978-94-007-1566-0