Chapter 2
Resistance to Tyrosine Kinase Inhibitors in Different Types of Solid Cancer

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Abstract Development of therapeutic resistance limits the efficacy of current cancer treatment. Understanding the molecular basis for therapeutic resistance should facilitate the identification of actionable targets and development of new combination therapies for cancer patients. Although extensive studies have been applied to elucidate the underlying mechanisms, evidence is far from enough to establish a well-defined picture to correct resistance. The unveiling point mutations within the kinase domain, gene amplification or overexpression, or modification of signaling pathway have been implicated in drug resistance. In the review, we will describe different currently developed strategies that have the potential to overcome drug resistance in different types of cancer therapies and facilitate prolonged anticancer effects of the first-line therapies. The knowledge obtained from these studies will allow to design better strategies to offer significant challenges on the path towards superior cancer treatment.

Keywords Epidermal growth factor receptor • Vascular endothelial growth factor • Solid cancers • Regorafinib • Gefitinib

Abbreviations

AIO	Arbeitsgemeinschaft Internistische Onkologie
AIP5	Apoptosis inhibitory protein 5
ATP	Adenosine triphosphate
Bcl-2	B-cell lymphoma 2

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BCR-ABL	Breakpoint cluster region-Abelson
BIRC2	Baculoviral IAP repeat-containing 2
B-Raf	v-Raf murine sarcoma viral oncogene homolog B
CBL	Casitas B-lineage lymphoma
Cdk	Cyclin-dependent kinase
c-FLIP	Caspase 8 and FAS-associated protein with death domain-like apoptosis regulator
CI	Confidence interval
CR	Complete response
CREB	cAMP-responsive element binding protein
CRTC2	CREB-regulated transcription coactivator 2
CXCR4	C-S-C chemokine receptor type 4
CYP3A4	Cytochrome P450 3A4
DDR1	Discoidin domain receptor 1
DFS	Disease free survival
EGFR	Epidermal growth factor receptor
ErbB2	Epidermal growth factor receptor II (Her 2)
EREG	Epiregulin
EZH2	Enhancer of zeste homolog 2
FGF2	Fibroblast growth factor
FLT1	FMI-like tyrosine kinase 1
FLT3	FMS-like tyrosine kinase 3
FOLFIRI	Folinic acid, Fluorouracil and Irinotecan
FOLFOX	Folinic acid (FA)-Fluorouracil (5FU)-Oxaliplatin (OX)
FOXO3a	Forkhead box O3 isoform a
GDNFR	Glial-cell-line-derived neurotrophic factor receptor
GSK	Glycogen synthase kinase
H2AFX	H2A histone family-member X
HR	Hazard ratio
HRG	Heregulin HGFR hepatocyte growth factor receptor
IGFR 1	Insulin-like growth factor receptor 1
IgG	Immunoglobulin G
IRS-1	Insulin receptor substrate 1
JAK	Janus kinase
KDR	Kinase insert domain-containing receptor tyrosine kinase
LA-HNSCC	Locally advanced head and neck squamous cell carcinoma
LOH	Loss of heterozygosity
m-AMSA	4′-(9-Acridinylamino)methanesulfon-m-aniside
MET	Mesenchymal epithelial transition
MMR	DNA mismatch repair
MR	Minor response
MSI	Microsatellite instability
MTD	Maximum tolerated dose
p27kip1 (p27)	A cyclin-dependent kinase inhibitor
p70S6K	(p70) S6 kinase

PDGFR	Platelet-derived growth factor receptor
PIK3CA	Phosphatidylinositol 3-kinase catalytic subunit
PLGF	Placental growth factor
PR	Partial response
RR	Relative risk
SCFR	Stem cell factor receptor
SD	Stable disease
SDF-1	Stromal-derived-factor-1
siRNA	Small interfering RNA
Skp2	S-phase kinase-associated protein 2
TAM	Tyro3/Axl/Mer
TGF-α	Transforming growth factor α
TOPO-II	Topoisomerase-II
TRIAP1	TP53 regulated inhibitor of apoptosis 1
VEGFR	Vascular endothelial growth factor receptors

2.1 Introduction

In the recent past, deregulated activity of kinases is shown to have significant role in several cancers making them an attractive targets for therapy. The development of ATP-competitive inhibitors to target oncogenic tyrosine kinases yielded significant success in treating certain cancer types [1]. The results achieved in treating such cancers arising due to mutated kinases with these inhibitors is significant [2]. The target specificity of these drugs is affected by the sequence/structural homology shared by most kinases [1]. Thus, several small molecule inhibitors have more than one target kinase [1]. For example, the ABL inhibitor imatinib that targets oncogenic BCR-ABL, also targets c-KIT and PDGFR kinase [3]. This resulted in testing of imatinib in c-KIT and PDGFR mutated cancers also with significant success [4, 5]. On the other hand, EGFR inhibitors like gefitinib and lapatinib are highly selective with few or no known additional targets. Most of the small molecule targeted drugs are ATP competitive reversible inhibitors although selective irreversible inhibitors were also reported [6]. In addition there are monoclonal antibodies, that they enter tumor cells and directly interfere with Tyrosine Kinase (TK) enzymes that are aberrantly activated in tumor cells and are critical to the growth of the tumor. Different targeted therapies have achieved various degrees of success in cancer treatment but in many cases, cancer patients develop primary or secondary drug resistance over time. Primary (intrinsic) resistance means lack of response to therapy, whereas secondary resistance emerges in cancer patients which become refractory to treatment, after an initial response. The possible mechanisms have been investigated in several studies which displayed that the coexistent genetic alterations of cancer-related genes could explain primary resistance or the activation of alternative signalling pathway could explain the development of secondary

resistance. This knowledge can be leveraged to facilitate the development of new therapeutic modules for future cancer patients.

2.2 Tyrosine Kinases and Their Signaling Pathways in Cancer

Tyrosine phosphorylation is an important type of protein modification for signal transduction when a cell receives extracellular stimuli such as hormones, cytokines and growth factors [7]. Tyrosine phosphorylation regulates numerous cellular processes such as cell proliferation, embryonic development, transcriptional activation, metabolism, cell migration, immune system function as well as neural transmission [7, 8]. The proteins that perform tyrosine phosphorylation are the tyrosine kinases. These kinases are enzymes that catalyze the transfer of the γ phosphate of ATP to tyrosine residues in a protein substrate. Phosphorylation of tyrosine residues serves two functions in a cell. First, it enables a protein to regulate its enzymatic activity [9]. Second, tyrosine phosphorylation generates binding sites for proteins containing Src homology-2 (SH2) and protein tyrosine-binding (PTB) domains [10]. These kinases consist of a glycosylated extracellular domain that is responsible for binding to ligands, a transmembrane helix and a cytoplasmic domain that harbor tyrosine kinase activity as well as additional regulatory residues that are subjected to phosphorylation (Fig. 2.1). Because tyrosine kinases play important roles in signal transduction that mediate numerous cellular processes, their activity is usually tightly regulated [8]. Activation of receptor tyrosine kinases (RTKs) is initiated when a ligand binds to its receptor. This process facilitates dimerization of the monomeric receptors. Because the two receptors are in close proximity, tyrosine residues on one receptor are now able to cross-phosphorylate each other. Receptor

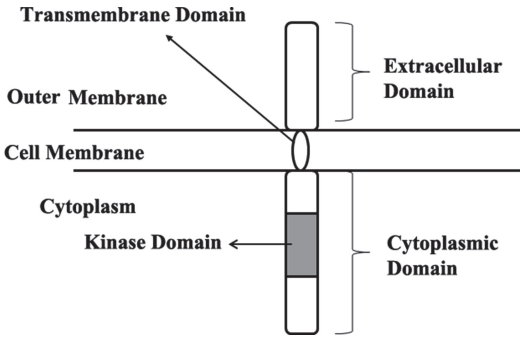

Fig. 2.1 Domain organization of receptor tyrosine kinases. Receptor tyrosine kinases consist of an extracellular domain that contains a ligand-binding site, a transmembrane helix and a cytoplasmic domain. The cytoplasmic domain contains a kinase active site that catalyzes tyrosine phosphorylation

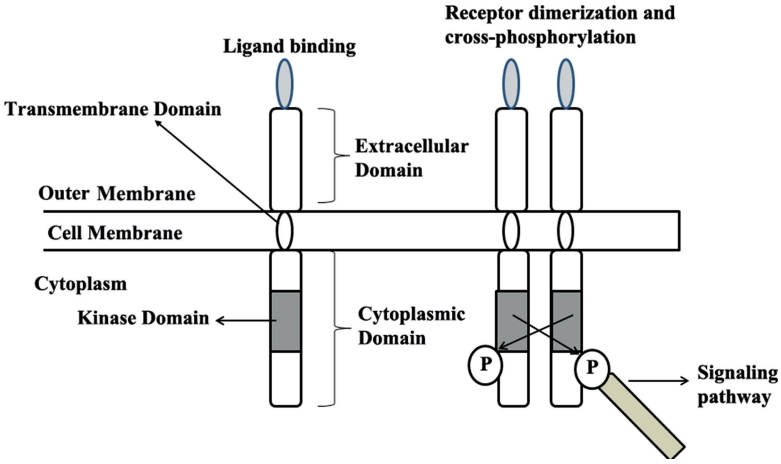

Fig. 2.2 Mechanism of receptor tyrosine kinase activation

cross-phosphorylation enhances the intrinsic kinase activity of the receptor tyrosine kinase as well as generating binding sites for the recruitment of downstream signaling proteins (Fig. 2.2) [9, 10]. Because of their substrate recognition sites, kinases are divided into two major classes-tyrosine kinases (TKs) and serine/threonine kinases (STKs) [11–13]. In humans, over 100 genes encode protein TKs, many of which are soluble, intracellular proteins, although others act as cell surface receptors such as EGFR and PDGFR. Fifty eight proteins encode transmembrane receptor protein tyrosine kinases (RPTKs) distributed into 20 subfamilies, and 32 encode cytoplasmic, non-receptor protein tyrosine kinases in 10 subfamilies [14, 15]. Activation of RTKs catalyzes phosphorylation of a range of cellular pathways controlling cell proliferation, differentiation and survival. Moreover, when RTKs bind their activating ligand they also catalyze autophosphorylation of their receptor domains, resulting in sustained receptor activation. Such constitutive activity is particularly important in the regulation of cellular homeostasis, e.g. cell proliferation, with the corollary being that its dysregulation is implicated in many cancer etiologies. Abnormalities in kinase activity, due to either changes in expression level or mutations in the protein sequence have been responsible in many disease pathologies and in the human genome, over 250 protein kinase genes map to disease loci è [16]. Many cancers are caused by kinase mutations, and so far, >100 dominant oncogenes are known of which protein kinases, in particular protein tyrosine kinases, comprise the largest group. Examples of dominant protein tyrosine kinase oncogenes are listed in Table 2.1. Since kinases regulate cell growth, differentiation and proliferation, abnormal functioning leads to uncontrolled growth, neoplasias or metastasis, and ultimately cancer. Many of the processes involved in tumor growth, progression and metastasis are mediated by signaling molecules acting downstream of activated RTKs. In particular, several members of the split kinase domain superfamily of RTKs are expressed on solid tumor cells and participate in autocrine loops

Table 2.1 Examples of dominant protein tyrosine kinase oncogenes [8]

PTK proto-oncogene	Viral oncogene (viral oncoproteins)	Oncogenic alteration	Tumor/cancer type
EGFR/ErbB1 C-ErbB	v-erB from AEV	v-ErB Truncated EGFR PTK c-erbB Overexpression (amplification) Extracellular domain deletion	v-ErbB fibrosarcoma c-ErbB mammary carcinoma, glioblastoma multiforme, ovarian, non-small-cell lung and other cancers
ErbB2/HER2/Neu		Overexpression (amplification)	Mammary ovarian, non-small-cell lung and other cancers
		No recurrent human mutations (V664G in rodents)	
ErbB3/HER3		Overexpression, constitutive tyrosine phosphorylation (heterodimer with ErbB2)	Mammary carcinoma
ErbB3/HER3		Overexpression	Mammary carcinoma, granulosa cell tumours
PDGFRα		Overexpression (amplification)	Glioma, glioblastoma, mammary carcinoma
PDGFRβ		Tel-PDGFRβ [t(5;12) translocation fusing Ets-like Tel with PDGFRβ PTK domain]	Tel-PDGFRβ: chronic myelomonocytic leukemia
			PDGFRβ glioma
FGFR1		ZNF198-FGFR1 [t(1;1) translocation fusing a novel Zn finger protein with FGFR1 PTK domain]	ZNF198-FGFR1: acute myelogenous leukemia (8p11 myeloproliferative syndrome), lymphoma
		Overexpression	Overexpression: various tumours
		Point mutations	Point mutations: autosomal skeletal/disorder and dysplasia
FGFR2/K-SAM		Overexpression, amplification and C-terminal truncation	Gastric carcinoma (mammary and prostate carcinomas)
VEGFR		IgH locus/MMSET translocation [t(4;14) translocation placing FGFR3 PTK downstream of IgH locus/MMSET]. Additional activation FGFR3 point mutations in skeletal dysplasia	Multiple myelomas (achondroplasia, thanatophoric dysplasia and hypocondronplasia)

(continued)

Table 2.1 (continued)

PTK proto-oncogene	Viral oncogene (viral oncoproteins)	Oncogenic alteration	Tumor/cancer type
FGFR4		Overexpression (amplification)	Mammalian, ovarian carcinoma
Scr c-Scr	v-Scr from RSV	v-Scr C-terminal truncation and point mutations (increased kinase activity) c-Scr C-terminal truncation increased kinase activity) Overexpression or/and increased kinase activity)	pp-60$^{v\text{-}scr}$ avian sarcoma c-Scr truncation: colon cancer c-Scr overexpression mammary and pancreatic cancers, neuroblastoma and others

implicated in cancer growth and survival (Fig. 2.3) (e.g. VEGF receptors in melanoma, PDGF receptors in gliomas, KIT in small cell lung cancer and EGFR in colonrectal cancer). The role of tyrosine kinases in cancer etiology was initially suggested by the observation that viral oncogenes express constitutively active protein kinases. In 1978, Ray Erikson found that the transforming factor of the Rous sarcoma virus (v-Src) was a protein kinase [17]. Already 2 years earlier, the Nobel laureates Michael Bishop and Harold Varmus described the first link of protein tyrosine phosphorylation with cancer. They found that the Rous sarcoma virus oncogene product is of cellular origin speculated that deregulation of this oncogene could lead to cancer [18]. This was confirmed in 1980 by the finding that v-Src is a protein tyrosine kinase [19, 20]. The breakthrough discovery of Bishop and Varmus that cancer-inducing genes of animal retroviruses such as v-Src and v-Ras represent mutated host genes that were recombined into the viral genome raised the question of whether the oncogenes concept was also relevant to human cancer [21]. The first cloning and sequence analysis of a cDNA encoding a cell surface protein, the human EGFR by Axel Ullrich in 1984, provided a partial answer to this question by revealing a close relationship with the v-erbB oncogenes [22, 23]. This first connection between a human gene product that regulates normal cell proliferation and a viral oncogene strongly suggested that human cancer development may also involve abnormalities in the expression and structure of endogenous genes that have regulatory roles in cell proliferation. A search for such genetic aberrations in tumor tissues using cDNA probes of EGFR and an EGFR-related gene, termed HER2 (human EGFR-related gene), resulted in the discovery that the gene encoding the HER2/neu receptor-like tyrosine kinase is amplified up to 100-fold in tumors of about 30 % of patients with invasive breast cancer. A significant clinical correlation was shown between HER2/neu gene amplification and overexpression and parameters of malignancy, including reduced survival and reduced time to relapse, relative to patients with normal receptor levels [24, 25]. Later it could be shown that EGFR expression is linked to activation of ErbB-2 in human breast cancers [26].

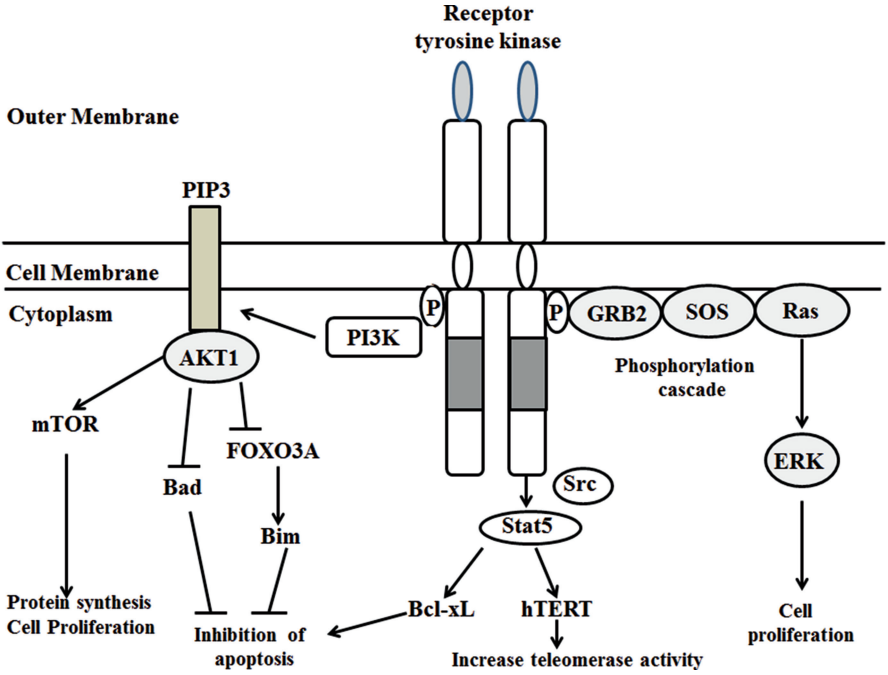

Fig. 2.3 Intracellular signalling pathways activated by receptor tyrosine kinases

Abnormalities in kinase activity have also been elucidated for many other protein tyrosine kinases such as Ret/GDNFR in multiple endocrine neoplasia, KIT/SCFR in gastrointestinal stromal tumors and acute myeloid leukaemia, Met/HGFR in papillary thyroid carcinomas and Src in colon cancer [27].

2.3 Protein Kinase Inhibitors in Targeted Cancer Therapy

Protein kinases mediate most of the signal transduction in eukaryotic cells by modification of substrate activity and they also control many other cellular processes, including metabolism, transcription, cell cycle progression, cytoskeletal rearrangement and cell movement, apoptosis, and differentiation. Protein phosphorylation also plays a critical role in intercellular communication during development, in physiological responses, in homeostasis, and in the functioning of the nervous and immune systems. Protein kinases are among the largest families of genes in eukaryotes [28, 29]. Mutations and dysregulation of protein kinases play causal roles in human diseases [27, 30]. As such, protein kinases are important targets in drug discovery aimed at treating many devastating diseases, including autoimmune disorders, diabetes, neurological disorders and cancer. The idea that one could actually

target protein kinases came up in the late 1980s with the discovery that rapamycin. Such molecule inhibits the protein kinase mTOR (mammalian target of rapamycin), a member of the phosphatidylinositide 3-kinase (PI-3 K) superfamily, which is required for interleukin-2-(IL-2)dependent T cell proliferation. In the field of cancer, Herceptin, an anti-HER2 monoclonal antibody (trastuzumab), was the first genomic research-based, targeted anti-kinase therapeutic approved for cancer therapy [31, 32]. In general, protein kinase inhibitors can be divided into two functional groups, namely therapeutic antibodies (biologics) and small-molecule kinase inhibitors, respectively, both in clinical use for cancer-specific targeted therapies of a broad range of different tumor indications. Most of the FDA approved TKIs for the treatment of cancer are multi-targeted inhibitors of several intracellular tyrosine kinases and a few specifically inhibit the members of a family. These inhibitors are commonly used in the treatment of cancers including: non-small-cell lung cancer (NSCLC), head and neck, colorectal, renal, prostate, breast, and primary brain cancer. This drug type has only been around since the 1980s, so some drugs are currently still in clinical trials while others are in current use. Small-molecule inhibitors of tyrosine kinases compete with the ATP binding site of the catalytic domain of several oncogenic kinases with the kinase activation loop in the active (type 1 inhibitor) or inactive (type 2 inhibitor) conformation. They are orally active, have a favourable safety profile and can be easily combined with other forms of chemotherapy or radiation therapy. To date, approximately 80 inhibitors have been advanced to some stage of clinical evaluation.

2.3.1 Epithelial Growth Factor Receptor (EGFR) Family and Specific/Selective TKIs

EGFR also is commonly overexpressed in many cancers such as glioblastoma, non-small cell lung, breast, colorectal, bladder, prostate, head-neck and ovarian carcinomas. Overexpression of EGFR correlates with poor prognosis, worse clinical outcome, and chemoresistance in a large number of the previous listed cancers. EGFR belongs to the ErbB receptor family of tyrosine kinases. There are four members of the ErbB family; EGF receptor (EGFR/erbB1), c-erbB2/HER2, c-erbB3-/HER3 and c-erbB4/HER4. EGFR is a 170-kd glycoprotein that consists of a glycosylated, extracellular-ligand binding domain, a transmembrane region, and an intracellular tyrosine kinase domain. There are many different ligands that are able to bind to the EGFR extracellular domain such as, epidermal growth factor (EGF), transforming growth factor-beta (TGF-β), amphiregulin, heparin-binding, and betacellulin. EGF and TGF-β are believed to be the most important ligands for EGFR [33]. In addition, increased receptor concentration is also associated with an increased production of EGFR activating ligands by the same tumor. This autocrine pathway leads to activation of the receptor and secondary downstream signaling. In the absence of a ligand, monomeric receptors reside within the cell membrane in an

inactive state distributed rather evenly over the cell membrane. Upon ligand binding, the ErbB receptors homo- or hetero- dimerize. This leads to activation of the intrinsic kinase domain, resulting in the auto-phosphorylation of tyrosine residues found within the cytosplasmic tail. It is now evident that further oligomerization of these receptors occurs in response to ligand binding and second messenger recruitment. Once dimerization occurs, the receptors serve as "signaling platforms" by recruiting different receptors and other secondary messengers [34]. The phosphorylated tyrosine residues serve as binding sites for a range of proteins. These proteins recognize the phosphorylated tyrosine residues on the EGFR cytoplasmic tail using their Src homology 2 (SH2) domain or phosphotyrosine (PTP) binding domain [34]. Important signaling effectors resulting from EGFR activation include signal transducer and activator of transcription (STAT), SRC tyrosine kinase, GRB2 (Growth Factor Receptor-Bound Protein-2), Nck (Nck Adaptor Protein), PLC-Gamma (Phospholipase-C-Gamma), and SHC (Src Homology-2 Domain Containing Transforming Protein) [34]. These effectors are involved in two main pathways activated by EGFR: the mitogen-activated protein kinase (MAPK) and the phosphotidylinositol 3-kinase (PI3K)-AKT pathway. When these pathways are activated they lead to cell growth, differentiation, proliferation, cell survival, and motility via up-regulation of transcription factors [35]. Therefore, specific/selective inhibition of EGFR is an ideal approach to cancer treatment. The approaches used to target EGFR signaling include EGFR blocking monoclonal antibodies targeting the extracellular ligand binding domain, and small molecular weight EGFR tyrosine kinase compounds [35]. Treatment of tumors with these inhibitors affects downstream signaling pathways that are essential for cancer development and progression [33]. The efficacy of these inhibitors has been shown when translated from mice to patients in clinical trials. These chemotherapeutics have been introduced into clinical practice by the development of the EGFR monoclonal antibody and the EGFR tyrosine kinase inhibitors. The best known monoclonal antibody is trastuzumab (proprietary name: Herceptin) developed by Gentech. It is a humanized monoclonal antibody which blocks overexpression of EGFR and HER2 in breast cancer.

Cetuximab is humanized monoclonal antibody targeted against HER-2/ErbB2 member of the EGFR family. In vitro experiment revealed that cetuximab effective against a wide range of human cancer including those of the pancreas, kidney, breast, colon, prostate, and head and neck. It was approved for treatment in patients with locally or regionally advanced HNSCC and EGFR-expressing, metastatic, colorectal cancer in 2006 and 2004 respectively [36].

Panitumumab is a fully humanized IgG2 monoclonal antibody that binds to the extracellular domain of the EGFR with high affinity but whose binding prevents cancer cells TK autophosphorylation process, cell growth and metastasis [37]. This also results in downregulation of EGF receptors through receptor internalization, induction of apoptosis, autophagy and inhibition of angiogenesis [38–40]. The response to panitumumab can be affected by KRAS mutations. A phase 3 trial that compared the effects of panitumumab monotherapy with those of best sup-

portive care in patients with metastatic Colon Rectal Cancer (mCRC) showed that panitumumab was only effective in patients with tumors with wild-type K-ras; response rates were 17 % vs. 0 % in patients with mutant vs. wild-type KRAS tumors [40].

Gefitinib (ZD1839, Iressa) and erlotinib (OSI-774, Tarceva) belong to the first generation of TKIs and are selective EGFR–TKIs that were approved on May 2003 and November 2004 for the treatment of NSCLC patients, respectively. Erlotinib was approved for use in non small lung cell carcinoma (NSCLC) in 2004 and pancreatic cancer 2005. Gefitinib was approved for use in locally advanced and metastatic NSCLC in 2003 [41, 42]. Furthermore, these drugs are been used in different trials on patients with different types of cancer, including gastric [43], gastroesophageal, esophageal [44], cervical [45] renal cell carcinoma [46], and hepatocellular carcinoma [47], but they have not demonstrated many clinical benefits [48].

Lapatinib is an orally active, low molecular weight TKI. It is a selective, reversible inhibitor of EGFR and HER2 and it binds to the ATP binding site of both receptors preventing signal transduction through both the MAPK and PI3K/AKT pathways, leading to an increase in apoptosis and decrease in cellular proliferation. Lapatinib is one of the most specific kinase inhibitors approved for the treatment of cancer. When 20 kinase inhibitors (at 10 μM) were compared in an in vitro binding assay against a panel of 113 substrates, lapatinib was one of the most specific, targeting HER2 and EGFR with 10-fold greater affinity than any off-target substrates. Lapatinib inhibits HER2 with an IC50 of 9.2 nM, EGFR with an IC50 of 10.8 nM, HER4 with an IC50 of 0.367 μM and c-Src with an IC50 of 3.5 μM making lapatinib a highly selective HER2/EGFR inhibitor [49]. Lapatinib inhibits the tyrosine kinase domain of both EGFR and the ErbB2 receptor and was approved for HER-2 overexpressing, metastatic breast cancer use in 2007 [50, 51]. In the first preclinical studies, the activity of such drug were evaluated in human cell lines over-expressing EGFR or HER2 including HN5 (head and neck), A-431 (vulva), BT474 (breast), CaLu-3 (lung), and, N87 (gastric) cell lines. In this cell lines, it was able to block the cell growth mediating the EGFR, HER2 and AKT dephosphorylation and to inhibit tumor xenograft growth of the HN5 and BT474 cells in mice [49]. Lapatinib was approved for HER-2 overexpressing, metastatic breast cancer use in 2007 [51] as the second-line treatment and for treatment of postmenopausal women with estrogen/HER2 receptor-positive breast cancer in combination with an aromatase inhibitor as first-line therapy.

Canertinib was designed as a pan-ErbB tyrosine kinase inhibitor. It inhibits all four ErbB receptor family members. Canertinib is an irreversible inhibitor that binds covalently to specific cysteine residues in the ATP-binding pocket such as cysteine 773 of EGFR, cysteine 784 of ErbB2 and cysteine 778 of ErbB4 thereby blocking the ATP binding site in the kinase domain of ErbB proteins, preventing their kinase activity and downstream signaling and additionally, it also prevents transmodulation of ErbB2 [52]. The covalent binding of canertinib results in prolonged suppression of ErbB activity [53]. Since canertinib blocks signaling through all members of the ErbB receptor family, it is more efficient than inhibitors

that only prevent signaling from one of the ErbB receptors [54, 55]. Canertinib has been shown to inhibit growth and induce apoptosis in several cancer cell lines and xenografts [56–58]. In clinical studies it has been shown to have acceptable side-effects. However, in phase II studies canertinib was only able to show modest effects on breast cancer and NSCLC patients [59, 60]. Such drug is able to dephosphorylate p70S6-kinase T389 in a dose-dependent manner as well as to block downstream signaling molecules in ALL cell lines. Additionally, it induces the apoptosis through an increased expression of the pro-apoptotic protein BIM (Bcl2-interacting mediato), caspase-3 cleavage and inhibits proliferation BCR/ABL-cells resistant to TKIs [61]. It is able to bind not only to the ErbB receptor family, but also to intracellular proteins. For instance, the Src kinase family consists of eight members, five of which are mainly expressed in hematopoeitic cells, Blk, Hck, Lck, Fyn, and Lyn, where the Lck protein seems to have a stronger binding to canertinib as shown in a protein binding assay [62].

Dacomitinib is an orally administered, highly selective irreversible pan-ErbB (EGFR, HER2, and HER4) TK-inhibitor. This inhibitor was active in preclinical studies against first-generation TKI-resistant tumor cells and human tumor xenograft models [63]. Furthermore, dacomitinib showed antitumor activity against tumors with activating EGFR mutations and tumors harbouring the T790M mutation, but very limited response was reported for K-RAS mutated tumors. It irreversibly inhibited tumor growth in H125, SKOV3 and A431 cells of xenografted mice models and its therapeutic activity ranged from delayed progression to complete regressions [64]. Furthermore, such TKI is able to inhibit HER2 phosphorylation at tyrosine residue (Tyr) 1248 in experiments using the A431 human squamous cell carcinoma xenografted model which over-expresses EGFR, HER2 and HER3 [65]. The preliminary data of a phase II clinical trial as a first-line agent in squamous cell carcinoma of the head and neck showed a median progression-free survival (PFS) of 2.8 months and overall survival (OS) of 8.3 months. Based on the results of this study, a phase III trial (ARCHER study) of second- or third-line dacomitinib vs. erlotinib has been started in patients with NSCLC and KRAS wild-type NSCLC (ClinicalTrials.gov, NCT01360554) [64]. Two phase I dose-escalation studies investigating dacomitinib pharmacokinetics in Western (NCT00225121) and Japanese (NCT007833328) patients with advanced solid tumors assessed the MTD in 45 mg once daily [65, 66]. In particular, the U.S. trial enrolled 121 patients, of whom 47 % were NSCLC patients and the majority of them had received a previous treatment with first-generation EGFR-TKIs. In agreement with the Western study, the Japanese trial showed that dacomitinib was generally safe and well tolerated. Antitumor activity was also reported, particularly in NSCLC [67]. A phase II trial evaluated dacomitinib efficacy in patients with NSCLC (wild-type K-RAS) who progressed after at least one prior chemotherapy regimen and erlotinib (NCT00548093), suggested diarrhea and mucositis as the most common adverse drug-related effects [68]. The safety and efficacy of the inhibitor were also investigated in a phase I/II study conducted in Asians refractory to chemotherapy, and erlotinib or gefitinib treatment. Preliminary data of the 30 evaluable patients showed

Progression-Free-Survival (PFS) (4 months) 35 %, OS (6 months) 87 %, overall RR 8 %, and clinical benefit rate (PR or SD ≥ 24 weeks) 20 % [69]. Based on the encouraging results provided during previous studies, a randomized, phase III trial (JBR.26) comparing dacomitinib to placebo as third-line setting is ongoing in advanced NSCLC patients with varying histology and molecular subtypes, who have failed chemotherapy and EGFR-TKIs (NCT01000025). A randomized phase II study comparing dacomitinib with erlotinib as second-line therapy in 188 patients with advanced NSCLC who progressed after upfront chemotherapy has been designed (NCT00769067) [70]. Baseline features were equally distributed between the two treatments with the exception of performance status 2 (19.1 % [dacomitinib] and 3.2 % [erlotinib]) and positive EGFR-mutation status (20.2 % [dacomitinib] and 11.7 % [erlotinib]). Among the overall population, longer median PFS was achieved with dacomitinib treatment compared to erlotinib (12.4 vs. 8.3 weeks, respectively; HR = 0.681; CI, 0.490-0.945; P = 0.019), as well as increased RR (17 % vs. 4 %) and clinical benefit (PR or SD for ≥ 24 weeks: 27.7 % vs. 13.8 %, respectively). Moreover, consistent benefits from dacomitinib treatment were observed across several subsets including wild-type EGFR patients (PFS: 11.1 weeks [dacomitinib] vs. 8.0 weeks [erlotinib]; HR = 0.624; CI, 0.389-1.002; P = 0.047). Thus, based on phase II data, a randomized phase III trial was launched to compare the efficacy of dacomitinib with erlotinib as second-line treatment setting in unselected advanced NSCLC patients (ARCHER 1009; NCT01360554) [68]. Furthermore, an open-label phase II study evaluating dacomitinib as first-line treatment in adenocarcinoma patients who were never-smokers/former light-smokers, or patients who harbour EGFR mutations is ongoing (NCT00818441) [71]. A total of 92 patients have been already enrolled. Thirty-four of 46 EGFR-activating mutation-positive patients had PR (74 %; CI: 59–86; exon 19 = 72 %; exon 21 = 76 %) and the reported median PFS was 17 months. PR rates and preliminary PFS were not significantly different for exons 19 and 21. Patients with wild-type EGFR had PR 7 % (n = 14; CI: 0–34) and PFS at 4 months 33 % (n = 14; CI: 11–58). Another multi-cohort, phase II safety study is now ongoing to assess the impact of daily dacomitinib as prophylactic treatment on the incidence of adverse events in advanced refractory NSCLC patients (ARCHER 1042-NCT01465802). Moreover, the impact of an interrupted drug dosing schedule in first-line treatment of EGFR mutation-positive NSCLC patients (EGFR mutation or HER2 mutation/amplification) will be investigated. More recently, a dose-related tumor shrinkage rate was observed in the combined analysis of multiple tumor size measurements collected from 200 patients from 4 clinical trials (3 phase I and 1 phase II) treated with 15–45 mg once daily dacomitinib [72]. Of note, 83 % less shrinkage was observed in patients with wild-type EGFR when compared with mutants. Thus, further research is ongoing to evaluate the potential of this inhibitor in the treatment of EGFR-mutated NSCLCs.

Afatinib (second-generation tyrosine kinase inhibitors BIBW-2992) binds EGFR and HER2, and inhibits the kinase activity of both wild-type and mutant receptors [73]. This drug is being examined extensively as a treatment for lung cancer in the LUX-Lung program, and early results indicate that afatinib significantly prolongs

Progression-FreeSurvival (PFS) compared with placebo in pretreated patients with clinically acquired resistance to gefitinib or erlotinib [74]. Afatinib is also being investigated in several trials in HER2 positive patients alone and in combination with paclitaxel or vinorelbine in patients who have progressed on prior HER2-targeted therapy [75], as a single agent vs. lapatinib or trastuzumab in HER2 positive treatment naïve patients [76] and also in HER2 positive Inflammatory Breast Cancer (IBC) [77]. A phase III trial of afatinib in combination with vinorelbine compared to trastuzumab and vinorelbine is currently underway in trastuzumab refractory breast cancer [78].

Neratinib (second-generation tyrosine kinase inhibitors HKI-272) inhibits HER2 phosphorylation in irreversible manner [79]. Preclinical studies have found the promising activity of neratinib in NSCLC and breast cancer. Different cell lines (breast cell lines transformed with HER2, NSCLC cell line and Calu-3, over-expressing HER2) and HER2 over-expressing BT474 cells xenografts were highly sensitive to the neratinib. In a phase II study, neratinib monotherapy showed clinical benefit among patients with HER2-positive breast cancer who were pre-treated with trastuzumab, the median PFS was 23 weeks and the objective response rate was 24 %. This study also evaluated neratinib as a monotherapy in HER2-positive breast cancer patients who had not been pre-treated with trastuzumab, interestingly the PFS doubled to 40 weeks and the objective response rate rose to 56 % [80]. Neratinib-monotherapy is being evaluated in a phase III trial in HER2 positive breast cancer following adjuvant trastuzumab therapy [81]. Neratinib is currently in multiple clinical trials including; a phase I/II study in combination with paclitaxel [82], in combination with trastuzumab [83], a phase II studying comparing neratinib and paclitaxel vs. trastuzumab and paclitaxel (NEFERTT) [84] and a phase II study evaluating neratinib vs. lapatinib and capecitabine in HER2 positive advanced breast cancer [85].

Nimotuzumab is an EGFR-specific monoclonal antibody which binds to an epitope in domain III of EGFR that highly overlaps with that of cetuximab, and inhibits ligand binding and EGFR activation [86]. Similar to cetuximab, nimotuzumab sensitizes cells to radiation. Treatment with nimotuzumab as a monotherapy administered following standard treatment in newly diagnosed glioblastoma patients demonstrated no significant improvement in overall survival, but may benefit a subset of patients with EGFR amplified tumors with non-methylated MGMT promoters. When combined with radiation therapy, nimotuzumab demonstrated significant survival benefit for patients with high grade gliomas (grade III anaplastic astrocytomas and grade IV glioblastomas) and excellent tolerability [87].

Zalutumumab is a fully human IgG1 monoclonal antibody (mAb) directed towards the EGFR. Specifically, zalutumumab is designed for the treatment of squamous cell carcinoma of the head and neck (SCCHN).

Although the advanced development of these clinical available EGFR TKIs shows some efficacy in certain cancers, the issue of poor response and/or constitutive resistance in a large number of patients and the development of acquired resistance in the responders remains an important area of research.

2.3.2 *Platelet-Derived Growth Factor Receptor (PDGFR) and Specific/Selective TKIs*

There are four types of PDGF ligands (PDGF-A to -D) and two different receptors exists (PDGFR alpha and beta). Overexpression of PDGF ligands or their receptors is frequent in malignancies, including NSCLC and glioblastoma [88]. PDGF signal activation can influence cell survival, proliferation, motility and also affect neighboring cells. In part, PDGF signaling maintains tumor growth by formation of intratumoral blood vessel network and also by recruiting stromal cell components to the tumor [89–91]. A cross-talk of PDGF and other secreted factors like VEGF and FGF2 was also observed, resulting in modulation of angiogenic properties. This is evident from the high expression levels of both PDGFR alpha and VEGFR2 in several human gliomas [92]. All approved PDGFR–TKIs are multi-targeted and a few specific/selective inhibitors are in preclinical and clinical settings. One of these inhibitors, imatinib (also named Glivec or STI 571), has been used clinically for treatment of chronic myeloid leukemia (CML), gastrointestitial stromal tumor (GIST), and gliomas [93]. Imatinib inhibits the kinase activities of PDGFR, c-KIT, BCR-ABL and c-ABL and was shown to inhibit the growth of dermatofibrosarcoma protuberans (DFSP) tumors by suppressing the activity of the PDGF receptor. Targeting PDGFRβ positive cancer-associated fibroblasts by imatinib was shown to dramatically reduce proliferation and angiogenesis of cervical carcinoma [94]. The multi-kinase targeting characteristic of imatinib makes it difficult to determine whether the effect seen is due to specific inhibition of PDGFR or to inhibition of other kinases. Therefore, more selective inhibitors specifically targeting PDGFR are urgently needed.

CP-673451 is an inhibitor of both PDGFRα and PDGFRβ. Different studies have observed that this drug blocked cell growth of mesenchymal-like NSCLC cell line H1703 with high expression of PDGFRα, but not in the epithelial cell line H292 lacking PDGFR [95]. Additionally, it dephosphorylated PDGFR, AKT, GSK-3α, GSK-3β, and impaired rhabdosphere-forming capacity in both RD and RUCH2 rhabdomyosarcoma cells [96]. It reduced proliferation, tumor growth and stromal cell infiltration in xenografted mice with RD and RUCH2 cell lines with high expression of PDGFR, whereas no effects were observed in PDGFR negative cell line RMS [96]. More studies are necessary on various PDGFR over-expressing tumors in clinical settings.

Crenolanib (CP-868596) inhibits cellular proliferation in different cell lines such as BaF3 D842V and EOL-1 cell lines with PDGFRα-dependent growth. In addition, several results indicated that it was significantly more potent than imatinib in inhibiting the kinase activity in imatinib-resistant mutated PDGFRα cell line with D842I, D842V, D842Y, DI842-843IM mutations and deletion I843. It showed to be more potent than imatinib against primary gastrointestinal stromal tumors cells expressing PDGFRα with D842V deletion [97].

2.3.3 Fibroblast Growth Factor Receptors (FGFRs) and Specific/Selective TKIs

There are five FGF receptors. FGFRs1-4 are single pass transmembrane tyrosine kinase receptors. FGFR5 lacks the intracellular tyrosine kinase domain and so although it has a high affinity for FGF, its function is not well understood [98]. Aberrant signalling from FGFRs is likely to cause the development and progression of tumours due to their effect on cell proliferation, anti-apoptotic and angiogenesis factors. Mutations causing ligand independent promotion of dimerization has been seen repeatedly with FGFR3 in bladder cancer and with FGFR2 in endometrial cancers [99]. Mutations in the tyrosine kinase domain of FGFRs can also cause ligand independent constitutive activation. Mutations of the kinase domain of FGFR4 have been identified as potential oncogenes in rhabdomyosarcoma (RMS), a childhood skeletal muscle cancer [100]. Constitutive activation of the kinase domain may also occur through the formation of a fusion protein such as the fusion of the kinase domain of FGFR1 to a dimerization domain of a separate protein seen in stem cell leukaemia lymphoma syndrome (SCLL) [101]. Single nucleotide polymorphism (SNP) found in FGFRs are also associated with cancers. The SNP G388R found in FGFR4 is thought to confer sustained signalling capabilities compared to wild type and therefore is suggested to contribute to the progression of the several forms of cancer [101]. SNPs found in FGFR2 causing increased expression of the receptor are also liked with increased risk of breast cancer. Amplification of the FGFR gene can cause overexpression of the receptor leading to more frequent ligand independent dimerization and therefore signalling. Roughly 10 % of breast cancers contains amplification of the chromosomal region 8p11-12 containing FGFR1 [102]. This amplification has been shown to correlate to overexpression of the receptor and increased downstream signalling [103]. Amplification of FGFR2 is seen in a further 1–3 % of breast cancers and has been shown to cause ligand independent signalling. Its inibition with siRNA or selective drug provoked a reduced survival of breast cancer cell line MFM223, with amplified FGFR2, compared to non-amplified cell lines. This suggests that the downstream kinase signalling from the amplified FGFR2 was required for the growth and survival of the cells and is a main component of the development and progression of the cancer. This all indicates how effective FGFRs may be as a therapeutic target in cancer and there are currently several FGFR inhibitors in clinical trials.

PD173074 is often referred to as a selective inhibitor of FGFRs with an IC50 of ~25 nM, whereas it effectively blocks VEGFR2 only at a four-fold higher concentration, which was reported in the original paper [104]. Nevertheless, PD173074 is used only in vitro and in experimental models, where it is shown to inhibit cancer cell proliferation, angiogenesis and tumour growth [105, 106]. TKI168 has been recently shown to be a more selective FGFR inhibitor in breast cancer cells and to effectively inhibit mammary tumour growth [107]. In a phase I clinical study, it caused a partial response in only a small portion (2/35) of cancer patients with advanced solid cancers [108]. Because of the problems in simultaneously targeting

several RTK families, more selective FGFR inhibitors are being developed, but no data on their efficacy or toxicity are available yet. Side-effects can also be expected with the selective FGFR inhibitors. In particular, the endocrine FGFs (FGF-19, -21 and -23) are important in regulating the metabolism and tissue calcification [109], so it is quite likely that inhibiting these FGFs will result in significant side effects.

2.3.4 MET Oncogene and Specific/Selective TKIs

The c-MET proto-oncogene is located on chromosome 7q31.2 [110], with its transcription being regulated by multiple transcription factors such as Ets (E-twenty six), Pax3 (paired box 3), AP2 (activator protein-2) and Tcf-4 (transcription factor 4) [111]. The protein product of this gene is the receptor for HGF, known as HGFR or more commonly as cMET. This cell-surface receptor tyrosine kinase is expressed in endothelial and epithelial cells during both embryogenesis and adulthood [112], while its ligand is expressed mainly in cells of mesenchymal origin, although some reports have shown that HGF is also expressed by some neoplastic epithelial cells [113]. c-MET is translated from single transcript, although the mature protein is formed by proteolytic processing into a disulphide-linked-heterodimer [114]. The extracellular portion of c-MET is composed of three domain types. The 500 N-terminal residues form the SEMA domain (semaphorins), which has been found to function as a protein-protein interaction domain [115–119]. Data collected from in vitro and in vivo tumour models suggest that oncogenic c-MET signalling typically occurs via one of three mechanisms: (a) the occurrence of specific genetic lesions, including translocations, gene amplifications and activating mutations; (b) transcriptional upregulation of the c-MET protein in the absence of gene amplification; or (c) ligand-dependent autocrine or paracrine mechanisms.

Proof of concept for the role of c-MET in human cancers was provided following the identification of activating point mutations in the germline of patients with hereditary papillary renal carcinomas [120, 121]. Activating mutations have been described mainly in gastric cancers, NSCLC, hereditary and spontaneous renal carcinomas, hepatocellular carcinomas, gliomas, squamous cell carcinoma of the head and neck, and breast carcinomas [122–126]. Potentially oncogenic point mutations that were reported in cancers include those that generate an alternative splice variant lacking exon 14, which encodes for the juxtamembrane domain of c-MET [124, 127]; point mutations in the kinase domain that render the enzyme constitutively active [122]; and a mutation at Y1003 that abrogates CBL binding leading to constitutive c-MET expression [128]. High protein expression, detected by immunohistochemistry, as a result of c-MET amplification has been associated with poor prognosis in colorectal cancer, NSCLC and gastric cancers [129–131]. Reports that c-MET is more frequently amplified in metastatic compared to primary tumours suggest a role for this gene in the late phases of malignant progression [129]. Additionally, increased protein expression as a consequence of transcriptional up-regulation in the absence of gene amplification is the most frequent cause of constitutive c-MET activation in

human tumours [112], and it has been reported in a growing number of carcinomas including thyroid, colorectal, ovarian, lung and breast [48]. Overall, reports tend to show that high levels of c-MET and/or HGF expression is found in a significant subset of primary patient samples, and, importantly, high levels of these proteins in distant metastases are often correlated with worse prognosis. c-MET has been implicated in the development and progression of a number of cancers, and is therefore being investigated as the target of anti-cancer therapies [48]. In pre-clinical animal models, inhibition of c-MET impairs tumourigenic and metastatic properties of cancer cells [132, 133]. c-MET inhibitors are being formulated as either small molecule tyrosine kinase inhibitors or antibodies that target either the ligand or the receptor [134]. Currently, the only phase II trial using c-MET inhibitors to treat metastatic colorectal cancer involves onartuzumab (MetMAb or OA-5D5), a human monovalent antagonistic anti-cMET antibody [135], in combination with FOLFOX and bevacizumab (a monoclonal VEGFR antibody) vs. placebo [136, 137]. Monovalent antibody treatment prevents ligand binding to the c-MET receptor, thereby preventing receptor dimerization and activation. So far, monoclonal antibodies in preclinical and clinical studies have only demonstrated partial or complete response in patients (or cell lines) with high c-MET levels or an HGF/c-MET autocrine loop [138–141]. As such, the MetMAb phase II mCRC trial has the goal of determining if patients expressing higher levels of c-MET mRNA or protein receive greater benefit from this treatment than those with lower levels. Another c-MET inhibitor in clinical trials is tivantinib (ARQ 197), a non-ATP competitive small molecule for which a mechanism of action is not yet fully described [142, 143].

Tivantinib recently completed a phase II study showing that it, in combination with erlotinib, showed an increase response rate and overall survival compared to erlotinib alone in KRAS mutation positive NSCLC [144]. However, the phase III MARQUEE trial that was created to further these results was terminated early, when it was concluded that although this combination significantly decreased progression-free survival, it did not appear to be affecting overall survival. Clinical trials testing the efficacy of tivantinib in mCRC include a phase I trial assessing the combination of tivantinib, cetuximab and irinotecan against irinotecan and cetuximab alone in wild type KRAS patients (NCT01075048 2010) and a phase I/II trial accepting patients with any type of solid tumour, including mCRC, to asses the efficacy of tivantinib + FOLFOX vs. FOLFOX alone [145]. Recently, two studies have shown that the inhibitory effect of tivantinib may be due to its targeting of microtubule dynamics, and may be completely or partially independent of c-MET inhibition [146, 147]. These studies suggest that negative results in clinical trials should not cast doubt on the effectiveness of "true" c-MET inhibitors, and that patients should not be selected for treatment with tivantinib based solely on c-MET expression status.

Rilotumumab (AMG102) is an anti-HGF monoclonal antibody that interferes with cMET activation by HGF. Rilotumumab is currently being evaluated in phase I/II studies alone or in combination with EGFR blocking antibody panitumumab [148] in WT KRAS mCRC. Previous studies have shown that rilotumumab decreases cMET phosphorylation and can stabilize the progression of certain solid tumours [140, 141].

EMD1214063 and EMD1204831 are two selective and ATP-competitive MET–TKIs that were identified recently [149]. Both drugs are able to inhibit HGF-induced MET phosphorylation in A549 cells and induced regression of human tumors in xenografted mice [149]. Ain one study, the authors found that EMD1214063 treatment in xenograft tumor model mice bearing NIH3T3 cells lead a complete regression of the sensitive H1112L mutant-derived tumors, but not in mice with L1213V tumors [150]. Such drug is able to trigger autophagy in gastric adenocarcinoma cell lines and additionally, the combination with autophagy inhibitors and EMD1214063 induced cell death [151]. This TKI reduced neuroblastoma tumor growth in immunocompromised xenografted mice [152].

2.3.5 TAM RTK Family: AXL and MER Inhibitors

AXL and MER are two receptor tyrosine kinases from the TAM family of receptor tyrosine kinases Both are important to signal transduction in many normal cell types and malignant (T-lymphoblastic lymphoma/leukemia) T-ALL cells. Signaling pathways downstream from the AXL and MER tyrosine kinases also activate other pathways that control platelet aggregation, pro-inflammatory cytokine production, regulation of the actin cytoskeleton, cell survival, growth, and metabolism [153, 154]. Therefore AXL and MER receptor tyrosine kinases play key roles in cancer development. Both AXL and MER are implicated in several human cancers, including MER over-expression seen in T-ALL lymphoblasts, but not during any developmental stages of normal T-cells [153].

R428 (BGB324) is a first-in-class, highly selective AXL–TKI that reduces the AXL dephosphorylation in both human MDA-MB-231 and murine 4 T1 breast cancer cell lines in vitro [155]. This drug blocks liver and lung metastases in a breast cancer mouse model in association with cisplatin [155]. It has an anti-proliferation effect on NCI-H1299 (mesenchymal, EGFR wild-type, erlotinib-resistant) human NSCLC cells and sensitizes the such cells xenografted mice to docetaxel. Moreover, treatment of A549 NSCLC cell line with R428 and in combination with either erlotinib or anti-VEGF human antibody bevacizumab displayed additive anti-tumor activity [156].

2.4 Vascular Endothelial Growth Factor Receptor (VEGFR) and Anti-Angiogenesis TKIs

The critical role of angiogenesis in cancer was first proposed more than 30 years ago by Folkman [157] and led to the development of the small-molecule kinase inhibitors. Angiogenesis is essential for tumors to grow beyond 1–2 mm^3, and switch from local vascular supply to novel microcapillary formation. It also allows tumor cells to enter the circulation, enabling the spread of cancer cells to multiple

organs (metastasis). Angiogenesis correlates with tumor progression and disease severity, and is controlled by pro-angiogenic factors such as VEGFs and PDGFs. The VEGF family (VEGF-A (usually referred to VEGF), VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PGF) are over-expressed by various solid tumors and bind to its receptors (VEGFRs: VEGFR1, VEGFR2 and VEGFR3) on the vascular endothelium and induce angiogenesis. At present, various selective VEGFR–TKIs, including vatalanib, tivozanib, cediranib, and lenvatinib, are under investigation for the treatment of various solid tumors.

Vatalanib, also named as PKT787/ZK 222584, is a novel, potent, orally bioavailable angiogenesis inhibitor which blocks all known VEGF receptor tyrosine kinases in the submicromolar range. Besides VEGF receptor tyrosine kinases, it can also inhibit the PDGFR-β tyrosine kinase, c-KIT, and c-Fms, but at higher concentrations. The mechanism of action of vatalanib is mainly through the specific inhibition of angiogenesis in solid tumors or hematological malignancies. Vascular changes. that have been detected using Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI), decreased plasma angiogenesis factor levels in a murine melanoma cell model have supported such mechanism of action [158]. Vatalanib inhibits the growth and metastasis of tumors in animal models. Due to this promising potential, vatalanib is being studied for the treatment of patients with solid tumors, including breast cancers, lung cancers, prostate cancers, gastrointestinal tract cancers, ovary cancers, liver cancers, brain cancers, renal cancers, and bladder cancers, and hematological malignancies, such as acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) [159]. In preclinical studies, vatalanib was well tolerated in animal models. In an in vitro study done by JM Wood et al., it was shown that vatalanib had no impairment on wound healing, no significant hematological effect on leukocytes in bone marrow or circulating blood cells, and no impairment on bone marrow recovery after a cytotoxic agent treatment [160].

Tivozanib (AV-951, KRN-951) is an oral quinoline urea derivative that suppresses angiogenesis by being selectively inhibitory against the VEGFR family. It inhibits the cellular proliferation in human breast, colon, liver, lung, ovarian, pancreas, and prostate cancer and in brain xenografted models. It attenuated VEGFR2 phosphorylation in tumor endothelium and displayed anti-tumor activity against a wide variety of human tumor xenografts. DCE-MRI has shown a reduction in tumor vascular hyper-permeability associated with the tivozanib anti-tumor activity [161]. This effects have been evaluated in a rat colon cancer RCN-9 syngeneic model in which the tumor cells have been transplanted into the peritoneal cavity of F344 rats. Treatment of transplanted mice model reduced angiogenesis, the formation of tumor nodules and the accumulation of malignant ascites. Such drug has been evaluated in different clinical trials in phases II and III for patients with renal cell carcinoma and breast cancer with a good results [162], and other clinical trials are ongoing for the treatment of different cancers using tivozanib alone or in combination [162, 163].

Cediranib, an indole-ether quinazoline, inhibits tyrosine kinase receptors, particularly all subtypes of the VEGF receptor, and has some activity against the PDGF and c-KIT receptors [164]. In gliomas cediranib was shown to induce nor-

malization of the vasculature by inhibiting pathological proliferation of endothelial cells and immature vessel [165–168]. This drug has also exhibited antitumor and antiangiogenic activity in various cell lines [169–171] and xenografts including colon, lung, prostate, breast and ovary [169, 172]. Its pharmacological effect has been evaluated in Phase II/III clinical trials for advanced non-small cell lung cancer, advanced colorectal cancer, metastatic renal cell carcinoma, and recurrent glioblastoma, etc [173].

Sunitinib is an orally available inhibitor of VEGFR, PDGFR, c-KIT, and FLT-3 kinase activity. In a phase II study in patients with immunotherapy refractory metastatic renal cell carcinoma treated with sunitinib (6-week cycles: 50 mg orally once daily for 4 weeks, followed by 2 weeks of), 40 % of patient showed a partial response and 27 % stable disease [174]. When the results were combined with a second study with an identical patient population, the total evaluable patient population was 168 patients. Objective responses were seen in 42 % and stable disease of 3 or more months in 24 %. Median progression free survival was 8.2 months [175]. These response rates were much higher than seen with any other systemic treatment in Renal Cell Carcinoma (RCC). Motzer reported the results of a phase III study comparing sunitinib (6-week cycles: 50 mg orally once daily for 4 weeks, followed by 2 weeks off) to IFN-alpha (6-week cycles: subcutaneous injection 9 MU given three times weekly) as first line therapy for metastatic renal cell cancer patients. There was a statistically significant improvement in median progression free survival (47.3 vs. 24.9 weeks) and objective response rate (24.8 % vs. 4.9 %) for sunitinib over IFN-α (Interferon) [176]. Sunitinib might therefore now be considered the new standard first-line treatment for advanced kidney cancer. In January 2006, sunitinib was not only approved by the FDA for advanced renal cell carcinoma, but also for imatinib-resistant and imatinib-intolerant GIST. This was based on the early results of a phase III trial in patients with documented progression of GIST on imatinib [177, 178]. Patients were treated with a starting dose of 50 mg sunitinib once daily for four weeks, followed by 2 weeks off treatment, in repetitive 6-week cycles (N = 207) or placebo (N = 105). Due to the positive results found at a planned interim analysis, the trial was unblinded and all patients started treatment with sunitinib. Partial response was seen in 6.8 % of sunitinib treated patients, compared to 0 % in the placebo group. Stable disease for more than 22 weeks occurred in 17.4 %, compared to 1.9 %. Time to progression was significantly longer in the sunitinib treated patients, 27.3 weeks compared to 6.4 weeks. The most common non-hematological adverse events were fatigue, diarrhea, nausea, sore mouth, and skin discoloration. From a biological point of view, continuous dosing of sunitinib seems more logical. A study in 28 patients with advanced imatinib-resistant GIST explored the continuous daily 37.5 mg dosing regimen, which was feasible and associated with similar tolerability as is seen with intermittent sunitinib dosing [179]. It showed a potentially beneficial effect in previously treated advanced NSCLC and unresectable neuroendocrine tumors in phase II studies [180, 181].

Sorafenib is a novel oral Raf-1 kinase, PDGFR and VEGFR kinase inhibitor with antitumor effects in colon, pancreas and breast cancer cell lines and in colon, breast and non-small-cell lung cancer xenograft models [182]. A phase I study in 69

patients with refractory solid tumors reported promising results [183]. In a recent phase II randomized discontinuation trial in patients with metastatic renal cell carcinoma, sorafenib showed anti-tumor activity and was well tolerated [184, 185]. An interim analysis of a phase III trial randomizing 769 patients with advanced RCC to sorafenib 400 mg bid or placebo reported an improvement of progression free survival from 12 weeks to 24 weeks in sorafenib treated patients compared to placebo [186]. Updated results reported at the ASCO 2006 meeting showed a survival benefit for sorafenib over placebo (median overall survival of 19.3 months vs. 15.9 months) [187]. Such drug was granted FDA fast track approval in December 2005. Phase III trials in stage III or IV melanoma and in advanced hepatocellular carcinoma, and phase II trials in multiple tumor types are currently ongoing. It has previously been suggested that rash commonly associated with EGF-pathway inhibitors could be predictive of treatment outcome, and that the onset of rash could be used for optimal dose titration [188]. This might also be effective in treatment with sorafenib, as it is an inhibitor of Raf kinase, which is a downstream effector molecule of the EGFR signaling pathway. A report combining data from four phase I trials supported this hypothesis. Patients receiving sorafenib dosed at or close to the recommended dose of 400 mg bid, and experiencing skin toxicity and/or diarrhea, had a significantly increased time to progression compared with patients without such toxicity [189].

Regorafenib is an oral multi kinase inhibitor targeting both tumor cell proliferation/survival pathways (Raf/MEK/Extracellular-Signal-Regulated Kinases-ERK) and selected RTKs such as VEGFR-2/3, TIE-2, PDGFR, RET, and c-KIT. In vitro, it potently inhibits a distinct set of kinases, including the angiogenic and stromal RTKs VEGFR-1–3 [190]. Regorafenib increases the overall survival of patients with metastatic CRC [191] and has been approved by the United States Food and Drug Administration in 2012. Furthermore, a recent study has shown that regorafenib alone or in combination with irinotecan significantly delayed tumor growth in CRC cell lines, patient-derived (PD) CRC xenografts and a murine CRC liver metastasis model [192]. Finally, Tang and al reported the clinical history of an elderly woman with KRAS wild-type colon cancer who was treated with different agents. Here, they described a protocol treatment with regorafenib in dose modification strategies to address adverse events in such patient who was able to remain on therapy for 11 months and achieve stable disease [193].

Bevacizumab, is a recombinant humanized IgG1 monoclonal antibody with multiple cancer indications. It has been approved in combination with chemotherapy for the treatment of metastatic colorectal cancer (CRC), metastatic breast cancer, unresectable advanced, metastatic or recurrent non-small cell lung cancer, advanced or metastatic renal cell cancer, advanced epithelial cancer of the ovary, the fallopian tube or the peritoneum and as a single agent for advanced glioblastoma [194]. Bevacizumab targets circulating vascular endothelial growth factor (VEGF or else VEGF-A), a vasculogenesis and angiogenesis regulator that is overexpressed in most human tumors. By blocking VEGF-A binding to its receptors (VEGFR-1 and VEGFR-2) on the surface of endothelial cells, this drug inhibits tumor angiogenesis, growth and metastases [195, 196]. Despite its efficacy, it has been associated

with significant risk of cardiovascular complications, such as hypertension, cardiac ischemia, and congestive heart failure [197–199].

Aflibercept was designed to block angiogenesis by binding VEGF A, VEGF B, PlGF1 and PlGF2 and prevent downstream biological effects [200]. It is a recombinant humanized fusion protein which consists of the extracellular domains of VEGFR1 and VEGFR2 with the constant region (Fc) of human immunoglobin G1 [200]. It has a higher VEGF A binding affinity than bevacizumab [dissociation constant (Kd) of ~1 pM] [201] compared with around 500 pM for bevacizumab [202]. Its ability to bind to VEGF B and PlGF in addition to the high binding affinity for VEGF A may provide more complete blockade of angiogenesis. Preclinically, treatment with aflibercept resulted in tumor growth inhibition in a variety of xenograft models, including human colon cancer [203, 204]. In addition, such drug in a phase III randomized trial in combination with FOLFIRI as second-line treatment of mCRC, has shown to improve overall survival and progression free survival compared to chemotherapy alone [205].

2.5 Resistance to TKIs in Different in Types of Cancer

2.5.1 Lung Cancer

2.5.1.1 Primary Resistance

This resistance is linked to various EGFR mutations: exon 19 deletions and L858R, exon 20 insertions or duplications [206–208] and secondary genetic alterations. For instance, a T790M mutation within EGFR has been occasionally found in tumor specimens containing classic EGFR mutations [209–211] whereas, MET amplification has been revealed in EGFR-mutant tumors before TKI exposure [211–213]. Both alterations are common mechanisms of acquired resistance, but they may trigger intrinsic resistance when there is high allelic frequencies. Alternatively, T790M or MET amplification may emerge as dominant clones early during therapy.

2.5.1.2 ALK

The anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase that is aberrant in a variety of malignancies. Multiple different ALK rearrangements have been identified in approximately 3–7 % of unselected NSCLC [214, 215] although some studies have found a slightly lower prevalence [215]. The majority of these ALK fusion variants result most commonly in fusions of portions of the echinoderm microtubule-associated protein-like 4 (EML4) gene with chromosome 2p the ALK gene. At least nine different EML4-ALK fusion variants have been identified in NSCLC [216–218]. The fusion proteins created by these rearranged genes have functions of both anaplastic lymphoma kinase and the partner protein. The presence

of the partner protein allows phosphorylation of anaplastic lymphoma kinase without dimerization. The oncogenic EML4-ALK fusion protein has a constitutively activated kinase and is linked to cell proliferation and inhibition of apoptosis mediated through the RAS/RAF/MAPK, PI3K/AKT and JAK3-STAT3 signaling pathways. ALK rearrangements are more commonly found in Adenocarcinoma (ADC) from younger patients who are never smokers or light smokers [215, 216]. ALK rearrangements are usually mutually exclusive with EGFR and KRAS mutations [215]. The United States Food and Drug Administration approved the ALK Break Apart fluorescence in situ hybridization (FISH) Probe Kit as a companion diagnostic for targeted therapy with Crizotinib in lung cancers [219]. In the near future, selection of patients with EML4-ALK fusion oncogene will be important, since crizotinib, an ALK targeted inhibitor, has shown very promising results in a phase I and III trials [220, 221].

2.5.1.3 Heterogeneity of TKI Response

The cancer cells can respond to ALK and EGFR TKIs involving differences within the cellular apoptotic machinery. In particular, recent data found that both Bim upregulation is correlated to the degree of apoptotic response in EGFR-mutant cell lines treated with EGFR [222–225]. Similarly, Bim inhibition can favor the selection of intrinsic resistance to EGFR TKIs. Consistent with these preclinical findings, pretreatment with specific BIM shRNA resulted in decreased tumor shrinkage in EGFR-mutant patients. Furthermore, one recent report shows that a genetic polymorphism in BIM is correlated with both alternative splicing and altered BIM function. Such association could contribute to intrinsic resistance in some patients [226].

2.5.1.4 Acquired Resistance

Mechanisms of acquired resistance can involve development of additional genetic alterations in the primary oncogene, which promotes downstream signaling. Alternatively, resistance can occur through activation of downstream signaling pathways, changes in tumor histology, or alterations in drug metabolism [221]. Unfortunately, virtually all patients who initially respond to EGFR-TKIs eventually develop acquired resistance throughout the course of their treatment [227]. Studies have shown in approximately 50 % of these cases that this resistance is due to a secondary mutation, T790M in exon 20 of the EGFR gene [228–230]. The EGFR T790M mutation is oncogenic by itself, but when present in conjunction with another EGFR activating mutation, the double mutant leads to a substantial increase in EGFR signalling and oncogenic transformation both in vivo and in vitro [227]. As such, the T790M mutation is associated with a short median progression free survival (PFS) of only 7.7 months in patients carrying the mutation compared with 16.5 months in those without the mutation [231]. Biochemical studies combined with the molecular analysis of tumour material from patients whom have

developed resistance have shown that the T790M mutation is a common cause of acquired resistance in NSCLC patients. The threonine at position 790 is the 'gatekeeper' residue as its location at the entrance of the hydrophobic pocket of the ATP binding cleft means it has important role in determining inhibitor specificity in the receptor [232]. As such, Yun et al. [232] carried out enzyme kinetic assays using an ATP/NADH coupled assay system whereby they could detect the rate of ATP hydrolysis in both the wild-type and T790M mutant. The kinetic characterisation of the WT and the mutant EGFR kinases revealed a marked decrease in the Michealis-Menton constant (Km) for ATP in the drug resistant T790M mutant compared with the drug sensitive L858R mutant. Consequently, it is now thought that the T790M restores the receptors affinity for ATP, enabling ATP to outcompete TKIs for the ATP binding cleft of the tyrosine kinase domain, re-establishing signalling from EGFR. Secondary mutations in the ALK kinase domain have found in approximately 30 % of ALK-positive patients with crizotinib resistance [233, 234]. One report shows that one ALK-positive patient developed disease progression after 5 months of treatment with crizotinib. Furthermore, analysis of pleural fluid from this patient found revealed two mutations, L1196M and C1156Y, within the ALK kinase domain. Both mutations caused crizotinib resistance in vitro. Since the initial case report of crizotinib resistance, additional second-site ALK mutations have been identified in patient-derived NSCLC specimens (1151Tins, L1152R, G1202R, S1206Y, and G1269A). Such mutations are present in the kinase domain, including the solvent front (G1202R, S1206Y), gatekeeper residue (L1196M), ATP-binding pocket (G1269A), and N-terminal to the C-helix (1151Tins, L1152R, and C1156Y). In vitro, they confer differential sensitivities to crizotinib and second-generation ALK TKIs. For example, the ALK S1206Y mutation confers lower degrees of crizotinib resistance compared with G1202R, L1196M, and 1151Tins mutations. It is noteworthy that ALK-positive patients develop multiple secondary mutations at the time of TKI resistance. This is in contrast to EGFR-mutant patients, in whom T790M is essentially the sole secondary mutation observed clinically. Gene amplification may also contribute to secondary resistance in correlation with mutations. In fact, a study on 37 EGFR-mutant patients with resistance to TKIs found EGFR amplification was identified in three patients (8 %) [235]. Such patients harbored simultaneous T790M mutations in EGFR [227, 235]. ALK fusion gene amplification has also been identified as a cause of crizotinib resistance [236, 237]. One study reported a crizotinib resistance in cell line models with amplification of wild-type EML4-ALK [237]. Other articles observed ALK fusion gene amplification in resistant clinical specimens [236]. In particular, a strong amplification was identified in 1 (7 %) of 15 specimens, whereas a separate study showed ALK copy number gain in 2 (18 %) of 11 patients [233]. One of these patients also had a secondary ALK G1269A mutation. Amplification of the MET receptor has also been shown to maintain ErbB3/PI3K/AKT signaling in the presence of gefitinib and cause resistance to EGFR targeted therapies in approximately 20 % of NSCLC patients [213]. In fact, clinical trials using combined EGFR and MET inhibitors in NSCLC patients with acquired resistance to gefitinib/erlotinib are currently underway. Activation of IGF-1R/IRS-1

signaling through loss of IGF binding proteins also drives gefitinib resistance in EGFR wild-type cancer cell lines [238]. Additionally, a recent study suggested that the MET ligand, HGF, can promote short-term resistance in two EGFR mutated cancer cell lines [239]. Both ligand-dependent resistance mechanisms maintain PI3K/AKT activation despite EGFR inhibition. However, differences between IGF and HGF ligand-driven resistance in terms of potency and activation of downstream signaling pathways have yet to be thoroughly examined. MET encodes a trans-membrane RTK for the HGF and is capable of activating ErbB receptors to drive cell migration, invasion, proliferation, survival and angiogenesis [240]. MET amplification has been detected in gastric and esophageal cancers [240, 241] and cell lines derived from such tumors display ligand-independent dependence on MET [242]. However, it remains unknown whether activation of MET by its ligand, HGF, is a mechanism utilized by human tumors to develop resistance to ERBB-targeted therapies. Recently, HER2 amplification was identified in 3 (12 %) of 26 EGFR-mutant patients with acquired resistance to TKIs [243]. Addional effectors downstream from EGFR have also been identified as potential mediators of resistance [235, 244, 245]. For example, PIK3CA mutations have been found in 5 % of EGFR-mutant patients with acquired resistance [235]. Different studies reported point mutations in BRAF in secondary resistance to TKIs [244]. Bypass tracts also contribute to resistance in ALK-positive NSCLC. Addional preclinical studies have shown that EGFR activation plays a crucial role in crizotinib resistance [236, 246]. In these models, two EGFR ligands such as EGF86 and amphiregulin are involved in the activation of EGFR [246]. In a latter report, one ALK-positive patient treated with crizotinib had an EGFR mutation and a negative ALK FISH test in a repeat biopsy specimen. Furthermore, KRAS mutations were also found in two ALK-positive patients during treatment with crizotinib, although one patient harbored this mutation pretreatment. Lastly, KIT gene amplification has been identified in 2 (15 %) of 13 crizotinib-resistant specimens, suggesting that this signaling pathway may also be co-opted to mediate resistance**.** Recently, Sequist and colleagues published a study detailing the histological and genetic alterations of 37 patients with drug-resistant NSCLC with EGFR mutations [235]. In this study, they identified the previously known T790M mutation and MET amplification as well as some novel mechanisms of resistance. The most surprising finding was the histological transition of 5 patients' tumours from NSCLC to Small Cell Lung Carcinoma (SCLC), and their subsequent sensitisation to standard SCLC treatments. Another rebiopsy study also revealed this phenomenon, albeit at a lower frequency. Arcila and colleagues [247] found 2 % of patients exhibited SCLC morphology in resistant tumours, where NSCLC had been observed before treatment, a substantially lower percentage than the 14 % identified by Sequist et al. [235]. Detection of EGFR-TKI refractory SCLC after an initial diagnosis of EGFR-mutant adenocarcinoma is rare but had been reported prior to the new studies. Zakowski describes one patient with adenocarcinoma who had a partial response to erlotinib treatment, but after disease progression was found to have SCLC, however this patient did not respond to etoposide, a common treatment for SCLC [248].

2.5.1.5 Other Mechanisms of Resistance

In preclinical models, EGFR TKI resistance has also been associated with insulin growth factor receptor signaling [249, 250] nuclear factor Nuclear Factor Kappa B (NFkB) activation, and loss of Phosphatase and tensin homolog (PTEN). Among ALK-positive patients, loss of the ALK fusion oncogene has also been raised as a potential mechanism of resistance [233]. In another study, Yu et al. have recently identified an patient with T790M mutation in one resistant sample and HER2 amplification [251]. Furthermore, EGFR mutant and ALK-positive patients can experience a disease "flare" on TKI discontinuation, presumably because of accelerated growth of TKI-sensitive clones [252, 253]. The dynamic nature of resistance underscores the value of repeat biopsies at each new phase of treatment to advance our understanding of resistance and guide clinical decision making [254, 255]. This also emphasizes the need to develop non invasive tools for monitoring resistance, such as mutational analysis of plasma DNA or circulating tumor cells [256].

2.5.2 Gastrointestinal Stromal Tumors

Treatment options for patients with advanced GIST were few until 2000s. Surgical resection has been the main therapy for GIST, with the main goal of complete resection and avoidance of tumor rupture [257]. The response rate to conventional chemotherapy agents was extremely low [258]. The resistance to chemotherapy in GIST might be due to the increased levels of P-glycoprotein and multidrug resistance protein [259]. Alternatively, oncogenic activation of tyrosine kinases might cause increased anti-apoptotic signaling and activation of other drug resistance pathways. Because of the diffuse pattern of recurrence in the liver or the peritoneum, radiation therapy was beneficial only to palliate patients with bleeding, but not for treatment. Median overall survival for patients with advanced disease was 18 months until imatinib was introduced [258]. Based on the discovery of KIT mutations in GIST by Hirota and co-workers in 1998 [260], many scientists investigated KIT activation as a crucial event in GIST pathogenesis. The mutations causing constitutive kinase activation and an uncontrolled cell growth behavior in GIST, was reminiscent of the mechanism of BCR-ABL in CML. These findings led to the hypothesis of KIT inhibition might be a therapeutic strategy for GIST. Concurrently, imatinib was shown not only specific to BCR-ABL, but also blocks the enzymatic activity of the transmembrane receptor tyrosine kinases KIT, PDGFRα and PDGFRβ [261, 262]. The inhibitory effect of imatinib on mutant KIT was functionally confirmed first in a mast leukemia cell line that harbors a similar mutation as clinically relevant GISTs [262, 263], then in the GIST cell line with a mutant KIT [264]. Inhibition of mutant KIT by imatinib led to GIST cell growth arrest and apoptosis [264]. Thereafter, clinical development of imatinib for GIST therapy rapidly progressed, and FDA approved imatinib therapy for advanced or metastatic GIST in 2002. In 2008, FDA approved adjuvant

use of imatinib for patients with high risk of recurrence. In 2000, imatinib treatment of the first patient with metastatic GIST started in Finland, and a dramatic response was observed [265]. The following Phase I and II trials reported partial response rates as 54 % and 68 %, respectively, and majority of the remaining patients achieved a stable disease [266]. Promising results led to two phase III trials, comparing the dose levels of imatinib (400 and 800 mg/day). These studies reported that imatinib achieved disease control in 70–85 % of patients with advanced GIST, median progression-free survival was 20–24 months, and median overall survival was 50 months [4]. A study evaluated the effect of imatinib therapy using positron emission tomography on fluorodeoxyglucose (FDG) levels revealed that tumors had a robust response to imatinib present a significant decrease in FDG signal, even within 24 h of the first dose [267]. This result suggests that a decrease in glycolytic mechanism is one of the initial effects of kinase inhibition. At molecular level, imatinib was shown to inhibit oncogenic signaling that down-regulated downstream survival pathways such as PI3K-AKT and MAPK [268], and to induce cell apoptosis through BIM [269] and soluble histone H2AX [270]. In addition, imatinib reduces the expression of indoleamine 2,3-dioxygenase (IDO), which is an enzyme produces immunosuppressive metabolites [271]. Reduction of IDO causes depletion of regulatory T cells and increase of tumor-infiltrating CD8+ T cells. Thus, imatinib stimulates an anti-cancer immune response by diminishing IDO-mediated immunosuppression. Clinical observations demonstrate that long-term imatinib treatment is not sufficient to eradicate GIST cells. In order to determine the optimal duration of imatinib therapy, an interesting clinical trial was conducted with patients who had continuous disease control after 3 years of imatinib treatment [272]. Patients were grouped as either to continue or to discontinue the treatment. The 2-year progression-free survival rates were 80 % in the continuous patient cohort and only 16 % in the discontinuous cohort. The relapse after the discontinuation of imatinib was due to persistent disease, showing that imatinib fails to eradicate cells although it stops their proliferation. The progression during continuous imatinib treatment was due to resistant disease. Another study investigated the histological responses of the tumors upon imatinib treatment [273]. After a range of 1–31 months of treatment, tumors showed a size reduction range between 10 and 90 %. Overall responses did not correlate with duration of treatment or KIT and PDGFRα mutational status. The residual tumor cells in the 75 % of the tumors were mitotically inactive, showing a quiescent state. These results demonstrate that GIST cells may avoid apoptosis by evading the cell cycle under imatinib exposure. Indeed, imatinib was shown to cause tumor cell quiescence through the APC/CDH1-SKP2-p27Kip1 signaling axis [274], and to induce autophagy that protects tumor cells from cell death [275]. Only a small percentage of patients (3–5 %) show a complete disappearance of their tumor upon imatinib treatment [276, 277]. However, it was reported that patients with tumors that shrink or remain stable in size show a similar clinical benefit from the treatment [278].

2.5.2.1 Imatinib Resistance

The majority of GIST patients with advanced disease achieve a clinical benefit from imatinib treatment. However, approximately 10 % of patients progress within 6 months of initial therapy, which is defined as primary resistance to imatinib [266, 278–280]. Approximately 50–60 % of the initially responding patients develop disease progression within 2 years. Such cases are regarded as secondary or acquired resistance to imatinib [266, 278–280].

2.5.2.2 Primary Imatinib Resistance

Primary resistance can be observed in GISTs with all kind of known mutations, however, it shows stronger correlation with certain genotypes [281–284]. For example, wild-type, KIT exon 9 mutated, and PDGFRα D842V mutated GISTs are more likely to show primary resistance. In experimental cell culture systems, GIST cells expressing exon 11 mutant KIT are highly sensitive to imatinib [285]. Correspondingly, patients with GISTs harboring KIT exon 11 mutations have a better progression-free and overall survival compared to patients with wild-type GISTs or GISTs harboring KIT exon 9 mutation [281, 284, 286]. The primary resistance arises in GISTs with no identifiable KIT or PDGFRA mutations is likely due to different mechanisms causing the disease development and activation of alternative signaling pathways. Therefore, treatment of these GISTs with the targeted agents other than imatinib, such as VEGFR, BRAF or MEK inhibitors, might be a better clinical alternative [287]. Mutations in exon 9 affect the extracellular KIT domain, mimicking the conformation change when Stem Cell Factor (SCF) binds to the receptor, which induces higher degree of dimerization [288]. Since this mutation does not interfere with the kinase domain, exon 9 mutated KIT has the kinase domain same as the wild-type KIT, in which decreased sensitivity to imatinib was observed in vitro compared to exon 11 mutant KIT [289]. Dose escalation is suggested for treatment of GISTs harboring these mutations [286]. Both clinical and in vitro studies have reported that PDGFRα D842V mutation is strongly resistant to imatinib [284, 290, 291]. This mutation results in a change in the kinase activation loop that strongly favors the active conformation of the kinase domain, which consequently disfavors imatinib binding [283, 292]. Patients with D842V mutant GISTs show low response rates and short progression-free and overall survival during imatinib treatment [293]. In addition to mutations, gene amplification of KIT or PDGFRα was shown as a potential mechanism leading to either primary or secondary resistance [294–296].

2.5.2.3 Secondary Imatinib Resistance

Secondary mutations in the same gene is the main known mechanism for developing secondary resistance [297–300]. A clinical trial revealed that 67 % of the patients whose tumors showed secondary resistance had a new mutation in KIT [299].

Secondary mutations involve two regions in the KIT and PDGFRα kinase domains: (i) the ATP-binding pocket (encoded by exons 13 and 14) that directly interfere with imatinib binding, and (ii) the kinase activation loop (encoded by exons 17 and 18) that can stabilize the kinase in the active conformation and hinder imatinib binding. It is noted that most of the imatinib-resistant tumors exhibit extensive intra- and inter-tumor heterogeneity [295, 301, 302]. Five different types of secondary mutations had been observed in different areas of the same tumor, and up to seven different secondary mutations across the multiple tumors of the same patient [295]. This heterogeneity has important implications in regard to the efficacy of second-line TKI therapy after the first-line imatinib treatment, because the diverse resistant subclones render the complete eradication of GIST cells by any particular TKI. Several alternative mechanisms of imatinib resistance have been described. Kinase switching is one of them and number of kinases have been involved in such mechanism. The first one is AXL, which is an oncogenic RTK that regulates the same downstream signaling pathways as KIT. GIST cells switch from KIT to AXL during acquisition of imatinib resistance in vitro and in vivo [303]. In addition, another study reported a switch from KIT to Focal Adhesion Kinase (FAK) and FYN activation in GIST cells upon acquisition of imatinib resistance, and pFAK inhibition can re-sensitize the resistant cells to imatinib-induced cell death [304]. FAK has also been implicated in growth and survival of imatinib-resistant GIST cells, and FAK inhibition induces apoptosis in GIST cells and decreases tumor size in mice [305].

2.5.3 Colorectal Cancer

2.5.3.1 Mechanisms of Primary Resistance to EGFR-Targeted Therapy

In human tumors activating KRAS mutations occur frequently in colorectal cancer (CRC) where approximately 35–40 % of patients have KRAS mutations, which are classically described as being associated with progression of a benign adenoma to a dysplastic adenocarcinoma [306]. From this early role as a candidate target for directed therapy, today mutated KRAS has emerged as a confirmed predictor of resistance to many targeted cancer therapies [307]. While, the effects of mutated KRAS on chemotherapy are still debated, in targeted treatment KRAS is widely accepted as a resistance mechanism for growth factor receptor directed therapies such as EGFR inhibitors, where it serves as a clinical marker which excludes such treatment for patients with colorectal cancer [308]. Mutations in KRAS occurring at codons 12 and 13 were the first to be causally implicated in resistance to EGFR-targeted monoclonal antibodies, initially in small patient cohorts [309, 310]. Randomized phase III studies has shown patients with mCRC bearing KRAS mutations are chemorefractory to treatment with single-agent cetuximab or panitumumab [311, 312]. Additional biomarkers of resistance are found through sequencing studies. For example, KRAS variants in exon 2 at codons 12 and 13 and

oncogenic mutations in KRAS codons 59, 61, 117, and 146 colorectal cancer samples are observed approximately 80 % of colorectal cancer samples [313–315]. Additional mutations of the NRAS isoform are found at codons 12, 13, and 61 in approximately 3–5 % of colorectal cancer samples [316]. Such mutations are able to activate the ERK signaling, even if EGFR is blocked. Furthermore, several studies have recently demostrated that mutations in KRAS exons 3 and 4 or NRAS exons 2 to 4 can also predict lack of clinical benefit to EGFR-targeted antibodies given in combination with first-line chemotherapy [317–319]. Thus, mutant NRAS or KRAS have shown to predict lack of response to EGFR-targeted antibodies. This is hypothesized to result from redundant growth and survival signals relayed simultaneously through RAS which activates multiple effecters simultaneously. This led to the idea that concurrent inhibition of multiple KRAS effects may lead to patient response in tumors with a mutant KRAS, currently being tested in clinical trials. In CRC, BRAF mutations, occurring in about 10 % of cases, are found in both hyperplastic polyps and serrated adenomas, suggesting that they represent an early and critical event in these types of lesions [320]. More importantly, BRAF mutations are frequently found in sporadic cases characterized by Microsatellite Instability (MSI), the hallmark of MMR gene deregulation (30 % vs. 5 % of patients without MMR alteration. Furthermore, it has been shown that BRAF mutations occur in CRC only when tumors do not carry any mutation in the KRAS gene [321]. These mutual exclusions have led to the assumption that BRAF and KRAS alterations could have the same functional effect in colorectal carcinogenesis, although mutated BRAF protein has 50-fold lower transforming activity than mutated RAS [322]. Several experiments studies in colorectal cancer cells and mouse models ahave shown that the presence of BRAFV600E is strongly associated with resistance to cetuximab or panitumumab [323–325]. The statistical significant correlation between BRAFV600E mutations and response to therapy (patients receiving EGFR-targeted monoclonal antibodies in combination with first-line chemotherapy) was not confirmed by different studies that have found an association between BRAFV600E mutations and poor prognosis in mCRC [316, 319]. KRAS gene amplification has been involved in resistance to cetuximab in functional genetics experiments and has been associated with lack of response to anti-EGFR treatment [326, 327]. It has recently been proposed that other mechanisms of KRAS activation, such as overexpression by amplification and/or transcriptional changes, may also contribute oncogenesis and/or therapeutic resistance. KRAS copy number has been examined in gastric and colon cancer cell lines [328] and non-small cell lung cancer [329, 330], and recently has been studied in a small cohort of metastatic CRC patients [327]. It is becoming clear that increased KRAS gene copy number is another way of deregulating KRAS activity, and is likely to play a role in resistance to targeted treatment, and/or worse prognosis. Furthemore, other gene amplification such as NRAS, BRAF, and C-RAF are found at at a very low prevalence (<1 % cases for individual genes) through analysis from the TCGA colorectal cancer database [331, 332], but the clinical relevance of these findings is unknown. A very recent study has found that that patients bearing BRAF codons

594 or 596 mutated tumors had markedly longer overall survival (OS) when compared with patients bearing BRAF V600E mutated tumors [333]. Genetic aberrations of the RTK ErbB2 and MET have been shown to bypass EGFR signaling and activate the MEK–ERK cascade. C-ErbB2 (HER-2/neu) gene amplification, and consequent overexpression on the protein level, is associated with a small fraction of RAS and BRAF wild-type mCRC patient-derived xenografts that were insensitive to cetuximab treatment [325]. Concordant data were obtained by Yonesaka and colleagues [334], who showed that activation of EebB2 signaling, dependent on either gene amplification or overproduction of the ErbB3 ligand heregulin, was present in a subset of patients with mCRC exhibiting de novo resistance to cetuximab-based therapy. The c-MET gene is amplified in small portion of RAS and BRAF wild-type mCRC patient–derived xenografts that were insensitive to cetuximab treatment mCRC [335]. Additional molecular alterations of genes including PI3K–AKT–PTEN could also involved in the response to EGFR-targeted monoclonal antibodies. In CRC, Phosphoinositide-3-kinase-Catalytic-alpha (PIK3CA) is altered mainly through activating point mutations in about 10–30 % of CRC [336] but also PIK3CA amplification, although very rare, was reported. A PIK3CA mutation was also observed in a very advanced tubulovillous adenoma greater than 5 cm in diameter, suggesting that PIK3CA mutations arise late in colorectal tumorigenesis. The large majority of PIK3CA mutations cluster in two conserved regions, the helical domain (encoded by exon 9) and the kinase domain (encoded by exon 20). The hotspot mutations E542K and E545K (in exon 9), and H1047R (in exon 20), are non-synonymous missense mutations that confer a constitutive kinase activity to the protein. Rare mutations may also occur in exons 6 and 7 [337]. In CRC PTEN is altered through a mixed genetic/epigenetic mechanisms (intragenic mutation/epigenetic or 10q23 loss of heterozigosity (LOH)/epigenetic), which lead to the biallelic inactivation of the protein in 20–30 % of cases. In addition to PTEN LOH and mutations, PTEN promoter hypermethylation is a frequent event in MSI sporadic CRC and may constitute an important epigenetic mechanism of PTEN inactivation in this setting [338]. Moreover, PIK3CA and PTEN alterations (around 10–15 % overall) often co-occur with KRAS or BRAF mutations [331, 332, 339, 340] a feature that further complicates their assessment. In summary, the role of PIK3CA mutation and PTEN status in conferring resistance to EGFR-directed therapy in colorectal cancer remains highly controversial. Finally, nongenetic mechanisms can play a crucial role in resistance to EGFR blockade. In one report, it has been demonstrated that only a fraction of cells derived from biopsies of patients who relapsed upon cetuximab or panitumumab therapy, carred RAS mutations, suggesting that wild-type cells can also survive the treatment [341]. This finding suggests that nongenetic mechanisms could also play a role in driving acquired resistance to EGFR blockade. For example, a recent report [342] shows that sensitive (wild-type) cells can survive during the cetuximab treatment in presence of their resistant derivatives. Notably, it was found that cells bearing acquired RAS mutations oversecrete the EGFR ligands TGFα and amphiregulin, which protect the surrounding wild-type cells [342, 343]. This paracrine network could potentially be targeted to increase the efficacy of anti-EGFR therapies.

2.5.3.2 Mechanisms of Secondary Resistance to Anti-EGFR Therapy

In a study, Montagut and colleagues [344] found a point mutation in the extracellular domain of EGFR (S492R) in a colorectal cancer cell line that resulted resistant to cetuximab. This mutation was also found in very few patients at relapse after cetuximab treatment. Patients, carrying S492R mutation at relapse, could be treated with panitumumab, since this mutation does not interfere with its binding. Additionally, these authors found a patients with such mutation after cetuximab treatment who responded transiently to panitumumab therapy.

ErbB2 or MET gene amplifications are involved in acquired resistance to EGFR blockade in cell models and patient samples [334, 335]. Several reports confirmed the initial results on the emergence of MET gene amplification in patients who develop acquired resistance to EGFR blockade [345, 346]. The secondary resistance to anti-EGFR therapy in colorectal cancer is linked to the KRAS gene point mutations and gene amplification such as to NRAS and BRAF mutations [347–349]. A study was performed on circulating cell-free tumor DNA derived from plasma samples of patients with colorectal cancer at relapse. Mutations in KRAS (codons 12 or 13) and BRAF (V600E) were found in two patients [349]. Furthermore, mutations in the kinase domain of EGFR (codons 714 and 794) were identified in other two patients.

2.5.3.3 Other Mechanisms of Resistance

Bertotti et al. have identified HER2 gene amplification as a potential mechanism of resistance to cetuximab in mCRC that harbor normal, wild type K-Ras/N-Ras/Braf/PIK3CA genes. Furthermore, the amplifications of HER2 and increased expression level of heregulin have been identified in cetuximab-resistant cell lines [325]. A role for Insulin-like Growth Factor Receptors (IGFR) in acquired resistance to EGFR targeting therapies is suggested by a study in A431 cells where exposure to increasing amount of gefitinib resulted in a gefitinib-resistant cell line with increased signalling via the Insulin-like Growth Factor 1 Receptor/Insulin Receptor Substrate *1/*(IGF-1R/IRS-1)/PI3K pathway [325]. Notably, gefitinib resistant cells were also resistant to erlotinib and cetuximab, suggesting that IGF-1R up regulation may confer resistance to both antibody and small molecule TKI EGFR inhibitors. Finally, the combinatorial inhibition of IGF-1R and EGFR has been shown to enhance the efficacy of EGFR inhibitors in a variety of cancer cells in vitro [350–353]. Importantly, as for c-MET, antibody-based therapies which target the IGFR such as AMG 479, are currently in clinical trials including a phase II study in combination with the EGFR-targeting antibody, panitumumab, in advanced colon. Many reports suggest that Sphingosine Kinase 1/Sphingosine 1-Phosphate (SphK1/S1P) signalling is involved in the resistance of cancer cell to both chemotherapy and radiotherapy. More recently, higher levels in SphK1 expression have been observed to correlate with worse outcomes in a cohort of breast cancer patients [354]. In addition, it has been shown that enforced overexpression of human SphK1 in MCF-7

cells increases the resistance to tamoxifen of breast cancer cells, whereas the initial sensitivity to drugs is replaced through SphK1 knockdown [354]. A recent studies elucidated the molecular mechanism by which SphK1 induces the acquisition of resistance to the anticancer agent cetuximab in colorectal cancer cell lines. SphK1 know-down by both pharmacological inhibitor or silencing via siRNA in resistant cells restores sensitivity to cetuximab, whereas exogenous SphK1 overexpression in sensitive cells confers resistance to these agents. Moreover, treatment of resistant cells with fingolimod (FTY720), a S1P receptor (S1PR) antagonist, resulted in resensitization to cetuximab both in vitro and in vivo, with inhibition of tumor growth, interference with signal transduction, induction of cancer cells apoptosis, and prolongation of mice survival [355, 356].

2.5.3.4 Resistance to Angiogenic Inhibitors

Despite proven clinical benefit, virtually all mCRC patients receiving antiangiogenic therapy in the front-line setting eventually relapse. It is now recognized that tumors employ a number of mechanisms to escape (evade) anti-VEGF therapy, which are distinct from classic chemotherapy resistance. Proposed mechanisms of anti-VEGF escape include: (1) Increased production of VEGF by the tumor in response to treatment; (2) Upregulation of other proangiogenic growth factors (e.g., platelet-derived growth factor [PDGF], fibroblast growth factors [FGF], Ephrin-A1, angiopoietin-1) with concurrent downregulation of endogenous angiogenic inhibitors (e.g., endostatin, thrombospondin); (3) Cooption of existing blood vessels that are less susceptible to VEGF blockade; (4) Transformation of the tumor vasculature towards a more mature, less VEGF-dependent phenotype; (5) Contribution of VEGF and other growth factors by host stroma; and (6) Genetic selection for tumor cells exhibiting increased resistance to both hypoxia and chemotherapy [357–361]. In order to underlie molecular mechanisms during the hypoxia, it has been demonstrated that the Hypoxia-inducible factor 1α (HIF-1α) increased expression level induces the release of *basic* Fibroblast Growth Factor (bFGF). Such release further augments these hypoxic inductions through the PI3K and MEK1/ERK pathway [362]. In addition, under hypoxic conditions, PD98059 (a MEK1,2 inhibitor) is able to suppress the HIF-1 activation, suggesting that the ras signaling cascade may also be involved in the resistance to anti-VEGF agents. Furthermore, a bFGF inhibitor (brivanib, BMS-582664) can significantly resume both angiogenesis and tumor progression in mouse resistant bevacizumab model of pancreatic islet [363]. Currently, only very few drugs are available to target the FGF receptor. Thus far, brivanib has been evaluated in combination with cetuximab in a phase I trial of patients with chemorefractory gastrointestinal malignancies. A recent study has been conducted on 34 patients with advanced or metastatic colorectal cancer (five patients (15 %) had a PR (palliative resection) and 19 patients (56 %) had stable disease) [364]. In a subsequent analysis, this effect was restricted to wild-type K-Ras tumors [365]. The phase III trial is evaluating the combined use of brivanib and cetuximab without chemotherapy in second/third-line therapy [366]. Thus, it is possible to improve the

efficacy of antiangiogenic therapies by suppressing bFGF-induced vascular regrowth. Another important cellular mechanism, underlying refractoriness to anti-VEGF therapy, has represented recruitment of CD11b+Gr1+ myeloid cells into the tumor tissue [367]. Broad-spectrum TK inhibitors, including sunitinib and sorafenib, also exhibit myeloid suppression by inhibiting c-KIT and flt-3 kinase (Fms-like tyrosine kinase 3) activation [368]. In addition, Granulocyte Colony-stimulating Factor (G-CSF) and Bv8 (prokineticin 2) show the strongest correlation with refractoriness to anti-VEGF in different tumor models [369]. Administration of anti-Bv8 mAb resulted in reduced tumor angiogenesis and growth, and was additive to anti-VEGF mAb in refractory tumors. Strikingly, anti-G-CSF treatment reduced circulating and tumor-associated myeloid cells to the levels detected in mice bearing sensitive tumors [370]. Another recent paper shows Notch-1 was significantly upregulated in regorafenib resistant colon cancer (Reg-R-SW480 cells) as well as HES1 (hairy enhancer of split 1) and HEY (Hairy/E (spl)-related (Hey) gene family members with YRPF motif). Additionally, inhibition of Notch-1 in resistant cells partially restored sensitivity to regorafenib treatment in vitro. Collectively, these data suggest a key role of Notch-1 in mediating the resistant effects of regorafenib in colorectal cancer cells [371]. Research has also shown that tumor vasculature can regrow aggressively soon after anti-VEGF therapy is halted. Therefore, discontinuing anti-VEGF therapy in the face of tumor growth could initiate even more rapid disease progression. Several strategies being explored to combat anti-VEGF treatment evasion include the use of dual antiangiogenic agents that target different growth pathways, sequencing of drugs, and using single agents that target multiple pathways such as regorafenib.

2.5.4 Kidney Cancer

With an incidence of about 2 % of all solid tumors, cancer of the kidney is not as common as breast or prostate cancer. However, it still claims 116,000 lives every year out of the 274,000 new cases diagnosed worldwide. The most common form of renal cancer is the conventional (clear cell) renal cell carcinoma (CCRCC). It constitutes 75 % of all renal carcinomas.

2.5.4.1 Intrinsic Resistance in ccRCC

Huang et al. have published an article where they believe that sunitinib targets mainly ccRCC endothelium and it inhibits tumor growth in this way [372]. However, Adelaiye et al. treated mice bearing two different patient-derived ccRCC xenografts (PDX) with a dose-escalation schema (40–60–80 mg/kg for 5 days in a week. Subsequently, they found that PDXs, although initially responsive to sunitinib at 40 mg/kg, eventually developed resistance. The tumor growth was blocked after an increased dose of sunitinib in PDXs. A resistant phenotype was correlated to both

with transient increase of tumor vasculature and changes in the expression of the methyltransferase EZH2. The stable transfected cells, 786-0shEZH2_D were treated with specific EZH2 inhibition, the antitumor effect of sunitinib increased in this cell line. Thi data suggest that epigenetic changes could be correlated with sunitinib resistance [373].

2.5.4.2 Epithelial-Mesenchymal Transition (EMT) Mechanism and Tumor Associated Macrophages (TAMs) and Resistance

In 2011, Gotink et al. have shown that resistance to sunitinib is transient in cell line models [374]. Such mechanism can be dependent on epithelial to mesenchymal transition (EMT), where polarized epithelial cells convert into motile mesenchymal cells. Reverse epithelial-mesenchymal transition and acquired resistance to sunitinib were investigated in ccRCC in xenograft studies. A histological examination from 2011 showed that EMT may be associated with acquired resistance to TKIs. Consistent with these findings, Hammers et al. [375] have demonstrated in transplanting sunitinib resistant cells (obtained from ccRCC skin metastasis biopsy) into nude mice, that density of microvessels was reduced. Moreover, mesenchymal marker, vimentin, as well as HIF-1α (which is involved in cell adaptation to the state of hypoxia; this, in return, induces VEGF and PDGF expression) had an elevated expression correlated with Sonic hedgehog signaling (Shh). Behnsawy et al. found that ccRCC is dependent on Shh signaling that induces EMT markers, such as E-cadherin [376]. Furthermore, Tumor-associated macrophages (TAMs) are also involved in the process of epithelial mesenchymal transition. Macrophages are a heterogeneous population of cells derived from monocytes. They show two different polarization states, M1 and M2 macrophages, in response to different microenvironmental signals. M1 macrophages are proinflammatory and characterized by the release of mainly inflammatory cytokines and microbicidal/tumoricidal activity. M2 macrophages have an immunosuppressive phenotype and are polarized by anti-inflammatory molecules such as Interleukin-4, -3 and -10 (IL-4, IL-13, and IL-10), apoptotic cells, and immune complexes. M2 macrophages release anti-inflammatory cytokines and have scavenging potentials as well as supporting angiogenesis and tissue repair. Monocyte/macrophage cells are important for tumour cell migration, invasion and metastasis. TAMs represent the M2-type and promote tumour progression [377, 378]. Monocytes are actively recruited to the tumour stroma, and high infiltration of TAMs in many tumour types correlates with lymph node involvement and distant metastasis [379]. Experimental studies show that inhibition of macrophage infiltration in tumours may inhibit metastasis and progression of secondary tumours [380, 381]. The clinical significance of macrophage infiltration in tumour stroma, however, is still controversial. High infiltration of TAMs is correlated to poor prognosis in different cancer including breast, prostatic, ovarian cervical carcinoma and ccRCC. TAMs contribute to angiogenesis, lymphangiogenesis and tumour progression by expressing pro-angiogenic growth factors such as Matrix Metalloproteinase 12 (MMP-12), IL-1, VEGF and IL-8 [382, 383]. Clinical studies

have shown that increased infiltration of TAMs in ccRCC tumours is associated with high micro-vessel density and poor prognosis. Dannenmann et al. state that ccRCC may even attract these macrophages when they are still undifferentiated and 'change' them into M2 phenotype. Their analysis confirmed that only M2, but not M1 macrophage phenotype is associated with more advanced tumor stage [384]. All in all, TAMs are key components in EMT process and represent a promising target for anti-ccRCC therapy.

2.5.4.3 Other Mechanisms of Resistance

Huang et al. showed that an elevated expression of interleukin-8 in sunitinib resistant ccRCC cells However, the co-administration of IL-8 neutralizing antibody and sunitinib rendered sensitive cells resistant to TKIs treatment [385]. In addition, the authors did not find mutations in FLT1 (VEGFR-1), KDR (VEGFR-2), FLT4 (VEGFR-3), PDGFR-α, PDGFR-β, c-KIT, and RET genes. This means that probably resistance to TKIs is mediated via VEGF/VEGFR mutation-independent mechanism [386]. The 'angiogenic switch' was documented not only in the in vitro, but in the in vivo studies as well in ccRCC animal models.

Pericytes physically surround the capillary endothelium, contacting and communicating with associated vascular endothelial cells via cell–cell and cell–matrix contacts. Pericyte–endothelial cell interactions thus have the potential to modulate growth and function of the microvasculature. They are well-known to stabilize blood-vessel endothelial cell function both during homeostasis and tumour-associated angiogenesi For this reason, they can play a crucial role in pathological over-angiogenesis of tumors and therefore are now potentially new therapeutic targets, especially for such well-vascularized tumors as clear-cell renal cell carcinoma [387, 388]. In 2013, Cao et al. have showed that higher number of pericyte-generated microvessels in serum is associated with much more aggressive type of ccRCC and with its higher resistance to treatment as well [389]. Recent data revealed that sunitinib is sequestered in lysosomes during treatment of clear-cell renal cell carcinoma. Gotink et al. have observed that this drug was concentrated in acidic lysosomes. This mechanism was assessed as reversible and mainly connected with sunitinib because of its chemical features [390].

2.5.5 Bladder Cancer

2.5.5.1 Mechanism of Resistance in Bladder Cancer

In one study, activation of glycogen synthase kinase-3β (GSK-3β) predicted sensitivity to gefitinib [391]. Sensitivity to gefitinib in gefitinib-resistant cell lines is associated with the inhibition of PDGFRβ. This study reported that the mechanism of resistance to gefitinib in a subset of bladder tumors is correlated with PDGFRβ

upregulation, that is able to switch off GSK-3β expression by passing EGFR signaling. In another study, the authors showed that growth inhibition during gefitinib treatment is correlated with both p27 protein expression and decreased cyclin-dependent kinase 2 (cdk2) activity [392]. Furthermore, it has also been found that some bladder cancer cell lines can respond differently to cetuximab treatment. These data can be associated with mutations in EGFR or differential expression of molecules that are able to modulate EGFR activity. In particular, the loss of E-cadherin correlates to resistance to cetuximab in some cell lines [393]. In another study, the authors were found that the resistance to gefitinib and lapatinib in UM-UC-5 cells is linked to inhibition of EGFR, HER3, MET and ERK1/2 and activation of p53 phosphorylation [394]. In addition, Knievel et al. reported the mechanism of resistance in different bladder cancer cell lines treated with sorafenib [395]. The results demonstrate that sorafenib inhibits MAPK signaling only to a limited degree in T24 cells that carry a known activating HRAS mutation. Such result may be attributed to the so called "RAF inhibitor paradox" [396, 397]. Even though RAS proteins are upstream activators of RAF proteins, RAF inhibitors are poorly active and can even exert paradoxical effects in cell lines with RAS mutations [396]. Of note, another study showed that efficacy of sorafenib was also lower in T24 cells when compared to two other Urothelial Cancer Cell Lines (UCCs) [398]. Moreover, a more detailed reports detected that this drug did not inhibit such pathway in the very sensitive cell line, VMCub1. These results were correlated with physiological feedback effects within the MAPK signaling pathway [399], which contribute to the RAF inhibitor paradox, but also occur during normal responses to growth factors. In addition, the crosstalk between the MAPK and the PI3K-pathway may attenuate sorafenib effects In an experiments study, this crosstalk resulted in an increased AKT phosphorylation in T24 cells [400]. Additionally, such drug is able to induce a cell cycle arrest but a limited degree of cell death in UCCs, when it is used at high concentrations. These results may depend on multiple mechanisms, including the expression of anti-apoptotic proteins like Myeloid cell leukemia-1(Mcl-1), B-cell lymphoma-extra large (Bcl-X_L) and c-FLIP [401].

2.5.6 Prostate Cancer

2.5.6.1 Mechanism of Resistance in Prostate Cancer

Prostate cancer (PC) has been mostly associated to the EGFR dysfunction, which after its activation induces cell survival, proliferation, invasion, metastasis, and thus, drug resistance [402, 403]. Furthermore, PC drug resistance can also occur through downstream EGFR genetic mutations, such as the KRAS, which in turn could activate the EGFR in an autocrine manner [404, 405]. One study found that the continuous in vitro exposure of the phosphatase and tensin homologue (deleted from chromosome 10)-negative prostate cancer PC3 cell line to gefitinib resulted in a sustained growth inhibition of 50 % for about 2 months, but afterwards the surviving

cells resumed their usual proliferation rate. During chronic treatment, gefitinib-treated cells developed drug resistance undergoing a G0/G1 cell cycle arrest, with a corresponding reduction in the G2/M cells without evident cell apoptosis, and thus a tyrosine kinase inhibitor-resistant (TKI-R) PC3 cell subline was isolated. TKI-R cells show (i) an increment in basal ERK activation, (ii) an epidermal growth factor-mediated and gefitinib insensitive ERK phosporylation, (iii) increased levels of Her2/Neu, (iv) a significant decrement in EGFR expression, (v) a very low sensitivity against EGFR TKIs and blocking antibodies, (vi) a moderate increase in the sensitivity to growth inhibition by the Her2 inhibitor, AG825 or by 2C4, the humanized monoclonal antibody which blocks Her2 heterodymerization, (vii) an increased expression of the neutrophine receptors, Tropomyosin-receptor-kinase A and B (TrkA and TrkB), and (viii) a significantly increased sensitivity to growth inhibition by the TrkA inhibitor, CEP701 [406]. IL-6 plays a critical role in prostate cancer pathogenesis at the molecular level [407] and its upregulation is associated with disease progression in patients [408, 409]. A report demonstrated that IL-6 increased in both in-vitro and in-vivo in the presence of TKIs in resistant PC-3 cells but not in TKI-sensitive DU-145 cells. These findings suggest that IL-6 may represent a biomarker for TKI resistance in patients with castration-resistant prostate cancer (CRPC) [410]. Finally, Siu et al. showed that miR-203 expression suppresses bone metastasis and induces apoptosis in TKIs-resistant Ras-activated prostate cancer cells in which miR-203 is down-regulated by EGFR signaling. The authors determined that the 3′ UTR of the mRNAs of EGFR ligands (EREG and TGFA) and anti-apoptotic proteins (API5, BIRC2, and TRIAP1) are direct targets of miR-203. Importantly, inhibition of miR-203 induced both cell invasion and resistance to TKIs treatment in prostate cancer cells, implying a dominant biological function for miR-203 in the EGFR network. These data suggest regulatory mechanisms whereby tumors with low miR-203 output, have an increase in EGFR ligands (EREG and TGFA) and anti-apoptotic proteins (API5, BIRC2, and TRIAP1) expression, resulting in prostate cancer bone metastasis and TKIs resistance [411]. However, different anti-angiogenic drugs have been tested in phase II and III trials in prostate cancer patients [412], including sunitinib and sorafenib. These two drugs are small molecule inhibitors of multiple intracellular and receptor protein kinases such as VEGFRs, PDGFRβ, cKIT and RET, expressed on the cell membrane. In addition, sorafenib is also able to directly target on the RAF intracellular pathway [413]. Several experiments have shown the difference between “normal” endothelial cells and endothelial cells within a tumor-context [414]. Studies have also revealed distinct gene expression profiles of tumor-derived endothelial cells (TECs) and identified cell-surface markers distinguishing tumor vs. normal endothelial cells [415]. In particular, the chromosomal instability is the most remarkable abnormality TECs [416], that express embryonic genes [417–419]. Finally, such cells show an increased survival, proliferation and angiogenic properties [417], as well as resistance to chemotherapeutics [418]. In a recent study, the authors have isolated and characterized three lines of TEC from three different prostate cancer human samples (PTEC). Moreover, they evaluated the effect of two anti-angiogenic drugs, sunitinib and sorafenib, on typical aspects of the angiogenic process such as the

ability to form functional blood vessels in vivo, in vitro proliferation, survival, tubulogenesis and motility. They found a resistant behavior of endothelial cells isolated from prostate cancer to sorafenib, but not sunitinib [419].

2.5.7 Breast Cancer

2.5.7.1 Mechanism of Resistance in Breast Cancer

The heterogeneity of breast cancer requires therapies tailored towards each patient's molecular profile. Such individual approaches are currently applied within precision medicine. As molecular profiles change with tumour development and under drug treatment, latest therapy approaches imply sequential application of targeted therapies guided by biomarker changes [420]. HER2 is such a biomarker including its downstream targets such as mTOR. Specific therapeutics were designed to prevent HER2 induced deregulated protein signalling, contributing to tumour progression. The monoclonal antibody drug trastuzumab has especially been designed to target HER2 [421]. Trastuzumab is a monoclonal antibody that binds to the extracellular domain of ErbB2. FDA approved the use of trastuzumab in clinics in 1998. Several publications have shown that in conjunction with adjuvant chemotherapy, trastuzumab lowers the risk of recurrence in ErbB2 positive breast cancer patients, compared to chemotherapy alone [422] and has significant effect on patient survivorship [423]. So it is in general combined with chemotherapy. The improved outcome due to the addition of trastuzumab is not completely understood. Besides inhibition of HER2, its dimerisation and cleavage, it has been associated with different mechanisms of action. These include inhibition of downstream signal transduction pathways like PI3K, antibody-dependent cellular cytotoxicity (ADCC), induction of cell cycle arrest and apoptosis or inhibition of tumour angiogenesis [424]. Pertuzumab prevents the formation of HER2 dimers, especially the most potent ones including HER3. The combination with trastuzumab-based chemotherapy is synergistically associated with improved clinical outcomes and was approved as neoadjuvant therapy for HER2-positive breast cancer in 2013 [425]. The small-molecule inhibitor erlotinib targets the intracellular tyrosine kinase domain of EGFR and is already in use against non-small cell lung cancer [426] and pancreatic cancer [427]. However, targeted therapeutics are limited in their success to inhibit the oncogenic signalling of overexpressed or mutated ErbB receptors. Frequently, therapy resistance occurs [428], often due to deregulated pathway activity [429, 430] or bypasses of pathway blockades via other RTKs, especially ErbB family dimers [431]. EGFR in particular plays a major role in overcoming HER2 targeting. Resistance to trastuzumab is a major problem in treating HER2-positive breast cancer. So-called intrinsic (also termed primary or innate) resistance is preexistent to drug treatment, e.g. due to mutations like PIK3CA [432]. Acquired resistance on the contrary is developed over time induced by diverse mechanisms, e.g. due to molecular changes, despite initial drug response. Possible resistance mechanisms involve

overexpression of EGFR, HER2 or HER3, which is accompanied by alternative cell signalling via different ErbB dimers. Alternative signalling pathways can be further induced by MET receptor or insulin-like growth factor 1 receptor (IGF-IR). Other mechanisms include constitutive PI3K pathway activation due to mutations in the PIK3CA gene or PTEN loss, steric hindrance of HER2-antibody interaction or overexpression of TGF-α, HRG or VEGF [424]. To overcome resistance in the treatment of HER2-positive breast cancer, diverse novel drugs are in development. The small molecule tyrosine kinase inhibitor lapatinib and the HER2/3 antibody pertuzumab for example paved the way for improved therapeutic strategies [433]. These drugs can target either EGFR or both EGFR and ErbB2 receptors. Among these inhibitors, gefitinib and erlotinib target only one receptor, EGFR, while newer FDA approved drugs, such as lapatinib target both EGFR and ErbB2. Increased expression of EGFR and ErbB2 occurs in about 30 % of breast cancers and since these two receptors are heterodimer partners, strategies in which the use of drugs like lapatinib or combination of drugs are being considered for clinical trials. Several studies have shown that targeting both EGFR and ErbB2 may have synergistic effects on proliferation for the BT474 and SkBr3 breast tumor cell lines [434]. In phase I clinical studies, lapatinib was tolerated up to 1800 mg once daily in breast cancer patients, with side effects of diarrhea, nausea, rash, fatigue, anorexia, and vomiting. Clinical activity was observed at a minimum of 650 mg/day [435]. A phase II trial showed that lapatinib was effective in approximately 20 % of patients with ErbB2-positive metastatic breast cancer who had not received first-line chemotherapy [436]. In a phase III trial, it was demonstrated that women with ErbB2-positive metastatic breast cancer benefit from lapatinib, whereas ErbB2-negative breast cancer did not [437]. In 2007, FDA approved lapatinib for use in combination with capecitabine for patients (previously treated with anthracycline, taxane, or trastuzumab) who have metastatic breast cancer that overexpresses ErbB2 [438], after several phase III trials that demonstrated the synergistic effect compared to either alone [439]. Lapatinib offers improvements over trastuzumab. Aside from its specificity to EGFR and ErbB2, lapatinib induces apoptosis in trastuzumab-resistant breast SkBr3 cancer cells [48]. In 2009, Scaltriti et al. showed that lapatinib enhances the effects of trastuzumab in MCF7 and SkBr3 breast cancer cell lines [440]. Additionally, lapatinib's anti-tumor activity was observed in Japanese patients with ErbB2-positive metastatic breast cancer that relapsed after trastuzumab-based therapy [441]. Furthermore, several studies demonstrated synergistic effects for lapatinib in combination with trastuzumab in xenograft tumor reduction [441, 442]. Therapeutic efficacy of lapatinib in patient populations is limited by both primary and acquired resistance. Multiple phase II trials have revealed that only 20–35 % of patients with ErbB2-positive metastatic breast cancer respond to lapatinib [442]. Similar to trastuzumab, the medium duration of response to lapatinib is less than 1 year [443]. Thus, lapatinib resistance is a vital issue, especially considering ErbB2 is used as a biomarker to initiate Lapatinib treatment in patients. However, the mechanisms of drug sensitivity and acquired resistance are not fully understood at this time. In an in vitro model, it was discovered that lapatinib resistance in BT474 breast tumor cells was mediated in part by estrogen receptor (ER)

and progesterone receptor (PR) signaling upregulations in response to lapatinib, with evidence in increased activity in FOXO3a and caveolin-1, as well as Bcl-2 anti-apoptotic protein [444]. Furthermore, ErbB2+/ER+ tumor biopsies after 14 days of lapatinib treatment also reflect increased expression of FOXO3a, PR, and Bcl-2. Consequently combinational treatment with tamoxifen demonstrated resistance prevention, suggesting such therapeutic approach is appropriate for ErbB2+/ER+ patients [444]. Within the past decade, many studies have investigated EGFR/ErbB2 tyrosine kinase inhibitors and the development of subsequent resistance following treatment in lung and breast cancer patients. The major contributing factor was identified as mutations in the kinase domain of EGFR and/or ErbB2. Another study by Tam et al. identified mutations in EGFR which confer different degree of sensitivities to gefitinib in lung adenocarcinomas [445]. These studies confirmed that mutations in EGFR and ErbB receptors may confer anti-ErbB drug resistance. Some groups proposed the activation of alternate pathways when EGFR and ErbB2 are inhibited as the sources of resistance. Recent studies have suggested a role for signal transducer and activator of transcription 3 (STAT3) in anti-ErbB resistance. In 2005, Greulich et al. observed that cell lines harboring EGFR mutations have increased levels of phosphorylated STAT3 which correlated with gefitinib sensitivity [208]. A recent study shows that both gefitinib and lapatinib block doxorubicin, etoposide, and m-AMSA induced apoptosis in SK-Br-3 HER2-amplified breast cancer cell line after longer exposure (48 h) by downregulation of the expression and activity of Topo IIα [446]. Additionally, in another study, mammary-derived growth inhibitor (MDGI) was shown to confer cetuximab resistance in both breast and lung cancer cell models by stimulating the intracellular localization of EGFR [447]. Finally, the gatekeeper T798M mutation in HER2 kinase domain has been observed to considerably shift drug sensitivity to HER2 in breast cancer therapy. A recent report found that TKIs can be grouped into three classes in terms of their response behavior to T798M mutation: class I inhibitors exhibit drug resistance upon the mutation, such as lapatinib; class II inhibitors are insensitive to the mutation, such as erlotinib and gefitinib; and class III inhibitors can be sensitized by the mutation, such as staurosporine. However, kinetic study indicated that the mutation has only a modest effect on the binding of substrate ATP to HER2 using a synthetic biology protocol for a drug response of clinical TKIs to the mutation [448].

2.5.8 Ovarian Cancer

2.5.8.1 Mechanism of Resistance in Ovarian Cancer

Ovarian cancer is a leading cause of cancer death among women. Currently, surgery and chemotherapy are the standard of care for ovarian cancer patients. Despite an initial response to chemotherapy, patients tend to develop chemoresistance over time, which leads to poor prognosis Understanding the molecular basis of chemoresistance will aid in identification of new targets for ovarian cancer treatment, which

will eventually improve patient outcomes. Platinum-based agents form the first-line chemotherapy for ovarian cancer patients. Ovarian cancer microenvironment contributes to tumor growth, angiogenesis, dissemination, and chemoresistance. One of the critical factors in ovarian cancer microenvironment is VEGF, which is a key regulator of angiogenesis and is associated with poor prognosis, and chemoresistance [449]. Bevacizumab, a monoclonal antibody against VEGF, has been tested in platinum-resistant recurrent ovarian cancer as an adjuvant therapy with chemotherapy in a phase III clinical trial. The combination therapy prolonged tumor progression free time even though it has not shown significant benefits in overall survival [450]. Clinical and molecular findings suggest that ovarian cancer represents a group of heterogeneous neoplasms [451]. Currently there are no differences in treatment strategies among various types of ovarian cancer, however it is becoming evident that the inherent variances of ovarian cancer influence treatment efficacy and development of therapeutic resistance [452]. Epithelial tumors account for approximately 90 % of primary malignant ovarian cancer. Historically, epithelial ovarian cancer is classified into four major subtypes based on cell type, which include serous, endometrioid, clear cell, and mucinous subtypes [453]. Next generation sequencing revealed that both high-grade serous ovarian carcinoma (HGS-OvCa) and endometriod carcinoma can be further divided into various groups according to gene expression profiles [454, 455]. Yet the genetic drivers and corresponding biological features have not been well characterized in these subgroups. Previous molecular data suggests that the four major subtypes have distinct genetic alterations. For example, K-RAS gene mutations are found in about 85 % mucinous ovarian adenocarcinomas, but are much less frequently observed in other subtypes of ovarian carcinoma [456]. In addition to the VEGF inhibitors, the EGFR has emerged as an attractive target. Several therapy that target the EGFR in gynecologic cancers have included monoclonal antibodies (trastuzumab, pertuzumab, EMD7200) and tyrosine kinases inhibitors (gefitinib, erlotinib, lapatinib and CI-1033). Different studies examined trastuzumab in patients with ovarian cancer who reported a low response rate [457–460]. Gordan et al. [461] showed the clinical activity of pertuzumab in patients with advanced ovarian cancer: there were five patients had a partial responses, eight patients had stable disease lasting at least 6 months, and 10 patients had a CA-125 reduction of at least 50 %. Different trials have been completed such as the EORTC (erlotinib as maintenance therapy following first-line chemotherapy in patients with ovarian cancer (NCT00263822) and a phase II open label trial of erlotinib and bevacizumab conducted in patients with advanced ovarian cancer (NCT00696670). Schilder et al. [462] reported that mutations in the EGFR tyrosine kinase domain occurred in 55 ovarian cancer patients following gefitinib treatment. Other studies [458–462] suggested that patients with platinum resistant disease and low levels of HER3 mRNA might benefit from pertuzumab. Several clinical studies are exploring the combination of EGFR inhibitors and VEGF inhibitors [463]. Furthermore, PDGFR is upregulated in 50–80 % of ovarian tumors [464] and for this reason different trials have been conducted in patients with ovarian cancer with PDGFr inhibitors [465].

2.5.9 Liver Cancer

2.5.9.1 Mechanism of Resistance in Liver Cancer

Acquired evasive resistance is a major limitation of hepatocellular carcinoma (HCC) treatment with the TKIs. Recent findings suggest that resistance to sorafenib may have a reversible phenotype. In addition, loss of responsiveness has been proposed to be due to a gradual decrease in sorafenib plasma levels in patients. Several studies have described the reversible mechanisms toward sorafenib in HCC using mice bearing intrahepatic human xenografts of Hep3B-hCG cells [466]. In these mice, sorafenib was able to block the cancer growth but the resistance to TKI occurred after 1 month, and it was associated with the levels of the secreted urinary protein tumor biomarker bhCG (b human chorionic gonadotropic hormone) and tumor weight changes [467]. When the resistant tumor cells were reimplanted into new hosts, the resultant tumors were completely resensitized to retreatment [467]. In a very recent article, the authors treated the Hep3B-hCG mice model of HCC with 30 mg/kg sorafenib. They found that drug level changes were tumor dependent and associated with the induction of tumoral CYP3A4 metabolism, In conclusion, tumor CYP3A4 induction by sorafenib can play a crucial role in the variability of systemic drug levels [468].

2.5.10 Glioma

Deregulation of EGFR signaling is associated with poor prognosis in various gliomas. There are multiple mechanisms that can lead to deregulation of EGFR signaling in gliomas. Of these mechanisms, increased EGFR abundance is frequently found in primary Gliobastomas (GBMs) and can be caused by gene amplification, increased translation of the EGFR gene, or both. EGFR amplification occurs in 40–70 % of primary GBMs, but is not observed in lower-grade astrocytomas [469], which indicates that EGFR activation may drive tumorigenesis in primary GBMs. Focal EGFR amplification occurs usually at an extremely high level (>20 copies). All primary GBMs with EGFR gene amplification have concurrent EGFR protein overexpression, but only a subset (70–90 %) of tumors with EGFR protein overexpression also show EGFR gene amplification, indicating that a fraction of GBM tumors show increased receptor abundance in the absence of gene amplification [470]. EGFR overexpression in primary GBMs is occasionally accompanied by increased abundance of its cognate ligands, EGF and TGFα. This suggests the existence of an autocrine loop that results in unregulated chronic EGFR signaling. In addition to increases in receptor and ligand abundance, activating mutations of EGFR have also been found in GBMs. A number of deletion mutations that occur in the EGFR extracellular domain are exclusively found in GBMs. These include the mutants that encode the EGFR type I and type II variants (EGFRvI and vII) [471, 472], which give rise to

truncated proteins that are believed to be oncogenic. Other point mutations that also reside primarily in the extracellular region of EGFR are identified in ~14 % of GBMs [472]. These mutations include R84K and A265V/D/T at the domain I/II interface, and P545L and G574V at the domain II/IV interface. Interestingly, these mutants are constitutively active but still capable of binding ligand [472]. The cytoplasmic tail deletion mutants EGFRvIV and vV are also found exclusively in GBMs [473]. These mutations are thought to occur at a low frequency (~15 % of EGFR-overexpressing GBMs) and may exhibit defects in receptor internalization. However, EGFRvIV and vV can still bind ligand and have the potential to modulate oncogenic signaling pathways commonly elicited by wt EGFR [474]. The most common and best-studied EGFR mutation found in GBM is the type III EGFR variant deletion mutant (EGFRvIII). This mutation has not been observed in normal tissue [475], but has been found in 20–30 % of overall GBM patients and 50–60 % in patients with EGFR amplification GBM [476]. However, EGFRvIII is not reported to be as prevalent in the secondary GBMs. Moreover, clinical studies have shown a correlation between the presence of the EGFRvIII receptor and poor prognosis in patients with GBM [477]. This mutant has similarities to the v-ErbB transforming protein of avian erythroblastosis virus, which also is an EGFR-related auto-activating oncogene generated by a large extracellular deletion. EGFRvIII activates several downstream pathways, but a considerable amount of evidence indicates that it preferentially activates the PI3K/AKT signal transduction pathway [478]. EGFRvIII expression is tightly correlated with the activation of downstream targets of PI3K/AKT, including the mammalian target of rapamycin (mTOR), the forkhead box (FOX) transcription factor family and S6 [479]. EGFRvIII could activate CRTC2 via the PI3K/AKT pathway, which in turn leads to stimulation of the NF-kB pathway and resistance to chemotherapy. Selective activation of the PI3K/AKT pathway by EGFRvIII is also thought to mediate the resistance to radiation in EGFRvIII-positive GBM [479, 480]. Moreover, EGFRvIII signaling via the PI3K/AKT pathway may be facilitated by associated loss or mutation of the PTEN gene, which occurs in approximately 40 % of patients with EGFRvIII mutant GBM [480, 481]. The EGFRvIII signaling is also thought to be associated with the enhanced signaling of angiogenesis in GBM cells. The tumorigenicity of GBM cell lines can be increased by EGFRvIII transfection [482–484]. Finally, both EGFRvIII and wt EGFR/ErbB family proteins have been identified in the nucleus and are thought to drive proliferation and DNA damage repair through both transcriptional and signaling functions [485]. Moreover, EGFR is also observed to translocate to the mitochondria [486]. All these provide evidence that the contributions of EGFR malignancy may not be limited to its conventional cell membrane location.

2.5.10.1 General Mechanisms of Resistance to EGFR-Targeted Therapies

The molecular heterogeneity of glioblastoma endows the ability to escape monotherapy targeted to inhibit EGFR activation, such as TK inhibitors and monoclonal antibodies, through activation of compensatory signaling through other receptor

tyrosine kinases (RTK), most commonly MET and PDGFRα and β [487, 488]. Expression of EGFR and PDGFRα in distinct subpopulations of glioblastoma cells demonstrates a mosaiclike pattern of intratumoral heterogeneity and inhibition of both required to completely abrogate PI3K signaling [488, 489]. Likewise, expression of MET has been shown to compensate EGFR inhibition in glioblastoma cell lines and inhibiting c-Met and EGFR restored sensitivity to treatment [489]. Another potential explanation for reduced activity of TKIs and monoclonal antibodies in glioblastoma relates to their relatively large size and difficulty crossing the blood-brain barrier (BBB). While the BBB is compromised at the site of the tumor, intact BBB surrounding normal tissue with infiltrating glioblastoma is more difficult to access by such therapies, reducing overall anti-tumor efficacy. There is mounting evidence that a population of glioma-initiating stem cells play an important role in resistance to chemotherapy and radiotherapy, due altered DNA checkpoint activation and enhanced capacity for DNA repair [490, 491]. For example, cotreatment with erlotinib and the hedgehog pathway inhibitor cyclopamine had an effect on sphere initiation in glioblastoma stem cell cultures [492]. In line with these data, two very recent studies have found two different proteins involved in acquired resistance to EGFR TKIs such as urokinase plasminogen activator (uPA). This proteins drives signaling through the MAPK pathway, which results in suppression of the proapoptotic BCL2-family member protein BIM (BCL2L11). In patient-derived GBM cells and genetic GBM models, urokinase plasminogen activator uPA is shown to suppress BIM levels through ERK1/2 phosphorylation, which can be reversed by siRNA-mediated knockdown of uPA. TKI-resistant GBMs are resensitized to EGFR TKIs by pharmacologic inhibition of MEK or a BH3 mimetic drug to replace BIM function. Whereas, GBM cells that overexpressing EGFR, became gefitinib-resistant after addition of this drug. These resistant clones were subject to RNAseq and the expression of several genes was found to be upregulated. These genes are mainly tyrosine kinase receptors and include Reactive Oxygen Species 1 (ROS1), DDR1 and PDGFRα and are known to control several downstream targets of EGFR.Treatment with a potent and highly specific pyrazole (ROS1) inhibitor in ROS1 overexpressing clones led to a sensitization of these cells to low concentrations of gefitinib. Combined treatment with gefitinib and ROS1 inhibitor induces massive cell death by apoptosis following a prolonged S phase cell cycle [493, 494].

2.5.10.2 Resistance to Therapeutic Approaches Specifically Targeting EGFRvIII

EGFRvIII has been described as a mediator of glioma cell resistance to chemotherapeutic drugs in vitro through upregulation of the anti-apoptotic protein B-cell lymphoma–extra large [495, 496]. A report elucidated the relationship between EGFRvIII expression and prolonged overall survival using tumor samples from 73 patients [497]. Furthermore, such study detected that EGFRvIII-negative neurosphere cells are more resistant to temozolomide than EGFRvIII-positive cells, suggesting that expression of EGFRvIII rather acts as a sensitizer to alkylating drugs.

A subgroup of tumor cells within a glioblastoma with stem cell characteristics expressed the putative stem cell marker CD133 in association with EGFRvIII [498]. Furthermore, in a small population of glioblastoma cells expressing the EGFRvIII mutant tumor growth is correlated with the increased expression of several cytokines such as interleukin-6 and leukemia inhibitory factor. Subsequently, these cytokines accelerated the proliferation of EGFRvIII-negative cells in a paracrine manner [499]. The expression of EGFRvIII is able to activate different downstream pathways in EGFRvIII-positive cells with respect to the EGFRvIII negative cells as revealed by proteomic analyses [500]. In this regard, preclinical data suggest that combination of EGFRvIII inhibitors with targeting of an additional pathway (such as c-MET signaling or the urokinase-type plasminogen activator receptor pathway) can act synergistically [501, 502]. Different studies revealed that that resistance to anti-EGFR strategies is correlated with increased expression of EGFRvIII, an induction of the expression of the regulatory 110-kDa delta subunit of PI3K (p110δ), and insulin-like growth factor receptor-I signaling via PI3K [503, 504]. Silencing of both EGFRvIII and p110δ subunit resulted in a sensitization to the EGFR inhibitor erlotinib.

2.5.10.3 VEGF-Targeting Therapy in Glioma

Inhibition of angiogenesis seems to be an attractive target for glioma therapy. One of the most best established anti-angiogenic cancer treatment modality in general is bevacizumab that is approved for recurrent glioblastoma in the USA since 2009, in the EU approval was rejected [165, 505]. In addition to antibody-based approaches targeting free VEGF, compounds have been developed to target the functioning of the VEGF receptor. An example of this class of inhibitors is cediranib inhibits tyrosine kinase receptors, particularly all subtypes of the VEGF receptor, and has some activity against the PDGF and c-KIT receptors In gliomas cediranib was shown to induce normalization of the vasculature by inhibiting pathological proliferation of endothelial cells and immature vessel [506]. However, the proven beneficial effects of angiogenesis inhibitors targeting the VEGF signaling pathway have not been translated into a survival benefit of similar extent [506]. Especially for treatment with bevacizumab, it has been reported that recurrence after therapy is more likely to be diffuse and distant to the primary tumor location [507]. Referring to this, increasing evidence suggests that impaired angiogenesis promotes infiltrative tumor growth. Impaired angiogenesis is therefore supposed to act as ‘detrimental driving force’ for enhanced tumor cell invasion into the surrounding tissue, a process commonly referred to as ‘evasive resistance’ Multiple adaptive mechanisms are supposed to be responsible for the evasive phenotype of increased invasiveness. In addition, anti-angiogenic therapy further increases hypoxia in the remaining tumor cells by constricting blood supply [506]. This in turn activates survival pathways like AKT/PI3K/mTOR and promotes glycolytic energy metabolism and autophagy [506]. Alternatively, anti-angiogenic therapy induces altered tumor cell invading patterns. For example, untreated glioblastoma cells often diffusely invade as single

cells into the surrounding brain tissue, whereas angiogenic impaired cells tend to invade as multicellular layers along blood vessels which is described as 'perivascular invasion' or 'vascular cooption' [508]. Of note, increased invasion is not the only mediator of 'evasive resistance' to angiogenesis inhibitors. Several others, all resulting in renewed angiogenesis and tumor growth are described as (I) activation and upregulation of alternative pro-angiogenic signaling effectors (e.g. fibroblast growth factor (FGF), angiopoietins), (II) recruitment of vascular progenitor cells from the bone marrow, and (III) increased pericyte coverage mediated blood vessel protection [508]. Furthermore, a novel mechanism for irradiation treatment induced evasive resistance was described by Kioi et al. [509] by suggesting that vasculogenesis and not angiogenesis is the key process of revascularization that occurs during glioma recurrence after irradiation treatment. For this it is important to mention, that in general two processes are responsible for the formation of new blood vessels: angiogenesis and vasculogenesis. The latter describes the process of a de novo formation of new blood vessels from endothelial precursor cells or bone marrow-derived hematopoietic cells (BMDCs), whereas angiogenesis involves the formation of new blood vessels by sprouting of loca pre-existing vessels via proliferation of endothelial cells. Prior to irradiation, tumor growth is mainly directed by angiogenesis. After irradiation, the tumor becomes more hypoxic due to a radiation-damaged vasculature, which leads to increased HIF-1 activity and subsequent CXCR4/SDF-1 mediated recruitment of BMDCs into the tumors to form new vessels [509]. Taken this into account the concept of anti-angiogenic treatment in recurrent glioblastoma needs to be reconsidered and probably more oriented on the signaling pathways involved in vasculogenesis (e.g. CXCR4/SDF-1 inhibitors) in order to counteract angiogenesis-stimulating side effects of radiation therapy. Taken together, an approach to overcome the problem of increasing infiltrative tumor growth lies in treatment modalities which combine anti-angiogenic and anti-invasive mechanisms. Applying such drugs, targeting evasive resistance mechanisms, are therefore promising options in modern glioblastoma therapy, since they comprise the probability to reach sustained efficacy of glioma treatment.

2.5.10.4 Resistance to Angiogenic Therapy

Patients with recurrent glioblastoma uncommonly exhibit primary resistance. In contrast, most new diagnoses either respond or stabilize initially but later develop acquired resistance. Different resistance mechanisms are acquired via upregulation of proangiogenic growth factors, mobilization/recruitment of pericytes or bone marrow-derived endothelial precursor cells, and tumor adaptions to increase invasion/migration or allow survival in a relatively hypoxic/acidotic environment [508]. An increase in circulating proangiogenic factors is observed during the angiogenic treatment in preclinical orthotopic glioblastoma models [510, 511]. Similar findings have been found in patients with recurrent glioblastoma treated with cediranib [166]. Additionally, two studies highlighted an increased tumor cell invasion in orthotopic glioblastoma xenograft tumors treated with angiogenic inhibitors

[512, 513], whereas a recent preclinical study demonstrated that a decreased tumor-associated edema and improved overall survival were associate with a single angiogenic inhibitor [514]. Consistent with these data, evidences have been raised regarding the emergence of an infiltrative phenotype after anti-VEGF/VEGFR therapy among some patients with glioblastoma [511].

2.5.11 Pancreatic Cancer

EGFR is over-expressed in 90 % of pancreatic tumors. Erlotinib is approved by the Federal Drug Agency (FDA) and the European Medicines Agency (EMA) or use in combination with gemcitabine for treatment of locally advanced, irresistible or metastatic pancreatic cancer, however with only a marginal improval of overall and progression free survival. Preclinical studies suggest additive effects when anti-EGFR agents are combined with inhibitors of VEGF [515]. In phase 2 studies of treatment-naive advanced pancreatic cancer, combining gemcitabine-based chemotherapywith bevacizumab (an antibody directed against VEGF) and anti-EGFR therapy (cetuximab or erlotinib) showed modest benefit,39,40 but these were not universally considered sufficient to warrant phase 3 evaluation [516]. A phase 3 study of patients with metastatic disease found that adding bevacizumab to gemcitabine plus erlotinib did not extend OS, but there was a statistically significant gain in PFS in the overall population and some evidence of survival benefit in patients with more aggressive disease [517].

2.5.11.1 Potential Predictors of Benefit from EGFR-Directed Therapies in Pancreatic Cancer

EGFR expression, EGFR gene amplification, EGFR intron 1 polymorphism, or the presence of the EGFR exon 12 R497K point mutation are not useful in predicting the survival benefit of these agents in patients with advanced pancreatic cancer [518]. Amphiregulin, can be a potential biomarker of response to EGFR-targeted therapy in patients with advanced pancreatic cancer [518].

2.5.11.2 KRAS Mutation in Pancreatic Cancer

The mutation of KRAS is the first notable genetic alteration identified in pancreatic cancer. Oncogenic KRAS is involved in the initiation or early phase of pancreatic tumorigenesis, and more than 85 % of the pancreatic cancer patients have KRAS gene mutation at the early stage of cancer development. In a recent retrospective analysis of 136 patients, KRAS wild type was correlated with a significant survival benefit among those treated with erlotinib but not among patients treated without erlotinib. Two different retrospective analysis conducted on patients with advanced

pancreatic, revealed that KRAS mutation status was neither prognostic for OS nor predictive of therapeutic response to erlotinib therapy [519, 520]. The phase 2 MARK trial (O21129; bio Marker trial), where patients with unresectable locally advanced or metastatic pancreatic cancer were recluted to receive erlotinib or placebo until disease progression, showed that KRAS mutation status was not associated with both progression-free survival and response to erlotinib. In the phase 3 AIO trial, KRAS mutations status was analyzed in tumor samples derived from patients in advanced pancreatic cancer. KRAS mutations was found in the 70 % of the samples but these mutations were not correlated with erlotinib efficacy [517]. Collectively, these studies failed to demonstrate a definite role for KRAS mutation status in predicting response to anti-EGFR therapies. Nevertheless, there was some evidence that KRAS status has a prognostic role in advanced pancreatic cancer.

2.5.11.3 Resistance Mechanisms to Anti-EGFR Therapy in Pancreatic Cancer

A key issue has been our poor understanding of resistance mechanisms of pancreatic cancer to traditional chemotherapy and radiotherapy, as well as to targeted therapies. In order to understand resistance to anti-EGFR agents it could be necessary a good patient selection by using appropriate including the dense desmoplastic stroma, a hypoxic microenvironment, and the presence of highly tumorigenic stem cells.

2.5.11.4 Alterations, Redundancies, and Crosstalk in EGFR-Related Pathways in Pancreatic Cancer

Activation of different pathway such as increased VEGF/VEGF receptor expression, the dysregulation of EGFR internalization, activation of HER dysregulation of the PI3K/Akt/mTOR Signaling, KRAS mutations or loss of PTEN, can contributes to the resistance of pancreatic cancer to EGFR TKIs [518]. Finally, high MET expression levels have been found in pancreatic cancer cells, and its activation via exogenous hepatocyte growth factor triggers proliferation and movement [518].

2.5.12 Head and Neck Cancers

Head and neck cancers comprise a spectrum of malignancies arising in the oral cavity, pharynx and larynx with squamous cell carcinoma representing the most common histology (~85 %). Worldwide, head and neck squamous cell carcinoma (HNSCC) is the/sixth most common cancer with an incidence of over 600,000 and over 350,000 deaths per year. Of newly diagnosed patients, about two-thirds present with advanced-stage disease, usually with regional lymph node involvement, and 10 % have distant metastases. The most common predisposing factors include

tobacco exposure and alcohol consumption. In addition, an increasing number of oropharyngeal cancers are linked with human papilloma virus (HPV) infection [521]. NSCC treatment generally involves several modalities including surgery, radiotherapy (RT) and chemotherapy (CT) The US FDA to date has approved six agents for the treatment of HNSCC including five conventional CT drugs (cisplatin, methotrexate, 5-flurouracil [5-FU], bleomycin and docetaxel) and one targeted agent (cetuximab). Platinums, including cisplatin and carboplatin are the most commonly used CT agents for HNSCC treatment with responses in 13–40 % of cases. Cetuximab, an EGFR mAb, is currently the only FDA approved EGFR-targeting strategy for HNSCC. It is approved based on a results obtained from a randomized Phase III trial where co-treatment with cetuximab and high-dose RT (211 patients) was compared with high-dose RT alone (213 patients) in patients with LA-HNSCC [522]. The combination of cetuximab and RT significantly improved median OS (49.0 vs. 29.3 months) and median progression-free survival (PFS; 17.1 vs. 12.4 months) vs. RT alone. A number of ongoing Phase III trials are attempting to expand the use of cetuximab in LA-HNSCC, (NCT00716391, NCT00999700 NCT01233843 NCT01086826). Although the final results have not been published, the latest report from a Phase III trial conducted by the Radiation Therapy Oncology Group 0522 investigating the addition of cetuximab to the radiation-cisplatin platform for LA-HNSCC demonstrated no significant improvement in mortality but higher local toxicity in the cetuximab arm [522]. Additionally, cetuximab has also been tested alone or in combination with CT in patients with R/M platinum refractory HNSCC. Four hundred and forty-two patients with R/M HNSCC in a Phase III EXTREME trial [523] were randomized to receive platinum-based therapy or in combination with cetuximab as a first-line palliative regimen. This trial has shown that improvement in median OS from 7.4 to 10.1 months ($p=0.04$) after cetuximab addition. The combination of cetuximab and platinum in patients with R/M HNSCC is more beneficial than treatment with a single-agent such as effective platinum [524–526]. Hence, a better understanding of the molecular mechanisms of resistance to cetuximab may provide insights in new drugs development and identifying predictive biomarkers to optimize treatment strategies and lead to personalized therapy.

2.5.12.1 EGFR-Targeted Therapy and Resistance in Head and Neck Squamous Cell Carcinoma

A classic example of such targeted therapies involve epidermal growth factor receptor (EGFR), whereby its overexpression has been associated with reduced overall survival and local regional control in HNSCC [527, 528]. Cetuximab has a RR of only 13 % as a single agent in HNSCC treatment. Other EGFR-targeted agents such as TKIs have not demonstrated improved survival in unselected populations. For example, zalutumumab was associated with a prolonged PFS over best supportive care (BSC) alone in patients with incurable Recurrent and/or Metastatic (R/M) HNSCC during a Phase III trial [529]. In another study, panitumumab in combination with paclitaxel, carboplatin and intensity-modulated RT had partial response in

all 19 LA-(Locally Advanced) HNSCC patients [530]. A randomized Phase III trial comparing panitumumab/RT with cisplatin/RT (NCT00820248) in locally advanced (LA)-HNSCC is ongoing. Several ongoing Phase II trials are evaluating combination of panitumumab with CT for R/M HNSCC (NCT00756444), as second-line monotherapy for R/M HNSCC (NCT00446446), or in combination with postoperative CRT for LA-HNSCC (NCT00798655) [530–532].

Nimotuzumab is approved for HNSCC in several countries outside the USA. Different trials have been performed and in one trial has been found a significant relationship between EGFR expression and OS in patients who received nimotuzumab plus CRT. Ongoing Phase III trials in LA-HNSCC are testing the addition of nimotuzumab to RT (NCT01345084) and to adjuvant CRT (NCT00957086). Moreover, ongoing Phase II trials may provide additional insights into the use of nimotuzumab when added to CT for incurable HNSCC (NCT01425736) and to CRT for LA-HNSCC (NCT01516996; NCT00702481). Erlotinib has demonstrated efficacy in patients with HNSCC in one trials [533]. In another randomized Phase II study, the combination of CRT and lapatinib (followed by lapatinib maintenance treatment) showed an increased CRR at 6 months post-CRT, and median PFS (55 % vs. 41 %) at 18 months post-CRT compared with CRT plus placebo in 67 unresected LA-HNSCC [534]. Another Phase II trial demonstrated that lapatinib monotherapy was able to stop the tumor progression in LA-HNSCC [535]. Conversely, this drug showed no CR or PR in either EGFR inhibitor-naive or refractory subjects in R/M HNSCC [536]. A randomized Phase III trial is currently ongoing to study the combination of RT plus platinum-based CT with lapatinib or placebo in postoperative setting (NCT00424255). Other Phase II trials are testing the use of lapatinib with RT for LA-HNSCC who cannot tolerate CRT (NCT00490061), in combination with primary CRT in LA-HNSCC (NCT00387127), and in combination with CRT (chemoradiotherapy) in HPV negative patients (NCT01711658). A new generation of TKIs, the irreversible small molecule pan-HER inhibitors, including afatinib and dacomitinib, have been developed. Afatinib was used on 124 patients with R/M HNSCC failed with platinum-based therapy. Such drug showed the same antitumor activity of cetuximab in this study [537]. The low efficacy suggests that other molecular mechanisms may exist that modulate intrinsic (primary) or acquired (secondary) resistance of EGFR inhibition. Identifying the mechanisms of resistance to EGFR-targeted agents may lead to strategies To overcome resistance it could be important to inhibit other receptor or non-receptor tyrosine kinases (e.g., MET, IGF-1R, sarcoma-family kinase [Src]), to block both VEGF/VEGFR pathway and downstream mediators in the EGFR signaling.

2.5.12.2 Angiogenic Therapy Head and Neck Squamous Cell Carcinoma

HNSCC relies upon angiogenesis in order to continue to proliferate and metastasize [538]. Normal keratinocytes and HNSCC cells are known to produce a variety of angiogenic factors including IL-8, VEGF, placental growth factor (PlGF) and FGF. IL-8 expression has been shown to be associated with tumor cells in HNSCC

samples using immunohistochemistry. In addition, in the closely related bronchogenic carcinomas, IL-8, the primary mediator of angiogenesis, has been found in fresh tumor homogenates. VEGF on the other hand, is considered as the prototypical proangiogenic factor whose biological activity is primarily associated with endothelial cells. These proangiogenic proteins can bind to their corresponding receptors located on the surface of endothelial cells and activate signaling cascades that lead to endothelial cell proliferation, directional migration and vessel formation. There can also be an indirect induction of angiogenesis by interaction of tumor cells with their surrounding stroma. HNSCC cells have been shown to attract monocytes and activate them to secrete angiogenic factors Also, macrophages are known to produce cytokines that stimulate the tumor cells (via paracrine signaling) to produce increased levels of IL-8 and VEGF [538]. In a study published in 2008, Hasina et al. [539] demonstrated that expression of VEGF, IL-8/CXCL8, FGF-2, and HGF as higher in samples derived from patients affected by HNSCC than in samples derived from normal and dysplastic mucosa of healthy subjects. Moreover, they identified two different clusters in HNSCC samples: tumors in Cluster A express high levels of VEGF and FGF-2 and low levels of IL-8/CXCL8 and HGF, whereas tumors in Cluster B, had a low expression levels of VEGF and FGF-2 and higher expression levels of IL-8/CXCL8 and HGF. In the same study the authors treated mice bearing different levels of expression of VEGF-derived several HNSCC cell lines with anti-VEGF antibody, with nonspecific human IgG antibody, or with PBS (phosphate-buffered saline, a buffer solution isotonic and nontoxic to cells). The growth of tumor with high levels of VEGF was inhibited by anti-VEGF treatment. Although bevacizumab is currently being evaluated in phase III clinical trials (NCT00588770), results from phase II clinical trials indicate that bevacizumab shows little activity as single agent in HNSCC. The single-agent response rate is less than 10 %, and even in patients who do respond, the duration of response is typically less than 3 months [540, 541]. VEGF has been shown to be a downstream target of EGFR signaling cascade and VEGF up-regulation through EGFR activation has been correlated with resistance to EGFR-targeting agents [542]. Using this rationale, a phase I/II study of bevacizumab in combination with elotinib was conducted by Vokes et al. involving 51 patients with R/M HNSCC [540]. An overall response rate of about 15 % was seen, which was significantly higher than that with either agent alone. However, median survival was similar to that with chemotherapy alone with less toxicity. Two (4 %) of the patients had complete response, five (10 %) had partial response, twenty-six (56 %) had stable disease and fifteen (30 %) had progressive disease. Interim analysis of an on-going phase II study of pemetrexed and bevacizumab in patients with R/M HNSCC at the University of Pittsburgh (544), showed that two (18 %) of the patients had complete response, three (27 %) had partial response, six (54 %) had stable disease and none (0 %) had progressive disease. Sorafenib and sunitinib have been tested in different studies in patients with R/M HNSCC. A Phase II study conducted to evaluate the tolerability and efficacy of sunitinib in metastatic and/or recurrent HNSCC patients, also concluded that sunitinib had low single agent activity [542]. Another study showed that sunitinib was not able to block the tumor progressoion in 11 out of 17 patients, affected by

M/R HNSCC [543]. Finally, a third study [544] showed that sunitinib was able to reduce the tumoral progression the 19 out of 38 patients with recurrent or metastatic HNSCC refractory to platinum-based treatment or unfit for platinum-based regimens. Sorafenib has shown anti-cancer activity in preclinical studies [545] Phase II trials in R/M HNSCC patients with single agent sorafenib showed stable disease in ten (38 %) patients and a median overall survival of 8 months [546]. The other published study, with sorafenib in first-line setting, was conducted on patients with persistent, recurrent, or metastatic HNSCC [547] and forty-one patients had stable disease

2.5.12.3 Resistance to VEGF-Targeted Therapeutics in Head and Neck Squamous Cell Carcinoma

Clinical trials with VEGF-targeted agents in HNSCC and several other cancers indicate that the therapeutic efficacy of these drugs is limited to date. Majority of the patients demonstrate an initial clinical response to the treatment but eventually exhibit progressive disease (acquired resistance). In addition, some patients show pre-existing indifference to angiogenesis inhibition (intrinsic resistance), such that tumor progression continues unabated. These incomplete drug responses are likely due to the complexity of signaling networks that the tumor cells can exploit in the setting of VEGF blockade. Currently, there are no reports that elucidate the molecular mechanisms of resistance to anti-VEGF therapy in HNSCC.

2.6 Conclusion

An improved molecular understanding of cancer has significantly facilitated the development of specific, targeted therapies. Such therapies refer to a new generation of anticancer drugs that are designed to interfere with a specific molecular target, usually a protein with a critical role in tumor growth or progression. In this sense, the concept of targeted therapy refers to a therapeutic strategy aimed at targeting those mutations that drive tumorigenesis. Additionally, targeted therapies provide a new approach for cancer therapy that has the potential for avoiding some of the drawbacks associated with cytotoxic chemotherapy. However, the task remains to balance the degree of empiric or preventative intensification of therapy for the purpose of subverting resistance with any risks of more potent therapies. Furthermore, emerging the ultimate goal of personalized medicine, knowing in advance what therapy will work, with the ultimate goal of cancer care, eradicating the disease without harming the rest of the body, is a positive step in the right direction. Advances in the understanding of the disease pathobiology, cancer stem cells, signal transduction have provided effective treatment options and survival benefits for patients with cancers. Furthermore, increasing evidence suggests that stemness results from the incessant convergence of cell-intrinsic features (genetic mutations

and epigenetic regulation), local signals (of a chemical, mechanical, and molecular nature), stochastic events, and population forces that continuously shape the stem cell pool. In this scenario, the future development of successful clinical strategies will be tightly linked to a deeper understanding of the dynamic, adaptable, and evolving nature of such cells.

Conflict of Interest Statement All authors have no conflicts of interest to declare.

References

1. Levitzki A, Mishani E. Tyrphostins and other tyrosine kinase inhibitors. Annu Rev Biochem. 2006;75:93–109.
2. Crean S, Boyd DM, Sercus B, Lahn M. Safety of multi-targeted kinase inhibitors as monotherapy treatment of cancer: a systematic review of the literature. Curr Drug Saf. 2009;4:143–54.
3. Wilhelm SM, Adnane L, Newell P, Villanueva A, Llovet JM, Lynch M. Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. Mol Cancer Ther. 2008;7:3129–40.
4. Blanke CD, Corless CL. State-of-the art therapy for gastrointestinal stromal tumors. Cancer Invest. 2005;23:274–80.
5. Ravegnini G, Nannini M, Sammarini G, et al. Personalized medicine in gastrointestinal stromal tumor (GIST): clinical implications of the somatic and germline DNA analysis. Int J Mol Sci. 2015;16:15592–608.
6. Moy B, Goss PE. Lapatinib: current status and future directions in breast cancer. Oncologist. 2006;11:1047–57.
7. Hubbard SR, Till JH. Protein tyrosine kinase structure and function. Annu Rev Biochem. 2000;69:373–98.
8. Blume-Jensen P, Hunter T. Oncogenic kinase signalling. Nature. 2001;411:355–65.
9. Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell. 2002;103:211–25.
10. Hunter T. Tyrosine phosphorylation: thirty years and counting. Curr Opin Cell Biol. 2009;21:140–6.
11. Edelman AM, Blumenthal DK, Krebs EG. Protein serine/threonine kinases. Annu Rev Biochem. 1987;56:567–613.
12. Ullrich A, Schlessinger J. Signal transduction by receptors with tyrosine kinase activity. Cell. 1990;61:203–12.
13. Yarden Y, Ullrich A. Growth factor receptor tyrosine kinases. Annu Rev Biochem. 1988;57:443–78.
14. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. Science. 2002;298:1912–34.
15. Robinson DR, Wu YM, Lin SF. The protein tyrosine kinase family of the human genome. Oncogene. 2000;19:5548–57.
16. Knuutila S, Bjorkqvist AM, Autio K, et al. DNA copy number amplifications in human neoplasms: review of comparative genomic hybridization studies. Am J Pathol. 1998;152:1107–23.
17. Collett MS, Erikson RL. Protein kinase activity associated with the avian sarcoma virus src gene product. Proc Natl Acad Sci U S A. 1978;75:2021–4.
18. Stehelin D. The transforming gene of avian tumor viruses. Pathol Biol (Paris). 1976;24:513–5.
19. Hunter T. Protein phosphorylated by the RSV transforming function. Cell. 1980;22:647–8.

20. Hunter T, Sefton BM. Transforming gene product of Rous sarcoma virus phosphorylates tyrosine. Proc Natl Acad Sci U S A. 1980;77:1311–5.
21. Varmus H, Bishop JM. Biochemical mechanisms of oncogene activity: proteins encoded by oncogenes. Introduction. Cancer Surv. 1986;5:153–8.
22. Downward J, Yarden Y, Mayes E, et al. Close similarity of epidermal growth factor receptor and v-erb-B oncogene protein sequences. Nature. 1984;307:521–7.
23. Ullrich A, Coussens L, Hayflick JS, et al. Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. Nature. 1984;309:418–25.
24. Chazin VR, Kaleko M, Miller AD, Slamon DJ. Transformation mediated by the human HER-2 gene independent of the epidermal growth factor receptor. Oncogene. 1992;7:1859–66.
25. Slamon DJ, Clark GM, Wong SG, et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science. 1987;235:177–82.
26. DiGiovanna MP, Stern DF, Edgerton SM, Whalen SG, Moore 2nd D, Thor AD. Relationship of epidermal growth factor receptor expression to ErbB-2 signaling activity and prognosis in breast cancer patients. J Clin Oncol. 2005;23:1152–60.
27. Tsatsanis C, Spandidos DA. Oncogenic kinase signaling in human neoplasms. Ann N Y Acad Sci. 2004;1028:168–75.
28. Hunter T. A thousand and one protein kinases. Cell. 1987;50:823–9.
29. Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. Nature. 2001;409:860–9.
30. Hunter T. Signaling-2000 and beyond. Cell. 2000;100:113–27.
31. Fendly BM, Winget M, Hudziak RM, Lipari MT, Napier MA, Ullrich A. Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor or HER2/neu gene product. Cancer Res. 1990;50:1550–8.
32. Hudziak RM, Lewis GD, Winget M, Fendly BM, Shepard HM, Ullrich A. p185HER2 monoclonal antibody has antiproliferative effects in vitro and sensitizes human breast tumor cells to tumor necrosis factor. Mol Cell Biol. 1989;9:1165–72.
33. Sasaki T, Hiroki K, Yamashita Y. The role of epidermal growth factor receptor in cancer metastasis and microenvironment. Biomed Res Int. 2013;2013:546318.
34. Bazley LA, Gullick WJ. The epidermal growth factor receptor family. Endocr Relat Cancer. 2005;12(Suppl):S17–27.
35. Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer. 2005;5:341–54.
36. Goldberg RM. Cetuximab. Nat Rev Drug Discov. 2005;Suppl:S10–1.
37. Yang B, Lum P, Chen A, et al. Pharmacokinetic and pharmacodynamic perspectives on the clinical drug development of panitumumab. Clin Pharmacokinet. 2010;49:729–40.
38. Giannopoulou E, Antonacopoulou A, Matsouka P, Kalofonos H. Autophagy: novel action of panitumumab in colon cancer. Anticancer Res. 2009;29:5077–82.
39. Keating G. Panitumumab: a review of its use in metastatic colorectal cancer. Drugs 2010;0:1059–78.
40. Hocking CM, Price TJ. Panitumumab in the management of patients with KRAS wild-type metastatic colorectal cancer. Therap Adv Gastroenterol. 2014;7:20–3.
41. Robinson KW, Sandler AB. EGFR tyrosine kinase inhibitors: difference in efficacy and resistance. Curr Oncol Rep. 2013;15:396–404.
42. O'Donnell P, Ferguson J, Shyu J, et al. Analytic performance studies and clinical reproducibility of a real-time PCR assay for the detection of epidermal growth factor receptor gene mutations in formalin-fixed paraffin-embedded tissue specimens of non-small cell lung cancer. BMC Cancer. 2013;13:210.
43. Dragovich T, McCoy S, Fenoglio-Preiser CM, et al. Phase II trial of erlotinib in gastroesophageal junction and gastric adenocarcinomas: SWOG 0127. J Clin Oncol. 2006;24:4922–7.
44. Wainberg ZA, Lin LS, DiCarlo B, et al. Phase II trial of modified FOLFOX6 and erlotinib in patients with metastatic or advanced adenocarcinoma of the oesophagus and gastro-oesophageal junction. Br J Cancer. 2011;105:760–5.

45. Schilder RJ, Sill MW, Lee YC, Mannel R. A phase II trial of erlotinib in recurrent squamous cell carcinoma of the cervix: a gynecologic oncology group study. Int J Gynecol Cancer. 2009;19:929–33.
46. Gordon MS, Hussey M, Nagle RB, et al. Phase II study of erlotinib in patients with locally advanced or metastatic papillary histology renal cell cancer: SWOG S0317. J Clin Oncol. 2009;27:5788–93.
47. Llovet JM, Hernandez-Gea V. Hepatocellular carcinoma: Reasons for phase III failure and novel perspectives on trial design. Clin Cancer Res. 2014;20:2072–9.
48. Hojjat-Farsangi M. Small-molecule inhibitors of the receptor tyrosine kinases: promising tools for targeted cancer therapies. Int J Mol Sci. 2014;15:13768–801.
49. Rusnak DW, Lackey K, Affleck K, et al. The effects of the novel, reversible epidermal growth factor receptor/ErbB-2 tyrosine kinase inhibitor, GW2016, on the growth of human normal and tumor-derived cell lines in vitro and in vivo. Mol Cancer Ther. 2001;1:85–94.
50. Xia W, Mullin RJ, Keith BR, et al. Anti-tumor activity of GW572016: A dual tyrosine kinase inhibitor blocks EGF activation of EGFR/erbB2 and downstream Erk1/2 and AKT pathways. Oncogene. 2002;21:6255–63.
51. Food and Drug Administration. FDA labelling information [online]. (2007). http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation
52. Fry DW, Bridges AJ, Denny WA, et al. Specific, irreversible inactivation of the epidermal growth factor receptor and erbB2, by a new class of tyrosine kinase inhibitor. Proc Natl Acad Sci U S A. 1998;95:12022–7.
53. Smaill JB, Palmer BD, Rewcastle GW, et al. Tyrosine kinase inhibitors. 15. 4-(Phenylamino) quinazoline and 4-(phenylamino)pyrido[d]pyrimidine acrylamides as irreversible inhibitors of the ATP binding site of the epidermal growth factor receptor. J Med Chem. 1999;42:1803–15.
54. Djerf EA, Trinks C, Abdiu A, Thunell LK, Hallbeck AL, Walz TM. ErbB receptor tyrosine kinases contribute to proliferation of malignant melanoma cells: inhibition by gefitinib (ZD1839). Melanoma Res. 2009;19:156–66.
55. Djerf Severinsson EA, Trinks C, Gréen H, et al. The pan-ErbB receptor tyrosine kinase inhibitor canertinib promotes apoptosis of malignant melanoma in vitro and displays anti-tumor activity in vivo. Biochem Biophys Res Commun. 2011;414:563–8.
56. Ako E, Yamashita Y, Ohira M, et al. The pan-erbB tyrosine kinase inhibitor CI-1033 inhibits human esophageal cancer cells in vitro and in vivo. Oncol Rep. 2007;17:887–93.
57. Nyati MK, Maheshwari D, Hanasoge S, et al. Radiosensitization by pan ErbB inhibitor CI-1033 in vitro and in vivo. Clin Cancer Res. 2004;10:691–700.
58. Slichenmyer WJ, Elliott WL, Fry DW. CI-1033, a pan-erbB tyrosine kinase inhibitor. Semin Oncol. 2001;28:80–5.
59. Rixe O, Franco SX, Yardley DA, et al. A randomized, phase II, dose-finding study of the pan-ErbB receptor tyrosine-kinase inhibitor CI-1033 in patients with pretreated metastatic breast cancer. Cancer Chemother Pharmacol. 2009;64:1139–48.
60. Janne PA, von Pawel J, Cohen RB, et al. Multicenter, randomized, phase II trial of CI-1033, an irreversible pan-ERBB inhibitor, for previously treated advanced non small cell lung cancer. J Clin Oncol. 2007;25:3936–44.
61. Irwin ME, Nelson LD, Santiago-O'Farrill JM, et al. Small molecule ErbB inhibitors decrease proliferative signaling and promote apoptosis in Philadelphia chromosome-positive acute lymphoblastic leukemia. PLoS One. 2013;8, e70608.
62. Fabian MA, Biggs 3rd WH, Treiber DK, et al. A small molecule-kinase interaction map for clinical kinase inhibitors. Nat Biotechnol. 2005;23:329–36.
63. Dorsey K, Agulnik M. Promising new molecular targeted therapies in head and neck cancer. Drugs. 2013;73:315–25.
64. Engelman JA, Zejnullahu K, Gale CM, et al. PF00299804, an irreversible pan-ERBB inhibitor, is effective in lung cancer models with EGFR and ERBB2 mutations that are resistant to gefitinib. Cancer Res. 2007;67:11924–32.

65. Gonzales AJ, Hook KE, Althaus IW, et al. Antitumor activity and pharmacokinetic properties of PF-00299804, a second-generation irreversible pan-erbB receptor tyrosine kinase inhibitor. Mol Cancer Ther. 2008;7:1880–9.
66. Janne PA, Boss DS, Camidge DR, et al. Phase I dose-escalation study of the pan-HER inhibitor, PF-00299804, in patients with advanced malignant solid tumors. Clin Cancer Res. 2011;17:1131–9.
67. Takahashi T, Boku N, Murakami H, et al. Phase I and pharmacokinetic study of dacomitinib (PF-00299804), an oral irreversible, small molecule inhibitor of human epidermal growth factor receptor-1, -2, and -4 tyrosine kinases, in Japanese patients with advanced solid tumors. Invest New Drugs. 2012;30:2352–63.
68. Ramalingam SS, Jänne PA, Mok TS, et al. Dacomitinib versus erlotinib in patients with advanced-stage, previously treated non-small-cell lung cancer (ARCHER 1009): a randomised, double-blind, phase 3 trial. Lancet Oncol. 2014;15:1369–78.
69. Park K, Heo DS, Cho B, et al. PF-00299804 (PF299) in Asian patients (pts) with non-small cell lung cancer (NSCLC) refractory to chemotherapy (CT) and erlotinib (E) or gefitinib (G): a phase (P) I/II study. J Clin Oncol. 2010;28 Suppl:abstract 7599.
70. Boyer MJ, Blackhall FH, Park K, et al. Efficacy and safety of PF299804 versus erlotinib: a global randomized phase 2 trial in patients with advanced non-small cell lung cancer (NSCLC) after failure of chemotherapy (CT). Oral presentation at: the 46th Annual Meeting of the American Society of Clinical Oncology, Chicago, IL, USA, 2010; June 4–8.
71. Kris MG, Mok T, Ou SH, et al. First-line dacomitinib (PF-00299804), an irreversible pan-HER tyrosine kinase inhibitor, for patients with EGFR-mutant lung cancers. J Clin Oncol. 2012:30 Suppl: abstract 7530.
72. Ruiz-Garcia A, Janne PA, Park K, et al. EGFR status and daily dose: Effect on tumor growth inhibition in cancer patients treated with dacomitinib (PF-00299804). J Clin Oncol. 2012;30 Suppl: abstract e18093.
73. Li D, Ambrogio L, Shimamura T, et al. BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. Oncogene. 2008;27:4702–11.
74. Metro G, Crinò L. The LUX-Lung clinical trial program of afatinib for non-small-cell lung cancer. Expert Rev Anticancer Ther. 2011;11:673–82.
75. clinicaltrials.gov. LUX-Breast 2; Afatinib in HER2 (Human Epidermal Growth Factor Receptor)-treatment failures NCT01271725 (2011–2016).
76. clinicaltrials.gov. 6 weeks treatment of locally advanced breast cancer with BIBW 2992 (Afatinib) or Lapatinib or Trastuzumab NCT00826267. http://www.clinicaltrials.gov/ct2/show/NCT00826267. Accessed 2 Feb 2012.
77. clinicaltrials.gov. Afatinib (BIBW2992) in HER2-overexpressing inflammatory breast cancer NCT013254 (2011–2016).
78. clinicaltrials.gov. LUX-Breast 1: BIBW 2992 (Afatinib) in HER2-positive metastatic breast cancer patients after one prior herceptin treatment NCT01125566 (2010–2016).
79. Rabindran SK, Discafani CM, Rosfjord EC, et al. Antitumor activity of HKI-272, an orally active, irreversible inhibitor of the HER-2 tyrosine kinase. Can Res. 2004;64:3958–65.
80. Burstein HJ, Sun Y, Dirix LY, et al. Neratinib, an irreversible ErbB receptor tyrosine kinase inhibitor, in patients with advanced ErbB2-positive breast cancer. J Clin Oncol. 2010;28:1301–7.
81. clinicaltrials.gov. Study evaluating the effects of neratinib after adjuvant trastuzumab in women with early stage breast cancer (ExteNET) NCT00878709. http://clinicaltrials.gov/ct2/show/NCT00878709. Accessed 1 Apr 2012.
82. clinicaltrials.gov. A phase 1/2 study of HKI-272 (Neratinib) in combination with paclitaxel (taxol) in subjects with solid tumors and breast cancer NCT00445458. http://clinicaltrials.gov/ct2/show/NCT00445458. Accessed 1 Apr 2012.
83. clinicaltrials.gov. A phase 1/2 Study Of HKI-272 (Neratinib) in combination with trastuzumab (herceptin) in subjects with advanced breast cancer NCT00398567. http://clinicaltrials.gov/ct2/show/NCT00398567. Accessed 1 Apr 2012.

84. clinicaltrials.gov. Study evaluating neratinib plus paclitaxel vs trastuzumab plus paclitaxel in ErbB-2 positive advanced breast cancer (NEFERTT) NCT00915018. http://clinicaltrials.gov/ct2/show/NCT00915018. Accessed 1 Apr 2012.
85. clinicaltrials.gov. Study evaluating neratinib versus lapatinib plus capecitabine for ErbB2 positive advanced breast cancer NCT00777101. http://clinicaltrials.gov/ct2/show/NCT00777101. Accessed 1 Apr 2012.
86. Berger C, Krengel U, Stang E, Moreno E, Madshus IH. Nimotuzumab and cetuximab block ligand-independent EGF receptor signaling efficiently at different concentrations. J Immunother. 2011;34:550–5.
87. Solomon MT, Selva IC, Figueredo J, et al. Radiotherapy plus nimotuzumab or placebo in the treatment of high grade glioma patients: results from a randomized, double blind trial. BMC Cancer. 2013;13:299.
88. Heinrich MC, Griffith D, McKinley A, et al. Crenolanib inhibits the drug-resistant PDGFRA D842V mutation associated with imatinib-resistant gastrointestinal stromal tumors. Clin Cancer Res. 2012;18:4375–84.
89. Lokker NA, Sullivan CM, Hollenbach SJ, Israel MA, Giese NA. Platelet-derived growth factor (PDGF) autocrine signaling regulates survival and mitogenic pathways in glioblastoma cells: evidence that the novel PDGF-C and PDGF-D ligands may play a role in the development of brain tumors. Cancer Res. 2002;62:3729–35.
90. Dong J, Grunstein J, Tejada M, et al. VEGF-null cells require PDGFR alpha signaling-mediated stromal fibroblast recruitment for tumorigenesis. Embo J. 2004;23:2800–10.
91. Pietras K, Pahler J, Bergers G, Hanahan D. Functions of paracrine PDGF signaling in the proangiogenic tumor stroma revealed by pharmacological targeting. PLoS Med. 2008;5, e19.
92. Puputti M, Tynninen O, Sihto H, et al. Amplification of KIT, PDGFRA, VEGFR2, and EGFR in gliomas. Mol Cancer Res. 2006;4:927–34.
93. Ostman A, Heldin CH. PDGF receptors as targets in tumor treatment. Adv Cancer Res. 2007;97:247–74.
94. Jain RK, Lahdenranta J, Fukumura D. Targeting PDGF signaling in carcinoma-associated fibroblasts controls cervical cancer in mouse model. PLoS Med. 2008;5, e24.
95. Thomson S, Petti F, Sujka-Kwok I, Epstein D, Haley JD. Kinase switching in mesenchymal-like non-small cell lung cancer lines contributes to EGFR inhibitor resistance through pathway redundancy. Clin Exp Metastasis. 2008;25:843–54.
96. Ehnman M, Missiaglia E, Folestad E, et al. Distinct effects of ligand-induced PDGFRα and PDGFRβ signaling in the human rhabdomyosarcoma tumor cell and stroma cell compartments. Cancer Res. 2013;73:2139–49.
97. Hayashi Y, Bardsley MR, Toyomasu Y, et al. Platelet-derived growth factor receptor-α regulates proliferation of gastrointestinal stromal tumor cells with mutations in KIT by stabilizing ETV1. Gastroenterology. 2015;149:420–32.
98. Brooks AN, Kilgour E, Smith PD. Molecular pathways: fibroblast growth factor signaling: a new therapeutic opportunity in cancer. Clin Cancer Res. 2012;18:1855–62.
99. Wesche J, Haglund K, Haugsten EM. Fibroblast growth factors and their receptors in cancer. Biochem J. 2011;437:199–213.
100. Taylor JG, Cheuk AT, Tsang PS, et al. Identification of FGFR4-activating mutations in human rhabdomyosarcomas that promote metastasis in xenotransplanted models. J Clin Invest. 2009;119:3395–407.
101. Tanner Y, Grose RP. Dysregulated FGF signalling in neoplastic disorders. Semin Cell Dev Biol. 2015; pii: S1084-9521(15)00209-8.
102. Gelsi-Boyer V, Orsetti B, Cervera N, Finetti P, et al. Comprehensive profiling of 8p11-12 amplification in breast cancer. Mol Cancer Res. 2005;3:655–67.
103. Turner N, Lambros MB, Horlings HM, et al. Integrative molecular profiling of triple negative breast cancers identifies amplicon drivers and potential therapeutic targets. Oncogene. 2010;20:2013–23.
104. Mohammadi M, Froum S, Hamby JM, et al. Crystal structure of an angiogenesis inhibitor bound to the FGF receptor tyrosine kinase domain. EMBO J. 1998;17:5896–904.

105. Koziczak M, Holbro T, Hynes NE. Blocking of FGFR signaling inhibits breast cancer cell proliferation through downregulation of D-type cyclins. Oncogene. 2004;23:3501–8.
106. Buchler P, Reber HA, Roth MM, Shiroishi M, Friess H, Hines OJ. Target therapy using a small molecule inhibitor against angiogenic receptors in pancreatic cancer. Neoplasia. 2007;9:119–27.
107. Dey JH, Bianchi F, Voshol J, Bonenfant D, Oakeley EJ, Hynes NE. Targeting fibroblast growth factor receptors blocks PI3K/AKT signaling, induces apoptosis, and impairs mammary tumor outgrowth and metastasis. Cancer Res. 2010;70:4151–62.
108. Sarker D, Molife R, Evans TR, et al. A phase I pharmacokinetic and pharmacodynamic study of TKI258, an oral, multitargeted receptor tyrosine kinase inhibitor in patients with advanced solid tumors. Clin Cancer Res. 2008;14:2075–81.
109. Shimada T, Urakawa I, Yamazaki Y, et al. FGF-23 transgenic mice demonstrate hypophosphatemic rickets with reduced expression of sodium phosphate cotransporter type IIa. Biochem Biophys Res Commun. 2004;314:409–14.
110. Dean M, Park M, Liu W, et al. The human met oncogene is related to the tyrosine kinase oncogenes. Nature. 1985;318:385–8.
111. Boon EM, van der Neut R, van de Wetering M, Clevers H, Pals ST. Wnt signaling regulates expression of the receptor tyrosine kinase met in colorectal cancer. Cancer Res. 2002;62:5126–8.
112. Comoglio PM, Giordano S, Trusolino L. Drug development of MET inhibitors: targeting oncogene addiction and expedience. Nat Rev Drug Discov. 2008;7:504–16.
113. Tsao MS, Yang Y, Marcus A, Liu N, Mou L. Hepatocyte growth factor is predominantly expressed by the carcinoma cells in non-small-cell lung cancer. Hum Pathol. 2001;32:57–65.
114. Trusolino L, Comoglio PM. Scatter-factor and semaphorin receptors: cell signalling for invasive growth. Nat Rev Cancer. 2002;2:289–300.
115. Love CA, Harlos K, Mavaddat N, et al. The ligand-binding face of the semaphorins revealed by the high-resolution crystal structure of SEMA4D. Nat Struct Biol. 2003;10:843–8.
116. Gherardi E, Love CA, Esnouf RM, Jones EY. The sema domain. Curr Opin Struct Biol. 2004;14:669–78.
117. Kong-Beltran M, Stamos J, Wickramasinghe D. The Sema domain of Met is necessary for receptor dimerization and activation. Cancer Cell. 2004;6:75–84.
118. Stamos J, Lazarus RA, Yao X, Kirchhofer D, Wiesmann C. Crystal structure of the HGF beta-chain in complex with the Sema domain of the Met receptor. EMBO J. 2004;23:2325–35.
119. Wickramasinghe D, Kong-Beltran M. Met activation and receptor dimerization in cancer: a role for the Sema domain. Cell Cycle. 2005;4:683–5.
120. Olivero M, Valente G, Bardelli A, et al. Novel mutation in the ATP-binding site of the MET oncogene tyrosine kinase in a HPRCC family. Int J Cancer. 1999;82:640–3.
121. Schmidt L, Junker K, Nakaigawa N, et al. Novel mutations of the MET proto-oncogene in papillary renal carcinomas. Oncogene. 1999;18:2343–50.
122. Giordano S, Maffe A, Williams TA, et al. Different point mutations in the met oncogene elicit distinct biological properties. FASEB J. 2000;14:399–406.
123. Lee JH, Han SU, Cho H, et al. A novel germ line juxtamembrane Met mutation in human gastric cancer. Oncogene. 2000;19:4947–53.
124. Ma PC, Tretiakova MS, MacKinnon AC, et al. Expression and mutational analysis of MET in human solid cancers. Genes Chromosomes Cancer. 2008;47:1025–37.
125. Seiwert TY, Jagadeeswaran R, Faoro L, et al. The MET receptor tyrosine kinase is a potential novel therapeutic target for head and neck squamous cell carcinoma. Cancer Res. 2009;69:3021–31.
126. Stella GM, Benvenuti S, Gramaglia D, et al. MET mutations in cancers of unknown primary origin (CUPs). Hum Mutat. 2011;32:44–50.
127. Lutterbach, Zeng Q, Davis LJ, et al. Lung cancer cell lines harboring MET gene amplification are dependent on Met for growth and survival. Cancer Res. 2007;67:2081–8.

128. Peschard P, Fournier TM, Lamorte L, et al. Mutation of the c-Cbl TKB domain binding site on the Met receptor tyrosine kinase converts it into a transforming protein. Mol Cell. 2001;8:995–1004.
129. Zeng ZS, Weiser MR, Kuntz E, et al. c-Met gene amplification is associated with advanced stage colorectal cancer and liver metastases. Cancer Lett. 2008;265:258–69.
130. Cappuzzo F, Jänne PA, Skokan M, et al. MET increased gene copy number and primary resistance to gefitinib therapy in non-small-cell lung cancer patients. Ann Oncol. 2009;20:298–304.
131. Go H, Jeon YK, Park HJ, Sung SW, Seo JW, Chung DH. High MET gene copy number leads to shorter survival in patients with non-small cell lung cancer. J Thorac Oncol. 2010;5:305–13.
132. Zou HY, Li Q, Lee JH, et al. An orally available small-molecule inhibitor of c-Met, PF-2341066, exhibits cytoreductive antitumor efficacy through antiproliferative and antiangiogenic mechanisms. Cancer Res. 2007;67:4408–17.
133. Corso S, Migliore C, Ghiso E, De Rosa G, Comoglio PM, Giordano S. Silencing the MET oncogene leads to regression of experimental tumors and metastases. Oncogene. 2008;27:684–93.
134. Eder JP, Vande Woude GF, Boerner SA, LoRusso PM. Novel therapeutic inhibitors of the c-Met signaling pathway in cancer. Clin Cancer Res. 2009;15:2207–14.
135. Jin H, Yang R, Zheng Z, et al. MetMAb, the one-armed 5D5 anti-c-Met antibody, inhibits orthotopic pancreatic tumor growth and improves survival. Cancer Res. 2008;68:4360–8.
136. NCT01418222 and Genentech. Randomized, double-blind, phase II study of FOLFOX/Bevacizumab with onartuzumab (MetMAb) versus placebo as first-line treatment for patients with metastatic colorectal cancer. 2011–2016; From http://clinicaltrials.gov/show/NCT01418222.
137. Bendell JC, Ervin TJ, Gallinson D, et al. Treatment rationale and study design for a randomized, double-blind, placebo-controlled phase II study evaluating onartuzumab (MetMAb) in combination with bevacizumab plus mFOLFOX-6 in patients with previously untreated metastatic colorectal cancer. Clin Colorectal Cancer. 2013;12:218–22.
138. Martens T, Schmidt NO, Eckerich C, et al. A novel one-armed anti-c-Met antibody inhibits glioblastoma growth in vivo. Clin Cancer Res. 2006;12:6144–52.
139. Vigna E, Pacchiana G, Mazzone M, et al. Active cancer immunotherapy by anti-Met antibody gene transfer. Cancer Res. 2008;68:9176–83.
140. Gordon MS, Sweeney CS, Mendelson DS, et al. Safety, pharmacokinetics, and pharmacodynamics of AMG 102, a fully human hepatocyte growth factor-neutralizing monoclonal antibody, in a first-in-human study of patients with advanced solid tumors. Clin Cancer Res. 2010;16:699–710.
141. Wen PY, Schiff D, Cloughesy TF, et al. A phase II study evaluating the efficacy and safety of AMG102 (rilotumumab) in patients with recurrent glioblastoma. Neuro Oncol. 2011;13:437–46.
142. Munshi N, Jeay S, Li Y, et al. ARQ 197, a novel and selective inhibitor of the human c-Met receptor tyrosine kinase with antitumor activity. Mol Cancer Ther. 2010;9:1544–53.
143. Eathiraj S, Palma R, Volckova E, et al. Discovery of a novel mode of protein kinase inhibition characterized by the mechanism of inhibition of human mesenchymal-epithelial transition factor (c-Met) protein autophosphorylation by ARQ 197. J Biol Chem. 2011;286:20666–76.
144. Schiller JH, Akerley WL, Brugge W, et al. Results from ARQ 197-209: a global randomized placebo-controlled phase II clinical trial of erlotinib plus ARQ 197 versus erlotinib plus placebo in previously treated EGFR inhibitor-naive patients with locally advanced or metastatic non-small cell lung cancer (NSCLC). J Clin Oncol. 2010;28(18s), LBA7502.
145. NCT01611857. A Phase I/II Trial of the c-Met inhibitor, tivantinib, in combination with FOLFOX for the treatment of patients with advanced solid tumors (phase I) and previously untreated metastatic adenocarcinoma of the distal esophagus, gastroesophageal (GE) junction, or stomach (phase II). 2012: From http://clinicaltrials.gov/ct2/show/NCT01611857.

146. Basilico C, Pennacchietti S, Vigna E, et al. Tivantinib (ARQ197) displays cytotoxic activity that is independent of its ability to bind MET. Clin Cancer Res. 2013;19:2381–92.
147. Katayama R, Aoyama A, Yamori T, et al. Cytotoxic activity of tivantinib (ARQ 197) is not due solely to c-MET inhibition. Cancer Res. 2013;73:3087–96.
148. NCT00788957 and Amgen. Panitumumab combination study with AMG 102 or AMG 479 in Wild-type KRAS mCRC. 2008; From http://clinicaltrials.gov/ct2/show/NCT00788957.
149. Bladt F, Faden B, Friese-Hamim M, et al. EMD 1214063 and EMD 1204831 constitute a new class of potent and highly selective c-Met inhibitors. Clin Cancer Res. 2013;19:2941–51.
150. Medova M, Pochon B, Streit B, et al. The novel ATP-competitive inhibitor of the MET hepatocyte growth factor receptor EMD1214063 displays inhibitory activity against selected MET-mutated variants. Mol Cancer Ther. 2013;12:2415–24.
151. Humbert M, Medova M, Aebersold DM, et al. Protective autophagy is involved in resistance towards MET inhibitors in human gastric adenocarcinoma cells. Biochem Biophys Res Commun. 2013;431:264–9.
152. Scorsone K, Zhang L, Woodfield SE, Hicks J, Zage PE. The novel kinase inhibitor EMD1214063 is effective against neuroblastoma. Investig New Drugs. 2014;32:815–24.
153. Graham DK, Salzberg DB, Kurtzberg J, et al. Ectopic expression of the proto-oncogene mer in pediatric T-cell acute lymphoblastic leukemia. Clin Cancer Res. 2006;12:2662–9.
154. Linger RMA, Keating AM, Earp HS, Graham DK. TAM receptor tyrosine kinases: Biologic functions, signaling, and potential therapeutic targeting in human cancer. Adv Cancer Res. 2008;100:35–83.
155. Holland SJ, Pan A, Franci C, et al. R428, a selective small molecule inhibitor of AXL kinase, blocks tumor spread and prolongs survival in models of metastatic breast cancer. Cancer Res. 2010;70:1544–54.
156. Wnuk-Lipinska K, Gausdal G, Sandal T, et al. Selective small molecule AXL inhibitor BGB324 overcomes acquired drug resistance in non-small cell lung carcinoma models. Clin Cancer Res. 2014;20:B30.
157. Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med. 1971;285:1182–6.
158. Morgan B, Thomas AL, Drevs J, et al. Dynamic contrast-enhanced magnetic resonance imaging as a biomarker for the pharmacological response of PTK787/ZK 222584, an inhibitor of the vascular endothelial growth factor receptor tyrosine kinases, in patients with advanced colorectal cancer and liver metastases: results from two phase I studies. J Clin Oncol. 2003;21:3955–64.
159. Jost LM, Gschwind HP, Jalava T, et al. Metabolism and disposition of vatalanib (PTK787/ZK-222584) in cancer patients. Drug Metab Dispos. 2006;34:1817–28.
160. Wood JM, Bold G, Buchdunger E, et al. PTK787/ZK 222584, a novel and potent inhibitor of vascular endothelial growth factor receptor tyrosine kinases, impairs vascular endothelial growth factor-induced responses and tumor growth after oral administration. Cancer Res. 2000;60:2178–89.
161. Nakamura K, Taguchi E, Miura T, et al. KRN951, a highly potent inhibitor of vascular endothelial growth factor receptor tyrosine kinases, has antitumor activities and affects functional vascular properties. Cancer Res. 2006;66:9134–42.
162. Mayer EL, Scheulen ME, Beckman J, et al. A Phase I dose-escalation study of the VEGFR inhibitor tivozanib hydrochloride with weekly paclitaxel in metastatic breast cancer. Breast Cancer Res Treat. 2013;140:331–9.
163. Eskens FA, de Jonge MJ, Bhargava P, et al. Biologic and clinical activity of tivozanib (AV-951, KRN-951), a selective inhibitor of VEGF receptor-1, -2, and -3 tyrosine kinases, in a 4-week-on, 2-week-off schedule in patients with advanced solid tumors. Clin Cancer Res. 2011;17:7156–63.
164. Batchelor TT, Duda DG, di Tomaso E, et al. Phase II study of cediranib, an oral pan-vascular endothelial growth factor receptor tyrosine kinase inhibitor, in patients with recurrent glioblastoma. J Clin Oncol. 2010;28:2817–23.
165. Wick W, Wick A, Weiler M, Weller M. Patterns of progression in malignant glioma following anti-VEGF therapy: perceptions and evidence. Curr Neurol Neurosci Rep. 2011;11:305–12.

166. Batchelor TT, Sorensen AG, di Tomaso E, et al. AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. Cancer Cell. 2007;11:83–95.
167. Rahman R, Smith S, Rahman C, Grundy R. Antiangiogenic therapy and mechanisms of tumor resistance in malignant glioma. J Oncol. 2010;2010:251231.
168. Winkler F, Kozin SV, Tong RT, et al. Kinetics of vascular normalization by VEGFR2 blockade governs brain tumor response to radiation: role of oxygenation, angiopoietin- 1, and matrix metalloproteinases. Cancer Cell. 2004;6:553–63.
169. Wedge SR, Kendrew J, Hennequin LF, et al. AZD2171: a highly potent, orally bioavailable, vascular endothelial growth factor receptor-2 tyrosine kinase inhibitor for the treatment of cancer. Cancer Res. 2005;65:4389–400.
170. Takeda M, Arao T, Yokote H, et al. AZD2171 shows potent antitumor activity against gastric cancer over-expressing fibroblast growth factor receptor 2/keratinocyte growth factor receptor. Clin Cancer Res. 2007;13:3051–7.
171. Siemann DW, Brazelle WD, Jurgensmeier JM. The vascular endothelial growth factor receptor-2 tyrosine kinase inhibitor cediranib (Recentin; AZD2171) inhibits endothelial cell function and growth of human renal tumor xenografts. Int J Radiat Oncol Biol Phys. 2009;73:897–903.
172. Medinger M, Esser N, Zirrgiebel U, Ryan A, Jurgensmeier JM, Drevs J. Antitumor and antiangiogenic activity of cediranib in a preclinical model of renal cell carcinoma. Anticancer Res. 2009;29:5065–76.
173. Dietrich J, Wang D, Batchelor TT. Cediranib: profile of a novel antiangiogenic agent in patients with glioblastoma. Expert Opin Investig Drugs. 2009;18:1549–57.
174. Motzer RJ, Michaelson MD, Redman BG, et al. Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. J Clin Oncol. 2006;24:16–24.
175. Motzer RJ, Rini BI, Bukowski RM, et al. Sunitinib in patients with metastatic renal cell carcinoma. JAMA. 2006;295:2516–24.
176. Motzer RJ, Hutson TE, Tomczak P, et al. Phase III ran domized trial of sunitinib malate (SU11248) versus interferon alfa (IFN-a) as first-line systemic therapy for patients with metastatic renal cell carcinoma (mRCC). J Clin Oncol. 2006;24 (Suppl; late breaking abstract 3).
177. Casali PG, Garrett CR, Blackstein ME, et al. Updated results from a phase III trial of sunitinib in GIST patients (pts) for whom imatinib (IM) therapy has failed due to resistance or intolerance. J Clin Oncol. 2006; 24 (Suppl; abstr 9513).
178. Demetri G, van Oosterom AT, Garrett C, et al. Improved survival and sustained clinical benefit with SU11248 (SU) in pts with GIST after failure of imatinib mesylate (IM) therapy in a phase III trial. 2006 Gastrointestinal Cancers Symposium 2006; Abstract No: 8.
179. George S, Casali PG, Blay J, et al. Phase II study of sunitinib administered in a continuous daily dosing regimen in patients (pts) with advanced GIST. J Clin Oncol. 2006;24 (Suppl; abstr 9532) 87.
180. Socinski MA, Novello S, Sanchez JM, et al. Efficacy and safety of sunitinib in previously treated, advanced non-small cell lung cancer (NSCLC): preliminary results of a multicenter phase II trial. J Clin Oncol. 2006;24 (Suppl; abstr 241):88.
181. Kulke M, Lenz HJ, Meropol NJ, et al. A phase 2 study to evaluate the efficacy and safety of SU11248 in patients (pts) with unresectable neuroendocrine tumors (NETs). J Clin Oncol. 2005;23 (Suppl; abstr 4008).
182. Sridhar SS, Hedley D, Siu LL. Raf kinase as a target for anticancer therapeutics. Mol Cancer Ther. 2005;4:677–85.
183. Strumberg D, Richly H, Hilger RA, et al. Phase I clinical and pharmacokinetic study of the Novel Raf kinase and vascular endothelial growth factor receptor inhibitor BAY 43-9006 in patients with advanced refractoryn solid tumors. J Clin Oncol. 2005;23:965–72.
184. Ratain MJ, Eisen T, Stadler WM, et al. Phase II placebo-controlled randomized discontinuation trial of sorafenib in patients with metastatic renal cell carcinoma. J Clin Oncol. 2006;24:2505–12.

185. Ratain MJ, Eisen T, Stadler WM, et al. Final findings from a Phase II, placebo-controlled, randomized discontinuation trial (RDT) of sorafenib (BAY 43-9006) in patients with advanced renal cell carcinoma (RCC). J Clin Oncol. 2005;23 (Suppl; abstr 4544).
186. Escudier B, Szczylic C, Eisen T, et al. Randomized Phase III trial of the Raf kinase and VEGFR inhibitor sorafenib (BAY 43-9006) in patients with advanced renal cell carcinoma (RCC). J Clin Oncol. 2005;23 (Suppl; late breaking abstract 4510).
187. Eisen T, Bukowski RM, Staehler M, et al. Randomized phase III trial of sorafenib in advanced renal cell carcinoma (RCC): impact of crossover on survival. J Clin Oncol. 2006;24 (Suppl; abstr 4524).
188. Perez-Soler R. Can rash associated with HER1/EGFR inhi bition be used as a marker of treatment outcome? Oncology (Williston Park). 2003;17:23–8.
189. Strumberg D, Awada A, Hirte H, et al. Pooled safety analysis of BAY 43-9006 (sorafenib) monotherapy in patients with advanced solid tumours: Is rash associated with treatment outcome? Eur J Cancer. 2006;42:548–56.
190. Wilhelm SM, Dumas J, Adnane L, et al. Regorafenib (BAY 73–4506): a new oral multikinase inhibitor of angiogenic, stromal and oncogenic receptor tyrosine kinases with potent preclinical antitumor activity. Int J Cancer. 2011;129:245–55.
191. Grothey A, Van Cutsem E, Sobrero A, et al. CORRECT Study Group. Regorafenib monotherapy for previously treated metastatic colorectal cancer: an international, multicentre, prospective, randomised, placebo controlled phase 3 trial (CORRECT). Lancet. 2013;381:303–12.
192. Schmieder R, Hoffmann J, Becker M, et al. Regorafenib (BAY 73-4506): antitumor and antimetastatic activities in preclinical models of colorectal cancer). Intern J Cancer. 2014;135:1487–96.
193. Tang R, Kain T, Herman J, Seer T. Durable response using regorafenib in an elderly patient with metastatic colorectal cancer: case report. Cancer Manag Res. 2015;7:357–60.
194. Parikh SS, Mehta HH, Desai BI. Advances in development of bevacizumab, a humanized antiangiogenic therapeutic monoclonal antibody targeting VEGF in cancer cells. Int J Pharm Biomed Sci. 2012;3:155–63.
195. Tabrizi M, Roskos LK. xposure–response relationships for therapeutic biologic products. In: Meibohm B, editor. Pharmacokinetics and pharmacodynamics of biotech drugs. Principles and case studies in drug development. Weinheim: Wiley-VCH; 2006. p. 295–327.
196. Gerber HP, Ferrara N. Pharmacology and pharmacodynamics of bevacizumab as monotherapy or in combination with cytotoxic therapy in preclinical studies. Cancer Res. 2005;65:671–80.
197. Schutz FA, Je Y, Azzi GR, Nguyen PL, Choueiri TK. Bevacizumab increases the risk of arterial ischemia: a large study in cancer patients with a focus on different subgroup outcomes. Ann Oncol. 2011;22:1404–12.
198. Economopoulou E, Kotsakis A, Kapiris I, Kentepozidis N. Cancer therapy and cardiovascular risk: focus on bevacizumab. Cancer Manag Res. 2015;15:133–43.
199. Mirone G, Shukla A, Marfe G. Signaling mechanisms of resistance to EGFR- and Anti-Angiogenic Inhibitors cancer. Crit Rev Oncol Hematol. 2016;97:85–95.
200. Holash J, Davis S, Papadopoulos N, et al. VEGF-Trap: a VEGF blocker with potent antitumor effects. Proc Natl Acad Sci U S A. 2002;99:11393–8.
201. Ferrara N, Hillan KJ, Gerber HP, Novotny W. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. Nat Rev Drug Discov. 2004;3:391–400.
202. Kim ES, Serur A, Huang J, Manley CA, et al. Potent VEGF blockade causes regression of coopted vessels in a model of neuroblastoma. Proc Natl Acad Sci U S A. 2002;99:11399–404.
203. Rudge JS, Holash J, Hylton D. Inaugural article: VEGF trap complex formation measures production rates of VEGF, providing a biomarker for predicting efficacious angiogenic blockade. Proc Natl Acad Sci U S A. 2007;104:18363–70.
204. Gomez-Manzano C, Holash J, Fueyo J. VEGF Trap induces antiglioma effect at different stages of disease. Neuro Oncol. 2008;10:940–5.

205. Van Cutsem E, Tabernero J, Lakomy R, et al. Addition of aflibercept to fluorouracil, leucovorin, and irinotecan improves survival in a phase III randomized trial in patients with metastatic colorectal cancer previously treated with an oxaliplatin based regimen. J Clin Oncol. 2012;30:3499–506.
206. Yasuda H, Kobayashi S, Costa DB. EGFR exon 20 insertion mutations in non-small-cell lung cancer: Preclinical data and clinical implications. Lancet Oncol. 2012;13:e23–31.
207. Wu JY, Wu SG, Yang CH, et al. Lung cancer with epidermal growth factor receptor exon 20 mutations is associated with poor gefitinib treatment response. Clin Cancer Res. 2008;14:4877–82.
208. Greulich H, Chen TH, Feng W, et al. Oncogenic transformation by inhibitor-sensitive and -resistant EGFR mutants. PLoS Med. 2005;2, e313.
209. Inukai M, Toyooka S, Ito S, et al. Presence of epidermal growth factor receptor gene T790M mutation as a minor clone in non-small cell lung cancer. Cancer Res. 2006;66:7854–8.
210. Tokumo M, Toyooka S, Ichihara S, et al. Double mutation and gene copy number of EGFR in gefitinib refractory non-small-cell lung cancer. Lung Cancer. 2006;53:117–21.
211. Sequist LV, Martins RG, Spigel D, et al. First-line gefitinib in patients with advanced non-small-cell lung cancer harboring somatic EGFR mutations. J Clin Oncol. 2008;26:2442–9.
212. Turke AB, Zejnullahu K, Wu YL, et al. Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. Cancer Cell. 2010;17:77–88.
213. Engelman JA, Zejnullahu K, Mitsudomi T, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science. 2007;316:1039–43.
214. Solomon B, Varella-Garcia M, Camidge DR. ALK gene rearrangements. A new therapeutic target in a molecularly defined subset of non-small cell lung cancer. J Thorac Oncol. 2009;4:1450–4
215. Selinger CI, Rogers TM, Russell PA, et al. Testing for ALK rearrangement in lung adenocarcinoma-a multicenter comparison of immunohistochemistry and fluorescent in situ hybridization. Mod Pathol. 2013;26:1545–53.
216. Koivunen JP, Mermel C, Zejnullahu K, et al. EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. Clin Cancer Res. 2008;14:4275–83.
217. Choi YL, Takeuchi K, Soda M, et al. Identification of novel isoform of the EML4-ALK transforming gene in non-small cell lung cancer. Cancer Res. 2008;68:4971–6.
218. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature. 2007;448:561–6.
219. Lindeman NI, Cagle PT, Beasley MB, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. J Thorac Oncol. 2013;8:823–59.
220. Méndez M, Custodio A, Provencio M. New molecular targeted therapies for advanced non-small-cell lung cancer. J Thorac Dis. 2011;3:30–56.
221. Gainor JF, Shaw AT. Emerging paradigms in the development of resistance to tyrosine kinase inhibitors in lung cancer. J Clin Oncol. 2013;31:3987–96.
222. Costa DB, Halmos B, Kumar A, et al. BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. PLoS Med. 2007;4:1669–79. discussion 1680.
223. Cragg MS, Kuroda J, Puthalakath H, et al. Gefitinib-induced killing of NSCLC cell lines expressing mutant EGFR requires BIM and can be enhanced by BH3 mimetics. PLoS Med. 2007;4:1681–9. discussion 1690.
224. Gong Y, Somwar R, Politi K, et al. Induction of BIM is essential for apoptosis triggered by EGFR kinase inhibitors in mutant EGFR-dependent lung adenocarcinomas. PLoS Med. 2007;4, e294.
225. Faber AC, Corcoran RB, Ebi H, et al. BIM expression in treatment-naive cancers predicts responsiveness to kinase inhibitors. Cancer Discov. 2011;1:352–65.
226. Ng KP, Hillmer AM, Chuah CT, et al. A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. Nat Med. 2012;18:521–8.

227. Ercan D, Zejnullahu K, Yonesaka K, et al. Amplification of EGFR T790M causes resistance to an irreversible EGFR inhibitor. Oncogene. 2010;29:2346–56.
228. Borras E, Jurado I, Hernan I, et al. Clinical pharmacogenomic testing of KRAS, BRAF and EGFR mutations by high resolution melting analysis and ultra-deep pyrosequencing. BMC Cancer. 2011;11:406.
229. Provencio M, Garcia-Campelo R, Isla D, de Castro J. Clinical molecular factors predicting response and survival for tyrosine-kinase inhibitors. Clin Transl Oncol. 2009;11:428–36.
230. Rosell R, Ichinose Y, Taron M, et al. Mutations in the tyrosine kinase domain of the EGFR gene associated with gefitinib response in non-small-cell lung cancer. Lung Cancer. 2005;50:25–33.
231. Rosell R, Moran T, Cardenal F, et al. Predictive biomarkers in the management of EGFR mutant lung cancer. Ann N Y Acad Sci. 2010;1210:45–52.
232. Yun CH, Mengwasser KE, Toms AV, et al. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. Proc Natl Acad Sci U S A. 2008;105:2070–5.
233. Doebele RC, Pilling AB, Aisner DL, et al. Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. Clin Cancer Res. 2012;18:1472–82.
234. Lovly CM, Pao W. Escaping ALK inhibition: mechanisms of and strategies to overcome resistance. Sci Transl Med. 2012;4:120.
235. Sequist LV, Waltman BA, Dias-Santagata D, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med. 2011;3:75ra26.
236. Katayama R, Shaw AT, Khan TM, et al. Mechanisms of acquired crizotinib resistance in ALK-rearranged lung cancers. Sci Transl Med. 2012;4:120ra17.
237. Katayama R, Khan TM, Benes C, et al. Therapeutic strategies to overcome crizotinib resistance in non-small cell lung cancers harboring the fusion oncogene EML4-ALK. Proc Natl Acad Sci U S A. 2011;108:7535–40.
238. Guix M, Faber AC, Wang SE, et al. Acquired resistance to EGFR tyrosine kinase inhibitors in cancer cells is mediated by loss of IGF-binding proteins. J Clin Invest. 2008;118:2609–19.
239. Yano S, Wang W, Li Q, et al. Hepatocyte growth factor induces gefitinib resistance of lung adenocarcinoma with epidermal growth factor receptor-activating mutations. Cancer Res. 2008;68:9479–87.
240. Christensen JG, Schreck R, Burrows J, et al. A selective small molecule inhibitor of c-Met kinase inhibits c-Met-dependent phenotypes in vitro and exhibits cytoreductive antitumor activity in vivo. Cancer Res. 2003;63:7345–55.
241. Miller CT, Lin L, Casper AM, et al. Genomic amplification of MET with boundaries within fragile site FRA7G and upregulation of MET pathways in esophageal adenocarcinoma. Oncogene. 2006;25:409–18.
242. Smolen GA, Sordella R, Muir B, et al. Amplification of MET may identify a subset of cancers with extreme sensitivity to the selective tyrosine kinase inhibitor PHA-665752. Proc Natl Acad Sci U S A. 2006;103:2316–21.
243. Takezawa K, Pirazzoli V, Arcila ME, et al. HER2 amplification: a potential mechanism of acquired resistance to EGFR inhibition in EGFR-mutant lung cancers that lack the second-site EGFRT790M mutation. Cancer Discov. 2012;2:922–33.
244. Ohashi K, Sequist LV, Arcila ME, et al. Lung cancers with acquired resistance to EGFR inhibitors occasionally harbor BRAF gene mutations but lack mutations in KRAS, NRAS, or MEK1. Proc Natl Acad Sci U S A. 2012;109:E2127–33.
245. Engelman JA, Mukohara T, Zejnullahu K, et al. Allelic dilution obscures detection of a biologically significant resistance mutation in EGFR-amplified lung cancer. J Clin Invest. 2006;116:2695–706.
246. Sasaki T, Koivunen J, Ogino A, et al. A novel ALK secondary mutation and EGFR signaling cause resistance to ALK kinase inhibitors. Cancer Res. 2011;71:6051–60.
247. Arcila ME, Oxnard GR, Nafa K, et al. Rebiopsy of lung cancer patients with acquired resistance to EGFR inhibitors and enhanced detection of the T790M mutation using a locked nucleic acid-based assay. Clin Cancer Res. 2011;17:1169–80.

248. Zakowski MF, Ladanyi KMG. EGFR mutations in small-cell lung cancers in patients who have never smoked. N Engl J Med. 2006;355:213–5.
249. Gong Y, Yao E, Shen R, et al. High expression levels of total IGF-1R and sensitivity of NSCLC cells in vitro to an anti-IGF-1R antibody (R1507). PLoS One. 2009;4, e7273.
250. Sharma SV, Lee DY, Li B, et al. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell. 2010;141:69–80.
251. Yu HA, Arcila ME, Rekhtman N, et al. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. Clin Cancer Res. 2013;19:2240–7.
252. Chaft JE, Oxnard GR, Sima CS, Miller VA, Riely GJ. Disease flare after tyrosine kinase inhibitor discontinuation in patients with EGFR-mutant lung cancer and acquired resistance to erlotinib or gefitinib: Implications for clinical trial design. Clin Cancer Res. 2011;17:6298–303.
253. Pop O, Pirvu A, Toffart AC, Moro-Sibilot D. Disease flare after treatment discontinuation in a patient with EML4-ALK lung cancer and acquired resistance to crizotinib. J Thorac Oncol. 2012;7:e1–2.
254. Overman MJ, Modak J, Kopetz S, et al. Use of research biopsies in clinical trials: Are risks and benefits adequately discussed? J Clin Oncol. 2013;31:17–22.
255. Peppercorn J. Toward improved understanding of the ethical and clinical issues surrounding mandatory research biopsies. J Clin Oncol. 2013;31:1–2.
256. Kuang Y, Rogers A, Yeap BY, et al. Noninvasive detection of EGFR T790M in gefitinib or erlotinib resistant non-small cell lung cancer. Clin Cancer Res. 2009;15:2630–6.
257. Hohenberger P, Ronellenfitsch U, Oladeji O, et al. Pattern of recurrence in patients with ruptured primary gastrointestinal stromal tumour. Br J Surg. 2010;97:1854–9.
258. Dematteo RP, Heinrich MC, El-Rifai WM, Demetri G. Clinical management of gastrointestinal stromal tumors: before and after STI-571. Hum Pathol. 2002;33:466–77.
259. Plaat BE, Hollema H, Molenaar WM, et al. Soft tissue leiomyosarcomas and malignant gastrointestinal stromal tumors: differences in clinical outcome and expression of multidrug resistance proteins. J Clin Oncol. 2000;18:3211–20.
260. Hirota S, Isozaki K, Moriyama Y, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science. 1998;279:577–80.
261. Buchdunger E, Cioffi CL, Law N, et al. Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors. J Pharmacol Exp Ther. 2000;295:139–45.
262. Heinrich MC, Griffith DJ, Druker BJ, Wait CL, Ott KA, Zigler AJ. Inhibition of c-kit receptor tyrosine kinase activity by STI 571, a selective tyrosine kinase inhibitor. Blood. 2000;96:925–32.
263. Heinrich MC, Wait CL, Yee KWH, Griffith DJ. STI571 inhibits the kinase activity of wild type and juxtamembrane c-kit mutants but not the exon 17 D816V mutation associated with mastocytosis. Blood. 2000;96:173b.
264. Tuveson DA, Willis NA, Jacks T, et al. STI571 inactivation of the gastrointestinal stromal tumor c-KIT oncoprotein: biological and clinical implications. Oncogene. 2001;20:5054–8.
265. Joensuu H, Roberts PJ, Sarlomo-Rikala M, et al. Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. N Engl J Med. 2001;344:1052–6.
266. van Oosterom AT, Judson IR, Verweij J, et al. Update of phase I study of imatinib (STI571) in advanced soft tissue sarcomas and gastrointestinal stromal tumors: a report of the EORTC Soft Tissue and Bone Sarcoma Group. Eur J Cancer. 2002;38 Suppl 5:S83–7.
267. Van den Abbeele AD, Badawi RD. Use of positron emission tomography in oncology and its potential role to assess response to imatinib mesylate therapy in gastrointestinal stromal tumors (GISTs). Eur J Cancer. 2002;38 Suppl 5:S60–5.
268. Bauer S, Yu LK, Demetri GD, Fletcher JA. Heat shock protein 90 inhibition in imatinib-resistant gastrointestinal stromal tumor. Cancer Res. 2006;66:9153–961.

269. Gordon PM, Fisher DE. Role for the proapoptotic factor BIM in mediating imatinib-induced apoptosis in a c-KIT-dependent gastrointestinal stromal tumor cell line. J Biol Chem. 2010;285:14109–14.
270. Liu Y, Tseng M, Perdreau SA, et al. Histone H2AX is a mediator of gastrointestinal stromal tumor cell apoptosis following treatment with imatinib mesylate. Cancer Res. 2007;67:2685–92.
271. Balachandran VP, Cavnar MJ, Zeng S, et al. Imatinib potentiates antitumor T cell responses in gastrointestinal stromal tumor through the inhibition of Ido. Nat Med. 2011;17:1094–100.
272. Le Cesne A, Ray-Coquard I, Bui BN, et al. Discontinuation of imatinib in patients with advanced gastrointestinal stromal tumours after 3 years of treatment: an open-label multicentre randomised phase 3 trial. Lancet Oncol. 2010;11:942–9.
273. Agaram NP, Besmer P, Wong GC, et al. Pathologic and molecular heterogeneity in imatinib-stable or imatinib-responsive gastrointestinal stromal tumors. Clin Cancer Res. 2007;13:170–81.
274. Liu Y, Perdreau SA, Chatterjee P, Wang L, Kuan SF, Duensing A. Imatinib mesylate induces quiescence in gastrointestinal stromal tumor cells through the CDH1-SKP2-p27Kip1 signaling axis. Cancer Res. 2008;68:9015–23.
275. Gupta A, Roy S, Lazar AJ, et al. Autophagy inhibition and antimalarials promote cell death in gastrointestinal stromal tumor (GIST). Proc Natl Acad Sci U S A. 2010;107:14333–8.
276. Abhyankar SA, Nair N. Highlighting the role of FDG PET scan in early response assessment of gastrointestinal stromal tumor treated with imatinib mesylate. Clin Nucl Med. 2008;33:213–4.
277. Choi H, Charnsangavej C, Faria SC, et al. Correlation of computed tomography and positron emission tomography in patients with metastatic gastrointestinal stromal tumor treated at a single institution with imatinib mesylate: proposal of new computed tomography response criteria. J Clin Oncol. 2007;25:1753–9.
278. Blanke CD, Demetri GD, von Mehren M, et al. Long-term results from a randomized phase II trial of standard- versus higher-dose imatinib mesylate for patients with unresectable or metastatic gastrointestinal stromal tumors expressing KIT. J Clin Oncol. 2008;26:620–5.
279. Blanke CD, Rankin C, Demetri GD, et al. Phase III randomized, intergroup trial assessing imatinib mesylate at two dose levels in patients with unresectable or metastatic gastrointestinal stromal tumors expressing the kit receptor tyrosine kinase: S0033. J Clin Oncol. 2008;26:626–32.
280. Verweij J, Casali PG, Zalcberg J, et al. Progression-free survival in gastrointestinal stromal tumours with high-dose imatinib: randomised trial. Lancet. 2004;364:1127–34.
281. Debiec-Rychter M, Sciot R, Le Cesne A, et al. KIT mutations and dose selection for imatinib in patients with advanced gastrointestinal stromal tumours. Eur J Cancer. 2006;42:1093–103.
282. Debiec-Rychter M, Wasag B, Stul M, et al. Gastrointestinal stromal tumours (GISTs) negative for KIT (CD117 antigen) immunoreactivity. J Pathol. 2004;202:430–8.
283. Heinrich MC, Corless CL, Demetri GD, et al. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. J Clin Oncol. 2003;21:4342–9.
284. Heinrich MC, Owzar K, Corless CL, et al. Correlation of kinase genotype and clinical outcome in the North American Intergroup Phase III Trial of imatinib mesylate for treatment of advanced gastrointestinal stromal tumor: CALGB 150105 Study by Cancer and Leukemia Group B and Southwest Oncology Group. J Clin Oncol. 2008;26:5360–7.
285. Heinrich MC, Maki RG, Corless CL, et al. Primary and secondary kinase genotypes correlate with the biological and clinical activity of sunitinib in imatinib-resistant gastrointestinal stromal tumor. J Clin Oncol. 2008;26:5352–9.
286. MetaGIST. Comparison of two doses of imatinib for the treatment of unresectable or metastatic gastrointestinal stromal tumors: a meta-analysis of 1,640 patients. J Clin Oncol. 2010;28:1247–53.

287. Janeway KA, Albritton KH, Van Den Abbeele AD, et al. Sunitinib treatment in pediatric patients with advanced GIST following failure of imatinib. Pediatr Blood Cancer. 2009;52:767–71.
288. Yuzawa S, Opatowsky Y, Zhang Z, Mandiyan V, Lax I, Schlessinger J. Structural basis for activation of the receptor tyrosine kinase KIT by stem cell factor. Cell. 2007;130:323–34.
289. Corless CL, Barnett CM, Heinrich MC. Gastrointestinal stromal tumours: origin and molecular oncology. Nat Rev Cancer. 2011;11:865–78.
290. Corless CL, Schroeder A, Griffith D, et al. PDGFRA mutations in gastrointestinal stromal tumors: frequency, spectrum and in vitro sensitivity to imatinib. J Clin Oncol. 2005;23:5357–564.
291. Weisberg E, Wright RD, Jiang J, et al. Effects of PKC412, nilotinib, and imatinib against GIST-associated PDGFRA mutants with differential imatinib sensitivity. Gastroenterology. 2006;131:1734–42.
292. Gajiwala KS, Wu JC, Christensen J, et al. KIT kinase mutants show unique mechanisms of drug resistance to imatinib and sunitinib in gastrointestinal stromal tumor patients. Proc Natl Acad Sci U S A. 2009;106:1542–7.
293. Biron P, Cassier PA, Fumagalli E, et al. Grp ESTBS. Outcome of patients (pts) with PDGFRA(D842V) mutant gastrointestinal stromal tumor (GIST) treated with imatinib (IM) for advanced disease. J Clin Oncol. 2010;28:15s (suppl; abstr 10051)
294. Debiec-Rychter M, Cools J, Dumez H, et al. Mechanisms of resistance to imatinib mesylate in gastrointestinal stromal tumors and activity of the PKC412 inhibitor against imatinib-resistant mutants. Gastroenterology. 2005;128:270–9.
295. Liegl B, Kepten I, Le C, et al. Heterogeneity of kinase inhibitor resistance mechanisms in GIST. J Pathol. 2008;216:64–74.
296. Miselli FC, Casieri P, Negri T, et al. c-Kit/PDGFRA gene status alterations possibly related to primary imatinib resistance in gastrointestinal stromal tumors. Clin Cancer Res. 2007;13:2369–77.
297. Antonescu CR, Viale A, Sarran L, et al. Gene expression in gastrointestinal stromal tumors is distinguished by KIT genotype and anatomic site. Clin Cancer Res. 2004;10:3282–90.
298. Grimpen F, Yip D, McArthur G, et al. Resistance to imatinib, low-grade FDG-avidity on PET, and acquired KIT exon 17 mutation in gastrointestinal stromal tumour. Lancet Oncol. 2005;6:724–7.
299. Heinrich MC, Corless CL, Blanke CD, et al. Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. J Clin Oncol. 2006;24:4764–74.
300. Wakai T, Kanda T, Hirota S, Ohashi A, Shirai Y, Hatakeyama K. Late resistance to imatinib therapy in a metastatic gastrointestinal stromal tumour is associated with a second KIT mutation. Br J Cancer. 2004;90:2059–61.
301. Loughrey MB, Waring PM, Dobrovic A, Demetri G, Kovalenko S, McArthur G. Polyclonal resistance in gastrointestinal stromal tumor treated with sequential kinase inhibitors. Clin Cancer Res. 2006;12(20 Pt 1):6205–6. author reply 6206-6207.
302. Wardelmann E, Merkelbach-Bruse S, Pauls K, et al. Polyclonal evolution of multiple secondary KIT mutations in gastrointestinal stromal tumors under treatment with imatinib mesylate. Clin Cancer Res. 2006;12:1743–9.
303. Mahadevan D, Cooke L, Riley C, et al. A novel tyrosine kinase switch is a mechanism of imatinib resistance in gastrointestinal stromal tumors. Oncogene. 2007;26:3909–19.
304. Takahashi T, Serada S, Ako M, et al. New findings of kinase switching in gastrointestinal stromal tumor under imatinib using phosphoproteomic analysis. Int J Cancer. 2013;133:2737–43.
305. Sakurama K, Noma K, Takaoka M, et al. Inhibition of focal adhesion kinase as a potential therapeutic strategy for imatinib-resistant gastrointestinal stromal tumor. Mol Cancer Ther. 2009;8:127–34.
306. Bos JL, Fearon ER, Hamilton SR, et al. Prevalence of ras gene mutations in human colorectal cancers. Nature. 1987;327:293–7.

307. Mascaux C, Iannino CN, Martin B, et al. The role of RAS oncogene in survival of patients with lung cancer: a systematic review of the literature with meta-analysis. Br J Cancer. 2005;92:131–9.
308. Linardou H, Dahabreh IJ, Kanaloupiti D, et al. Assessment of somatic k-RAS mutations as a mechanism associated with resistance to EGFR-targeted agents: a systematic review and meta-analysis of studies in advanced non-small-cell lung cancer and metastatic colorectal cancer. Lancet Oncol. 2008;9:962–72.
309. Lievre A, Bachet JB, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res. 2006;66:3992–5.
310. Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F, et al. Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. Cancer Res. 2007;67:2643–8.
311. Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol. 2008;26:1626–34.
312. Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med. 2008;359:1757–65.
313. Edkins S, O'Meara S, Parker A, et al. Recurrent KRAS codon 146 mutations in human colorectal cancer. Cancer Biol Ther. 2006;5:928–32.
314. Janakiraman M, Vakiani E, Zeng Z, et al. Genomic and biological characterization of exon 4 KRAS mutations in human cancer. Cancer Res. 2010;70:5901–11.
315. Smith G, Bounds R, Wolf H, Steele RJ, Carey FA, Wolf CR. Activating K-Ras mutations out with 'hotspot' codons in sporadic colorectal tumours – implications for personalised cancer medicine. Br J Cancer. 2010;102:693–703.
316. Vaughn CP, Zobell SD, Furtado LV, Baker CL, Samowitz WS. Frequency of KRAS, BRAF, and NRAS mutations in colorectal cancer. Genes Chromosomes Cancer. 2011;50:307–12.
317. Douillard JY, Oliner KS, Siena S, et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. N Engl J Med. 2013;369:1023–34.
318. Schwartzberg LS, Rivera F, Karthaus M, et al. PEAK: a randomized, multicenter phase II study of panitumumab plus modified fluorouracil, leucovorin, and oxaliplatin (mFOLFOX6) or bevacizumab plus mFOLFOX6 in patients with previously untreated, unresectable, wild-type KRAS exon 2 metastatic colorectal cancer. J Clin Oncol. 2014;32:2240–7.
319. Heinemann V, von Weikersthal LF, Decker T, et al. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer (FIRE-3): a randomised, open-label, phase 3 trial. Lancet Oncol. 2014;15:1065–75.
320. Rosenberg DW, Yang S, Pleau DC, et al. Mutations in BRAF and KRAS differentially distinguish serrated versus non-serrated hyperplastic aberrant crypt foci in humans. Cancer Res. 2007;67:3551–4.
321. Rajagopalan H, Bardelli A, Lengauer C, Kinzler KW, Vogelstein B, Velculescu VE. Tumorigenesis: RAF/RAS oncogenes and mismatch repair status. Nature. 2002;418:934.
322. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature. 2002;417:949–95.
323. Di Nicolantonio F, Martini M, Molinari F, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. J Clin Oncol. 2008;26:5705–12.
324. Jhawer M, Goel S, Wilson AJ, et al. PIK3CA mutation/PTEN expression status predicts response of colon cancer cells to the epidermal growth factor receptor inhibitor cetuximab. Cancer Res. 2008;68:1953–61.
325. Bertotti A, Migliardi G, Galimi F, et al. A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. Cancer Discov. 2011;1:508–23.
326. Valtorta E, Misale S, Sartore-Bianchi A, et al. KRAS gene amplification in colorectal cancer and impact on response to EGFR-targeted therapy. Int J Cancer. 2013;133:1259–65.
327. Mekenkamp LJ, Tol J, Dijkstra JR, et al. Beyond KRAS mutation status: influence of KRAS copy number status and microRNAs on clinical outcome to cetuximab in metastatic colorectal cancer patients. BMC Cancer. 2012;12:292.

328. Mita H, Toyota M, Aoki F, et al. A novel method, digital genome scanning detects KRAS gene amplification in gastric cancers: involvement of overexpressed wild-type KRAS in downstream signaling and cancer cell growth. BMC Cancer. 2009;9:198.
329. Wagner PL, Perner S, Rickman DS, et al. In situ evidence of KRAS amplification and association with increased p21 levels in non-small cell lung carcinoma. Am J Clin Pathol. 2009;132:500–5.
330. Sasaki H, Hikosaka Y, Kawano O, Moriyama S, Yano M, Fujii Y. Evaluation of Kras gene mutation and copy number gain in non-small cell lung cancer. J Thorac Oncol. 2011;6:15–20.
331. Laurent-Puig P, Cayre A, Manceau G, et al. Analysis of PTEN, BRAF, and EGFR status in determining benefit from cetuximab therapy in wild-type KRAS metastatic colon cancer. J Clin Oncol. 2009;27:5924–30.
332. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature. 2012;487:330–7.
333. Cremolini C, Di Bartolomeo M, Amatu A, et al. BRAF codons 594 and 596 mutations identify a new molecular subtype of metastatic colorectal cancer at favorable prognosis. Ann Oncol. 2015;26:2092–7.
334. Yonesaka K, Zejnullahu K, Okamoto I, et al. Activation of ERBB2 signaling causes resistance to the EGFR-directed therapeutic antibody cetuximab. Sci Transl Med. 2011;3:99ra86.
335. Bardelli A, Corso S, Bertotti A, et al. Amplification of the MET receptor drives resistance to anti-EGFR therapies in colorectal cancer. Cancer Discov. 2013;3:658–73.
336. Samuels Y, Wang Z, Bardella A, et al. High frequency of mutations of the PIK3CA gene in human cancer. Apoptosis. 2004;9:667–76.
337. Karakas B, Bachman KE, Park BH. Mutation of the PIK3CA oncogene in human cancers. Br J Cancer. 2006;94:455–9.
338. Goel A, Arnold CN, Niedzwiecki D, et al. Frequent inactivation of PTEN by promoter hypermethylation in microsatellite instability-high sporadic colorectal cancers. Cancer Res. 2004;64:3014–21.
339. De Roock W, Claes B, Bernasconi D, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemo-therapy refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol. 2010;11:753–62.
340. Sartore-Bianchi A, Di Nicolantonio F, Nichelatti M, et al. Multi-determinants analysis of molecular alterations for predicting clinical benefit to EGFR-targeted monoclonal antibodies in colorectal cancer. PLoS One. 2009;4, e7287.
341. Misale S, Yaeger R, Hobor S, et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature. 2012;486:532–6.
342. Hobor S, Van Emburgh BO, Crowley E, Misale S, Di Nicolantonio F, Bardelli A. TGF-α and amphiregulin paracrine network promotes resistance to EGFR blockade in colorectal cancer cells. Clin Cancer Res. 2014;20:6429–38.
343. Misale S, Di Nicolantonio F, Sartore-Bianchi A, Siena S, Bardelli A. Resistance to Anti-EGFR Therapy in Colorectal Cancer: From Heterogeneity to Convergent Evolution Cancer Discov. 2014;4:1269–80.
344. Montagut C, Dalmases A, Bellosillo B, et al. Identification of a mutation in the extracellular domain of the Epidermal Growth Factor Receptor conferring cetuximab resistance in colorectal cancer. Nat Med. 2012;18:221–3.
345. Diaz Jr LA, Sausen M, Fisher GA, Velculescu VE. Insights into therapeutic resistance from whole-genome analyses of circulating tumor DNA. Oncotarget. 2013;4:1856–7.
346. Mohan S, Heitzer E, Ulz P, et al. Changes in colorectal carcinoma genomes under anti-EGFR therapy identified by whole-genome plasma DNA sequencing. PLoS Genet. 2014;10, e1004271.
347. Diaz LA, Williams RT, Wu J, et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature. 2012;486:537–40.

348. Misale S, Arena S, Lamba S, et al. Blockade of EGFR and MEK intercepts heterogeneous mechanisms of acquired resistance to anti-EGFR therapies in colorectal cancer. Sci Transl Med. 2014;6:224ra26.
349. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med. 2014;6:224ra24.
350. Jones HE, Goddard L, Gee JM, et al. Insulin-like growth factor-I receptor signalling and acquired resistance to gefitinib (ZD1839; Iressa) in human breast and prostate cancer cells. Endocr Relat Cancer. 2004;11:793–814.
351. Camirand A, Zakikhani M, Young F, Pollak M. Inhibition of insulin-like growth factor-1 receptor signaling enhances growth-inhibitory and proapoptotic effects of gefitinib (Iressa) in human breast cancer cells. Breast Cancer Res. 2005;7:R570–9.
352. Desbois-Mouthon C, Baron A, Blivet-Van Eggelpoël MJ, et al. Insulin-like growth factor-1 receptor inhibition induces a resistance mechanism via the epidermal growth factor receptor/HER3/AKT signaling pathway: rational basis for cotargeting insulin like growth factor-1 receptor and epidermal growth factor receptor in hepatocellular carcinoma. Clin Cancer Res. 2009;15:5445–56.
353. Ruckhäberle E, Rody A, Engels K, et al. Microarray analysis of altered sphingolipid metabolism reveals prognostic significance of sphingosine kinase 1 in breast cancer. Breast Cancer Res. Treat. 2008;112:41–52.
354. Sukocheva O, Wang L, Verrier E, Vadas MA, Xia P. Restoring endocrine response in breast cancer cells by inhibition of the sphingosine kinase-1 signaling pathway. Endocrinology. 2000;150:4484–92.
355. Rosa R, Marciano R, Malapelle U, et al. Sphingosine kinase 1 overexpression contributes to cetuximab resistance in human colorectal cancer models. Clin Cancer Res. 2013;19:138–47.
356. Gao Y, Gao F, Chen K, Tian ML, Zhao DL. Sphingosine kinase 1 and resistance. Drug Des Devel Ther. 2015;9:3239–45.
357. Jia Y, Liu M, Huang W, et al. Recombinant human endostatin Endostar inhibits tumor growth and metastasis in a mouse xenograft model of colon cancer. Pathol Oncol Res. 2012;18:315–23.
358. Jitawatanarat P, Wee W. Update on antiangiogenic therapy in colorectal cancer: aflibercept and regorafenib. J Gastrointest Oncol. 2013;4:231–8.
359. Edelman MJ, Mao L. Resistance to anti-angiogenic agents: a brief review of mechanisms and consequences. Transl Lung Cancer Res. 2013;2:304–7.
360. Prager GW, Poettler M, Unseld M, Zielinski CC. Angiogenesis in cancer: Anti-VEGF escape mechanisms. Transl Lung Cancer Res. 2012;1:14–25.
361. Zhao Y, Adjei AA. Targeting angiogenesis in cancer therapy: moving beyond vascular endothelial growth factor. Oncologist. 2015;20:660–73.
362. Shi YH, Bingle L, Gong LH, Wang YX, Corke KP, Fang WG. Basic FGF augments hypoxia-induced HIF-1-alpha expression and VEGF release in T47D breast cancer cells. Pathology. 2007;39:396–400.
363. Casanovas O, Hicklin DJ, Bergers G, Hanahan D. Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. Cancer Cell. 2005;8:299–309.
364. Ayers M, Awad M, Malone D, et al. Association of K-ras status with efficacy end points from a phase 1/2 study of brivanib in combination with cetuximab in patients with advanced or metastatic colorectal cancer (CRC). American Society of Clinical Oncology Gastrointestinal Cancers (ASCO-GI) Symposium, San Francisco, 2009; Abstract no. 375.
365. Garrett CR, Siu LL, El-Khoueiry A, et al. Phase I dose escalation study to determine the safety, pharmacokinetics and pharmacodynamics of brivanib alaninate in combination with full dose cetuximab in patients with advanced gastrointestinal malignancies who have failed prior therapy. Br J Cancer. 2011;105:44–52.
366. Clinicaltrials.gov. Cetuximab with or without brivanib in treating patients with metastatic colorectal cancer. http://clinicaltrials.gov/ct2/. Accessed 30 Nov 2010.

367. Shojaei F, Wu X, Malik AK, et al. Tumor refractoriness to anti-VEGF treatment is mediated by CD11b+ Gr1+ myeloid cells. Nat Biotechnol. 2007;25:911–20.
368. Arora A, Scholar EM. Role of tyrosine kinase inhibitors in cancer therapy. J Pharmacol Exp Ther. 2005;315:971–9.
369. Faivre S, Demetri G, Sargent W, Raymond E. Molecular basis for sunitinib efficacy and future clinical development. Nat Rev Drug Discov. 2007;6:734–45.
370. Shojaei F, Wu X, Qu X, et al. GCSF-initiated myeloid cell mobilization and angiogenesis mediate tumor refractoriness to anti-VEGF therapy in mouse models. Proc Natl Acad Sci U S A. 2009;106:6742–7.
371. Mirone G, Perna S, Shukla A, Marfe G. Involvement of Notch-1 in resistance to regorafenib in colon cancer cells. J Cell Physiol. 2015;231:1097–105.
372. Huang D, Ding Y, Li Y, et al. Sunitinib acts primarily on tumor endothelium rather than tumor cells to inhibit the growth of renal cell carcinoma. Cancer Res. 2010;70:1053–62.
373. Adelaiye R, Ciamporcero E, Miles KM, et al. Sunitinib dose escalation overcomes transient resistance in clear cell renal cell carcinoma and is associated with epigenetic modifications. Mol Cancer Ther. 2015;14:513–22.
374. Gotink KJ, Verheul HMW. Anti-angiogenic tyrosine kinase inhibitors what is their mechanism of action? Angiogenesis. 2010;13:1–14.
375. Hammers HJ, Verheul HM, Salumbides B, et al. Reversible epithelial to mesenchymal transition and acquired resistance to sunitinib in patients with renal cell carcinoma evidence from a xenograft study. Mol Cancer Ther. 2010;9:1525–35.
376. Behnsawy HM, Shigemura K, Meligy FY, et al. Possible role of sonic hedgehog and epithelial-mesenchymal transition in renal cell cancer progression. Korean J Urol. 2013;54:547–54.
377. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol. 2002;23:549–55.
378. Condeelis J, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell. 2006;124:263–6.
379. Cecconi O, Calorini L, Mannini A, Mugnai G, Ruggieri S. Enhancement of lungcolonizing potential of murine tumor cell lines co-cultivated with activated macrophages. Clin Exp Metastasis. 1997;15:94–101.
380. Oosterling SJ, van der Bij GJ, Meijer GA, et al. Macrophages direct tumour histology and clinical outcome in a colon cancer model. J Pathol. 2005;207:147–55.
381. Miselis NR, Wu ZJ, Van Rooijen N, Kane AB. Targeting tumor-associated macrophages in an orthotopic murine model of diffuse malignant mesothelioma. Mol Cancer Ther. 2008;7:788–99.
382. Chen JJ, Yao PL, Yuan A, et al. Upregulation of tumor interleukin-8 expression by infiltrating macrophages: its correlation with tumor angiogenesis and patient survival in non-small cell lung cancer. Clin Cancer Res. 2003;9:729–37.
383. Sundar SS, Ganesan TS. Role of lymphangiogenesis in cancer. J Clin Oncol. 2007;25:4298–307.
384. Dannenmann SR, Thielicke J, Stöckli M, et al. Tumor-associated macrophages subvert T-cell function and correlate with reduced survival in clear cell renal cell carcinoma. Oncoimmunology. 2013;2, e23562.
385. Huang D, Ding Y, Zhou M, et al. Interleukin-8 mediates resistance to antiangiogenic agent. Cancer Res. 2010;70:1063–71.
386. Bielecka ZF, Czarnecka AM, Solarek W, Kornakiewicz A, Szczylik C. Mammalian target of rapamycin inhibitors resistance mechanisms in clear cell renal cell carcinoma. Curr Signal Transduct Ther. 2014;8:218–28.
387. Armulik A, Genové G, Betsholtz C. Pericytes developmental, physiological, and pathological perspectives, problems, and promises. Dev Cell. 2011;21:193–215.
388. Hall AP. Review of the pericyte during angiogenesis and its role in cancer and diabetic retinopathy. Toxicol Pathol. 2006;34:763–75.

389. Cao Z, Shang B, Zhang G, et al. Tumor cell-mediated neovascularization and lymphangiogenesis contrive tumor progression and cancer metastasis. Biochim Biophys Acta. 1836;2013:273–86.
390. Gotink KJ, Broxterman HJ, Labots M, et al. Lysosomal sequestration of sunitinib a novel mechanism of drug resistance. Clin Cancer Res. 2011;17:7337–46.
391. Kassouf W, Dinney CP, Brown G, et al. Uncoupling between epidermal growth factor receptor and downstream signals defines resistance to the antiproliferative effect of Gefitinib in bladder cancer cells. Cancer Res. 2005;65:10524–35.
392. Shrader M, Pino MS, Brown G, et al. Molecular correlates of gefitinib responsiveness in human bladder cancer cells. Mol Cancer Ther. 2007;6:277–85.
393. Black PC, Brown GA, Inamoto T, et al. Sensitivity to epidermal growth factor receptor inhibitor requires E-cadherin expression in urothelial carcinoma cells. Clin Cancer Res. 2008;14:1478–86.
394. Li J, Lin B, Li X, Tang X, He Z, Zhou K. Biomarkers for predicting response to tyrosine kinase inhibitors in drug-sensitive and drug-resistant human bladder cancer cells. Oncol Rep. 2015;33:951–7.
395. Knievel J, Schulz WA, Greife A, et al. Multiple mechanisms mediate resistance to sorafenib in urothelial cancer. Int J Mol Sci. 2014;15:20500–17.
396. Cox AD, Der CJ. The RAF inhibitor paradox: unexpected consequences of targeted drugs. Cancer Cell. 2010;17:221–3.
397. Pinto-Leite R, Carreira I, Melo J, et al. Genomic characterization of three urinary bladder cancer cell lines: understanding genomic types of urinary bladder cancer. Tumour Biol. 2014;35:4599–617.
398. Rose A, Grandoch M, vom Dorp F, et al. Stimulatory effects of the multi-kinase inhibitor sorafenib on human bladder cancer cells. Br J Pharmacol. 2010;160:1690–8.
399. Sturm OE, Orton R, Grindlay J, et al. The mammalian MAPK/ERK pathway exhibits properties of a negative feedback amplifier. Sci Signal. 2010;3:ra90.
400. Nawroth R, Stellwagen F, Schulz WA, et al. S6K1 and 4E-BP1 are independent regulated and control cellular growth in bladder cancer. PLoS One. 2011;6, e27509.
401. Ewald F, Ueffing N, Brockmann L, et al. The role of c-FLIP splice variants in urothelial tumours. Cell Death Dis. 2011;2, e245.
402. Di Lorenzo G, Tortora G, D'Armiento FP, et al. Expression of epidermal growth factor receptor correlates with disease relapse and progression to androgen-independence in human prostate cancer. Clin Cancer Res. 2002;8:3438–44.
403. Traish AM, Morgentaler A. Epidermal growth factor receptor expression escapes androgen regulation in prostate cancer: a potential molecular switch for tumour growth. Br J Cancer. 2009;101:1949–56.
404. Nazarian R, Shi H, Wang Q, et al. Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. Nature. 2010;468:973–7.
405. Schulze A, Nicke B, Warne PH, Tomlinson S, Downward J. The transcriptional response to Raf activation is almost completely dependent on Mitogen-activated Protein Kinase Kinase activity and shows a major autocrine component. Mol Biol Cell. 2005;15:3450–63.
406. Festuccia C, Gravina GL, Millimaggi D, et al. Uncoupling of the epidermal growth factor receptor from downstream signal transduction molecules guides the acquired resistance to gefitinib in prostate cancer cells. Oncol Rep. 2007;18:503–11.
407. Culig Z, Bartsch G, Hobisch A. Interleukin-6 regulates androgen receptor activity and prostate cancer cell growth. Mol Cell Endocrinol. 2002;197:231–8.
408. Chen T, Wang LH, Farrar WL. Interleukin 6 activates androgen receptor-mediated gene expression through a signal transducer and activator of transcription 3-dependent pathway in LNCaP prostate cancer cells. Cancer Res. 2000;60:2132–5.
409. Michalaki V, Syrigos K, Charles P, Waxman J. Serum levels of IL-6 and TNF-alpha correlate with clinicopathological features and patient survival in patients with prostate cancer. Br J Cancer. 2004;90:2312–6.

410. Kutikov A, Makhov P, Golovine K, et al. Interleukin-6: a potential biomarker of resistance to multitargeted receptor tyrosin kinase inhibitors in castration-resistant prostate cancer. Urology. 2011;78:968.e7–11.
411. Siu MK, Abou-Kheir W, Yin JJ, et al. Loss of EGFR signaling regulated miR-203 promotes prostate cancer bon metastasis and tyrosine kinase inhibitors resistance. Oncotarget. 2014;5:3770–84.
412. Antonarakis ES, Carducci MA. Targeting angiogenesis for the treatment of prostate cancer. Expert Opin Ther Targets. 2012;16:365–76.
413. Blanc J, Geney R, Menet C. Type II kinase inhibitors: an opportunity in cancer for rational design. Anticancer Agents Med Chem. 2013;13:731–47.
414. Bussolati B, Grange C, Camussi G. Tumor exploits alternative strategies to achieve vascularization. FASEB J. 2001;25:2874–82.
415. Seaman S, Stevens J, Yang MY, Logsdon D, Graff-Cherry C, St Croix B. Genes that distinguish physiological and pathological angiogenesis. Cancer Cell. 2007;11:539–54.
416. Hida K, Hida Y, Amin DN, et al. Tumor-associated endothelial cells with cytogenetic abnormalities. Cancer Res. 2004;64:8249–55.
417. Bussolati B, Deambrosis I, Russo S, Deregibus MC, Camussi G. Altered angiogenesis and survival in human tumor-derived endothelial cells. FASEB J. 2003;17:1159–61.
418. Xiong YQ, Sun HC, Zhang W, et al. Human hepatocellular carcinoma tumor-derived endothelial cells manifest increased angiogenesis capability and drug resistance compared with normal endothelial cells. Clin Cancer Res. 2009;15:4838–46.
419. Fiorio Pla A, Brossa A, Bernardini M, et al. Differential sensitivity of prostate tumor derived endothelial cells to sorafenib and sunitinib. BMC Cancer. 2014;14:939.
420. Sahin O, Wang Q, Brady SW, et al. Biomarker-guided sequential targeted therapies to overcome therapy resistance in rapidly evolving highly aggressive mammary tumors. Cell Res. 2014;24:542–59.
421. Tinoco G, Warsch S, Glück S, Avancha K, Montero AJ. Treating breast cancer in the 21st century: emerging biological therapies. J Cancer. 2013;4:117–32.
422. Valabrega G, Montemurro F, Aglietta M. Trastuzumab: mechanism of action, resistance and future perspectives in HER2-overexpressing breast cancer. Ann Oncol. 2007;18:977–84.
423. Hudis CA. Trastuzumab-mechanism of action and use in clinical practice. N Engl J Med. 2007;357:39–51.
424. Arteaga CL, Sliwkowski MX, Osborne CK, Perez EA, Puglisi F, Gianni L. Treatment of HER2-positive breast cancer: current status and future perspectives. Nat Rev Clin Oncol. 2012;9:16–32.
425. Reynolds K, Sarangi S, Bardia A, Dizon DS. Precision medicine and personalized breast cancer: combination pertuzumab therapy. Pharmgenomics Pers Med. 2014;7:95–105.
426. Pallis AG, Syrigos KN. Epidermal growth factor receptor tyrosine kinase inhibitors in the treatment of NSCLC. Lung Cancer. 2013;80:120–30.
427. Moore MJ, Goldstein D, Hamm J, National Cancer Institute of Canada Clinical Trials Group, et al. Erlotinib Plus Gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials. Group J Clin Oncol. 2007;25:1960–6.
428. Wilson TR, Fridlyand J, Yan Y, et al. Widespread potential for growth-factor-driven resistance to anticancer kinase inhibitors. Nature. 2012;487:505–9.
429. Gallardo A, Lerma E, Escuin D, et al. Increased signalling of EGFR and IGF1R, and deregulation of PTEN/PI3K/Akt pathway are related with trastuzumab resistance in HER2 breast carcinomas. Br J Cancer. 2012;106:1367–73.
430. Wang L, Zhang Q, Zhang J, et al. PI3K pathway activation results in low efficacy of both trastuzumab and lapatinib. BMC Cancer. 2011;11:248.
431. Diermeier S, Horváth G, Knuechel-Clarke R, Hofstaedter F, Söllosi J, Brockhoff G. Epidermal growth factor receptor coexpression modulates susceptibility to herceptin in HER2/neu over-

expressing breast cancer cells via specific erbB-receptor interaction and activation. Exp Cell Res. 2005;304:604–19.
432. Kataoka Y, Mukohara T, Shimada H, Saijo N, Hirai M, Minami H. Association between gain-of-function mutations in PIK3CA and resistance to HER2-targeted agents in HER2-amplified breast cancer cell lines. Ann Oncol. 2010;21:255–62.
433. Figueroa-Magalhães MC, Jelovac D, Connolly RM, Wolff AC. Treatment of HER2-positive breast cancer. Breast. 2014;23:128–36.
434. D'Alessio A, De Luca A, Maiello MR, et al. Effects of the combined blockade of EGFR and ErbB-2 on signal transduction and regulation of cell cycle regulatory proteins in breast cancer cells. Breast Cancer Res Treat. 2009;123:387–96.
435. Versola MB, Burris S, Jones H, et al. Clinical activity of GW572016 in EGF10003 in patients with solid tumors. J Clin Oncol. 2004;22 (14S July 15 Suppl):3047.
436. Gomez HL, Doval DC, Chavez MA, et al. Efficacy and safety of lapatinib as first-line therapy for ErbB2-amplified locally advanced or metastatic breast cancer. J Clin Oncol. 2008;26:2999–3005.
437. Press MF, Finn RS, Cameron D, et al. HER-2 gene amplification, HER-2 and epidermal growth factor receptor mRNA and protein expression, and lapatinib efficacy in women with metastatic breast cancer. Clin Cancer Res. 2008;14:7861–70.
438. Ryan QA, Ibrahim MH, Cohen J, et al. FDA drug approval summary: lapatinib in combination with capecitabine for previously treated metastatic breast cancer that overexpresses HER-2. Oncologist. 2008;13:1114–9.
439. Zhou X, Cella D, Cameron D, et al. Lapatinib plus capecitabine versus capecitabine alone for HER2+ (ErbB2+) metastatic breast cancer: quality-of-life assessment. Breast Cancer Res Treat. 2009;117:577–89.
440. Scaltriti M, Verma C, Guzman M, et al. Lapatinib, a HER2 tyrosine kinase inhibitor, induces stabilization and accumulation of HER2 and potentiates trastuzumab-dependent cell cytotoxicity. Oncogene. 2009;28:803–14.
441. Toi M, Iwata M, Fujiwara Y, et al. Lapatinib monotherapy in patients with relapsed, advanced, or metastatic breast cancer: efficacy, safety, and biomarker results from Japanese patients phase II studies. Br J Cancer. 2009;101:1676–82.
442. Xia W, Gerard CM, Liu L, Baudson NM, Ory TL, Spector NL. Combining lapatinib (GW572016), a small molecule inhibitor of ErbB1 and 73 ErbB2 tyrosine kinases, with therapeutic anti-ErbB2 antibodies enhances apoptosis of ErbB2-overexpressing breast cancer cells. Oncogene. 2005;24:6213–21.
443. Vazquez-Martin A, Oliveras-Ferraros C, del Barco S, Martín-Castillo B, Menéndez JA. mTOR inhibitors and the anti-diabetic biguanide metformin: new insights into the molecular management of breast cancer resistance to the HER2 tyrosine kinase inhibitor lapatinib (Tykerb). Clin Transl Oncol. 2009;11:455–9.
444. Xia W, Bacus S, Hegde P, et al. A model of acquired autoresistance to a potent ErbB2 tyrosine kinase inhibitor and a therapeutic strategy to prevent its onset in breast cancer. Proc Natl Acad Sci U S A. 2006;103:7795–800.
445. Tam IY, Leung EL, Tin VP, et al. Double EGFR mutants containing rare EGFR mutant types show reduced in vitro response to gefitinib compared with common activating missense mutations. Mol Cancer Ther. 2009;8:2142–51.
446. Bhosle J, Kiakos K, Porter AC, et al. Treatment with gefitinib or lapatinib induces drug resistance through downregulation of topoisomerase IIα expression. Mol Cancer Ther. 2013;12:2897–908.
447. Nevo J, Mattila E, Pellinen T, et al. Mammary-derived growth inhibitor alters traffic of EGFR and induces a novel form of cetuximab resistance. Clin Cancer Res. 2009;15:6570–81.
448. Meng X, Li Y, Tang H, et al. Drug response to HER2 gatekeeper T798M mutation in HER2-positive breast cancer. Amino Acids. 2015;48:487–97.
449. Vecchione A, Belletti B, Lovat F, et al. A microRNA signature defines chemoresistance in ovarian cancer through modulation of angiogenesis. Proc Natl Acad Sci U S A. 2013;110:9845–50.

450. Pujade-Lauraine E, Hilpert F, Weber B, et al. Bevacizumab combined with chemotherapy for platinum-resistant recurrent ovarian cancer: The AURELIA open-label randomized phase III trial. J Clin Oncol. 2014;32:1302–8.
451. Bast Jr RC, Hennessy B, Mills GB. The biology of ovarian cancer: new opportunities for translation. Nat Rev Cancer. 2009;9:415–28.
452. Verhaak RG, Tamayo P, Yang JY, et al. Cancer genome atlas research network prognostically relevant gene signatures of high-grade serous ovarian carcinoma. J Clin Invest. 2013;123:517–25.
453. Cho KR, Shih IM. Ovarian cancer. Annu Rev Pathol. 2009;4:287–313.
454. Cancer Genome Atlas Research, Networks. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474:609–15.
455. Cancer Genome Atlas Research, Networks, Kandoth C, Schultz N, Cherniack AD, et al. Integrated genomic characterization of endometrial carcinoma. Nature. 2013;497:67–73.
456. Cuatrecasas M, Villanueva A, Matias-Guiu X, Prat J. K-ras mutations in mucinous ovarian tumors – a clinicopathologic and molecular study of 95 cases. Cancer. 1997;79:1581–6.
457. Bookman MA. Biological therapy of ovarian cancer: current directions. Semin Oncol. 1998;25:381–96.
458. Kaye SB, Poole CJ, Dańska-Bidzińska A, et al. A randomized phase II study evaluating the combination of carboplatin-based chemotherapy with pertuzumab versus carboplatin based chemotherapy in patients with relapsed platinum sensitive ovarian cancer. Ann Oncol. 2013;24:145–52.
459. Amler L, Makhija S, Januario T, et al. Her 2 pathway gene expression analysis in a phase II study of pertuzumab + gemcitabine vs. gemcitabine + placebo in patients with platinum resistant epithelial ovarian cancer. J Clin Oncol. 2008;26:abstract 5562.
460. Makhija S, Glenn D, Ueland F, et al. Results from a phase II randomized, placebo-controlled, double-blind trial suggest improved PFS with the addition of pertuzumab to gemcitabine in patients with platinum-resistant ovarian, fallopian tube, or primary peritoneal cancer. J Clin Oncol. 2007;25:18S-abstract 5507.
461. Gordon MS, Matei D, Aghajanian C, et al. Clinical activity of pertuzumab (rhuMAb2C4), a HER dimerization inhibitor, in advanced ovarian cancer: potential predicative relationship between tumor HER 2 activation status. J Clin Oncol. 2006;24:4324–32.
462. Schilder RJ, Sill MW, Chen X, et al. Phase II study of Getinib in patients with relapsed or persistent ovarian or primary peritoneal carcinoma and evaluation of epidermal growth factor receptor mutations and immunohistochemical expression: a Gynecological Oncology Group Study. Clinical Cancer Res. 2005;11:5539–48.
463. Nimeiri HS, Oza AM, Morgan RJ, et al. Efficacy and safety of bevacizumab plus erlotinib for patients with recurrent ovarian, primary peritoneal, and fallopian tube cancer: a trial of the Chicago, PMH, and California Phase II Consortia. Gynecol Oncol. 2008;110:49–55.
464. Apte SM, Bucana CD, Killion JJ, Gershenson DM, Fidler IJ. Expression of platelet-derived growth factor and activated receptor in clinical specimens of epithelial ovarian cancer and ovarian carcinoma cell lines. Gynecol Oncol. 2004;93:78–86.
465. Della Pepa C, Tonini G, Pisano C, et al. Ovarian cancer standard of care: are there real alternatives? Chin J Cancer. 2015;34:17–27.
466. Berasain C. Hepatocellular carcinoma and sorafenib: too many resistance mechanisms? Gut. 2013;62:1674–5.
467. Tang TC, Man S, Xu P, et al. Development of a resistance-like phenotype to sorafenib by human hepatocellular carcinoma cells is reversible and can be delayed by metronomic UFT chemotherapy. Neoplasia. 2010;12:928–40.
468. Kuczynski EA, Lee CR, Man S, Chen E, Kerbel RS. Effects of sorafenib dose on acquired reversible resistance and toxicity in hepatocellular carcinoma. Cancer Res. 2015;75:2510–9.
469. Ohgaki H, Dessen P, Jourde B, et al. Genetic pathways to glioblastoma: a population-based study. Cancer Res. 2004;64:6892–9.
470. Ohgaki H, Kleihues P. Genetic pathways to primary and secondary glioblastoma. Am J Pathol. 2007;170:1445–53.

471. Humphrey PA, Gangarosa LM, Wong AJ, et al. Deletion-mutant epidermal growth factor 76 receptor in human gliomas: effects of type II mutation on receptor function. Bioch and Biop Res Comm. 1991;178:1413–20.
472. Lee JC, Vivanco I, Beroukhim R, et al. Epidermal growth factor receptor activation in glioblastoma through novel missense mutations in the extracellular domain. PLoS Med. 2006;3, e485.
473. Frederick L, Wang XY, Eley G, James CD. Diversity and frequency of epidermal growth factor receptor mutations in human glioblastomas. Cancer Res. 2000;60:1383–7.
474. Peschard P, Park M. From Tpr-Met to Met, tumorigenesis and tubes. Oncogene. 2007;26:1276–85.
475. Moscatello DK, Holgado-Madruga M, Godwin AK, et al. Frequent expression of a mutant epidermal growth factor receptor in multiple human tumors. Cancer Res. 1995;55:5536–9.
476. Furnari FB, Fenton T, Bachoo RM, et al. Malignant astrocytic glioma: genetics, biology, and paths to treatment. Genes and Dev. 2007;21:2683–710.
477. Downward J, Parker P, Waterfield MD. Autophosphorylation sites on the epidermal growth factor receptor. Nature. 1984;311:483–5.
478. Mendelsohn J, Baselga J. The EGF receptor family as targets for cancer therapy. Oncogene. 2000;19:6550–65.
479. Choe G, Horvath S, Cloughesy TF, et al. Analysis of the phosphatidylinositol 3'-kinase signaling pathway in glioblastoma patients in vivo. Cancer Res. 2003;63:2742–6.
480. Tanaka K, Babic I, Nathanson D, et al. Oncogenic EGFR signaling activates an mTORC2-NF-kappaB pathway that promotes chemotherapy resistance. Cancer Discov. 2011;1:524–38.
481. Mellinghoff IK, Wang MY, Vivanco I, et al. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. N Engl J Med. 2005;353:2012–24.
482. Wang SI, Puc J, Li J, et al. Somatic mutations of PTEN in glioblastoma multiforme. Cancer Res. 1997;57:4183–6.
483. Bonavia R, Inda MM, Vandenberg S, et al. EGFRvIII promotes glioma angiogenesis and growth through the NF-kappaB, interleukin-8 pathway. Oncogene. 2012;31:4054–66.
484. Wu JL, Abe T, Inoue R, Fujiki M, Kobayashi H. IkappaBalphaM suppresses angiogenesis and tumorigenesis promoted by a constitutively active mutant EGFR in human glioma cells. Neurol Res. 2004;26:785–91.
485. Wang SC, Hung MC. Nuclear translocation of the epidermal growth factor receptor family membrane tyrosine kinase receptors. Clin Cancer Res. 2009;15:6484–9.
486. Boerner JL, Demory ML, Silva C, Parsons SJ. Phosphorylation of Y845 on the epidermal growth factor receptor mediates binding to the mitochondrial protein cytochrome c oxidase subunit II. Mol Cell Biol. 2004;24:7059–71.
487. Snuderl M, Fazlollahi L, Le LP, et al. Mosaic amplification of multiple receptor tyrosine kinase genes in glioblastoma. Cancer Cell. 2011;20:810–7.
488. Little SE, Popov S, Jury A, et al. Receptor tyrosine kinase genes amplified in glioblastoma exhibit a mutual exclusivity in variable proportions reflective of individual tumor heterogeneity. Cancer Res. 2012;72:1614–20.
489. Jun HJ, Acquaviva J, Chi D, J et al. Acquired MET expression confers resistance to EGFR inhibition in a mouse model of glioblastoma multiforme. Oncogene. 2012;31:3039–50.
490. Bao SQ, Wu Q, McLendon RE, et al. Glioma stem cells promote radio resistance by preferential activation of the DNA damage response. Nature. 2006;444:756–60.
491. Tamura K, Aoyagi M, Ando N, et al. Expansion of CD133-positive glioma cells in recurrent de novo glioblastomas after radiotherapy and chemotherapy. J Neuros. 2013;119:1145–55.
492. Eimer S, Dugay F, Airiau K, et al. Cyclopamine cooperates with EGFR inhibition to deplete stem-like cancer cells in glioblastoma-derived spheroid cultures. Neuro Oncol. 2012;14: 1441–51.
493. Wykosky J, Hu J, Gomez GG, et al. A urokinase receptor-Bim signaling axis emerges during EGFR inhibitor resistance in mutant EGFR glioblastoma. Cancer Res. 2015;15(75):394–404.

494. Aljohani H, Koncar RF, Zarzour A, Park BS, Lee SH, Bahassi el M. ROS1 amplification mediates resistance to gefitinib in glioblastoma cells Oncotarget. 2015;6:20388–95.
495. Roth P, Weller M. Challenges to targeting epidermal growth factor receptor in glioblastoma: escape mechanisms and combinatorial treatment strategies. Neuro Oncol. 2014;16:viii14-19.
496. Kang CS, Pu PY, Li YH, et al. An in vitro study on the suppressive effect of glioma cell growth induced by plasmid-based small interference RNA (siRNA) targeting human epidermal growth factor receptor. J Neurooncol. 2005;74:267–73.
497. Nagane M, Levitzki A, Gazit A, Huang HJ. Drug resistance of human glioblastoma cells conferred by a tumor-specific mutant epidermal growth factor receptor through modulation of Bcl-XL and caspase-3-like proteases. Proc Natl Acad Sci U S A. 1998;95:5724–9.
498. Fan QW, Cheng CK, Gustafson WC, et al. EGFR phosphorylates tumor-derived EGFRvIII driving STAT3/5 and progression in glioblastoma. Cancer Cell. 2013;24:438–49.
499. Inda MM, Bonavia R, Mukasa A, et al. Tumor heterogeneity is an active process maintained by a mutant EGFR-induced cytokine circuit in glioblastoma. Genes Dev. 2010;24:1731–45.
500. Johnson H, Del Rosario AM, Bryson BD, Schroeder MA, Sarkaria JN, White FM. Molecular characterization of EGFR and EGFRvIII signaling networks in human glioblastoma tumor xenografts. Mol Cell Proteomics. 2012;11:1724–40.
501. Lal B, Goodwin CR, Sang Y, et al. EGFRvIII and c-Met pathway inhibitors synergize against PTEN-null/EGFRvIII+ glioblastoma xenografts. Mol Cancer Ther. 2009;8:1751–60.
502. Hu J, Jo M, Cavenee WK, Furnari F, VandenBerg SR, Gonias SL. Crosstalk between the urokinase-type plasminogen activator receptor and EGF receptor variant III supports survival and growth of glioblastoma cells. Proc Natl Acad Sci U S A. 2011;108:15984–9.
503. Chakravarti A, Loeffler JS, Dyson NJ. Insulin-like growth factor receptor I mediates resistance to anti–epidermal growth factor receptor therapy in primary human glioblastoma cells through continued activation of phosphoinositide 3-kinase signaling. Cancer Res. 2002;62:200–7.
504. Lotsch D, Steiner E, Holzmann K, et al. Major vault protein supports glioblastoma survival and migration by upregulating the EGFR/PI3K signalling axis. Oncotarget. 2013;4:1904–18.
505. Wick W, Weller M, van den Bent M, Stupp R. Bevacizumab and recurrent malignant gliomas: a European perspective. J Clin Oncol. 2010;28:e188–9; author reply e190–182.
506. Zhang M, Ye G, Li J, Wang Y. Recent advance in molecular angiogenesis in glioblastoma: the challenge and hope for anti-angiogenic therapy. Brain Tumor Pathol. 2015;32:229–36.
507. Zuniga RM, Torcuator R, Jain R, et al. Efficacy, safety and patterns of response and recurrence in patients with recurrent high-grade gliomas treated with bevacizumab plus irinotecan. J Neurooncol. 2009;91:329–36.
508. Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. Nat Rev Cancer. 2008;8:592–603.
509. Kioi M, Vogel H, Schultz G, Hoffman RM, Harsh GR, Brown JM. Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice. J Clin Invest. 2010;120:694–705.
510. Ebos JM, Lee CR, Christensen JG, Mutsaers AJ, Kerbel RS. Multiple circulating proangiogenic factors induced by sunitinib malate are tumor-independent and correlate with antitumor efficacy. Proc Natl Acad Sci U S A. 2007;104:17069–74.
511. Reardon DA, Turner S, Peters KB, et al. A review of VEGF/VEGFR-targeted therapeutics for recurrent glioblastoma. J Natl Compr Canc Netw. 2011;9:414–27.
512. Paez-Ribes M, Allen E, Hudock J, et al. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer Cell. 2009;15:220–31.
513. Kunkel P, Ulbricht U, Bohlen P, et al. Inhibition of glioma angiogenesis and growth in vivo by systemic treatment with a monoclonal antibody against vascular endothelial growth factor receptor- 2. Cancer Res. 2001;61:6624–8.
514. Kamoun WS, Ley CD, Farrar CT, et al. Edema control by cediranib, a vascular endothelial growth factor receptor-targeted kinase inhibitor, prolongs survival despite persistent brain tumor growth in mice. J Clin Oncol. 2009;27:2542–52.

515. Ciardiello F, Bianco R, Damiano V, et al. Antiangiogenic and antitumor activity of anti–epidermal growth factor receptor C225 monoclonal antibody in combination with vascular endothelial growth factor antisense oligonucleotide in human GEO colon cancer cells. Clin Cancer Res. 2000;6:3739–47.
516. Ko AH, Youssoufian H, Gurtler J, et al. A phase II randomized study of cetuximab and bevacizumab alone or in combination with gemcitabine as first-line therapy for metastatic pancreatic adenocarcinoma. Invest New Drugs. 2012;30:1597–606.
517. Heinemann V, Vehling-Kaiser U, Waldschmidt D, et al. Gemcitabine plus erlotinib followed by capecitabine versus capecitabine plus erlotinib followed by gemcitabine in advanced pancreatic cancer: final results of a randomised phase 3 trial of the 'Arbeitsgemeinschaft Internistische Onkologie' (AIO-PK0104). Gut. 2013;62:571–9.
518. Philip PA, Lutz MP. Targeting Epidermal Growth Factor Receptor-Related Signaling Pathways in Pancreatic Cancer. Pancreas. 2015;44:1046–52.
519. Kim ST, Lim do H, Jang KT. Impact of KRAS mutations on clinical outcomes in pancreatic cancer patients treated with first-line gemcitabine-based chemotherapy. Mol Cancer Ther. 2011;10:1993–9.
520. Propper D, Davidenko I, Bridgewater J, et al. Phase II, randomized, biomarker identification trial (MARK) for erlotinib in patients with advanced pancreatic carcinoma. Ann Oncol. 2014;25:1384–90.
521. Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med. 2010;363:24–35.
522. Bonner JA, Harari PM, Giralt J, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med. 2006;354:567–78.
523. Vermorken JB, Mesia R, Rivera F, et al. Platinum-based chemotherapy plus cetuximab in head and neck cancer. N Engl J Med. 2008;359:1116–27.
524. Vermorken JB, Trigo J, Hitt R, et al. Open-label, uncontrolled, multicenter phase II study to evaluate the efficacy and toxicity of cetuximab as a single agent in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck who failed to respond to platinum-based therapy. J Clin Oncol. 2007;25:2171–7.
525. Baselga J, Trigo JM, Bourhis J, et al. Phase II multicenter study of the antiepidermal growth factor receptor monoclonal antibody cetuximab in combination with platinum-based chemotherapy in patients with platinumrefractory metastatic and/or recurrent squamous cell carcinoma of the head and neck. J Clin Oncol. 2005;23:5568–77.
526. Herbst RS, Arquette M, Shin DM, et al. Phase II multicenter study of the epidermal growth factor receptor antibody cetuximab and cisplatin for recurrent and refractory squamous cell carcinoma of the head and neck. J Clin Oncol. 2005;23:5578–87.
527. Ang KK, Berkey BA, Tu X, et al. Impact of epidermal growth factor receptor expression on survival and pattern of relapse in patients with advanced head and neck carcinoma. Cancer Res. 2002;62:7350–6.
528. Bentzen SM, Atasoy BM, Daley FM, et al. Epidermal growth factor receptor expression in pretreatment biopsies from head and neck squamous cell carcinoma as a predictive factor for a benefit from accelerated radiation therapy in a randomized controlled trial. J Clin Oncol. 2005;23:5560–7.
529. Machiels JP, Subramanian S, Ruzsa A, et al. Zalutumumab plus best supportive care versus best supportive care alone in patients with recurrent or metastatic squamous-cell carcinoma of the head and neck after failure of platinum-based chemotherapy: an open-label, randomised phase 3 trial. Lancet Oncol. 2011;12:333–43.
530. Wirth LJ, Allen AM, Posner MR, et al. Phase I dose-finding study of paclitaxel with panitumumab, carboplatin and intensity-modulated radiotherapy in patients with locally advanced squamous cell cancer of the head and neck. Ann Oncol. 2010;21:342–7.
531. Ramakrishnan MS, Eswaraiah A, Crombet T, et al. Nimotuzumab, a promising therapeutic monoclonal for treatment of tumors of epithelial origin. MAbs. 2009;1:41–8.
532. Soulieres D, Senzer NN, Vokes EE, Hidalgo M, Agarwala SS, Siu LL. Multicenter phase II study of erlotinib, an oral epidermal growth factor receptor tyrosine kinase inhibitor, in

patients with recurrent or metastatic squamous cell cancer of the head and neck. J Clin Oncol. 2004;22:77–85.
533. Martins RG, Parvathaneni U, Bauman JE, et al. Cisplatin and radiotherapy with or without erlotinib in locally advanced squamous cell carcinoma of the head and neck: a randomized phase II trial. J Clin Oncol. 2013;31:1415–21.
534. Harrington K, Berrier A, Robinson M, et al. Randomised Phase II study of oral lapatinib combined with chemoradiotherapy in patients with advanced squamous cell carcinoma of the head and neck: rationale for future randomised trials in human papilloma virus-negative disease. Eur J Cancer. 2013;49:1609–18.
535. Del Campo JM, Hitt R, Sebastian P, et al. Effects of lapatinib monotherapy: results of a randomised phase II study in therapy-naive patients with locally advanced squamous cell carcinoma of the head and neck. Br J Cancer. 2011;105:618–27.
536. de Souza JA, Davis DW, Zhang Y, et al. A phase II study of lapatinib in recurrent/metastatic squamous cell carcinoma of the head and neck. Clin Cancer Res. 2012;18:2336–43.
537. Seiwert TY, Fayette J, Cupissol D, et al. A randomized, phase II study of afatinib versus cetuximab in metastatic or recurrent squamous cell carcinoma of the head and neck. Ann Oncol. 2014;25:1813–20.
538. Carla C, Daris F, Cecilia B, Francesca B, Francesca C, Paolo F. Angiogenesis in head and neck cancer: a review of the literature. J Oncol. 2012;2012:358472.
539. Hasina R, Whipple M, Martin L, Kuo WP, Ohno-Machado L, Lingen MW. Angiogenic heterogeneity in head and neck squamous cell carcinoma: biological and therapeutic implications Lab. Invest. 2008;88:342–53.
540. Vokes EE, Cohen EEW, Mauer AM, et al. A phase I study of erlotinib and bevacizumab for recurrent or metastatic squamous cell carcinoma of the head and neck (HNC). J Clin Oncol. 2005;23:(16 Suppl):5504.
541. Karamouzis MV, Friedland D, Johnson R, Rajasenan K, Branstetter B, Argiris A. Phase II trial of pemetrexed (P) and bevacizumab (B) in patients (pts) withrecurrent or metastatic head and neck squamous cell carcinoma (HNSCC): an interiman analysis. J Clin Oncol. 2007;25(18 Suppl):6049.
542. Choong NW, Kozloff M, Taber D, et al. Phase II study of sunitinib malate in head and neck squamous cell carcinoma. Invest New Drugs. 2010;28:677–83.
543. Fountzilas G, Fragkoulidi A, Kalogera-Fountzila A, et al. A phase II study of sunitinib in patients with recurrent and/or metastatic non-nasopharyngeal head and neck cancer. Cancer Chemoth Pharmacol. 2010;65:649–60.
544. Machiels JP, Henry S, Zanetta S, et al. Phase II study of sunitinib in recurrent or metastatic squamous cell carcinoma of the head and neck: GORTEC 2006-01. J Clin Oncol. 2010;28:21–8.
545. Kim S, Yazici YD, Calzada G, et al. Sorafenib inhibits the angiogenesis and growth of orthotopic anaplastic thyroid carcinoma xenografts in nude mice. Mol Cancer Ther. 2007;6:1785–92.
546. Elser C, Siu LL, Winquist E, et al. Phase II trial of sorafenib in patients with recurrent or metastatic squamous cell carcinoma of the head and neck or nasopharyngeal carcinoma. J Clin Oncol. 2007;25:3766–73.
547. Williamson SK, Moon J, Huang CH, et al. Phase II evaluation of sorafenib in advanced and metastatic squamous cell carcinoma of the head and neck: southwest Oncology Group Study S0420. J Clin Oncol. 2010;28:3330–5.

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