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Tailoring Tyrosine Kinase Inhibitors to Fit the Lung Cancer Genome

Brendan D. Looyenga*, Irene Cherni†, Jeffrey P. MacKeigan* and Glen J. Weiss†,‡

*Van Andel Research Institute, Systems Biology, Grand Rapids, MI, USA; †The Translational Genomics Research Institute, Lung Cancer Unit, Cancer & Cell Biology Division, Phoenix, AZ, USA; ‡Virginia G. Piper Cancer Center at Scottsdale Healthcare, Scottsdale, AZ, USA

Abstract

Tyrosine kinase inhibitors (TKIs) have been in use as cancer therapeutics for nearly a decade, and their utility in targeting specific malignancies with defined genetic lesions has proven to be remarkably effective. Recent efforts to characterize the spectrum of genetic lesions found in non–small cell lung carcinoma (NSCLC) have provided important insights into the molecular basis of this disease and have also revealed a wide array of tyrosine kinases that might be effectively targeted for rationally designed therapies. The findings of these studies, however, also provide a cautionary tale about the limitations of single-agent therapies, which fail to account for the genetic heterogeneity and pathway redundancy that characterize advanced NSCLC. Emergence of drug resistance mechanisms to specific TKIs, such as gefitinib and erlotinib, suggests that more sophisticated chemotherapeutic paradigms that target multiple pathways at the same time will be required to effectively treat this disease.

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Introduction

Recent large-scale efforts to characterize the genetic basis of cancer have provided significant insights into the etiology of this disease [1–7]. Sequencing of several distinct "cancer genomes" has confirmed the importance of key tumor suppressors and oncogenes, but it has also turned up an abundance of additional mutations that have yet to be characterized with regards to their transforming potential and effect on tumor behavior during disease progression. Although most of these mutations are likely to be nonfunctional sequence changes, a significant number of them undoubtedly play important roles in tumor initiation and progression. The remarkable variation in mutational profiles seen across patient samples suggests that each tumor represents a distinct disease state that can only be effectively treated with precision therapy that targets the specific combination of genetic changes unique to each tumor.

This concept of "personalized medicine" has found particular traction in the treatment of non–small cell lung carcinoma (NSCLC), which is one of the best-characterized solid tumor types owing to its high rate of incidence in Western society [8–12]. Even before the advent of genome sequencing, many of the more common genetic alterations that characterize NSCLC had been discovered by traditional molecular biology approaches [13–15]. One theme that emerged from this work was the prevalence of genetic alterations to key growth factor signaling pathways that regulate cell proliferation, survival, and migration [3,12]. These pathways largely depend on signal propagation by kinase cascades, suggesting that they would be excellent targets for rationally designed chemotherapies aimed at the inhibition of kinase enzymatic function.

One of the more effectively targeted classes of kinases to date has been the tyrosine kinase family of signaling enzymes (Figure 1). This large class of molecules includes both receptor and nonreceptor protein tyrosine kinases and has been extensively reviewed in many excellent articles elsewhere [16–18]. Several members of this kinase family have been successfully targeted for treatment of a relatively narrow range of solid and hematologic malignancies, suggesting that further development of novel tyrosine kinase inhibitors (TKIs) may be useful for targeting a broader assortment of tumor types [19].

Whereas this approach to cancer therapy has undoubtedly provided some significant success stories, it has also highlighted the inherent difficulty of treating genetically heterogeneous patient populations with targeted therapeutics. In this review, we will discuss the various

Address all correspondence to: Brendan D. Looyenga, PhD, Van Andel Research Institute, 333 Bostwick Ave NE, Grand Rapids, MI 49503. E-mail: brendan.looyenga@vai.org
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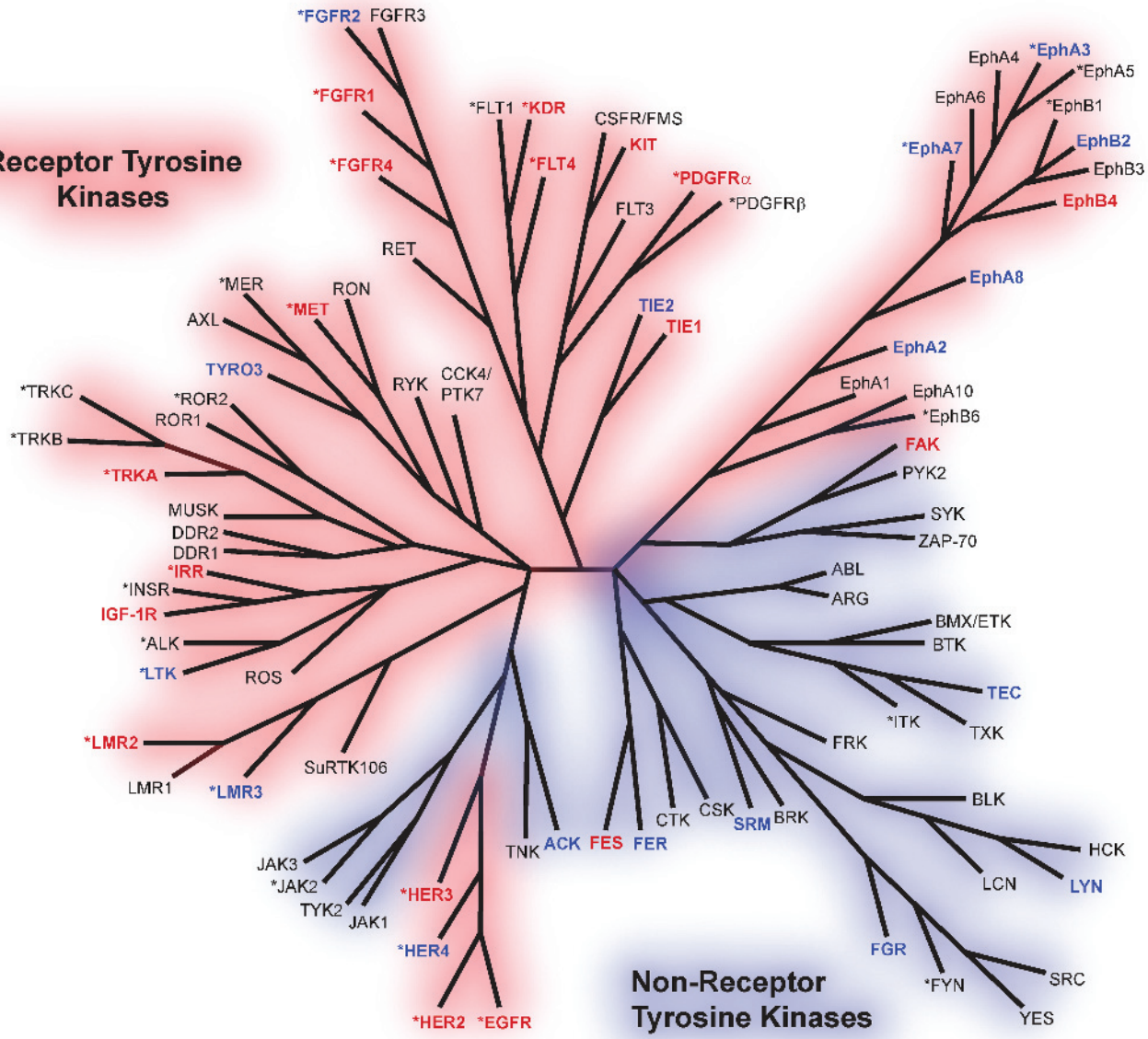

Figure 1. Genetic alterations of the tyrosine kinase family in non–small cell lung cancer. Alignment of all tyrosine kinases encoded in the human genome according to the similarity of their kinase domains reveals a unique pattern of mutagenic changes associated with non–small cell lung carcinoma. Significant genetic alterations tend to occur in multiple members of individual kinase subfamilies, with receptor tyrosine kinases being more commonly mutated than NRTKs. Genetic alterations include amplification (red font), deletion (blue font), and point mutation (asterisk), all of which have been identified at significant rates in primary patient samples. See Table 1 for statistical evaluation of copy number changes.

TKIs that have been used for the treatment of NSCLC or are currently undergoing clinical validation, as well as the resistance mechanisms that have limited the success of these drugs in the clinic. Finally, we will also discuss future prospects of TKI in the treatment of NSCLC and suggest how these drugs may be used for successful personalized therapies.

The Genetic Basis of Primary Resistance to TKIs in NSCLC

Use of rationally targeted therapies for cancer treatment by definition presumes that the specific target of interest has been correctly identified. In the case of single agent therapies, the default assumption is that hitting a single target—or at least a narrow range of defined targets—will be sufficient to treat a given tumor. This model of "oncogene addiction" has gained wide acceptance with the identification of single mutational events that both initiate and maintain transformation in most patients with a specific subtype of cancer [20–22]. Examples of this phenomenon include the *BCR-ABL* translocation in chronic myelogenous leukemia and the *ERBB2/Her2* mutations in breast cancer [23,24]. Treatment of these cancers with single-agent TKIs targeted at BCR-ABL or *ERBB2*, respectively, has shown remarkable efficacy in the clinic, and it leads to the hope that correct identification of driving mutations in other cancers will yield similar results [25–27].

The findings of recent tumor genome sequencing studies—as well as the results of manifold clinical trials—unfortunately suggest that hope for broadly successful monotherapy in NSCLC may be largely unfounded [3,28]. These studies have clearly demonstrated that no single genetic event occurs in more than 15% to 20% of the patient population and that most patients instead display a unique combination of mutations to several known oncogenes and tumor suppressors. This is particularly true of potential tyrosine kinase targets, of which more than 30 different molecules have been reported to be amplified or mutationally activated in NSCLC (Figure 1) [2–5]. Although a few kinase mutations are certainly more common than others—for example, mutations to epidermal growth factor receptor (EGFR)—these mutations rarely occur in isolation and may be compensated for by genetic alterations to other RTKs and/or downstream effectors [29].

In this context, it has become increasingly clear that the central mechanism of primary resistance to TKI therapy in NSCLC is the heterogeneity and mutational redundancy of this disease. In genetically undefined patient populations, a given molecular target may only be present in the tumors of a small percentage of patients. Furthermore, even when the target is present, it may not be absolutely necessary for tumor maintenance, especially in patients with advanced disease. This finding likely underlies the poor response rate of NSCLC to most targeted therapies, which rarely exceeds a 5% to 10% objective response rate in patients with advanced disease [9,12]. Unfortunately, most clinical trials are designed around the use of single agents in genetically undefined patient populations, making it highly unlikely that any given drug will achieve a statistically significant response during the study. In fact, such designs are inherently flawed because the overall benefit to a small fraction of eligible subjects is diluted over a nonselected population, thus skewing the result of such a randomized study toward incorrect conclusion about a given therapy [30].

Framework for Genetic Classification of Therapeutic Subgroups in NSCLC

As stated above, the success of rationally targeted therapies—including TKI—depends heavily on the correct identification of key driver mutations in each patient. In the absence of reliable genetic information, patients diagnosed with NSCLC can be directed toward the best therapy based on the histology (squamous *vs* adenocarcinoma) and natural histology (age, smoking status) of their disease. Because the prevalence of specific driver mutations in NSCLC varies dramatically in tumors with different histologies and natural histories, use of these criteria is helpful in eliminating treatments that are unlikely to succeed. For example, patients with squamous cell tumors are poor candidates for first-line EGFR inhibitor therapy because this tumor type rarely contains activating EGFR mutations and is more likely to respond to standard platinum-based therapies than targeted agents currently available [31].

When genetic information is available in addition to tumor histology and patient history, more reliable and precise decisions can be made regarding the choice of targeted therapy for NSCLC patients. This is particularly true for patients with the more common adenocarcinoma histology, which is consistently characterized by distinct driver mutations that can be broadly segregated into three classes: 1) tumors with activating mutations and/or amplification of growth factor receptor tyrosine kinases (RTKs), 2) tumors with activating mutations of the *KRAS* oncogene, and 3) tumors with EML-ALK fusions. Although this classification scheme is clearly too simplistic in that it ignores mutations to other well-defined tumor suppressors (*PTEN*, *LKB1*) and oncogenes (*PIK3CA*, *BRAF*), it provides a reliable framework for the classification of most NSCLC. Furthermore, the three classes in this framework tend to be mutually exclusive, whereas mutations to other known tumor suppressors and oncogenes are commonly found across all three classes [3,32–34].

Proper segregation of patients into one of these three classes has significant implications for targeted therapy and for primary resistance to these therapies. For instance, use of an EGFR TKI to treat a *KRAS* or EML4-ALK driven tumor will almost certainly result in a poor outcome [35]. In an unselected patient population, this result would be classified as primary resistance, whereas in a genetically defined NSCLC population, it would be considered a successful negative control. On the basis of this example, it is clear that more complete genetic data are required for the advancement of targeted therapy, particularly when a relatively selective kinase inhibitor is being used.

Assuming that NSCLC patients can be properly segregated into the classes noted above based on genetic data, the next question that clinicians must address is what therapy should be used to treat each class of tumors. Activating *KRAS* mutations are the single most common genetic driver in lung cancer, represented about 15% to 20% of all NSCLCs [3,32,35]. Attempts to target activated Ras variants have historically failed for a wide variety of reasons, and despite decades of research on this oncogene, there is no clear answer for how to treat mutant Ras-driven tumors [36]. The most promising avenues for treating this class of cancers lie in targeting the downstream signaling effectors or metabolic byproducts of Ras transformation, although these targets tend to be highly cancer-specific [37,38]. New advances in targeting Ras-driven tumors have been reviewed elsewhere and will not be extensively discussed in this review [36,39].

The two other known classes of NSCLC collectively depend on activating mutations to a variety of tyrosine kinases, including both receptor and nonreceptor members of this family (Figure 1 and Table 1). This population of tumors represents anywhere from 40% to 80% of all NSCLCs, which implies that up to 175,000 new lung cancer patients each year in the United States alone could benefit from some form of targeted TKI therapy, provided that the correct target for each tumor could be identified [40]. This alone is a daunting task because more than 30 different tyrosine kinases have been implicated in the cause of NSCLCs (Figure 1 and Table 1). With faster and more accessible genome sequencing technologies on the horizon, however, identifying the correct targets for TKI therapy is becoming increasingly tangible.

The remainder of this review will focus on TKIs that have been introduced into the clinic or are in clinical testing, with a specific emphasis on their performance in clinical trials and on the understanding of resistance mechanisms that will aid in the refinement of TKI therapy.

Growth Factor Receptor TKIs

EGFR Inhibitors

The most well-studied small-molecule TKIs used for treatment of NSCLCs are the quinazoline class of compounds targeted to EGFR. Two drugs in this class, erlotinib and gefitinib, were approved for the treatment of NSCLC in 2004 and 2005, respectively (Figure 2*A*). Since then, several thousand patients have received these drugs as

Table 1. Significant Copy Number Changes of Key Tyrosine Kinases in Non–Small Cell Lung Cancer.

Name	Family	GISTIC Score (*q*)	Frequency (%)
Significantly amplified			
EGFR	EGFR	9.29*e* − 25	10.0
FGFR1	FGFR	3.61*e* − 23	10.8
INSRR	InsR	3.29*e* − 12	15.4
TRKA	Trk	3.29*e* − 12	15.6
HER2/ErbB2	EGFR	9.76*e* − 10	10.9
FAK	Fak	1.61*e* − 06	12.2
PDGFRa	PDGFR	4.67*e* − 04	8.9
HER3/ErbB3	EGFR	6.98*e* − 04	12.0
MET	Met	1.08*e* − 03	6.8
KIT	PDGFR	1.29*e* − 03	8.9
IGF1R	InsR	1.75*e* − 03	10.9
FLT4	VEGFR	1.99*e* − 03	10.5
KDR	VEGFR	3.84*e* − 03	8.5
FGFR4	FGFR	8.80*e* − 03	10.4
LMR2	Lmr	9.02*e* − 03	7.6
EphB4	Eph	4.10*e* − 02	7.2
TIE1	Tie	5.26*e* − 02	4.2
FES	Fer	7.71*e* − 02	8.9
Significantly deleted			
LTK	Alk	5.34*e* − 11	9.3
TIE2	Tie	2.37*e* − 08	6.1
TYRO3	Axl	3.48*e* − 08	9.1
JAK2	JakA	4.08*e* − 06	4.2
SRM	Src	1.74*e* − 05	8.1
FGR	Src	3.67*e* − 05	7.8
EphB2	Eph	3.70*e* − 05	8.1
EphA8	Eph	6.55*e* − 05	7.9
LMR3	Lmr	2.51*e* − 04	7.2
EphA2	Eph	4.19*e* − 04	7.2
EphA7	Eph	3.68*e* − 02	4.4
FER	Fer	3.90*e* − 02	4.1
TEC	Tec	5.26*e* − 02	4.2
LYN	Src	7.26*e* − 02	4.0
EphA3	Eph	7.82*e* − 02	3.4
FGFR2	FGFR	8.41*e* − 02	4.9
ACK	Ack	1.91*e* − 01	4.2
HER4/ErbB4	EGFR	1.97*e* − 01	4.8

Copy number variation (CNV) of tyrosine kinases in NSCLC was assessed using the GISTIC algorithm and SNP array data obtained from the Broad Institute Tumorscape program [2,5]. The significance of amplification or deletion was assessed for all human tyrosine kinases. Those exceeding the GISTIC significance score (*q*) of 0.25 are included in each table. Frequency values indicate the percentage of 384 non–small cell lung cancer samples that contain focal amplification or homozygous deletion of the indicated tyrosine kinase.

first-, second-, or third-line therapy, providing a large amount of clinical data for retrospective analysis of drug efficacy [41,42].

Initial studies with erlotinib and gefitinib in unsegregated patient populations demonstrated a relatively low objective response rate (8%-9%), which was somewhat surprising given the strong efficacy of these drugs in preclinical models and the high prevalence of EGFR overexpression in lung cancer [43,44]. In the past 5 years, it has become increasingly clear that patients who respond well to these drugs almost universally display tumors with activating mutations to EGFR. More specifically, activating mutations involving exon 19 deletions or exon 21 point mutations were found to be particularly sensitive to these drugs and were strong predictors of patient outcome in EGFR TKI therapy [41,45]. Nevertheless, patients' tumors that display elevated expression of wild-type EGFR generally fail to show radiographic responses to treatment with these agents, suggesting that careful genetic prescreening of patients for specific EGFR mutations will be required to gain full advantage in the clinic [46,47].

As is the case in most advanced cancers, NSCLC patients on EGFR inhibitor therapy who initially respond to treatment eventually develop secondary resistance, in this case with a median progression-free survival time of 8 to 10 months [28,48,49]. Several mechanisms to account for secondary resistance to EGFR TKI have been elucidated, providing new insights into how anti-EGFR therapy might be improved in the future. These mechanisms and their implications for NSCLC treatment are briefly detailed below.

Resistance Mechanism 1: Secondary Mutations to EGFR

One of the more common mechanisms of kinase inhibitor resistance is the acquisition of secondary mutations to the target kinase. This mechanism has been previously demonstrated in chronic myelogenous leukemia patients bearing the BCR-ABL fusion oncogene, who eventually develop resistance to the TKI imatinib after prolonged periods of stable disease [50,51]. In the case of NSCLC patients, resistance to EGFR TKI therapy has been observed in the presence of primary or secondary point mutations to exon 20 of EGFR (Figure 2*B*). The most common of these mutations—T790M—decreases the affinity

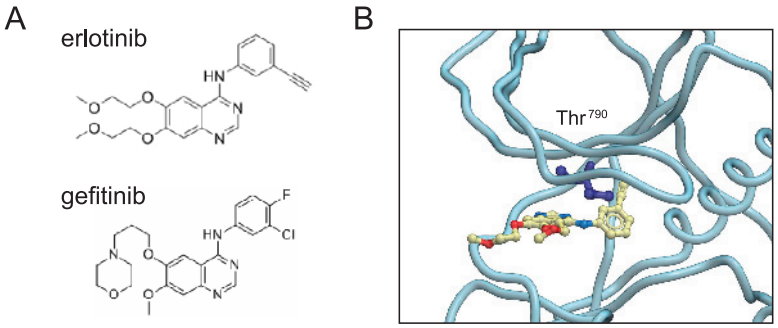

Figure 2. Resistance to EGFR inhibitors mediated by gatekeeper residue mutations. (A) Chemical structures of erlotinib and gefitinib, both of which are members of the quinazoline class of EGFR inhibitors. (B) The crystal structure of erlotinib bound to EGFR demonstrates its physical orientation within the ATP binding pocket of the enzyme. Both erlotinib and gefitinib function as competitive inhibitors by preventing the binding of ATP into this cleft. The threonine at position 790, commonly referred to as a "gatekeeper" residue owing to its location within the binding pocket, is commonly altered in NSCLC. Mutation of this residue to methionine (T790M) prevents incorporation of both erlotinib and gefitinib owing to steric constraints induced by decreased pocket size. Structure information was obtained from Research Collaboratory for Structural Bioinformatics (accession number: PDB 1M17) [55].

of TKI for the receptor, thus rendering tumors insensitive to chemotherapy [52–55].

Because exon 20 point mutations may occur as the primary or secondary mechanism for constitutive EGFR activation in NSCLC, drugs that can inhibit EGFR kinase activity in the presence of these and other activating mutations, while leaving wild-type EGFR unaffected, have become a major area of focus for TKI development [56,57]. Promising results with a new pyrimidine-based class of drugs that meet these criteria have recently been reported in preclinical models, suggesting that improved EGFR TKI therapies may soon be available [58].

Resistance Mechanism 2: Activation of Compensatory Oncogenes

In addition to being a key form of primary resistance, amplification and/or mutation of oncogenes with redundant function to EGFR have emerged as another key mode of resistance to EGFR TKI. The best-characterized mechanism of this type is amplification of MET, another RTK that has been implicated in a wide range of solid tumors, including NSCLC [59]. Recent data suggest that cells harboring low-level MET amplification may exist in patients with EGFR-driven tumors and that these cells are heavily selected for after long-term treatment with erlotinib or gefitinib [60]. Emergence of drug-resistant secondary tumors is particularly accelerated in the presence of the MET ligand hepatocyte growth factor, which has also been shown to be amplified or overexpressed in many solid tumors.

Activation of other RTK with similar redundancy to EGFR may also play an important role in acquired resistance to EGFR TKI (Figure 1 and Table 1). Insulin-like growth factor receptor (IGF1R), for instance, has been shown to mediate resistance in cultured lung cancer cells and is known to be amplified in a subset of NSCLC [61,62]. Although no solid evidence for compensation of EGFR inhibition by RTKs other than MET and IGF1R has been reported to date, it is likely that current large-scale sequencing studies of EGFR TKI-resistant tumors will uncover similar compensatory mechanisms by RTK or other signaling oncoproteins [29,63,64]. Identification of these compensatory oncoproteins will provide new targets for combined treatment that may be used to prevent the emergence of TKI-resistant cell populations from EGFR TKI-sensitive tumors.

It is important to note that activating mutations to *KRAS*—although an important mode of primary resistance—have not been associated with secondary resistance to EGFR TKI [32,35,65]. Although more studies are required to confirm that *KRAS* activation is insufficient to compensate for loss of EGFR activity, this observation is consistent with differences in the natural history of *KRAS*-mutant and *EGFR*-mutant tumors, which are differentially enriched in smokers and nonsmokers, respectively [47,66]. Both findings suggest that the EGFR and K-Ras oncoproteins are not redundant with respect to the molecular mechanisms by which they induce and maintain transformation. As such, it might be predicted that efforts to target signaling pathways required for RTK-driven tumors will fail in patients with Ras-driven tumors—and *vice versa*—owing to the nonoverlapping modes of transformation used by these oncoproteins.

Resistance Mechanism 3: Epigenetic Identity Switching

Perhaps the most interesting mechanism for EGFR TKI resistance that has been uncovered to date is described in a recent report by Sharma et al [67]. In their study, the authors exposed EGFR TKI-sensitive cell lines to gefitinib for several weeks and then selected the subclones that emerged from this treatment for *in vitro* expansion. Surprisingly, the various cell lines selected by this method seemed to have developed stable resistance to EGFR TKI by undergoing a multistep process of nonrandom epigenetic switching to an alternative cellular identity. This identity did not seem to be due to compensatory genetic changes in other RTK and was reversible after several passages in TKI-free medium.

Whereas the underlying mechanism(s) that allow NSCLC cells to transiently change their cellular identity are incompletely understood, it is clear that identity switching can be prevented by inhibition of histone deacetylases (HDACs), which are responsible for mediating the nonrandom changes in gene expression observed in EGFR TKI-resistant cells. The epigenetic switch also seems to depend on signaling through the IGF1R, suggesting that activation of an alternate RTK to EGFR may be a key feature of both genetic and epigenetic resistance mechanisms [67]. Further studies in patient-derived tumor samples and a broader array of cell lines will be required to determine the extent to which this mechanism actually occurs in EGFR TKI-resistant NSCLC; although based on results in drug-resistant cell lines derived from other cancer types, it seems that coordinate treatment of tumors with cytotoxic or targeted agents and HDAC inhibitors may present a feasible method to prevent the emergence of drug resistance tumor subclones.

It is worth noting that the drug-resistant cells described by Sharma et al. are reminiscent of tumor-initiating cells—also known as cancer stem cells (CSCs)—which have been identified in a variety of solid tumors [68,69]. Both cell types exist as a small population of slow-cycling, self-renewing tumor cells that are significantly more resistant to both cytotoxic and targeted therapy than other tumor cells [70,71]. Although it was initially thought that CSCs exist as a stable population in solid tumors, it has been recently shown that the CSC identity may instead represent a reversible state that is transiently maintained by a small population of cells in a given tumor [72]. Interestingly, the phenotypic switching mechanisms for CSCs and TKI-resistant cells seem to be driven by similar epigenetic mechanisms involving the JARID family of chromatin-modifying proteins. These findings suggest that reversion of tumor cells to a CSC-like cellular identity may represent a universal drug resistance mechanism that must be accounted for regardless of the chemotherapeutic regimen being used for cancer treatment.

MET Inhibitors

Owing to its role in EGFR TKI resistance and as a primary driving oncogene in several solid tumors, MET has become a key target of interest for rationally designed chemotherapy [73,74]. Although MET kinase inhibitors have yet to be approved for use by the Food and Drug Administration, several different agents are being tested in clinical trials alone or in combination with EGFR TKI for the treatment of NSCLC (Table 2). These agents include drugs that are specifically targeted to MET, as well as drugs that target both MET and other cancer-associated RTKs [75].

Clinical data regarding resistance to MET inhibitors have yet to be thoroughly documented, although an *in vitro* study using the selective MET inhibitor PHA-665257 to derive tumor cell lines with secondary resistance has been performed [76]. Data from this study suggest that resistance to MET inhibition is driven by the emergence of EGFR pathway alterations, either at the level of EGFR itself or at the other receptors from the ERBB family of RTKs. This finding provides additional confirmation of the functional redundancy between

Table 2. Tyrosine Kinase Targets in Development for NSCLC Treatment.

Target	Drug
RTKs	
EGFR	Vandetanib, lapatinib, BIBW2992, PF299804 (pan ERBB family)
MET	Foretinib, crizotinib, ARQ197, XL184
ALK	Crizotinib
FGFR	Intedanib, BGJ398 (pan), dovitinib, AZD4547
IGF1R	BMS754807, XL228
VEGFR	Sorafenib, sunitinib, vandetanib, pazopanib, vatalanib, foretinib, intedanib
PDGFR	Pazopanib, intedanib
KIT	Imatinib
EPHB4	XL647
NRTKs	
SRC	Dasatinib, XL228
FAK	GSK2256098, PF04554878, PF562271
JAK	INCB018424, lestaurtinib

Tyrosine kinases that are commonly altered in NSCLC are being targeted for therapy with several different TKIs. Drugs that are currently approved for therapy or are in clinical development are listed at the right of the indicated tyrosine kinase.

MET and ERBB family receptors and also underscores the clinical importance of targeting both classes of RTKs simultaneously to prevent the acquisition of secondary resistance to TKI therapy.

Anaplastic Lymphoma Kinase Inhibitors

Anaplastic lymphoma kinase (ALK) is an RTK that has been previously implicated in a number of solid and hematologic malignancies, most prominently anaplastic large cell lymphoma (ALL) and neuroblastoma [77,78]. Like EGFR and MET, ALK may be amplified or point mutated to achieve activation. In addition, ALK can also be activated by chromosomal rearrangements, as is the case in subsets of ALL and NSCLC [34,79]. Whereas ALL-associated rearrangements primarily involve chromosomal translocation that result in the fusion of ALK to the various nuclear or cytoplasmic proteins such as nucleophosmin (NPM1), NSCLC-associated rearrangements more commonly involve small chromosome 2p inversions that link the C-terminal intracellular kinase domain of ALK to the N-terminal domain of the microtubule-associated protein EML4 [80]. The resulting fusion protein is constitutively active because of autodimerization, making it highly oncogenic and capable of independently transforming NIH-3T3 fibroblasts based on xenograft assays.

NSCLC cell lines harboring EML4-ALK fusions are highly sensitive to ALK TKI *in vitro*, providing strong evidence that this genetic rearrangement is the primary driving force for their transformation [81]. Interestingly, *EML4-ALK* translocations are found in roughly 4% of all NSCLC but are particularly enriched (9.4%) in younger patients with no history of smoking [82,83]. Owing to this relatively high prevalence and encouraging preclinical data in cell culture models, ALK TKIs are rapidly being introduced into the clinic in parallel with genetic screening for chromosome 2 rearrangements that produce the EML4-ALK fusion. Early reports suggest that patients with these genetic lesions respond favorably to the dual TKI crizotinib, which selectively targets both ALK and MET [34,84,85]. Ongoing clinical trials will provide a more complete picture of the efficacy and durability of patient responses to ALK TKI therapy in the near future [86].

Other RTK Targets in Clinical Development for NSCLC Therapy

Although tumors bearing amplification and/or mutation of EGFR, MET, and ALK represent the largest and best-characterized cohort of RTK-driven NSCLC, large-scale sequencing studies have demonstrated that several other growth factor receptors are recurrently mutated as well (Figure 1). These include IGF1R, stem cell factor receptor (SCFR/KIT) and members of the fibroblast growth factor receptor (FGFR), neurotrophin receptor (NTRK), and ephrin receptor (EPHA and EPHB) families [3,87,88]. TKIs that target several of these RTKs are in early stages of clinical testing for NSCLC treatment, although results of these studies have yet to be reported (Table 2). Providing that these drugs are able to sufficiently inhibit their targets at pharmacologically relevant doses and with limited toxicity, they are likely to prove useful in the treatment of patients with genetically defined NSCLC.

Antiangiogenic Receptor TKIs

A second class of RTKs that has received significant attention for targeted chemotherapy is the proangiogenic RTKs. This group of receptors is primarily composed of the vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR) families, each of which contains three distinct members [89,90]. The FGFR family may also be considered in this category, although its effects in lung cancers are more traditionally associated with tumor cell proliferation and survival rather than endothelial cell proliferation and angiogenesis [91,92]. Several TKIs targeted to the FGFR family are in clinical development (Table 2), only results for dovitinib, which selectively targets FGFR1-3, in addition to VEGFR, PDGFR, and other tyrosine kinases, have been reported to date [93]. As such, discussion of antiangiogenic TKI can be limited to VEGFR and PDGFR families because these receptors have been the major focus of ongoing antiangiogenic therapy [94].

The first Food and Drug Administration–approved small-molecule angiogenesis inhibitors were the TKIs sorafenib and sunitinib, which are both currently indicated for the treatment of metastatic renal tumors and gastrointestinal stromal tumors and hepatocellular carcinoma (sorafenib only) [95]. Whereas both drugs are primarily intended to target VEGF-dependent endothelial cell proliferation and angiogenesis owing to their potent inhibition of the VEGFR family, they also inhibit a number of other tyrosine kinases including KIT and members of the PDGFR family (Table 3). In addition, both drugs have been

Table 3. Polypharmacologic TKIs in Development for NSCLC Treatment.

Name	Manufacturer	Known Targets	Developmental Phase
Sorafenib	Bayer/Onyx	VEGFR, PDGFR, RAF, KIT	2/3
Sunitinib	Pfizer	VEGFR, PDGFR, KIT, RET, FLT3	3
Imatinib	Novartis	BCR-ABL, PDGFRb, KIT, RET	2
Vandetanib	AstraZeneca	VEGFR, EGFR	3
Intedanib	Boehringer Ingelheim	FGFR, VEGFR, PDGFR	2/3
Dasatinib	Bristol-Myers Squibb	SRC family kinases	1/2
Pazopanib	GlaxoSmithKline	VEGFR, PDGFR, KIT	2
Lapatinib	GlaxoSmithKline	EGFR, HER2	2
XL-184	Exelixis	MET, RET, VEGFR2	1/2
BIBW2992	Boehringer Ingelheim	EGFR, HER2	2
Foretinib	GlaxoSmithKline	MET, VEGFR2	1/2
Crizotinib	Pfizer	MET, ALK	3
XL-647	Exelixis	EGFR, HER2, VEGFR, EPHB4	2
XL-228	Exelixis	IGF1R, SRC, BCR-ABL	1
Lestaurtinib	Cephalon	JAK2, FLT4, TRK	1/2
PF04554878	Pfizer	FAK, PYK2	1

Many of the TKIs currently in clinical development target multiple kinases. Validated targets for each drug are indicated to the right, along with the stage of clinical testing each drug was in at the time this article was prepared.

shown to inhibit different serine-threonine kinases at physiologically relevant levels for cancer therapy [96,97]. This is particularly important for sorafenib, which was originally designed as a BRAF inhibitor. It is likely that this activity—and not its antiangiogenic potential—explains recent reports of modest sorafenib efficacy in *KRAS* mutant lung cancers in phase 2 clinical trials [98–100].

In addition to sorafenib and sunitinib, several other TKIs are in various stages of clinical development for antiangiogenic NSCLC therapy. All of these compounds target various members of the VEGFR family—most prominently *KDR/VEGFR2*—as well as other RTKs that have been implicated in NSCLC or other solid tumors (Tables 2 and 3) [101]. One of these agents, pazopanib (approved for use in kidney cancer), shows selectivity for *VEGFR*, *PDGFR*, and *KIT* and may prove to be useful for treating tumors that develop secondary resistance to treatment with sorafenib or sunitinib [102,103]. Newer TKIs that target both VEGFR and growth factor receptors include vandetanib and foretinib, which demonstrate selectivity for EGFR and MET, respectively [104–107]. Whereas both of these drugs are still in the early phases of clinical testing, they hold significant promise as dual inhibitors of primary tumor cell proliferation/survival and angiogenesis.

In addition to serving as traditional antiangiogenic compounds, drugs that target PDGFR family members may also be beneficial as adjuvant therapies owing to their ability to promote delivery of standard cytotoxic chemotherapies to tumors. Recent studies in NSCLC xenografts have demonstrated that combined treatment of tumors with imatinib—which inhibits PDGFRβ—and cytotoxic drugs including taxanes, doxorubicin, or etoposide leads to more effective tumor cell killing than any of the cytotoxic drugs alone [108]. This effect likely results from loss of interstitial vascular pressure and microvessel leakiness induced by imatinib treatment, which allows better penetration of drugs into tumors. A phase 2/3 clinical trial with pazopanib intended to test this preclinical model in stage I NSCLC patients is ongoing and should provide answers in the next year [109].

Although no information on resistance of NSCLC to antiangiogenic TKIs is available because of the relative infancy of this therapy in lung cancer patients, potential mechanisms for acquired resistance to sunitinib have been described in the context of renal carcinoma where this drug has been in use for a longer period. These mechanisms are briefly described below.

Resistance Mechanism 4: Angiogenic Compensation

Traditional models of tumor angiogenesis suggest that primary tumor cells promote increased vascularization by producing proangiogenic factors such as VEGF, which recruit adjacent endothelial cells to dissociate from stable vessels, migrate into the tumor, and proliferate to from new blood vessels. Antiangiogenic TKIs such as sunitinib disrupt this paracrine signaling loop by inhibiting VEGFR and PDGFR activation in endothelial cells, resulting in decreased endothelial cell recruitment and/or collapse of immature tumor vasculature. Because endothelial cells—and not cancer cells—are the target of drug treatment, secondary mutations to angiogenic receptors are unlikely to account for resistance.

A recent xenograft model of acquired resistance to sunitinib has been described for renal cell carcinoma in which tumor cells instead compensate for loss of VEGF signaling by upregulating signaling by the cytokine interleukin-8 (IL-8) [110]. This mechanism fits with previous work showing that various cytokines including IL-8 can elicit an alternative mode of angiogenesis independent of VEGF [111,112]. Whether this mechanism plays a role in patients who relapse after initial response to sunitinib is unclear at this point, although results from this study and others suggest that IL-8 levels may be enhanced in the tumors of patients who demonstrated primary resistance to antiangiogenic therapy [113].

Resistance Mechanism 5: Reversible Cellular Identity Switching

The ability of epithelial-derived cancer cells to detach from primary tumors and metastasize to distant locations is associated with a reversible change in cellular identity termed an *epithelial-mesenchymal transition* (EMT) [114]. EMT is characterized by broad changes in gene expression, cellular morphology, and migratory behavior in which stationary epithelial cells assume the characteristics of migratory mesenchymal cells. Whereas EMT can be induced in a controlled manner by the exposure of transformed cells to specific signaling ligands such as those from the transforming growth factor beta (TGFβ) family, several studies have shown that EMT also occurs spontaneously in tumors and may be associated with drug resistance [115].

Evidence that reversible EMT may underlie acquired sunitinib resistance was described in a recent study in which cells derived from a sunitinib-resistant renal tumor metastasis with mesenchymal features were explanted to tissue culture and then xenografted into athymic nude mice [116]. Surprisingly, xenografted cells reverted to a sunitinib-sensitive state and demonstrated phenotypic reversion to an epithelial identity. Although the precise reason for the reversion was not explored, it is likely that the tumor cells reverted to an epithelial phenotype during tissue culture because of the enhanced proliferative capacity associated with this cellular identity.

It is interesting to note that the reversible changes in cellular phenotype that encompass EMT, such as decreased cellular proliferation and nonrandom epigenetic alterations, are strikingly similar in nature to those described for resistance to erlotinib [67,72]. Because these changes in identity also promote resistance to cytotoxic chemotherapies, it might be suggested that a single mechanism is responsible for driving several of the behaviors associated with aggressive solid tumor growth [115]. The clinical implications of this hypothesis are critically important because they suggest that prevention of identity switching in primary tumors may prove to be an effective therapy to inhibit drug resistance and seeding of secondary tumors to distant locations [117]. Further studies combining HDAC inhibitors with targeted agents including TKI will be necessary to test this hypothesis in the clinic. One phase 2 study of a HDAC inhibitor (entinostat) in combination with erlotinib has completed accrual, and results are pending [118].

Nonreceptor TKIs

Although the RTK class of kinases has received significantly greater attention as targets for NSCLC therapy, several nonreceptor tyrosine kinases (NRTKs) are also implicated in the cause and progression of this disease [3]. NRTKs primarily serve as secondary messengers to relay extracellular signals from growth factor, cytokine, and adhesion receptors to a wide array of targets in the cytoplasm and nucleus. Similar to RTK, these kinases can be activated by different genetic mechanisms including genomic amplification, point mutation, and translocation (Figure 1 and Table 1) [16]. In addition, they can also be strongly activated by dysregulated RTK signaling in the absence of genomic alteration. These mechanisms are proposed to alter kinase

activity in a manner that promotes tumor cell proliferation and survival or changes in tumor cell adhesion and migratory capacity.

For the purposes of this discussion, we will focus on three classes of NRTKs that are currently under development as targets for TKI therapy. Although only two of these classes are currently being targeted in ongoing clinical trials specific to NSCLC, all three are in various stages of clinical testing as chemotherapeutic targets. Both the rationale for targeting these kinases and the TKIs that are being used for this purpose are discussed below.

SRC Family Inhibitors

Seminal work by Spector et al. [119] in the late 1970s demonstrated that cancer is initiated by mutagenic changes to normal genes ("proto-oncogenes") found in the human cell. The focus of their work was the sarcoma viral oncogene (*v-Src*), which was first associated with transforming avian leukosis viruses, and then later found to have a normal cellular counterpart in human cells called *c-Src*, or simply *SRC* in current genomic nomenclature [120]. Since then, genome sequencing studies from several species have demonstrated that *SRC* is part of a larger family of nine related NRTKs, all of which have been implicated in various cancer-relating signaling processes in a wide variety cell and tissue types [16,121].

While activating mutations to SRC family members have been reported for a wide variety of solid tumors, it is now recognized that Src family oncoproteins are more commonly activated downstream of other signaling pathways—particularly activated RTK—in cancer [122]. Because Src plays an important role in tumor cell survival, adhesion, migration, and invasion, it is an attractive target for rationally designed NSCLC therapy [123]. Two small-molecule inhibitors of Src—dasatinib and XL-228—are currently in clinical development as single or combined agents for treatment of NSCLC (Table 3) [124,125]. Although dasatinib also shows selectivity for the tyrosine kinase ABL, it is primarily being developed as a *SRC* family kinase inhibitor for cancer therapy [126]. XL-228 shows similar selectivity for SRC but is primarily being developed as an inhibitor of the BCR-ABL fusion protein and IGF1R owing to its higher specificity for these kinases. Both preclinical models and early clinical trials with dasatinib suggest that coordinate targeting of EGFR and Src may be a useful therapeutic paradigm for the treatment of EGFR-positive NSCLC [127,128].

Focal Adhesion Kinase Inhibitors

Signaling by the integrin family of adhesion receptors is critical for survival of both normal and transformed cells [129]. Activated integrins usually transmit these signals in the context of large protein complexes such as focal adhesions, which contain a variety of signaling enzymes including serine/threonine and tyrosine kinases [130]. Key mediators of survival signaling downstream of integrins include the closely related tyrosine kinases FAK and PYK2. Inhibition of signaling by these kinases induces cell death in a variety of tumor lines, consistent with their necessity for cellular viability [131].

Three different TKIs targeted to FAK and PYK2 are currently being evaluated in phase 1 clinical trials as single-agent therapies, although none of these drugs are specifically being used for the treatment of NSCLC (Table 2) [132,133]. Because FAK signaling has been implicated survival of several NSCLC cell lines, however, these drugs may find use as single or combined therapeutics for the treatment of this disease.

Janus Kinase Inhibitors

The Janus kinase (JAK) family of proteins is composed of four separate tyrosine kinases that primarily function to transmit signals from cytokine receptors to their cytoplasmic and nuclear effectors. The most well-characterized targets of JAK family kinases are the signal transducer and activator of transcription (STAT) family of proteins, which translocate to the nucleus and induce transcription of key target genes in response to tyrosine phosphorylation [134]. Both JAK and STAT proteins are implicated in a wide variety of cancer-associated processes, most notably cell survival, angiogenesis, and immune surveillance.

JAK family kinases have primarily been implicated in hematologic malignancies, particularly in the case of JAK2, which is mutated in myeloproliferative disorders [135]. Emerging evidence suggests, however, that JAK/STAT signaling may play a wider role in NSCLC than has been previously appreciated because many NSCLC cell lines and patient tumors demonstrate elevated levels of JAK2 activity or STAT phosphorylation [136,137]. Two potential mechanisms to explain this effect are the selective epigenetic silencing of the suppressor of cytokine signaling-3 (SOCS3) promoter or genetic loss of the phosphatase PTPRD in NSCLC cells, both of which effectively increase JAK activity by removing critical negative feedback loops required to silence the activity of STAT proteins [138–140]. These findings have yet to be translated into the clinic for NSCLC treatment, although multiple JAK inhibitors are currently undergoing phase 1/2 testing for the treatment of other malignancies (Table 2) [141–144].

Future Directions in TKI Therapy for NSCLC

Although many of the TKI discussed above are in relatively early stages of clinical testing, data that have already emerged from these studies suggest that rationally designed use of these agents will likely be effective in treating subsets of patients with specific combinations of genetic "driver mutations" [145]. Much work will be required to effectively determine which patients are the correct candidates for any given therapy, however, because it is now clear that simple expression and/or activation of a given target—for example, EGFR—is insufficient to predict patient responsiveness to inhibitors. This will require extensive genetic comparison of responders and non-responders, which in turn depends on further advances in sequencing technology that make such comparisons feasible [146].

A key theme that has clearly emerged from clinical studies of various TKIs is the necessity of inhibiting multiple targets in parallel to prevent the acquisition of drug resistance. In some respects, this should have been obvious based on the wealth of literature regarding antibiotic resistance in bacteria and viruses, which, in some instances, occurs by mechanisms that are remarkably similar to those discovered for EGFR TKI resistance [147,148]. Targeting of multiple kinases in parallel can be achieved by combining several highly specific drugs or by use of inhibitors that selectively target multiple targets [149]. The latter class of drugs has become especially popular in recent years because most kinase inhibitors function as nucleotide mimetics and, as a result, often demonstrate selectivity for more than one target (Table 3) [150]. Intentional use of these so-called "polypharmacologics" to interfere with multiple RTKs or multiple cancer-related processes such as tumor cell proliferation and angiogenesis has recently gained wider acceptance and is likely to see further development in the near future [151].

Another approach to rationally designed chemotherapy that has seen increased attention of late focuses on combination of compounds that target commonly activated pathways rather than individual

oncogenic molecules [38]. Because many of the RTKs that have been implicated in NSCLC activate similar downstream effectors, targeting common pathways rather than multiple receptors may be a more effective alternative for chemotherapy [152,153]. Several drugs that inhibit the Ras/MAPK, PI3K/Akt, mTOR, and JAK/STAT pathways are in clinical development as single or combined agents. Use of these drugs may turn out to be more effective than targeting individual RTKs because the mutational spectrum for receptors is significantly wider than the number of signaling pathways activated by these molecules.

The question of whether targeted therapeutics will provide additional benefit in combination with radiotherapy (XRT) or cytotoxic chemotherapy (CC) is also of great clinical interest because XRT and/or CC remain the standard of care for unresectable or advanced NSCLC. Several phase 2/3 clinical trials have begun to address this question by concurrent use of sorafenib or various EGFR inhibitors with CC agents, including platinum-based drugs, DNA-damaging agents, and taxanes [47,154–157], or in combination with XRT [158,159]. Results from these studies have been almost uniformly disappointing and demonstrate little, if any, additional benefit of combined therapy over CC or combined CC and XRT alone. Because most of these studies were performed in unselected patient populations, however, it remains to be seen whether combinational treatment with CC or XRT and targeted therapeutics will improve outcome in patients with genetically defined lesions. It has also been suggested that any potential benefit from combinatorial treatment is more likely to emerge from serial—rather than concurrent—treatment or maintenance CC because targeted drugs including TKIs often induce a cytostatic response that can antagonize the proliferation-dependent mechanisms of cell killing by traditional cytotoxic therapies when used concurrently [160].

Regardless of which therapeutic paradigm turns out to be most effective, it is clear that NSCLC will have to be treated with a "personalized medicine" approach if chemotherapy is to be widely successful in the clinic. This approach requires that each patient be segregated into a specific treatment group according to the constellation of molecular alterations that define his or her disease [161]. Whereas the most tangible methods for segregation currently rely on limited sequencing and gene expression data, it is likely that more sophisticated approaches including whole-genome sequencing, epigenome profiling, proteome profiling, and microRNA profiling (miRome profiling) will eventually be used to provide a more complete picture of lung cancer biology. Although this entire panel of analyses is unlikely to find clinical utility in the near future, incremental introduction of each technique into clinical practice will help to better focus rationally designed therapies on the patients who will most benefit from them.

References

[1] ICGC (2010). International network of cancer genome projects. *Nature* **464**, 993–998.

[2] Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, Barretina J, Boehm JS, Dobson J, Urashima M, et al. (2010). The landscape of somatic copy-number alteration across human cancers. *Nature* **463**, 899–905.

[3] Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, Cibulskis K, Sougnez C, Greulich H, Muzny DM, Morgan MB, et al. (2008). Somatic mutations affect key pathways in lung adenocarcinoma. *Nature* **455**, 1069–1075.

[4] Lee W, Jiang Z, Liu J, Haverty PM, Guan Y, Stinson J, Yue P, Zhang Y, Pant KP, Bhatt D, et al. (2010). The mutation spectrum revealed by paired genome sequences from a lung cancer patient. *Nature* **465**, 473–477.

[5] Weir BA, Woo MS, Getz G, Perner S, Ding L, Beroukhim R, Lin WM, Province MA, Kraja A, Johnson LA, et al. (2007). Characterizing the cancer genome in lung adenocarcinoma. *Nature* **450**, 893–898.

[6] Ding L, Ellis MJ, Li S, Larson DE, Chen K, Wallis JW, Harris CC, McLellan MD, Fulton RS, Fulton LL, et al. (2010). Genome remodelling in a basal-like breast cancer metastasis and xenograft. *Nature* **464**, 999–1005.

[7] Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, et al. (2008). An integrated genomic analysis of human glioblastoma multiforme. *Science* **321**, 1807–1812.

[8] Gazdar AF (2009). Personalized medicine and inhibition of EGFR signaling in lung cancer. *N Engl J Med* **361**, 1018–1020.

[9] Wakelee H, Loo BW Jr, Kernstine KH, Putnam JB Jr, Edelman MJ, Vokes EE, Schiller JH, Baas P, Saijo N, Adjei A, et al. (2009). Cooperative group research efforts in thoracic malignancies 2009: a review from the 10th Annual International Lung Cancer Congress. *Clin Lung Cancer* **10**, 395–404.

[10] Hutchinson L and DeVita VT Jr (2008). The era of personalized medicine: back to basics. *Nat Clin Pract Oncol* **5**, 623.

[11] Perez-Soler R (2009). Individualized therapy in non–small-cell lung cancer: future *versus* current clinical practice. *Oncogene* **28**(suppl 1), S38–S45.

[12] Herbst RS, Heymach JV, and Lippman SM (2008). Lung cancer. *N Engl J Med* **359**, 1367–1380.

[13] Bergh JC (1990). Gene amplification in human lung cancer. The *myc* family genes and other proto-oncogenes and growth factor genes. *Am Rev Respir Dis* **142**, S20–S26.

[14] Siegfried JM, Gillespie AT, Mera R, Casey TJ, Keohavong P, Testa JR, and Hunt JD (1997). Prognostic value of specific *KRAS* mutations in lung adenocarcinomas. *Cancer Epidemiol Biomarkers Prev* **6**, 841–847.

[15] Shiraishi M, Noguchi M, Shimosato Y, and Sekiya T (1989). Amplification of protooncogenes in surgical specimens of human lung carcinomas. *Cancer Res* **49**, 6474–6479.

[16] Blume-Jensen P and Hunter T (2001). Oncogenic kinase signalling. *Nature* **411**, 355–365.

[17] Lemmon MA and Schlessinger J (2010). Cell signaling by receptor tyrosine kinases. *Cell* **141**, 1117–1134.

[18] Vlahovic G and Crawford J (2003). Activation of tyrosine kinases in cancer. *Oncologist* **8**, 531–538.

[19] Natoli C, Perrucci B, Perrotti F, Falchi L, and Iacobelli S (2010). Tyrosine kinase inhibitors. *Curr Cancer Drug Targets* **10**, 462–483.

[20] Jonkers J and Berns A (2004). Oncogene addiction: sometimes a temporary slavery. *Cancer Cell* **6**, 535–538.

[21] Weinstein IB and Joe AK (2006). Mechanisms of disease: oncogene addiction—a rationale for molecular targeting in cancer therapy. *Nat Clin Pract Oncol* **3**, 448–457.

[22] Weinstein IB (2002). Cancer. Addiction to oncogenes—the Achilles heal of cancer. *Science* **297**, 63–64.

[23] Ren R (2005). Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. *Nat Rev Cancer* **5**, 172–183.

[24] Hudziak RM, Lewis GD, Winget M, Fendly BM, Shepard HM, and Ullrich A (1989). p185HER2 monoclonal antibody has antiproliferative effects *in vitro* and sensitizes human breast tumor cells to tumor necrosis factor. *Mol Cell Biol* **9**, 1165–1172.

[25] Shawver LK, Slamon D, and Ullrich A (2002). Smart drugs: tyrosine kinase inhibitors in cancer therapy. *Cancer Cell* **1**, 117–123.

[26] Schneider-Merck T and Trepel M (2010). Lapatinib. *Recent Results Cancer Res* **184**, 45–59.

[27] Sharma SV and Settleman J (2007). Oncogene addiction: setting the stage for molecularly targeted cancer therapy. *Genes Dev* **21**, 3214–3231.

[28] Engelman JA and Janne PA (2008). Mechanisms of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non–small cell lung cancer. *Clin Cancer Res* **14**, 2895–2899.

[29] Xu AM and Huang PH (2010). Receptor tyrosine kinase coactivation networks in cancer. *Cancer Res* **70**, 3857–3860.

[30] Stewart DJ, Whitney SN, and Kurzrock R (2010). Equipoise lost: ethics, costs, and the regulation of cancer clinical research. *J Clin Oncol* **28**, 2925–2935.

[31] Selvaggi G and Scagliotti GV (2009). Histologic subtype in NSCLC: does it matter? *Oncology (Williston Park)* **23**, 1133–1140.

[32] Pao W, Wang TY, Riely GJ, Miller VA, Pan Q, Ladanyi M, Zakowski MF, Heelan RT, Kris MG, and Varmus HE (2005). *KRAS* mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. *PLoS Med* **2**, e17.

[33] Tam IY, Chung LP, Suen WS, Wang E, Wong MC, Ho KK, Lam WK, Chiu SW, Girard L, Minna JD, et al. (2006). Distinct epidermal growth factor receptor and

KRAS mutation patterns in non–small cell lung cancer patients with different tobacco exposure and clinicopathologic features. *Clin Cancer Res* **12**, 1647–1653.

[34] Solomon B, Varella-Garcia M, and Camidge DR (2009). *ALK* gene rearrangements: a new therapeutic target in a molecularly defined subset of non–small cell lung cancer. *J Thorac Oncol* **4**, 1450–1454.

[35] Mao C, Qiu LX, Liao RY, Du FB, Ding H, Yang WC, Li J, and Chen Q (2009). *KRAS* mutations and resistance to EGFR-TKIs treatment in patients with non–small cell lung cancer: a meta-analysis of 22 studies. *Lung Cancer* **69**, 272–278.

[36] Young A, Lyons J, Miller AL, Phan VT, Alarcon IR, and McCormick F (2009). Ras signaling and therapies. *Adv Cancer Res* **102**, 1–17.

[37] Legrier ME, Yang CP, Yan HG, Lopez-Barcons L, Keller SM, Perez-Soler R, Horwitz SB, and McDaid HM (2007). Targeting protein translation in human non small cell lung cancer via combined MEK and mammalian target of rapamycin suppression. *Cancer Res* **67**, 11300–11308.

[38] Faivre S, Djelloul S, and Raymond E (2006). New paradigms in anticancer therapy: targeting multiple signaling pathways with kinase inhibitors. *Semin Oncol* **33**, 407–420.

[39] Walker K and Olson MF (2005). Targeting Ras and Rho GTPases as opportunities for cancer therapeutics. *Curr Opin Genet Dev* **15**, 62–68.

[40] American Cancer Society (2010). *Cancer Facts & Figures, 2010*. American Cancer Society, Atlanta, GA.

[41] Jackman DM, Miller VA, Cioffredi LA, Yeap BY, Janne PA, Riely GJ, Ruiz MG, Giaccone G, Sequist LV, and Johnson BE (2009). Impact of epidermal growth factor receptor and *KRAS* mutations on clinical outcomes in previously untreated non–small cell lung cancer patients: results of an online tumor registry of clinical trials. *Clin Cancer Res* **15**, 5267–5273.

[42] Tsujino K, Kawaguchi T, Kubo A, Aono N, Nakao K, Koh Y, Tachibana K, Isa S, Takada M, and Kurata T (2009). Response rate is associated with prolonged survival in patients with advanced non–small cell lung cancer treated with gefitinib or erlotinib. *J Thorac Oncol* **4**, 994–1001.

[43] Fuster LM and Sandler AB (2004). Select clinical trials of erlotinib (OSI-774) in non–small-cell lung cancer with emphasis on phase III outcomes. *Clin Lung Cancer* **6**(suppl 1), S24–S29.

[44] Milano G, Spano JP, and Leyland-Jones B (2008). EGFR-targeting drugs in combination with cytotoxic agents: from bench to bedside, a contrasted reality. *Br J Cancer* **99**, 1–5.

[45] Rosell R, Taron M, Reguart N, Isla D, and Moran T (2006). Epidermal growth factor receptor activation: how exon 19 and 21 mutations changed our understanding of the pathway. *Clin Cancer Res* **12**, 7222–7231.

[46] Dahabreh IJ, Linardou H, Siannis F, Kosmidis P, Bafaloukos D, and Murray S (2010). Somatic EGFR mutation and gene copy gain as predictive biomarkers for response to tyrosine kinase inhibitors in non–small cell lung cancer. *Clin Cancer Res* **16**, 291–303.

[47] Bell DW, Lynch TJ, Haserlat SM, Harris PL, Okimoto RA, Brannigan BW, Sgroi DC, Muir B, Riemenschneider MJ, Iacona RB, et al. (2005). Epidermal growth factor receptor mutations and gene amplification in non–small-cell lung cancer: molecular analysis of the IDEAL/INTACT gefitinib trials. *J Clin Oncol* **23**, 8081–8092.

[48] Wheeler DL, Dunn EF, and Harari PM (2010). Understanding resistance to EGFR inhibitors—impact on future treatment strategies. *Nat Rev Clin Oncol* **7**, 493–507.

[49] Jackman D, Pao W, Riely GJ, Engelman JA, Kris MG, Janne PA, Lynch T, Johnson BE, and Miller VA (2010). Clinical definition of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non–small-cell lung cancer. *J Clin Oncol* **28**, 357–360.

[50] La Rosee P and Hochhaus A (2010). Molecular pathogenesis of tyrosine kinase resistance in chronic myeloid leukemia. *Curr Opin Hematol* **17**, 91–96.

[51] Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, and Sawyers CL (2001). Clinical resistance to STI-571 cancer therapy caused by *BCR-ABL* gene mutation or amplification. *Science* **293**, 876–880.

[52] Kobayashi S, Boggon TJ, Dayaram T, Janne PA, Kocher O, Meyerson M, Johnson BE, Eck MJ, Tenen DG, and Halmos B (2005). EGFR mutation and resistance of non–small-cell lung cancer to gefitinib. *N Engl J Med* **352**, 786–792.

[53] Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, Kris MG, and Varmus H (2005). Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* **2**, e73.

[54] Bean J, Riely GJ, Balak M, Marks JL, Ladanyi M, Miller VA, and Pao W (2008). Acquired resistance to epidermal growth factor receptor kinase inhibitors associated with a novel T854A mutation in a patient with EGFR-mutant lung adenocarcinoma. *Clin Cancer Res* **14**, 7519–7525.

[55] Stamos J, Sliwkowski MX, and Eigenbrot C (2002). Structure of the epidermal growth factor receptor kinase domain alone and in complex with a 4-anilinoquinazoline inhibitor. *J Biol Chem* **277**, 46265–46272.

[56] Kwak EL, Sordella R, Bell DW, Godin-Heymann N, Okimoto RA, Brannigan BW, Harris PL, Driscoll DR, Fidias P, Lynch TJ, et al. (2005). Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. *Proc Natl Acad Sci USA* **102**, 7665–7670.

[57] Sos ML, Rode HB, Heynck S, Peifer M, Fischer F, Kluter S, Pawar VG, Reuter C, Heuckmann JM, Weiss J, et al. (2010). Chemogenomic profiling provides insights into the limited activity of irreversible EGFR inhibitors in tumor cells expressing the T790M EGFR resistance mutation. *Cancer Res* **70**, 868–874.

[58] Zhou W, Ercan D, Chen L, Yun CH, Li D, Capelletti M, Cortot AB, Chirieac L, Iacob RE, Padera R, et al. (2009). Novel mutant-selective EGFR kinase inhibitors against EGFR T790M. *Nature* **462**, 1070–1074.

[59] Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, Lindeman N, Gale CM, Zhao X, Christensen J, et al. (2007). MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* **316**, 1039–1043.

[60] Turke AB, Zejnullahu K, Wu YL, Song Y, Dias Santagata D, Lifshits E, Toschi L, Rogers A, Mok T, Sequist L, et al. (2010). Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. *Cancer Cell* **17**, 77–88.

[61] Guix M, Faber AC, Wang SE, Olivares MG, Song Y, Qu S, Rinehart C, Seidel B, Yee D, Arteaga CL, et al. (2008). Acquired resistance to EGFR tyrosine kinase inhibitors in cancer cells is mediated by loss of IGF-binding proteins. *J Clin Invest* **118**, 2609–2619.

[62] Dziadziuszko R, Merrick DT, Witta SE, Mendoza AD, Szostakiewicz B, Szymanowska A, Rzyman W, Dziadziuszko K, Jassem J, Bunn PA Jr, et al. (2010). Insulin-like growth factor receptor 1 (IGF1R) gene copy number is associated with survival in operable non–small-cell lung cancer: a comparison between IGF1R fluorescent *in situ* hybridization, protein expression, and mRNA expression. *J Clin Oncol* **28**, 2174–2180.

[63] Kono SA, Marshall ME, Ware KE, and Heasley LE (2009). The fibroblast growth factor receptor signaling pathway as a mediator of intrinsic resistance to EGFR-specific tyrosine kinase inhibitors in non–small cell lung cancer. *Drug Resist Updat* **12**, 95–102.

[64] Thomson S, Petti F, Sujka-Kwok I, Epstein D, and Haley JD (2008). Kinase switching in mesenchymal-like non–small cell lung cancer lines contributes to EGFR inhibitor resistance through pathway redundancy. *Clin Exp Metastasis* **25**, 843–854.

[65] Raponi M, Winkler H, and Dracopoli NC (2008). *KRAS* mutations predict response to EGFR inhibitors. *Curr Opin Pharmacol* **8**, 413–418.

[66] Amler LC, Goddard AD, and Hillan KJ (2005). Predicting clinical benefit in non–small-cell lung cancer patients treated with epidermal growth factor tyrosine kinase inhibitors. *Cold Spring Harb Symp Quant Biol* **70**, 483–488.

[67] Sharma SV, Lee DY, Li B, Quinlan MP, Takahashi F, Maheswaran S, McDermott U, Azizian N, Zou L, Fischbach MA, et al. (2010). A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell* **141**, 69–80.

[68] Glinsky GV (2008). "Stemness" genomics law governs clinical behavior of human cancer: implications for decision making in disease management. *J Clin Oncol* **26**, 2846–2853.

[69] Visvader JE and Lindeman GJ (2008). Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nat Rev Cancer* **8**, 755–768.

[70] Dean M, Fojo T, and Bates S (2005). Tumour stem cells and drug resistance. *Nat Rev Cancer* **5**, 275–284.

[71] Elrick LJ, Jorgensen HG, Mountford JC, and Holyoake TL (2005). Punish the parent not the progeny. *Blood* **105**, 1862–1866.

[72] Roesch A, Fukunaga-Kalabis M, Schmidt EC, Zabierowski SE, Brafford PA, Vultur A, Basu D, Gimotty P, Vogt T, and Herlyn M (2010). A temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumor growth. *Cell* **141**, 583–594.

[73] Birchmeier C, Birchmeier W, Gherardi E, and Vande Woude GF (2003). Met, metastasis, motility and more. *Nat Rev Mol Cell Biol* **4**, 915–925.

[74] Stellrecht CM and Gandhi V (2009). MET receptor tyrosine kinase as a therapeutic anticancer target. *Cancer Lett* **280**, 1–14.

[75] Eder JP, Vande Woude GF, Boerner SA, and LoRusso PM (2009). Novel therapeutic inhibitors of the c-Met signaling pathway in cancer. *Clin Cancer Res* **15**, 2207–2214.

[76] McDermott U, Pusapati RV, Christensen JG, Gray NS, and Settleman J (2010). Acquired resistance of non–small cell lung cancer cells to MET kinase inhibition

is mediated by a switch to epidermal growth factor receptor dependency. *Cancer Res* **70**, 1625–1634.

[77] Chiarle R, Voena C, Ambrogio C, Piva R, and Inghirami G (2008). The anaplastic lymphoma kinase in the pathogenesis of cancer. *Nat Rev Cancer* **8**, 11–23.

[78] Mosse YP, Laudenslager M, Longo L, Cole KA, Wood A, Attiyeh EF, Laquaglia MJ, Sennett R, Lynch JE, Perri P, et al. (2008). Identification of ALK as a major familial neuroblastoma predisposition gene. *Nature* **455**, 930–935.

[79] Cheng M and Ott GR (2010). Anaplastic lymphoma kinase as a therapeutic target in anaplastic large cell lymphoma, non–small cell lung cancer and neuroblastoma. *Anticancer Agents Med Chem* **10**, 236–249.

[80] Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, Fujiwara S, Watanabe H, Kurashina K, Hatanaka H, et al. (2007). Identification of the transforming EML4-ALK fusion gene in non–small-cell lung cancer. *Nature* **448**, 561–566.

[81] McDermott U, Iafrate AJ, Gray NS, Shioda T, Classon M, Maheswaran S, Zhou W, Choi HG, Smith SL, Dowell L, et al. (2008). Genomic alterations of anaplastic lymphoma kinase may sensitize tumors to anaplastic lymphoma kinase inhibitors. *Cancer Res* **68**, 3389–3395.

[82] Shaw AT, Yeap BY, Mino-Kenudson M, Digumarthy SR, Costa DB, Heist RS, Solomon B, Stubbs H, Admane S, McDermott U, et al. (2009). Clinical features and outcome of patients with non–small-cell lung cancer who harbor EML4-ALK. *J Clin Oncol* **27**, 4247–4253.

[83] Sasaki T, Rodig SJ, Chirieac LR, and Janne PA (2010). The biology and treatment of EML4-ALK non–small cell lung cancer. *Eur J Cancer* **46**, 1773–1780.

[84] Yamazaki S, Skaptason J, Romero D, Lee JH, Zou HY, Christensen JG, Koup JR, Smith BJ, and Koudriakova T (2008). Pharmacokinetic-pharmacodynamic modeling of biomarker response and tumor growth inhibition to an orally available cMet kinase inhibitor in human tumor xenograft mouse models. *Drug Metab Dispos* **36**, 1267–1274.

[85] Koivunen JP, Mermel C, Zejnullahu K, Murphy C, Lifshits E, Holmes AJ, Choi HG, Kim J, Chiang D, Thomas R, et al. (2008). EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. *Clin Cancer Res* **14**, 4275–4283.

[86] Bang Y, Kwak EL, Shaw AT, Camidge DR, Iafrate AJ, Maki RG, Solomon BJ, Ou SI, Salgia R, and Clark JW (2010). Clinical activity of the oral ALK inhibitor PF-02341066 in ALK-positive with non–small cell lung cancer (NSCLC). In *ASCO Annual '10 Meeting; Chicago, IL.* Abstract No. 3.

[87] Pasquale EB (2010). Eph receptors and ephrins in cancer: bidirectional signalling and beyond. *Nat Rev Cancer* **10**, 165–180.

[88] Antoniu SA and Kolb MR (2010). Intedanib, a triple kinase inhibitor of VEGFR, FGFR and PDGFR for the treatment of cancer and idiopathic pulmonary fibrosis. *IDrugs* **13**, 332–345.

[89] Kim DW, Lu B, and Hallahan DE (2004). Receptor tyrosine kinase inhibitors as anti-angiogenic agents. *Curr Opin Investig Drugs* **5**, 597–604.

[90] Heath VL and Bicknell R (2009). Anticancer strategies involving the vasculature. *Nat Rev Clin Oncol* **6**, 395–404.

[91] Marek L, Ware KE, Fritzsche A, Hercule P, Helton WR, Smith JE, McDermott LA, Coldren CD, Nemenoff RA, Merrick DT, et al. (2009). Fibroblast growth factor (FGF) and FGF receptor–mediated autocrine signaling in non–small-cell lung cancer cells. *Mol Pharmacol* **75**, 196–207.

[92] Berger W, Setinek U, Mohr T, Kindas-Mugge I, Vetterlein M, Dekan G, Eckersberger F, Caldas C, and Micksche M (1999). Evidence for a role of FGF-2 and FGF receptors in the proliferation of non–small cell lung cancer cells. *Int J Cancer* **83**, 415–423.

[93] Angevin E, Lin C, Pande AU, Lopez JA, Gschwend J, Harzstark AL, Shi M, Anak O, and Escudier BJ (2010). A phase I/II study of dovitinib (TKI258), a FGFR and VEGFR inhibitor, in patients (pts) with advanced or metastatic renal cell cancer: phase I results. In *ASCO Annual '10 Meeting; Chicago, IL.* Abstract No. 3057.

[94] Lee CB and Socinski MA (2007). Vascular endothelial growth factor receptor tyrosine kinase inhibitors in non–small cell lung cancer: a review of recent clinical trials. *Rev Recent Clin Trials* **2**, 117–120.

[95] Ivy SP, Wick JY, and Kaufman BM (2009). An overview of small-molecule inhibitors of VEGFR signaling. *Nat Rev Clin Oncol* **6**, 569–579.

[96] Atkins M, Jones CA, and Kirkpatrick P (2006). Sunitinib maleate. *Nat Rev Drug Discov* **5**, 279–280.

[97] Wilhelm S, Carter C, Lynch M, Lowinger T, Dumas J, Smith RA, Schwartz B, Simantov R, and Kelley S (2006). Discovery and development of sorafenib: a multikinase inhibitor for treating cancer. *Nat Rev Drug Discov* **5**, 835–844.

[98] Lind JS, Dingemans AM, Groen HJ, Thunnissen FB, Bekers O, Heideman DA, Honeywell RJ, Giovannetti E, Peters GJ, Postmus PE, et al. (2010). A multicenter phase II study of erlotinib and sorafenib in chemotherapy-naive patients with advanced non–small cell lung cancer. *Clin Cancer Res* **16**, 3078–3087.

[99] Takezawa K, Okamoto I, Yonesaka K, Hatashita E, Yamada Y, Fukuoka M, and Nakagawa K (2009). Sorafenib inhibits non–small cell lung cancer cell growth by targeting *B-RAF* in *KRAS* wild-type cells and *C-RAF* in *KRAS* mutant cells. *Cancer Res* **69**, 6515–6521.

[100] Kim ES, Herbst R, Lee JJ, Blumenschein GR, Tsao A, Alden CM, Tang X, Liu S, Stewart DJ, Heymach JV, et al. (2010). The BATTLE trial (Biomarker-integrated Approaches of Targeted Therapy for Lung Cancer Elimination): personlazing therapy for lung cancer. In *2010 American Association for Cancer Research Annual Meeting; Washington, DC.* Abstract No. LB-1.

[101] Scagliotti G and Govindan R (2010). Targeting angiogenesis with multitargeted tyrosine kinase inhibitors in the treatment of non–small cell lung cancer. *Oncologist* **15**, 436–446.

[102] Bukowski RM, Yasothan U, and Kirkpatrick P (2010). Pazopanib. *Nat Rev Drug Discov* **9**, 17–18.

[103] Jain RK, Duda DG, Clark JW, and Loeffler JS (2006). Lessons from phase III clinical trials on anti-VEGF therapy for cancer. *Nat Clin Pract Oncol* **3**, 24–40.

[104] Heymach JV, Paz-Ares L, De Braud F, Sebastian M, Stewart DJ, Eberhardt WE, Ranade AA, Cohen G, Trigo JM, Sandler AB, et al. (2008). Randomized phase II study of vandetanib alone or with paclitaxel and carboplatin as first-line treatment for advanced non–small-cell lung cancer. *J Clin Oncol* **26**, 5407–5415.

[105] Natale RB, Bodkin D, Govindan R, Sleckman BG, Rizvi NA, Capo A, Germonpre P, Eberhardt WE, Stockman PK, Kennedy SJ, et al. (2009). Vandetanib *versus* gefitinib in patients with advanced non–small-cell lung cancer: results from a two-part, double-blind, randomized phase II study. *J Clin Oncol* **27**, 2523–2529.

[106] Eder JP, Shapiro GI, Appleman LJ, Zhu AX, Miles D, Keer H, Cancilla B, Chu F, Hitchcock-Bryan S, Sherman L, et al. (2010). A phase I study of foretinib, a multi-targeted inhibitor of c-Met and vascular endothelial growth factor receptor 2. *Clin Cancer Res* **16**, 3507–3516.

[107] Byers LA and Heymach JV (2007). Dual targeting of the vascular endothelial growth factor and epidermal growth factor receptor pathways: rationale and clinical applications for non–small-cell lung cancer. *Clin Lung Cancer* **8**(suppl 2), S79–S85.

[108] Vlahovic G, Rabbani ZN, Herndon JE II, Dewhirst MW, and Vujaskovic Z (2006). Treatment with imatinib in NSCLC is associated with decrease of phosphorylated PDGFR-β and VEGF expression, decrease in interstitial fluid pressure and improvement of oxygenation. *Br J Cancer* **95**, 1013–1019.

[109] Besse B and Soria JC (2008). A randomized, double-blind, placebo-controlled phase II-III multi-centre study to evaluate the effect of adjuvant pazopanib (GW786034) versus placebo on post-surgical disease-free survival in patients with stage I non small cell lung cancer and tumor size equal or inferior to 5 cm. First Received: October 17, 2008. Last Updated: April 28, 2010. Available at: http://www.clinicaltrials.gov/ct2/show/NCT00775307?term=adjuvant+lung+cancer+pazopanib&rank=1.

[110] Huang D, Ding Y, Zhou M, Rini BI, Petillo D, Qian CN, Kahnoski R, Futreal PA, Furge KA, and Teh BT (2010). Interleukin-8 mediates resistance to antiangiogenic agent sunitinib in renal cell carcinoma. *Cancer Res* **70**, 1063–1071.

[111] Koch AE, Polverini PJ, Kunkel SL, Harlow LA, DiPietro LA, Elner VM, Elner SG, and Strieter RM (1992). Interleukin-8 as a macrophage-derived mediator of angiogenesis. *Science* **258**, 1798–1801.

[112] Strieter RM, Belperio JA, Burdick MD, Sharma S, Dubinett SM, and Keane MP (2004). CXC chemokines: angiogenesis, immunoangiostasis, and metastases in lung cancer. *Ann N Y Acad Sci* **1028**, 351–360.

[113] Hanrahan EO, Lin HY, Kim ES, Yan S, Du DZ, McKee KS, Tran HT, Lee JJ, Ryan AJ, Langmuir P, et al. (2010). Distinct patterns of cytokine and angiogenic factor modulation and markers of benefit for vandetanib and/or chemotherapy in patients with non–small-cell lung cancer. *J Clin Oncol* **28**, 193–201.

[114] Thiery JP, Acloque H, Huang RY, and Nieto MA (2009). Epithelial-mesenchymal transitions in development and disease. *Cell* **139**, 871–890.

[115] Singh A and Settleman J (2010). EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. *Oncogene* **29**, 4741–4751.

[116] Hammers HJ, Verheul HM, Salumbides B, Sharma R, Rudek M, Jaspers J, Shah P, Ellis L, Shen L, Paesante S, et al. (2010). Reversible epithelial to mesenchymal transition and acquired resistance to sunitinib in patients with renal cell carcinoma: evidence from a xenograft study. *Mol Cancer Ther* **9**, 1525–1535.

[117] Barr S, Thomson S, Buck E, Russo S, Petti F, Sujka-Kwok I, Eyzaguirre A, Rosenfeld-Franklin M, Gibson NW, Miglarese M, et al. (2008). Bypassing cellular EGF receptor dependence through epithelial-to-mesenchymal-like transitions. *Clin Exp Metastasis* **25**, 685–693.

[118] Witta S, Konduri K, and Raju J (2008). A randomized, placebo-controlled, double-blind, multicenter phase 2 study with a lead in phase of erlotinib with

or without SNDX-275 in patients with non–small cell lung carcinoma after failure in up to two prior chemotherapeutic regimens for advanced disease. First Received: January 25, 2008. Last Updated: February 2, 2010. Available at: http://clinicaltrials.gov/ct2/show/NCT00602030?term=MS-275&rank=2.

[119] Spector DH, Varmus HE, and Bishop JM (1978). Nucleotide sequences related to the transforming gene of avian sarcoma virus are present in DNA of uninfected vertebrates. *Proc Natl Acad Sci USA* **75**, 4102–4106.

[120] Oppermann H, Levinson AD, Varmus HE, Levintow L, and Bishop JM (1979). Uninfected vertebrate cells contain a protein that is closely related to the product of the avian sarcoma virus transforming gene (*src*). *Proc Natl Acad Sci USA* **76**, 1804–1808.

[121] Kim LC, Song L, and Haura EB (2009). Src kinases as therapeutic targets for cancer. *Nat Rev Clin Oncol* **6**, 587–595.

[122] Elsberger B, Stewart B, Tatarov O, and Edwards J (2010). Is Src a viable target for treating solid tumours? *Curr Cancer Drug Targets* **10**, 683–694.

[123] Zhang J, Kalyankrishna S, Wislez M, Thilaganathan N, Saigal B, Wei W, Ma L, Wistuba II, Johnson FM, and Kurie JM (2007). SRC-family kinases are activated in non–small cell lung cancer and promote the survival of epidermal growth factor receptor–dependent cell lines. *Am J Pathol* **170**, 366–376.

[124] Song L, Morris M, Bagui T, Lee FY, Jove R, and Haura EB (2006). Dasatinib (BMS-354825) selectively induces apoptosis in lung cancer cells dependent on epidermal growth factor receptor signaling for survival. *Cancer Res* **66**, 5542–5548.

[125] Schenone S, Brullo C, Musumeci F, and Botta M (2010). Novel dual Src/Abl inhibitors for hematologic and solid malignancies. *Expert Opin Investig Drugs* **19**, 931–945.

[126] Johnson FM, Saigal B, Talpaz M, and Donato NJ (2005). Dasatinib (BMS-354825) tyrosine kinase inhibitor suppresses invasion and induces cell cycle arrest and apoptosis of head and neck squamous cell carcinoma and non–small cell lung cancer cells. *Clin Cancer Res* **11**, 6924–6932.

[127] Haura EB, Tanvetyanon T, Chiappori A, Williams C, Simon G, Antonia S, Gray J, Litschauer S, Tetteh L, Neuger A, et al. (2010). Phase I/II study of the Src inhibitor dasatinib in combination with erlotinib in advanced non–small-cell lung cancer. *J Clin Oncol* **28**, 1387–1394.

[128] Yoshida T, Okamoto I, Okamoto W, Hatashita E, Yamada Y, Kuwata K, Nishio K, Fukuoka M, Janne PA, and Nakagawa K (2010). Effects of Src inhibitors on cell growth and epidermal growth factor receptor and MET signaling in gefitinib-resistant non–small cell lung cancer cells with acquired MET amplification. *Cancer Sci* **101**, 167–172.

[129] Mitra SK and Schlaepfer DD (2006). Integrin-regulated FAK-Src signaling in normal and cancer cells. *Curr Opin Cell Biol* **18**, 516–523.

[130] Schwock J, Dhani N, and Hedley DW (2010). Targeting focal adhesion kinase signaling in tumor growth and metastasis. *Expert Opin Ther Targets* **14**, 77–94.

[131] Meng XN, Jin Y, Yu Y, Bai J, Liu GY, Zhu J, Zhao YZ, Wang Z, Chen F, Lee KY, et al. (2009). Characterisation of fibronectin-mediated FAK signalling pathways in lung cancer cell migration and invasion. *Br J Cancer* **101**, 327–334.

[132] Roberts WG, Ung E, Whalen P, Cooper B, Hulford C, Autry C, Richter D, Emerson E, Lin J, Kath J, et al. (2008). Antitumor activity and pharmacology of a selective focal adhesion kinase inhibitor, PF-562,271. *Cancer Res* **68**, 1935–1944.

[133] Wendt MK and Schiemann WP (2009). Therapeutic targeting of the focal adhesion complex prevents oncogenic TGF-β signaling and metastasis. *Breast Cancer Res* **11**, R68.

[134] Yu H, Pardoll D, and Jove R (2009). STATs in cancer inflammation and immunity: a leading role for STAT3. *Nat Rev Cancer* **9**, 798–809.

[135] Levine RL, Pardanani A, Tefferi A, and Gilliland DG (2007). Role of JAK2 in the pathogenesis and therapy of myeloproliferative disorders. *Nat Rev Cancer* **7**, 673–683.

[136] Cortas T, Eisenberg R, Fu P, Kern J, Patrick L, and Dowlati A (2007). Activation state EGFR and STAT-3 as prognostic markers in resected non–small cell lung cancer. *Lung Cancer* **55**, 349–355.

[137] Wu HY, Tseng VS, Chen LC, Chang HY, Chuang IC, Tsay YG, and Liao PC (2010). Identification of tyrosine-phosphorylated proteins associated with lung cancer metastasis using label-free quantitative analyses. *J Proteome Res* **9**, 4102–4112.

[138] He B, You L, Uematsu K, Zang K, Xu Z, Lee AY, Costello JF, McCormick F, and Jablons DM (2003). SOCS-3 is frequently silenced by hypermethylation and suppresses cell growth in human lung cancer. *Proc Natl Acad Sci USA* **100**, 14133–14138.

[139] Baltayiannis G, Baltayiannis N, and Tsianos EV (2008). Suppressors of cytokine signaling as tumor repressors. Silencing of SOCS3 facilitates tumor formation and growth in lung and liver. *J BUON* **13**, 263–265.

[140] Veeriah S, Brennan C, Meng S, Singh B, Fagin JA, Solit DB, Paty PB, Rohle D, Vivanco I, Chmielecki J, et al. (2009). The tyrosine phosphatase PTPRD is a tumor suppressor that is frequently inactivated and mutated in glioblastoma and other human cancers. *Proc Natl Acad Sci USA* **106**, 9435–9440.

[141] Lasho TL, Tefferi A, Hood JD, Verstovsek S, Gilliland DG, and Pardanani A (2008). TG101348, a JAK2-selective antagonist, inhibits primary hematopoietic cells derived from myeloproliferative disorder patients with JAK2V617F, MPLW515K or JAK2 exon 12 mutations as well as mutation negative patients. *Leukemia* **22**, 1790–1792.

[142] Pardanani A, Hood J, Lasho T, Levine RL, Martin MB, Noronha G, Finke C, Mak CC, Mesa R, Zhu H, et al. (2007). TG101209, a small molecule JAK2-selective kinase inhibitor potently inhibits myeloproliferative disorder–associated JAK2V617F and MPLW515L/K mutations. *Leukemia* **21**, 1658–1668.

[143] Verstovsek S (2009). Therapeutic potential of JAK2 inhibitors. *Hematology Am Soc Hematol Educ Program* **1**, 636–642.

[144] Wernig G, Kharas MG, Okabe R, Moore SA, Leeman DS, Cullen DE, Gozo M, McDowell EP, Levine RL, Doukas J, et al. (2008). Efficacy of TG101348, a selective JAK2 inhibitor, in treatment of a murine model of JAK2V617F-induced polycythemia vera. *Cancer Cell* **13**, 311–320.

[145] Janku F, Stewart DJ, and Kurzrock R (2010). Targeted therapy in non–small-cell lung cancer—is it becoming a reality? *Nat Rev Clin Oncol* **7**, 401–414.

[146] Janne PA (2008). Challenges of detecting EGFR T790M in gefitinib/erlotinib–resistant tumours. *Lung Cancer* **60**(suppl 2), S3–S9.

[147] Menendez-Arias L (2010). Molecular basis of human immunodeficiency virus drug resistance: an update. *Antiviral Res* **85**, 210–231.

[148] Ren J and Stammers DK (2008). Structural basis for drug resistance mechanisms for non-nucleoside inhibitors of HIV reverse transcriptase. *Virus Res* **134**, 157–170.

[149] Naumov GN, Nilsson MB, Cascone T, Briggs A, Straume O, Akslen LA, Lifshits E, Byers LA, Xu L, Wu HK, et al. (2009). Combined vascular endothelial growth factor receptor and epidermal growth factor receptor (EGFR) blockade inhibits tumor growth in xenograft models of EGFR inhibitor resistance. *Clin Cancer Res* **15**, 3484–3494.

[150] Knight ZA and Shokat KM (2005). Features of selective kinase inhibitors. *Chem Biol* **12**, 621–637.

[151] Knight ZA, Lin H, and Shokat KM (2010). Targeting the cancer kinome through polypharmacology. *Nat Rev Cancer* **10**, 130–137.

[152] Yagui-Beltran A, He B, Raz D, Kim J, and Jablons DM (2006). Novel therapies targeting signaling pathways in lung cancer. *Thorac Surg Clin* **16**, 379–396, vi.

[153] Zhang Q, Feng W, Zhou H, and Yan B (2009). Advances in preclinical small molecules for the treatment of NSCLC. *Expert Opin Ther Pat* **19**, 731–751.

[154] Herbst RS, Prager D, Hermann R, Fehrenbacher L, Johnson BE, Sandler A, Kris MG, Tran HT, Klein P, Li X, et al. (2005). TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non–small-cell lung cancer. *J Clin Oncol* **23**, 5892–5899.

[155] Herbst RS, Sun Y, Eberhardt WE, Germonpre P, Saijo N, Zhou C, Wang J, Li L, Kabbinavar F, Ichinose Y, et al. (2010). Vandetanib plus docetaxel *versus* docetaxel as second-line treatment for patients with advanced non–small-cell lung cancer (ZODIAC): a double-blind, randomised, phase 3 trial. *Lancet Oncol* **11**, 619–626.

[156] Ready N, Janne PA, Bogart J, Dipetrillo T, Garst J, Graziano S, Gu L, Wang X, Green MR, and Vokes EE (2010). Chemoradiotherapy and gefitinib in stage III non–small cell lung cancer with epidermal growth factor receptor and *KRAS* mutation analysis: cancer and leukemia group B (CALEB) 30106, a CALGB-stratified phase II trial. *J Thorac Oncol* **5**, 1382–1390.

[157] Scagliotti G, Novello S, von Pawel J, Reck M, Pereira JR, Thomas M, Abrao Miziara JE, Balint B, De Marinis F, Keller A, et al. (2010). Phase III study of carboplatin and paclitaxel alone or with sorafenib in advanced non–small-cell lung cancer. *J Clin Oncol* **28**, 1835–1842.

[158] Kelly K, Chansky K, Gaspar LE, Albain KS, Jett J, Ung YC, Lau DH, Crowley JJ, and Gandara DR (2008). Phase III trial of maintenance gefitinib or placebo after concurrent chemoradiotherapy and docetaxel consolidation in inoperable stage III non–small-cell lung cancer: SWOG S0023. *J Clin Oncol* **26**, 2450–2456.

[159] Okamoto I, Takahashi T, Okamoto H, Nakagawa K, Watanabe K, Nakamatsu K, Nishimura Y, Fukuoka M, and Yamamoto N (2010). Single-agent gefitinib with concurrent radiotherapy for locally advanced non–small cell lung cancer harboring mutations of the epidermal growth factor receptor. *Lung Cancer*. Epub ahead of print.

[160] Baselga J (2004). Combining the anti-EGFR agent gefitinib with chemotherapy in non–small-cell lung cancer: how do we go from INTACT to impact? *J Clin Oncol* **22**, 759–761.

[161] John T, Liu G, and Tsao MS (2009). Overview of molecular testing in non–small-cell lung cancer: mutational analysis, gene copy number, protein expression and other biomarkers of EGFR for the prediction of response to tyrosine kinase inhibitors. *Oncogene* **28**(suppl 1), S14–S23.