

Mechanisms of resistance to EGFR targeted therapies

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EGFR is a validated therapeutic target in many human cancers. EGFR targeted therapies are in widespread clinical use in patients with non-small cell lung cancer and other tumor types. Despite the clinical success of EGFR targeted therapy, resistance to treatment is a significant barrier to the optimized use of EGFR inhibitors to cure patients with lung and other cancers. Here, we review established and emerging mechanisms of resistance to EGFR targeted therapy and highlight strategies that could overcome treatment resistance and therefore enhance clinical outcomes.

Introduction

Lung cancer accounted for approximately one-third of all cancer deaths in the United States in 2011, making it the foremost cause of cancer mortality in the United States and worldwide. Unfortunately, less than 15% of patients with lung cancer survive five years.^{1–3} Thus, conventional approaches to the diagnosis and management of lung cancer patients have achieved limited success. Until recently, lung cancer was primarily diagnostically classified on a histological basis as either non-small cell (NSCLC, ~85% of cases) or small cell (SCLC, ~15% of cases). NSCLC encompasses adenocarcinoma, squamous cell carcinoma and large-cell carcinoma.⁴ Patients with either advanced stage NSCLC or SCLC traditionally have been treated with systemic cytotoxic chemotherapy. This approach has been largely unsuccessful and resulted in heterogeneous, incomplete and transient clinical responses (with few notable exceptions) and little progress in reducing lung cancer-related mortality. Over the past decade, the identification of specific genetic alterations (oncogene drivers) in lung (and other) cancers has paved the way for a new classification of lung cancer wherein distinct clinical subsets are defined by molecular criteria. These discoveries have simultaneously led to the development of more effective therapies that capitalize on the key tumor-specific genetic changes upon which tumor growth is dependent (oncogene dependence).⁵ Thus, clinical practice has moved into an era in which individualized, precision approaches to the diagnosis and treatment of many lung cancer patients are

leading to improved clinical outcomes, although resistance to these personalized approaches also develops. In this review, we will focus on mechanisms of drug resistance in EGFR-driven lung cancers and briefly address the role of EGFR signaling and EGFR-directed therapies in other cancers.

Somatic activating mutations in the epidermal growth factor receptor (EGFR) gene occur in approximately 15–30% of NSCLCs.⁶ EGFR belongs to the ErbB/HER family of ligand-activated receptor tyrosine kinases (RTKs), whose members play important roles in the regulation of cellular processes including cell proliferation, survival and migration. Regulation of EGFR kinase activity can be disrupted by several mechanisms, including increased production of ligands (i.e., EGF, TGF α and Heregulin), upregulation of EGFR expression, impaired down-regulation of EGFR, cross-talk with other receptors (i.e., other ErbB family members, RTKs, cell adhesion molecules, cytokine receptors, ion channels and G-protein coupled receptors) and activating mutations.⁷ Since 2004, several activating lesions in *EGFR* have been identified in exons 18 to 21 and are localized around the ATP-binding site of the kinase domain.^{8,9} The most common mutations are an in-frame deletion within exon 19 that removes residues amino acids 746–750 (del746–750) and an exon 21 missense mutation replacing leucine 858 with arginine (L858R).¹⁰ The crystal structures of the L858R and G719S EGFR mutants provide evidence that these mutations increase kinase activity by 50- and 10-fold, respectively, through abrogation of autoinhibitory interactions proximal to the ATP-binding cleft of the EGFR protein.¹¹ Additionally, the EGFR juxtamembrane domain functions differently from other receptor tyrosine kinases in that it activates, rather than inhibits, the kinase domain through stabilization of an asymmetric kinase dimer.^{12,13} Indeed, a lung cancer mutation (V665M) identified within the activating region of the EGFR juxtamembrane domain has been shown to activate EGFR constitutively through increased receptor dimerization.¹⁴ Thus far, the crystal structure of EGFR (del746–750) has not been determined.

Targeting EGFR Mutations

EGFR-mutant NSCLC cells are a characteristic example of oncogene dependence because these cells are addicted to the aberrant signaling of the mutant kinase for their growth and survival. This characteristic provides a tumor-specific vulnerability and

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therapeutic opportunity to target mutant EGFR specifically in the tumor cells while sparing normal cells. The ATP-competitive EGFR tyrosine kinase inhibitors (TKIs), erlotinib (and gefitinib in Europe and Asia), are currently approved for first-line use in lung cancer patients whose tumors harbor an EGFR activating mutation. These small molecule inhibitors of EGFR achieve clinical efficacy by suppressing the activity of the mutant kinase and downregulating downstream signaling.¹⁵⁻¹⁷ Importantly, activating EGFR mutations show significantly greater sensitivity to treatment with EGFR TKIs than wild type EGFR. For example, the EGFR L858R mutant is approximately 100 times more sensitive to EGFR TKI inhibition than wild type EGFR.¹⁸⁻²⁰ This exquisite response is attributed to the preferential binding of TKI-sensitive mutants to erlotinib or gefitinib vs. ATP.⁴ Since the presence of activating EGFR mutations is associated with enhanced clinical responsiveness to EGFR TKI therapy,^{15-17,21} initial molecular analysis of NSCLC tumors for activating EGFR mutations is now standard of care for patients with advanced NSCLC.²²

Another approach to targeted EGFR inhibition is the use of monoclonal antibodies that bind to the extracellular domain of EGFR. Antibody binding prevents ligand-induced receptor dimerization and activation or induces receptor endocytosis and/or degradation. Cetuximab is a monoclonal human-murine chimeric antibody approved for the treatment of colorectal cancer and head and neck squamous cell carcinoma (HNSCC), but its effectiveness in EGFR-mutant NSCLC has been limited to date.²³⁻²⁵ Cetuximab interferes with EGFR ligand-binding and activation of downstream signaling cascades and may also activate the complement pathway to induce antibody-dependent cytotoxicity. Panitumumab is a similar, but fully humanized, monoclonal antibody directed against EGFR that has been approved for the treatment of colorectal cancers that harbor wild type KRAS. Panitumumab also acts by blocking the binding of EGFR ligands to EGFR, but in contrast with cetuximab does not lead to activation of the complement pathway.^{26,27} Unlike the positive correlation between *EGFR* mutations and response to EGFR TKIs, the role of *EGFR* mutations as predictive markers for sensitivity to anti-EGFR antibodies has not been demonstrated. While antibodies are capable of blocking ligand-induced receptor activation and promoting antibody-mediated receptor downregulation, mutations that confer ligand independence, such as those found in EGFR in NSCLCs, may abrogate the efficacy of anti-EGFR antibody therapy.²⁸

Mechanisms of Resistance to EGFR-Targeted Therapies

Despite the effectiveness of EGFR TKIs in NSCLC patients with EGFR activating mutations, the success of EGFR-targeted therapy is limited by the challenge of drug resistance (Fig. 1). Some patients exhibit de novo resistance to EGFR inhibition and are refractory to therapy that is expected to be effective based on the biology or genetics of the cancer. Furthermore, patients who initially respond to therapy invariably develop acquired resistance to treatment.

Secondary genetic alterations in EGFR. NSCLCs harboring mutant EGFR can exhibit de novo resistance to EGFR TKI therapy due to the nature of the primary activating lesion or the presence of a secondary drug-resistant EGFR mutation (Table 1). NSCLCs that are initially sensitive to EGFR TKI treatment have EGFR mutations primarily in exons 18, 19 and 21, whereas NSCLCs with infrequent exon 20 insertions or duplications are less responsive to EGFR TKI treatment both in vitro¹⁸ and in patients.²⁹ Likewise, the T790M substitution in exon 20 is occasionally detected in a minor cell population in tumors displaying de novo drug resistance³⁰ and can also be found at low frequency in circulating tumor cells captured from EGFR TKI naïve patients with metastatic disease.³¹ Interestingly, the presence of a germline EGFR T790M mutation associated with familial NSCLC may enhance the effect of other EGFR activating mutations that occur in cis,³² as well as increase the risk of developing NSCLC.³³⁻³⁵ In addition to T790M, other secondary mutations in EGFR may cause primary resistance. For example, the presence of D761Y in cis with the drug-sensitive L858R mutation reduces EGFR sensitivity to EGFR TKIs.³⁶ Altogether, these findings suggest that early detection of small fractions of second-site mutant alleles could be used for predicting response to NSCLCs that harbor drug-sensitive *EGFR* mutations. It is also becoming apparent that the intrinsic nature of different EGFR mutants elicits varying responses to EGFR TKI therapy,¹¹ and that successful treatment of lung cancers with drug-resistant mutations will require alternative strategies for EGFR kinase inhibition.

The second-site T790M mutation accounts for acquired EGFR TKI resistance in over 50% of NSCLC patients with EGFR-mutant tumors who initially respond to erlotinib or gefitinib. This amino acid change of a highly conserved “gatekeeper” residue near the kinase active site is frequently detected in TKI-resistant tumors. The T790M mutation in EGFR is analogous to the T315I substitution found in BCR-ABL in chronic myelogenous leukemia patients who have acquired resistance to the ABL kinase inhibitor imatinib.³⁷ While T315I causes a steric clash that blocks imatinib from binding in the ATP pocket of ABL kinase,³⁸ EGFR T790M most likely confers drug resistance primarily by enhancing the ATP affinity of EGFR L858R and thus reducing the efficacy of an ATP-competitive kinase inhibitor.³⁹ Although EGFR T790M mutations are rarely detected in EGFR TKI naïve patients, continuous treatment with erlotinib or gefitinib may promote positive selection and proliferation of even a small fraction of T790M-harboring cells present at the start of EGFR TKI therapy.³⁰

Other secondary mutations in EGFR have been reported in cases of acquired resistance, but at much lower frequency than EGFR T790M. These EGFR mutations include the exon 19 mutations L747S⁴⁰ and D761Y,³⁶ and the exon 21 mutation T854A.⁴¹ Crystallographic studies indicate that L747S could affect the binding of ATP and small molecules or shift the conformational equilibrium of EGFR toward the active state, while D761Y is situated in the putative α -C helix and predicted to affect the kinase activation loop.⁴² The T854A substitution in the ATP-binding site may cause loss of contact and binding affinity

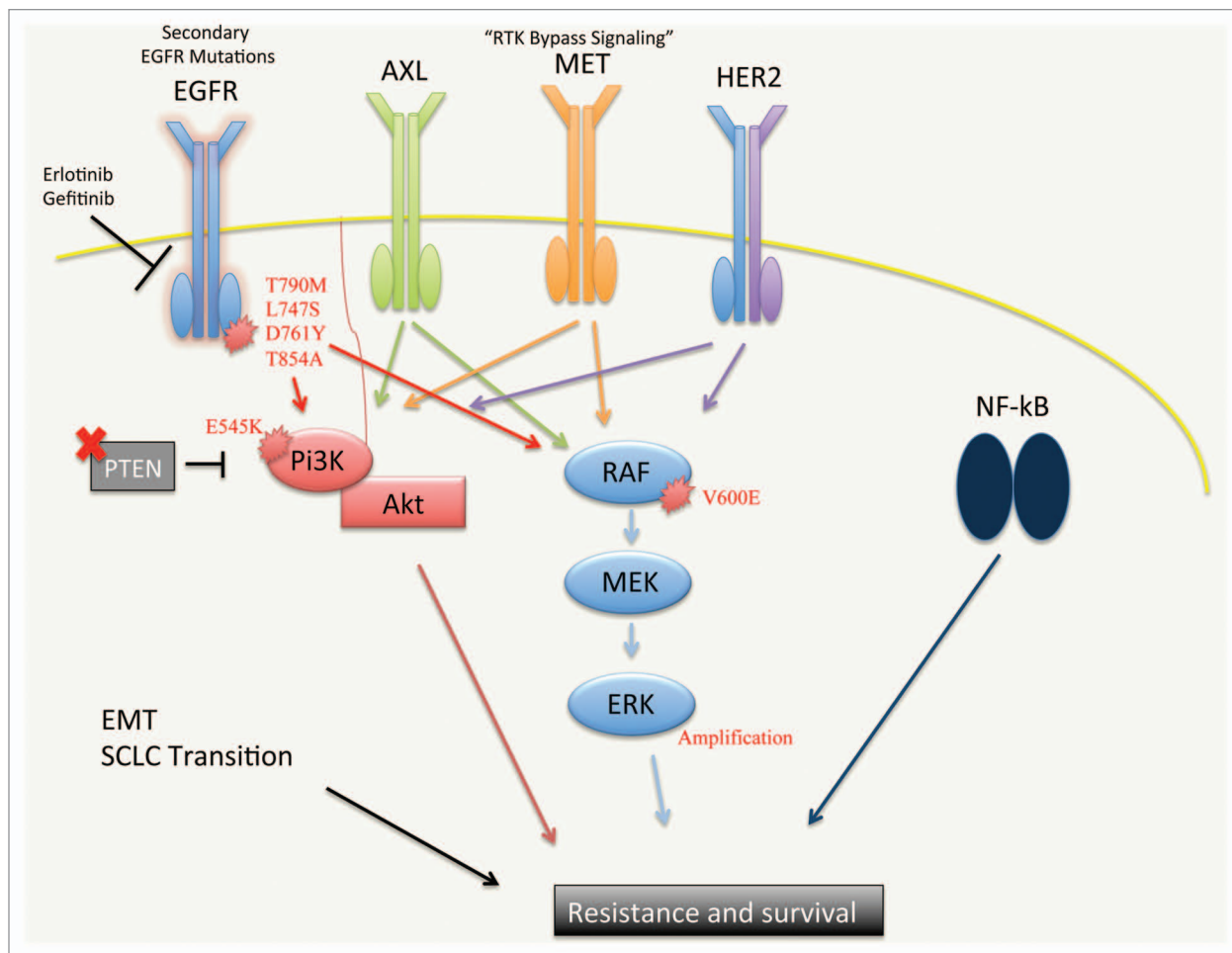


Figure 1. A schematic of the molecular pathways implicated in resistance to EGFR TKIs. Mutations in the EGFR kinase domain (predominantly T790M, which accounts for 50–60% of EGFR inhibitor resistance) abrogate the ability of first generation EGFR TKIs to inhibit mutant EGFR. Second generation EGFR TKIs BIBW2992, PF299804 and WZ4002 are currently in clinical trials and show promise as inhibitors of EGFR T790M. EGFR TKI resistance can also occur via upregulation or activation of other RTKs, such as AXL (20–25%), MET (5%) and HER2, which can bypass the inhibition of oncogenic EGFR signaling and activate downstream effector pathways. RTK-independent activation of some of these downstream effectors can also occur, leading to resistance. PTEN loss and activating PI3K mutations (i.e., E545K) have been observed, leading to constitutive AKT activation. Activating BRAF V600E mutations and *MAPK1* amplification have been reported which lead to hyper-activation of MAPK signaling and resistance. NFκB pathway activation has been associated with resistance to EGFR TKI treatment. The epithelial-mesenchymal transition (EMT) and transition to small-cell neuroendocrine phenotype have each been associated with resistance to EGFR TKIs, though the mechanistic underpinnings of these observations are unclear.

to EGFR TKIs due to the replacement of the bulky threonine with the smaller alanine. Alternatively, the T854A mutation could induce a local conformational change in the kinase that abrogates EGFR TKI efficacy.

Attempts have been made to inhibit EGFR T790M using second-generation irreversible quinazoline-based EGFR TKIs, including afatinib (BIBW2992), dacomitinib (PF299804), canertinib (CI-1033) and neratinib (HKI-272).⁴³⁻⁴⁵ While clinical studies showed that afatinib did not increase survival in NSCLC patients who developed resistance to erlotinib or gefitinib,⁴⁵ dual targeting of EGFR with afatinib and the monoclonal antibody cetuximab has recently been reported to be effective at targeting T790M-driven tumors in mouse models of T790M-mediated resistance and in some patients.^{46,47} Unfortunately, preclinical studies indicate that EGFR T790M mutant tumor cells quickly acquire resistance to dacomitinib through amplification of the

T790M allele.⁴⁸ The limited clinical efficacy of these inhibitors may be attributed to the increased potency of these agents against both mutant and wild type EGFR. As the ATP affinity of EGFR T790M is similar to that of wild type EGFR, the inhibitor concentration needed to suppress EGFR T790M also inhibits the wild type EGFR protein, resulting in dose-limiting toxicity in the form of skin rash and diarrhea.⁴⁹ Consequently, targeting EGFR T790M with these irreversible inhibitors has had limited clinical efficacy thus far.

The toxicity ascribed to the concurrent inhibition of wild type and T790M-mutant EGFR proteins underscores the need to develop small molecule inhibitors of EGFR that selectively target tumor cells that express mutant EGFR while sparing cells that express wild type EGFR. In contrast to the quinazoline-based EGFR inhibitors, the pyrimidine-based EGFR TKI WZ4002 is approximately 100-fold more potent against EGFR T790M and

Table 1. EGFR alterations in cancer and mechanisms of resistance to EGFR TKIs

Cancer	Predominant EGFR alteration	EGFR inhibitors	Resistance mechanisms
Non-small cell lung cancer	EGFR activating mutations	Erlotinib*	Secondary EGFR mutations, PI3K/AKT, MAPK, MET, AXL, HER2, NFκB, BRCA1, BIM IGF1R, FGFR (preclinical)
		Gefitinib	
		Afatinib	
		Dacomitinib	
		Canertinib	
		Neratinib	
		CO-1686	
		WZ4002	
Cetuximab			
Glioma	EGFRvIII	Dacomitinib	PTEN loss
Colorectal cancer	EGFR overexpression	Cetuximab* Panitumumab*	KRAS mutations
Head and neck squamous cell carcinoma	EGFR overexpression	Cetuximab* Afatinib	Preclinical
Pancreatic cancer	EGFR and ligand upregulation	Erlotinib*	Preclinical

*FDA approved.

100-fold less effective against wild type EGFR.⁴⁹ This suggests that EGFR T790M can be sufficiently inhibited at physiological doses that will not target wild type EGFR and result in toxicity, making WZ4002 a promising therapeutic candidate.⁴

Activation of PI3K/AKT signaling. Cell death in EGFR-mutant cancer cells is associated with a decrease in phosphoinositide-3-kinase (PI3K)/AKT signaling upon EGFR inhibition. The ability to survive in the face of inhibition of oncogenic EGFR often involves re-activation of the PI3K/AKT pathway through a variety of mechanisms in tumor cells. Loss of PTEN expression (found in approximately 4% of EGFR-mutant cases) is associated with resistance to EGFR TKI treatment through activation of the PI3K/AKT signaling pathway.^{51,52} In patients who develop EGFR TKI resistance, approximately 5% have been found to have a PIK3CA (p110α catalytic subunit of PI3K) mutation.⁵⁴ This is consistent with in vitro work showing that introduction of activated PIK3CA can confer resistance to NSCLC cell lines initially sensitive to EGFR TKI treatment.^{53,54} As we will discuss in detail below, many of the mechanisms that cause resistance to EGFR TKI treatment, including the EGFR T790M gatekeeper mutation and RTK bypass signaling, lead to activation of PI3K/AKT signaling.

Activation of MAPK signaling. Although many studies implicate PI3K/AKT as a critical pathway mediating EGFR-mutant NSCLC cell survival and drug resistance, the role of the MAPK signaling pathway in EGFR TKI resistance has been less clear until recently. However, *MAPK1* amplification was recently observed in EGFR-mutant lung cancer cells that developed resistance to the irreversible EGFR inhibitor WZ4002 and also in one patient specimen with acquired resistance to erlotinib.⁵⁰ In these drug resistant models, treatment with a MEK or ERK

inhibitor alone was not able to induce apoptosis, but required combination therapy with an EGFR inhibitor. Importantly, in these studies, combining PI3K or AKT inhibition with WZ4002 was also unable to induce apoptosis in EGFR-mutant NSCLC cells resistant to WZ4002 treatment, suggesting that therapeutic strategies must be personalized according to the specific molecular event through which resistance occurs. Additionally, there have been recent reports of patients whose resistant tumors harbor an activating BRAF V600E or G469A mutation.⁵⁵ Together, these findings suggest that resistance to EGFR TKI treatment can occur through reactivation of MAPK signaling.^{50,56}

“Bypass signaling” via receptor tyrosine kinases (RTKs). Upregulation of specific RTKs can promote lung tumorigenesis and drive therapeutic resistance in several cancers, including NSCLCs harboring mutant EGFR or the EML4-ALK fusion, CRC, HNSCC, breast cancer and others. Here, we review the roles of several of these RTKs, including MET, IGF1R, AXL and HER2 (ERbB2) in resistance to EGFR inhibitor treatment in NSCLC.

MET amplification. Approximately 5% of lung cancer specimens from patients who develop resistance to gefitinib or erlotinib harbor amplification of the MET RTK.⁵⁷ MET (also known as hepatocyte growth factor receptor, HGF-R) activation bypasses EGFR inhibition through sustained activation of the PI3K/AKT signaling pathway.^{53,58} Interestingly, MET amplification and the EGFR T790M resistance mutation can coexist in resistant tumor specimens.⁵³ EGFR-mutant lung cancer cells exposed to HGF, the ligand for MET, are resistant to EGFR TKI treatment, suggesting that MET activation is sufficient to confer resistance in otherwise drug-sensitive cells.⁵⁹ Interestingly, these studies have shown that MET can

also act through ErbB3-mediated activation of PI3K. Further reports have shown that MET-mediated resistance to EGFR TKI treatment can be overcome by inhibition of PI3K alone or in combination with erlotinib in vitro and in vivo.⁶⁰ It is unclear whether EGFR-mutant cells actively “acquire” MET amplification or whether a pre-existent, minor subpopulation of tumor cells with MET amplification are selected for during EGFR TKI treatment. The latter hypothesis may be supported by the clinical observation that MET amplification is seen at low frequency in EGFR-mutant lung cancers prior to treatment and was associated with resistance to EGFR TKI treatment in these patients.⁶¹

Presently, it is uncertain whether MET inhibition will be effective in overcoming acquired EGFR TKI resistance in the clinic; however, clinical trials are underway. Recently, the MET inhibitor ARQ197 showed a statistically significant increase in progression free survival in combination with erlotinib in a Phase III trial, but the trial was ultimately discontinued due to failure to satisfy the primary endpoint of overall survival benefit in this patient cohort.^{62,63} Additionally, a Phase I clinical trial combining erlotinib and the MET/VEGFR2/RET inhibitor XL184 has been completed.⁶⁴ In this trial, one NSCLC patient with acquired resistance to erlotinib associated with MET copy number gain was able to achieve a partial response to the combination.

IGF1R. Insulin-like growth factor receptor 1 (IGF1R) is a RTK that is known to engage in cross-talk with EGFR and activates both the PI3K/AKT and MAPK pathways.⁶⁵ Combined inhibition of IGF1R and EGFR induces apoptosis in a subset of EGFR-mutant lung cancer cell lines whereas inhibition of IGF1R or EGFR alone induces only cell cycle arrest.⁶⁶ Furthermore, loss of the inhibitory IGF-binding proteins (leading to increased IGF1R signaling) has been associated with acquired resistance to EGFR TKI treatment in squamous cell lung cancer models that overexpress wild type EGFR. Thus, IGF1R may contribute to resistance to EGFR TKI treatment in some NSCLCs. However, IGF1R has yet to be validated as a bona fide driver of resistance to EGFR TKI treatment in human clinical samples.

Activation of AXL kinase. AXL is a RTK previously implicated in resistance to imatinib in some KIT-driven gastrointestinal stromal tumor cells and in resistance to lapatinib treatment in some breast cancer cells.^{67,68} More recently, activation of AXL has been shown to drive resistance to EGFR TKI treatment in EGFR-mutant NSCLC as well. Preclinical studies using multiple in vitro and in vivo EGFR-mutant NSCLC models of acquired EGFR TKI resistance revealed that activation of AXL either through overexpression or upregulation of its ligand GAS6 conferred drug resistance.⁶⁹ AXL activation resulted in a restoration of MAPK, PI3K/AKT and NFκB signaling in the EGFR-mutant NSCLC models with acquired erlotinib resistance. Combined treatment with erlotinib and an AXL inhibitor restored sensitivity in the resistant models by inducing cell death, whereas single agent treatment was ineffective. Conversely, forced overexpression of AXL in an EGFR TKI-sensitive EGFR-mutant NSCLC cell line was sufficient to induce erlotinib resistance. Increased expression of AXL and

GAS6 was observed in approximately 25% of EGFR-mutant NSCLCs obtained from patients that developed acquired resistance to erlotinib or gefitinib.⁶⁹ The data showed that AXL upregulation is a clinically important mechanism of acquired resistance to EGFR inhibitor treatment. AXL is a novel rational therapeutic target that when inhibited may enhance response to EGFR TKI treatment in selected NSCLC patients. Current efforts are focused on the development of specific AXL inhibitors and the design of clinical trials testing AXL and EGFR inhibition in the clinic.

HER2 amplification. Studies examining the mechanisms of acquired resistance to the anti-EGFR monoclonal antibody cetuximab have led to the identification of upregulation of other ErbB family members as a potential mechanism of resistance to EGFR targeted therapies.⁷⁰ In these studies, initially sensitive NSCLC cell lines that developed resistance to cetuximab in vitro harbored amplification of *HER2*. *HER2* overexpression or knockdown conferred resistance or sensitivity, respectively, to cetuximab in some NSCLC models. Interestingly, inhibition or disruption of *HER2/HER3* hetero-dimerization via treatment with the monoclonal antibody pertuzumab was sufficient to restore sensitivity to cetuximab both in vitro and in xenograft models.⁷¹ Furthermore, using FISH analysis, Takezawa et al. revealed that *HER2* was amplified in approximately 12% of tumors with acquired resistance to EGFR TKI treatment, whereas *HER2* amplification was present in only 1% of EGFR TKI naïve tumors.⁷⁰ Of note, *HER2* amplification was not found to coexist with the EGFR T790M gatekeeper mutation in these clinical specimens.

Activation of NFκB signaling. The NFκB signaling pathway is implicated in a wide spectrum of biological functions, including immune and inflammatory responses and cancer.⁷² In a search to better define the molecular determinants of EGFR oncogene dependence and of response and resistance to EGFR targeted therapy in NSCLC, a novel genetic approach demonstrated that NFκB pathway activation can promote primary resistance to EGFR TKI treatment in EGFR-mutant NSCLCs.⁷³ This study applied a RNA interference based screening approach using a pooled shRNA screening library to identify genes that, when knocked down, restored sensitivity to an erlotinib-resistant EGFR-mutant NSCLC cell line not harboring the EGFR T790M resistance mutation. Unexpectedly, 18 of the 36 screening hits were previously implicated in NFκB signaling, including CD95/FAS. Furthermore, NFκB activation levels were shown to be a specific predictive biomarker of response to treatment with an EGFR TKI, but not chemotherapy, in EGFR-mutant NSCLC patients. High NFκB activity as indicated by low levels of IκB, a negative regulator of NFκB signaling, identified EGFR-mutant NSCLC patients that responded poorly to EGFR TKI treatment. These results indicate that the NFκB pathway plays an important role in modulating response to EGFR inhibition by enabling EGFR-mutant tumor cells to bypass dependence on oncogenic EGFR. Combined therapy with an EGFR TKI and an inhibitor of NFκB signaling can enhance responses in some EGFR-mutant NSCLC models and is currently being pursued in clinical trials.^{73,74}

The DNA Damage Response and Efficacy of EGFR TKI Treatment

Recent data suggest a role for the DNA damage response (DDR) in modulating sensitivity to EGFR inhibitor treatment. In breast cancer, it has been observed that low BRCA1 levels can be used as a biomarker for sensitivity to PARP inhibitor treatment.^{75,76} Additionally, erlotinib has been shown to suppress DNA repair in some breast cancer lines and cetuximab treatment can modulate the expression of DDR genes and sensitize to PARP inhibitor treatment in some HNSCC cell lines.⁷⁷ These data suggest that there could be a link between EGFR inhibition and the DDR in some EGFR-driven tumors.

In NSCLC, emerging evidence indicates that the DDR could be an important regulator of response to EGFR inhibitor treatment in some patients. Recent clinical evidence showed that EGFR-mutant NSCLC patients whose tumors harbored low BRCA1 levels achieve significantly longer progression free survival during erlotinib treatment.⁷⁸ A possible explanation for these clinical observations is that EGFR may activate elements of the DDR. EGFR inhibition may therefore decrease activation of the DDR, create a potential synthetically lethal relationship between EGFR inhibition and the DDR in tumor cells in which elements of the DDR, such as BRCA1, are suppressed through EGFR-independent mechanisms. Furthermore, a genetic screen identified several DDR genes that, when knocked down, can confer erlotinib sensitivity in EGFR-mutant cell lines that are otherwise resistant to the drug.⁷³ These observations provided rationale for the initiation of clinical trials assessing the efficacy of PARP inhibitor treatment in NSCLC patients with EGFR TKI-resistant tumors. Further investigation is required to explain mechanistically the clinical response observed in EGFR-mutant NSCLC patients whose tumors harbor low BRCA1 levels and to understand the molecular basis for the interplay between components of the DDR and EGFR oncogene dependence. The studies to date provide rationale to determine whether additional DDR components regulate EGFR oncogene dependence and EGFR inhibitor sensitivity.

Morphological Changes, Cellular Reprogramming and Other Factors Associated with EGFR TKI Resistance

Histological transition to small cell lung cancer (SCLC). Whether drug resistance mechanisms observed in NSCLCs occur due to the selection of minor resistant sub-clones that exist prior to therapy, focal genetic alterations induced during therapy or more global epigenetic reprogramming events that occur in response to therapy remains unclear in most cases. Interesting insight into this unresolved issue comes from the recent demonstration that some EGFR-mutant lung adenocarcinomas can transform to small cell lung cancer histology and aberrantly express neuroendocrine biomarkers during the acquisition of resistance to EGFR TKI therapy.^{54,62,79-81} This histological transformation could have resulted from an epigenetic reprogramming event that occurred in the context of the original genetic landscape of these tumors

because each of the resistant specimens harbored the EGFR-mutant allele prior to therapy. Additionally, no specimen had the EGFR T790M gatekeeper mutation or MET amplification. The histological transformation was clinically important because the EGFR TKI resistant tumors with SCLC transformation were sensitive to standard SCLC treatments.⁵⁴ To our knowledge, a small cell transition has not been demonstrated in *in vitro* models of acquired EGFR TKI resistance. However, pulmonary alveolar cells can transition to a neuroendocrine morphology upon p53 and Rb1 loss, although at low frequency.⁸²

Epithelial-mesenchymal transition (EMT). In addition to SCLC transformation, another histological transformation, EMT, has also been reported in human tumors with acquired resistance to EGFR TKI treatment.⁸³ EMT is implicated in enhanced migratory capacity, resistance to apoptosis and a stem cell like phenotype. However, the function and mechanistic underpinnings of EMT in the setting of EGFR TKI resistance are incompletely understood. High expression of Slug, a key transcriptional regulator of the EMT program, has been correlated with increased recurrence and decreased survival in NSCLC patients.^{84,85} Furthermore, E-cadherin expression was shown to be a biomarker predicting erlotinib response in some NSCLC patients.⁸⁶ Similar observations have been made in NSCLC *in vitro* models as well. NSCLC cells initially sensitive to erlotinib that acquire resistance *in vitro* express biomarkers of an EMT including loss of E-Cadherin and upregulation of vimentin, Twist, Slug and enhanced migration. Interestingly, vimentin has been shown to modulate AXL expression in erlotinib-resistant EGFR-mutant NSCLC cell lines driven by AXL.⁶⁹ Further studies have shown that Slug overexpression alone can induce EMT in EGFR-mutant NSCLC lines and protect cells from gefitinib-induced apoptosis. Conversely, ectopic expression of E-cadherin enhances sensitivity to gefitinib.⁸⁷ Likewise, low levels of miR-200, a suppressor of EMT, can promote EGFR inhibitor resistance.⁸⁸ The role of EMT in acquired EGFR TKI resistance remains to be fully clarified. Altogether, the data raise the intriguing possibility that therapies targeting Slug or other EMT regulators in combination with EGFR may overcome EGFR TKI resistance in some NSCLCs.

Whether the emergence of EMT and SCLC phenotypes in acquired resistance is the result of broad epigenetic changes or whether inhibition of oncogenic EGFR regulates epigenetic modifiers themselves, is unclear. However, emerging evidence has linked epigenetic alterations such as CpG methylation to drug resistance in multiple models.⁸⁹ In support of this, EGFR-mutant NSCLC cells that persist after EGFR TKI treatment exhibit a distinctive chromatin state that is regulated by histone demethylase and other epigenetic modifiers.⁹⁰ Overall, the broader genomic, epigenetic and proteomic changes occurring during acquired resistance have been challenging to study and remain poorly defined. Thus, further investigation is necessary to clarify how acquired resistance evolves in EGFR-mutant NSCLC, particularly in the context of histologically transformed phenotypes. Furthermore, recent data indicate that the tumor microenvironment and paracrine signaling may contribute to drug resistance in NSCLC and some other tumor types.^{91,92} Given the abundance of

RTK bypass mechanisms and the previously unappreciated role of NF κ B signaling in EGFR TKI resistance, factors produced by neighboring cells, stromal elements, the ECM and infiltrating immune cells could all potentially promote resistance to EGFR targeted therapies in patients.

The Role of BIM in Drug Resistance

Upregulation of the pro-apoptotic B-cell CLL/lymphoma 2 (BCL2) family member, BIM, is required for some TKIs to induce apoptosis in kinase-dependent malignancies such as EGFR-mutant NSCLC. One group found that cell lines sharing a common oncogenic driver and treated with the corresponding kinase inhibitor exhibited varying degrees of TKI-mediated apoptosis despite comparable inhibition of the target kinase and downstream effectors.⁵⁴ Interestingly, *BIM* RNA levels prior to TKI treatment correlated positively with the ability of the appropriate TKIs to induce apoptosis in EGFR-mutant, *HER2*-amplified and *PIK3CA*-mutant cancers. Similarly, *BIM* RNA levels in *EGFR*-mutant lung cancer primary specimens were also predictive of clinical response to EGFR inhibitors. In addition, paired-end DNA sequencing recently identified a common intronic deletion in *BIM* that results in expression of *BIM* isoforms that lack the pro-apoptotic BCL2-homology domain 3 (BH3).⁹³ In NSCLC cell lines, this polymorphism was shown to be sufficient to confer EGFR TKI resistance that was overcome by treatment with small molecule BH3 mimetics. Notably, NSCLC patients harboring the *BIM* intronic deletion exhibited poorer responses to EGFR TKI treatment. These data provide rationale for the clinical testing of BH3 mimetics to overcome drug resistance mediated by the *BIM* polymorphism. Altogether, these recent studies suggest impaired function of BIM by various mechanisms could account for some of the heterogeneity of response to TKI treatment in patients.

EGFR as a Target Beyond Lung Cancers

In addition to NSCLC, EGFR has been implicated as an oncogenic driver in a number of other cancers, including glioma, colorectal cancer, head and neck squamous cell carcinoma and pancreatic cancer.

Malignant glioma is commonly characterized by amplification of a truncated EGFR protein, EGFRvIII, that lacks part of the extracellular ligand-binding domain due to genomic deletion of exons 2 to 7 and is thus ligand-independent and constitutively active.⁹⁴⁻⁹⁶ Unlike NSCLC patients who harbor mutant EGFR, glioma patients with EGFRvIII generally respond poorly to EGFR TKI treatment despite evidence indicating that erlotinib is able to cross the blood-brain barrier.⁹⁷ This disparity in response has been attributed to mutant-specific differences in the kinetics of kinase site occupancy by EGFR TKIs. Relative to wild type EGFR, the glioma-derived EGFRvIII releases erlotinib more quickly from its active site, while the EGFR L858R and EGFR del746-750 mutants typically found in NSCLC release erlotinib more slowly. These data suggest that sensitivity to EGFR TKI therapy may depend at least in part on the nature of the *EGFR*

activating mutation.⁹⁸ Furthermore, studies have found that the common loss of PTEN in gliomas is associated with significantly diminished responsiveness to EGFR kinase inhibitors.⁹⁹

In contrast to lung cancer and glioma, *EGFR* mutations are uncommon in colorectal cancers.^{100,101} Instead, overexpression of EGFR is commonly detected in colorectal cancer cell lines and tumors.¹⁰² EGFR expression is positively correlated with increased malignant properties in colorectal cancers,¹⁰³⁻¹⁰⁵ and is increased in most colorectal cancer metastases.^{106,107} The clinical significance of EGFR overexpression and its influence on patient prognosis, however, remains unclear.^{103,108-110} Treatment with the anti-EGFR antibodies cetuximab and panitumumab has shown anti-tumor effects and blocking of EGFR signaling in some colorectal cancer patients with metastatic disease, and is clinically approved for use in those patients whose tumors harbor wild type KRAS.¹¹¹⁻¹¹³ However, response to EGFR-directed antibody therapy does not uniformly correlate with EGFR expression levels in colorectal cancers.^{114,115}

EGFR is overexpressed in 80-90% of HNSCC cases and increased EGFR expression is associated with decreased survival.¹¹⁶⁻¹²⁰ Thus, the EGFR signaling pathway is a therapeutic target in HNSCC. Clinical trials employing both anti-EGFR monoclonal antibodies and TKIs have produced promising results. Although these inhibitors have had limited success in HNSCC as single agents, there is evidence that they can act synergistically with radiotherapy in locally advanced disease and with platinum-based chemotherapy in metastatic or recurrent disease.^{23,121,122} Thus far only cetuximab has been approved for the treatment of HNSCC, but the use of other monoclonal antibodies and EGFR TKIs (including erlotinib and gefitinib) is currently being investigated.¹²³

Recent studies indicate that EGFR plays a critical role in KRAS-induced tumorigenesis in pancreatic ductal adenocarcinoma (PDAC). Current models suggest that oncogenic driver mutations within an individual signaling pathway do not co-exist in a given cancer cell. Consistent with this theory, KRAS and EGFR mutations are purported to be mutually exclusive in NSCLC tumors.¹²⁴ Similarly, clinical trials have shown that EGFR inhibitors are only effective in CRC patients harboring non-mutant RAS.¹²⁵ EGFR mutations occur in less than 3% of human pancreatic cancers. Intriguingly, however, these mutations have been observed to co-occur with KRAS mutations in PDAC.¹²⁶ Furthermore, the presence of oncogenic KRAS mutations in non-tumorigenic human pancreata suggests that secondary lesions are required for PDAC tumorigenesis.¹²⁷ Injury-induced inflammation or activation of KRAS induces acinar-to-ductal metaplasia (ADM), an early step in PDAC in which damaged acini are replaced with duct-like cells.¹²⁸ Pancreatic overexpression of the EGFR ligand, TGF α , has been shown to induce ADM and tumorigenesis in mouse models of PDAC¹²⁹ by a mechanism dependent on the EGFR ligand sheddase ADAM17.¹³⁰ Furthermore, upregulation of EGFR expression in ADM has been reported in KRAS-driven mouse models and human pancreatitis samples.^{131,132} Studies indicate that EGFR is required for both tumor initiation and maintenance of early neoplastic lesions and that genetic ablation or pharmacologic

inhibition of EGFR can prevent KRAS-driven PDAC development.^{130,132} These results support a model in which EGFR signaling is necessary to sustain the threshold of RAS activity needed for tumorigenesis.^{130,133} In 2007, a clinical trial combining erlotinib with gemcitabine provided only modest benefit to a limited number of unselected PDAC patients.¹³⁴ This clinical evidence and the recent data from preclinical models of PDAC suggest that appropriately selected patients whose PDAC harbors activation of EGFR could benefit from EGFR inhibitor treatment.

Conclusion

In recent years, tremendous advances in our genetic and biological understanding of cancer, the development of numerous targeted cancer therapies, and the pace of translational research has led to a paradigm shift in oncology toward a targeted, precision approach to diagnosis and treatment. An increased understanding of the molecular basis of mechanisms of primary and acquired resistance to EGFR-targeted therapy in NSCLC and other cancers has contributed to this remarkable transformation in oncology. This knowledge has also led to the development of effective and promising therapeutics for the clinical subset of patients with mutant EGFR-driven lung cancers. The challenge now is to characterize completely the molecular events that regulate response and resistance to EGFR inhibition in patients and

to translate this knowledge into the clinic to optimize outcomes for patients by preventing or overcoming drug resistance. While NSCLC has served as the archetype for EGFR-driven cancer, ongoing studies will hopefully shed light on the biological mechanisms by which EGFR promotes the growth of other tumor types, perhaps in cooperation with other molecular lesions. This information will allow us to use EGFR-directed therapies more effectively to improve outcomes for a wider spectrum of cancer patients.

Disclosure of Potential Conflicts of Interest

T.G.B. is a consultant and advisory board member of the Cancer Therapeutics Innovation Group. The other authors have no disclosures to report.

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