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Overcoming Molecular Mechanisms of Resistance to First-Generation Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors

Jair Bar, Amir Onn

Abstract

The epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib have provided substantial benefits to patients with advanced non–small cell lung cancer (NSCLC). However resistance to these agents has emerged as a significant clinical issue; most patients who initially respond to treatment eventually experience relapse. The mechanisms underlying gefitinib and erlotinib resistance are multifactorial and several have been described. Clearly there is a need for novel and more effective therapies that can overcome resistance to the currently available TKIs. Several agents are in clinical development, including irreversible EGFR TKIs, inhibitors of the MET pathway, and others. In this review we discuss the various underlying mechanisms of gefitinib and erlotinib resistance and highlight the agents currently in clinical development that may have potential for overcoming this resistance.

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Keywords: EGFR, Irreversible tyrosine kinase inhibitors, NSCLC, Resistance, Reversible tyrosine kinase inhibitors

Introduction

Lung cancer remains the primary cause of cancer deaths worldwide and is classified as small-cell or non–small-cell lung cancer (NSCLC) on the basis of its biological characteristics and different responses to therapy and prognosis. NSCLC accounts for approximately 85% of all lung cancers and is classified into 3 major subtypes: adenocarcinoma, squamous cell (epidermoid) carcinoma, and large-cell carcinoma. Adenocarcinoma in situ (AIS, formerly called pure bronchioloalveolar carcinoma [BAC]) accounts for 4% of NSCLCs, and tumors mixed with some lepidic growth pattern component (also formerly called BAC) account for > 20% of NSCLCs. More than two thirds of patients with lung cancer present with advanced-stage disease (stage III or stage IV). The long-term survival of these patients is poor, with 5-year survival rates of 24% for those with regional disease and 3.5% for those with distant metastases.

Targeting the epidermal growth factor receptor (EGFR) has become the focus of many treatment strategies that have emerged during the past decade. Gefitinib and erlotinib were the first EGFR tyrosine kinase inhibitors (TKIs) to be approved for the second-line treatment of patients with advanced NSCLC. In the European Union, erlotinib has been recommended for first-line treatment of EGFR mutation–positive patients, whereas gefitinib is approved generally for such patients. These agents have demonstrated effectiveness, but their clinical benefits are limited by the fact that only a subset of patients with NSCLC respond to therapy, and those who do eventually acquire resistance. In this review we discuss the known and putative molecular mechanisms of resistance to EGFR TKIs and highlight the agents currently in clinical development that may overcome this resistance.

EGFR Family: Target for Lung Cancer Therapy

The EGFR family is composed of 4 receptor tyrosine kinases (RTKs): EGFR (ERBB1), HER2 (ERBB2), HER3 (ERBB3), and HER4 (ERBB4). Each of these monomeric receptors contains an intracellular tyrosine kinase (TK) domain, a transmembrane domain, and an extracellular ligand-binding domain (Figure 1A). The ligand-binding domains of EGFR, HER3, and HER4 receptors bind to a specific set of ligands; no ligand has been identified for HER2 to date (Figure 1B). In the absence of a ligand, receptor monomers are inactive. However on ligand binding, the receptors form functionally active dimers, either with another receptor of the same type (a homodimer, such as EGFR-EGFR) or with a different type...

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Keywords: EGFR, Irreversible tyrosine kinase inhibitors, NSCLC, Resistance, Reversible tyrosine kinase inhibitors
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The EGFR family plays important roles in coordinating many normal processes, including growth and development, normal tissue turnover, and wound healing. Conversely, aberrant EGFR family signaling is known to play a role in tumor growth and progression, providing a rationale for targeting these receptors as an anticancer strategy. EGFR, as well as some of its ligands, are overexpressed in a large proportion of NSCLC tumors. Mutations in the EGFR TK domain can be found in 10%-15% of NSCLC in white patients and in around 30% or more of NSCLC in East Asian patients. These mutations confer EGFR with oncogenic properties in tissue culture and animal models, enhancing the downstream prosurvival and proliferative effects of the receptor. Gefitinib and erlotinib are reversible EGFR TKIs that have demonstrated activity in NSCLC. The US Food and Drug Administration (FDA) granted fast-track approval to gefitinib and erlotinib for the second-line treatment of advanced NSCLC in 2003 and 2004, respectively (Table 1). Early clinical data showed that approximately 10% of patients with NSCLC experience a response to gefitinib or erlotinib. In a double-blind phase II trial, patients with advanced NSCLC who had previously undergone at least 2 chemotherapy regimens were randomly

Abbreviations: Akt = protein kinase B; EGF = epidermal growth factor; EGFR = epidermal growth factor receptor; HB-EGF = heparin-binding epidermal growth factor; HER = human epidermal growth factor receptor; JM = juxtamembrane domain; MAPK = mitogen-activated protein kinase; MEK = mitogen-activated protein kinase kinase; NRG = neuregulin; P = phosphate; PI3K = phosphatidylinositol-3-kinase; Raf = v-ras H11005 murine leukemia viral oncogene homolog 1; Ras = rat sarcoma viral oncogene homolog; SOS = son of sevenless; TGF = transforming growth factor.

Figure 1 EGFR and Downstream Signaling. (A) Structure of EGFR. The EGFR Monomer has an Extracellular Domain Consisting of 2 Ligand-Binding Subdomains (L1 and L2) and 2 Cysteine-Rich Domains (S1 and S2); S1 Permits EGFR Dimerization With a Second HER Receptor. SH1 is the Protein Tyrosine Kinase Domain and Resides in the Cytoplasmic Domain Above the 6 Tyrosine Residues Available for Transphosphorylation. The Transmembrane Domain and Juxtamembrane Domain are Required for Targeting of EGFR to Caveolae. (B) Ligands, Dimerization Partners, and Downstream Signaling Pathways of EGFR. The EGFR, HER3, and HER4 Receptors Recognize and Bind to Specific Ligands. HER2 Has No Known Ligand and HER3 Does Not Have a Functional Tyrosine Kinase Domain. After Ligand Binding, the Ligand-Bound Receiver Forms a Functionally Active Homodimer (such as EGFR-EGFR) or Heterodimer (EGFR-HER2, EGFR-HER3, or EGFR-HER4), Which Leads to the Activation (phosphorylation) of Certain Tyrosine Residues in the Intracellular Domain of EGFR. This Phosphorylation Triggers the Activation of Downstream Signaling Pathways, Primarily the Ras-Raf-MEK-MAPK and PI3K-Akt Pathways, Which Promote Cellular Proliferation and Survival. (A) Adapted from Bazley LA, Gullick WJ. The Epidermal Growth Factor Receptor Family. Endocr Relat Cancer 2005; 12(Suppl 1):S17-27. © Society for Endocrinology (2005). Reproduced by Permission. (B) Adapted from Ciardiello F, Tortora G. EGFR Antagonists in Cancer Treatment. N Engl J Med, 2008; 358:1160-74. © Massachusetts Medical Society. Reprinted With Permission.
assigned to receive 250 or 500 mg/day of gefitinib. Symptom improvement was observed in 43% and 35% of patients in the 2 treatment arms, respectively. Furthermore, in most patients these improvements occurred rapidly after the initiation of treatment. Partial responses (PRs) were observed in 12% and 9% of patients who received 250 and 500 mg/day of gefitinib, respectively, the majority of whom experienced symptom improvement. However in a subsequent randomized phase III study (ISEL [Iressa Survival Evaluation in Lung cancer]) that recruited patients refractory to chemotherapy treatments, gefitinib did not result in a survival benefit compared with placebo. In the United States, patients who previously benefited from treatment; Europe: approved for advanced NSCLC with an EGFR-activating mutation (n = 176), PFS was longer with chemotherapy than did placebo (889 patients = OS, 12 vs. 11 months; HR, 0.81; 95% CI, 0.70-0.95; p = .0088). Gefitinib maintenance was also demonstrated to prolong PFS and sustain quality of life in INFORM, a Chinese study of 296 patients (PFS, 2.6 vs. 4.8 months; HR, 0.42; 95% CI, 0.32-0.54; p < .0001).

Several molecular features have been identified that are predictive of response to EGFR TKIs. In particular, EGFR-activating mutations (e.g., exon 19 deletions and the L858R mutation) have emerged as important predictive biomarkers for EGFR TKI sensitivity. Recent results from IPASS (Iressa Pan-Asia Study) demonstrated the value of these mutations for predicting response to EGFR TKIs. In this trial, 1217 Asian patients with advanced pulmonary adenocarcinoma and no substantial smoking history were randomly assigned to receive first-line gefitinib or carboplatin/paclitaxel. A subanalysis of 437 patients (36% of the trial population) revealed that among patients whose tumors had an EGFR-activating mutation (n = 261), PFS was significantly longer with gefitinib than with chemotherapy (HR, 0.48; p < .001). Conversely, in patients whose tumors lacked an EGFR-activating mutation (n = 176), PFS was longer with che-

### Table 1: First-Generation EGFR TKIs Gefitinib and Erlotinib for the Treatment of NSCLC

<table>
<thead>
<tr>
<th>Structure</th>
<th>Gefitinib</th>
<th>Erlotinib</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Gefitinib Structure" /></td>
<td><img src="image2.png" alt="Erlotinib Structure" /></td>
<td></td>
</tr>
<tr>
<td><strong>IC&lt;sub&gt;50&lt;/sub&gt; Against EGFR</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33 nM</td>
<td>2 nM</td>
</tr>
<tr>
<td><strong>Indication</strong></td>
<td>US labeling limits its use to patients who, in the opinion of their treating physician, have previously benefited from treatment; Europe: approved for advanced NSCLC with an EGFR activating mutation</td>
<td>Treatment of patients with locally advanced or metastatic NSCLC after failure of at least 1 previous chemotherapy regimen; maintenance therapy after first-line chemotherapy</td>
</tr>
<tr>
<td><strong>Recommended Daily Dose</strong></td>
<td>One 250-mg tablet orally, once daily, with or without food</td>
<td>150 mg orally, once daily at least 1 hour before or 2 hours after the ingestion of food</td>
</tr>
<tr>
<td><strong>Most Common AEs</strong></td>
<td>Diarrhea, rash, acne, dry skin, nausea, and vomiting</td>
<td>Rash and diarrhea</td>
</tr>
<tr>
<td><strong>Serious AEs Reported With Treatment</strong></td>
<td>Interstitial lung disease</td>
<td>Interstitial lung disease-like events; hepatic failure and hepatorenal syndrome (including fatalities); acute renal failure (including fatalities); and renal insufficiency</td>
</tr>
<tr>
<td><strong>Food</strong></td>
<td>May alter bioavailability and potentially increase AE risk</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** AE = adverse event; EGFR = epidermal growth factor receptor; IC<sub>50</sub> = half-maximal inhibitory concentration; NSCLC = non–small-cell lung cancer; TKI = tyrosine kinase inhibitor.

<sup>a</sup>IC<sub>50</sub> values shown are from cell-free in vitro kinase assays of gefitinib and erlotinib against wild-type EGFR.

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motherapy (HR, 2.85; \( p < .001 \)). Final OS results of this trial were recently presented and showed no significant difference in OS in the overall population with gefitinib (18.8 months) compared with carboplatin/paclitaxel (17.4 months; HR, 0.90; 95% CI, 0.79–1.02; \( p = .109 \)). In addition, no significant difference in OS was observed between gefitinib and carboplatin/paclitaxel in patients with tumors that were EGFR mutation–positive (21.6 vs. 21.9 months; HR, 1.00; 95% CI, 0.76–1.33; \( p = .990 \)) or EGFR mutation–negative (11.2 vs. 12.7 months; HR, 1.18; 95% CI, 0.86–1.63; \( p = .309 \)). The authors noted that these results may be partly due to the high percentage of patients in this study who received subsequent therapies. In the gefitinib arm, 49% of patients received subsequent carboplatin/paclitaxel and 52% of patients in the carboplatin/paclitaxel arm received subsequent EGFR TKI therapy, 64.3% if they had an EGFR mutation–positive tumor. Erlotinib was also compared with chemotherapy as first-line treatment for patients with advanced NSCLC with an EGFR-activating mutation and was found to be superior in terms of PFS in the OPTIMAL trial, a Chinese study of 165 patients (PFS; HR, 0.16; \( p < .0001 \); OS data not yet mature) and EURTAC (European Randomised Trial of Tarceva vs. Chemotherapy), a European study of 174 patients (PFS; HR, 0.42; \( p < .0001 \); OS currently similar; HR, 0.80; \( p = .42 \); 22.9 vs. 18.8 months). Despite these clinical benefits, most patients who are treated with reversible EGFR TKIs (erlotinib or gefitinib) are initially refractory to treatment or eventually experience resistance. Therapeutic responses to reversible EGFR TKIs have been reported for up to 2 to 3 years in patients with NSCLC, but the mean duration of OS achieved with these treatments is only approximately 8 months. Furthermore, almost all patients who initially respond to these agents will experience progressive disease. Unraveling the genetic heterogeneity of NSCLC that underlies this resistance and developing strategies to delay or prevent its emergence are paramount to the successful implementation of EGFR-targeted therapies.

Molecular Mechanisms of Resistance

Resistance to EGFR inhibitors can be categorized as primary or acquired. Patients with primary resistance are initially refractory to treatment, whereas acquired resistance occurs after an initial response. Investigators have attempted to elucidate the molecular underpinnings that cause or contribute to resistance and, to date, multiple mechanisms have been identified.

Primary Resistance to EGFR TKIs

Patients whose tumors lack a predictive biomarker of response to EGFR TKIs, such as an EGFR-activating mutation, are more likely to demonstrate primary resistance. A first-line study of EGFR TKIs vs. chemotherapy demonstrated chemotherapy to be superior in patients with wild-type EGFR. However, EGFR mutation status was not predictive of benefit from erlotinib when given as second- or third-line treatment. Erlotinib given as maintenance treatment improved OS in patients with wild-type EGFR (HR, 0.77; \( p = .0243 \)). Therefore EGFR TKIs as maintenance or advanced-line treatment is an option for patients with wild-type EGFR. Specific types of mutations such as insertions in exon 20 of the EGFR gene and several additional molecular mechanisms have been described that may contribute to primary resistance (Table 2).

### Table 2

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EGFR</strong></td>
<td></td>
</tr>
<tr>
<td>Exon 19</td>
<td>D761Y (P/A), L747S (P/A)</td>
</tr>
<tr>
<td>Exon 19</td>
<td>T790M (P/A)</td>
</tr>
<tr>
<td>Exon 20</td>
<td>D770_N771 (ins NPG) (P)</td>
</tr>
<tr>
<td>Exon 20</td>
<td>D770_N771 (ins SVQ) (P)</td>
</tr>
<tr>
<td>Exon 21</td>
<td>N771T (P)</td>
</tr>
<tr>
<td>Exon 21</td>
<td>V769L (P)</td>
</tr>
<tr>
<td>Exon 21</td>
<td>S768I (P)</td>
</tr>
<tr>
<td><strong>KRAS</strong></td>
<td></td>
</tr>
<tr>
<td>Exon 2</td>
<td>G12C</td>
</tr>
<tr>
<td>Exon 2</td>
<td>G12D</td>
</tr>
<tr>
<td>Exon 2</td>
<td>G12S</td>
</tr>
<tr>
<td>Exon 2</td>
<td>G12V</td>
</tr>
<tr>
<td>Exon 2</td>
<td>G12A</td>
</tr>
<tr>
<td>Exon 2</td>
<td>G13C</td>
</tr>
</tbody>
</table>

Abbreviations: EGFR = epidermal growth factor receptor; KRAS = Kirsten rat sarcoma viral oncogene homolog; NSCLC = non–small-cell lung cancer; TKI = tyrosine kinase inhibitor; P = primary resistance; A = acquired resistance.

Mutations in Kirsten rat sarcoma viral oncogene homolog (KRAS), a downstream mediator of EGFR signaling, have also been associated with primary resistance to EGFR TKIs in patients with NSCLC (Table 2). In particular, patients whose tumors have a KRAS mutation tend to have lower overall RRs to EGFR inhibitors than do those with KRAS wild-type tumors. In the NSCLC patient population, EGFR and KRAS mutations are considered to be mutually exclusive. In a prospective phase II trial (N = 102) of patients with AIS who received erlotinib, of 84 evaluable patients, 18 (21%) had a PR or a complete response (CR); patients whose tumors had a KRAS mutation had a significantly reduced RR (0% vs. 30%; \( p = .0084 \)). In an analysis of pulmonary adenocarcinoma samples from patients treated with erlotinib or gefitinib, 38 samples were from tumors refractory to these agents; 9 (24%) of these 38 tumors had a KRAS mutation. In contrast, of 21 samples from erlotinib-sensitive or gefitinib-sensitive tumors, none had a detectible KRAS mutation (\( p = .02 \)). In addition, in an analysis of 206 samples from patients enrolled in the BR.21 trial of erlotinib vs. placebo (N = 731), Zhu et al found that among 118 patients evaluable for response to erlotinib, those whose tumors had wild-type KRAS had a significant survival benefit compared with those with mutant KRAS (HR, 0.69; \( p = .03 \)). It has also been hypothesized that loss of phosphatase and tensin homolog (PTEN) may contribute to primary resistance to EGFR TKIs. PTEN functions as a negative regulator of phosphatidylinos...
sitol-3-kinase (PI3K) by dephosphorylating phosphatidylinositol (3,4,5)-tri-phosphate (PIP3), resulting in the downregulation of Akt (protein kinase B) and other survival and proliferation mediators. Loss of PTEN expression is an independent indicator of poor prognosis in patients with NSCLC and may be used to identify an aggressive subset of tumors. In clinical studies, loss of PTEN expression was found to be an independent negative prognostic indicator and was associated with reduced OS and PFS, poor differentiation, lymph node involvement, distant metastases, and late-stage disease in patients with NSCLC.\(^45\),\(^46\) In a preclinical study, homozygous genomic loss of a C-terminal portion of PTEN was identified in an erlotinib-resistant lung cancer cell line.\(^47\) When these cells were reconstituted with wild-type PTEN and treated with erlotinib and an Akt inhibitor, reduced viability was observed, suggesting that PTEN may be necessary for the coupling of EGFR inhibition to downstream Akt signaling. Homozygous loss of PTEN was also found in a small subset of EGFR mutation–positive primary lung cancers. Hemizygous deletion may be more common, possibly accompanied by a mutation in the remaining allele in cases of acquired resistance to EGFR TKIs.\(^47\)

Epithelial cancer cells commonly undergo epithelial-to-mesenchymal transition (EMT), a critical developmental process consisting of a change in the expression profile of cell-cell and cell-matrix adhesion molecules that is associated with cancer progression. In cell culture and xenograft models, EMT has been associated with EGFR TKI resistance, perhaps because of a reduction in cellular dependence on EGFR signaling.\(^48\) It has also been hypothesized that EMT may contribute to EGFR TKI resistance through EGFR-independent activation of Akt/Pi3K or insulin-like growth factor receptor-1, an RTK with several downstream targets that overlap with those of EGFR.\(^48\) However, survival signals may promote EGFR TKI resistance and cause EMT as an unrelated outcome. The role of EMT in clinical resistance to EGFR TKIs in patients with NSCLC has yet to be determined.

A recent report points to NF-\(\kappa\)B as a pathway that when activated rescues NSCLC cells bearing a mutant EGFR from EGFR inhibitors.\(^49\) The authors of the study screened for genes that when knocked down enhanced the efficacy of EGFR inhibitors; a majority of the hits were components of the NF-\(\kappa\)B pathway. In a set of erlotinib-resistant, EGFR-mutant NSCLCs, low \(\kappa\)B expression (indicative of high NF-\(\kappa\)B activity) was associated with shorter PFS and OS. The in vitro data indicated NF-\(\kappa\)B activity could also be involved in secondary resistance. Presumably more data about this pathway and its clinical relevance will become available in the near future.

**Acquired Resistance to EGFR TKIs**

Similar to mechanisms leading to primary resistance, EGFR alterations and compensatory signaling of overlapping or downstream mediators may contribute to acquired resistance to EGFR TKIs in NSCLC (Figures 2 and 3).\(^5\),\(^3\),\(^6\),\(^5\) Several secondary mutations in exons 19 and 20 of the TK domain of EGFR have been associated with resistance to reversible EGFR TKIs (Table 2).\(^5\),\(^5\) Of these, T790M is the most common (Figure 2)\(^5\),\(^5\) and is detected in approximately 50% of NSCLC tumors with acquired gefitinib or erlotinib resistance.\(^5\) T790M mutation results in an enhanced affinity of the kinase to adenosine triphosphate (ATP).\(^5\) This mutation has also recently been detected in 10 (38%) of 26 NSCLC tumor samples before treatment with erlotinib or gefitinib. These patients had shorter PFS compared with those who did not have the T790M mutation (7.7 vs. 16.5 months; HR, 11.5; \(p < .001\)). It should be noted that in this study, the T790M mutation was identified using an allele-specific polymerase chain reaction method and that detection required a high number of amplification cycles, suggesting that the mutation was present in only a small number of cells.\(^5\) When pretreatment biopsy specimens from 129 patients with NSCLC who received erlotinib were analyzed for T790M mutations, no difference was observed in the initial response to erlotinib in patients with (63.6% RR) and those without (72.3% RR; \(p\) value not reported) detectible T790M mutations.\(^5\) However median PFS was 12 months in patients with T790M mutations and 18 months in patients without T790M mutations (\(p = .05\)). These observations support the idea that clonal selection during treatment may play an important role in the development of acquired resistance. Interestingly, in a recent study of patients who had acquired resistance to erlotinib (\(n = 76\)), gefitinib (\(n = 24\)), or XL647 (\(n = 6\)), the T790M EGFR mutation was detected in 59% of rebiopsy tumor samples; the presence of the T790M mutation was associated with longer survival after the development of acquired resistance compared with acquired resistance in the absence of this mutation (HR, 0.55; \(p = .046\)).\(^5\)

Other less common second-site mutations, such as D761Y in exon 19 and T854A mutation in exon 21 have also been associated with resistance to EGFR TKIs. In addition, an in-frame deletion mutation at exons 2 through 7, \(EGFRvIII\), has been detected in a number of malignancies, including NSCLC, and may play a role in resistance to EGFR inhibition.\(^5\) Ji et al reported that \(EGFRvIII\) led to the development of NSCLC in a mouse model and was detectable in 3 of 56 human pulmonary squamous cell carcinomas but not in any of 123 adenocarcinoma samples tested.\(^5\) In vitro analyses demonstrated that cells transformed with the \(EGFRvIII\) mutation were relatively insensitive to gefitinib and erlotinib. Additional studies will be required to further define the role of the \(EGFRvIII\) mutation in the pathogenesis and drug sensitivity of NSCLC.

Amplification of the mesenchymal-epithelial transition factor (MET) protooncogene has also been shown to confer acquired resistance to EGFR TKIs (Figure 3). Engelman et al reported that MET amplification was detected in 4 (22%) of 18 lung cancer biopsy samples from patients who had acquired resistance to gefitinib or erlotinib.\(^6\) They also found that MET amplification mediated resistance to EGFR TKIs in a gefitinib-sensitive lung cancer cell line. Furthermore this resistance occurred through the MET-mediated activation of HER3, which in turn activated the PI3K pathway. HER3 might be a central node in the resistance to EGFR TKI, and agents targeting this molecule are being developed (NCT01211483). Enhanced levels of hepatocyte growth factor (HGF), the ligand for MET, in the cancer microenvironment may also cause activation of the MET-PI3K-Akt pathway and lead to EGFR TKI resistance.\(^6\) In this scenario, resistance may occur independently of EGFR and may not be treatable with an irreversible EGFR inhibitor. For example, results in a preclinical model demonstrated that the presence of HGF desensitized human lung cancer cells to an irreversible EGFR inhibitor.\(^6\)

The results of in vitro studies suggest that the insulin-like growth factor-1 (IGF-1) pathway may also contribute to EGFR TKI resistance (Figure 3). Morgillo et al reported that the sensitivity of
NSCLC cell lines to gefitinib was inversely related to IGF1R expression. Moreover, IGF1R inhibition sensitized cells resistant to gefitinib. In apparent contradiction, a recent clinical study of previously treated patients with advanced NSCLC demonstrated increased IGF1R expression to be correlated with improved responsiveness to gefitinib. Additional data from recent studies regarding IGF1R inhibition are discussed further on.

Additional potential mechanisms through which acquired resistance to EGFR TKIs may develop include altered EGFR trafficking, amplification or activation of downstream or overlapping signaling pathways, and expression of the ATP-binding cassette subfamily G member 2 (ABCG2) drug-efflux transporter. Small cell lung cancer (SCLC) histologic type was found in 5 of 37 (14%) patients with NSCLC who initially responded to erlotinib or gefitinib and acquired resistance, some of them with a clinical course and response to treatment typical of those seen in SCLC. This report suggests dedifferentiation or selection of a different histologic type as a possible novel mechanism of EGFR TKI resistance. It also stresses the importance of repeated biopsies when treatment decisions are required. Additional mechanisms of resistance are highlighted in a recent review.
Overcoming Resistance

Clearly, novel strategies are needed to prevent and overcome resistance to reversible EGFR TKIs. Therapeutic strategies using the concomitant administration of 2 available therapies as well as a number of novel agents are currently in development for NSCLC treatment. Importantly, a clinical definition of acquired resistance to EGFR TKIs has recently been proposed; this may enhance reproducibility of results of studies including these patients. According to this definition, patients classified as having acquired resistance to EGFR TKIs must have been previously treated with a single-target EGFR TKI (erlotinib or gefitinib), have a tumor harboring an EGFR mutation associated with EGFR TKI sensitivity or have demonstrated objective clinical benefit from treatment with an EGFR TKI, and have systemic progression of disease during continuous treatment with erlotinib/gefitinib within the previous 30 days. Objective clinical benefit is defined as attainment of a CR, PR, or durable stable disease (SD) (≥ 6 months) after initiation of gefitinib or erlotinib. The clinical course of 106 patients with acquired resistance to EGFR TKIs showed a median survival of 16 months after the development of EGFR TKI resistance, stressing the importance of optimizing the treatment of this subgroup of patients.

Alternating Reversible EGFR TKIs

Although the mechanisms of action of gefitinib and erlotinib are similar, it has been postulated that patients who acquire resistance to gefitinib may experience a response to erlotinib. A phase II study of 21 patients with NSCLC treated with erlotinib after the failure of gefitinib reported a disease control rate (DCR) and RR for all patients of 28.6% and 9.5%, respectively. In patients with SD receiving erlotinib, erlotinib treatment resulted in a significantly higher DCR (75.0% vs. 17.6% in patients without SD; \( p = .050 \)) and RR (50% vs. 0% in patients without SD; \( p = .029 \)). For all patients, the median duration of disease control was 125 days, and the median time to progression and duration of OS were 60 and 158 days, respectively. A larger phase II trial conducted in Japan reported similar findings: of 48 patients, PR and SD with erlotinib after gefitinib failure were observed in 5 and 29 patients, respectively (RR, 10.4%; DCR, 70.8%). The DCR in patients who received gefitinib treatment for > 1 year was significantly higher compared with patients who were treated for < 1 year (81.5% vs. 57.1%; \( p = .018 \)). Erlotinib may be a viable therapeutic option for patients with advanced NSCLC and wild-type EGFR who experienced a prolonged SD with gefitinib.

Combination Therapies

A role for continuing treatment with erlotinib or gefitinib after emergence of resistance has been suggested, possibly in combination with other agents. Combining a reversible EGFR TKI and an anti-EGFR antibody may be a relevant strategy for overcoming EGFR TKI resistance. In xenograft models of human lung cancer, greater tumor regression and prolonged regrowth delay were demonstrated for cetuximab, an anti-EGFR antibody, and gefitinib or erlotinib compared with either agent alone. In a recent phase II trial evaluating erlotinib in combination with cetuximab, an unfavorable result of no response in 13 patients was reported. Afatinib (BIBW 2992) an irreversible inhibitor of EGFR, HER2, and HER4, in combination with cetuximab, was reported to have significant activity in a phase Ib trial of patients with acquired resistance to EGFR TKIs (see discussion further on).

Combination therapy with erlotinib and the cyclooxygenase-2 inhibitor celecoxib was recently evaluated in a phase I trial of 22 patients with stage IIIB or stage IV NSCLC. This dose-escalation study determined the optimal biological dose of celecoxib in combination with a fixed dose of erlotinib 150 mg/day. Of 21 evaluable patients, 7 (33%) had a PR and 5 (24%) had SD. A randomized phase II study is currently recruiting patients to evaluate the addition of celecoxib to erlotinib in patients with advanced NSCLC (NCT00499655).

Histone acetylation status, maintained by histone acetylases and histone deacetylases (HDACs), affects the abundance of transcribed genes, and these enzymes may be abnormally regulated in cancer cells. In vitro, synergism between an HDAC inhibitor and an EGFR TKI has been reported in NSCLC cell lines. A phase I/II trial to evaluate vorinostat, an HDAC inhibitor, in combination with gefitinib in patients with advanced NSCLC has been initiated (NCT01027676). A randomized phase II study (ENCORE [E)ntinostat Combinations Overcoming REsistance] 401) comparing erlotinib with placebo or with an HDAC inhibitor (entinostat) has recently been reported. No improved outcome was demonstrated for the patients treated with entinostat. However a subset of patients with high E-cadherin expression revealed by immunohistochemical analysis did better with erlotinib and entinostat compared with erlotinib and placebo (OS, 9.2 vs. 4.2 months; HR, 0.46; \( p = .08 \)). The relevant molecular mechanism whereby an HDAC inhibitor would improve the efficacy of erlotinib in tumors with high expression of E-cadherin was not elucidated.

V-src sarcoma viral oncogene homolog (SRC) is a non-receptor tyrosine kinase that may be involved in EGFR activation. Agents that inhibit SRC have been developed that have the potential to reduce EGFR-dependent cell proliferation and inhibit EGFR mutants that are resistant to EGFR TKIs. Dasatinib, a dual breakpoint cluster region-v-abl Abelson murine leukemia viral oncogene homolog 1 (BCR-ABL)/SRC inhibitor, was tested in a phase I trial in combination with erlotinib as a treatment for advanced lung cancer and resulted in a DCR of 63%. The combination of dasatinib and erlotinib is currently being tested in a phase I/Ii trial in patients with NSCLC (NCT00826449), and dasatinib alone is being evaluated in a phase II trial in patients with EGFR TKI–resistant NSCLC (NCT00570401).

Emerging Agents

Irreversible Inhibitors of EGFR Family Members. In recent years, several irreversible inhibitors of the EGFR receptor family have emerged (Table 3) that may have activity in patients with erlotinib- or gefitinib-resistant NSCLC harboring an EGFR mutation. Experimental models demonstrate that resistance to an irreversible EGFR TKI can occur through amplification of a T790M-containing allele. However other preclinical studies have shown that resistance to reversible inhibitors may be overcome with irreversible EGFR TKIs. Several of these agents have activity against multiple members of the EGFR family. This activity allows them to interrupt the cooperative signaling that occurs between HER family members,
which may lead to improved efficacy.\textsuperscript{86} TKIs that selectively inhibit T790M EGFR but not wild-type EGFR are also in development and may be more effective and less toxic, as EGFR in non-cancerous tissues would be unaffected.\textsuperscript{87} Following is a discussion of the recently and currently evaluated irreversible TKIs.

**Afatinib.** This agent, mentioned earlier, inhibits the kinase activity of wild-type and mutant EGFR, including erlotinib-resistant isoforms, as well as the kinase activity of HER2. It has also been shown to induce the regression of tumor growth in xenograft and transgenic lung cancer models.\textsuperscript{77}

Afatinib was evaluated in a single-arm phase II trial (LUX-Lung 2) in patients with advanced pulmonary adenocarcinoma harboring an activating EGF\textsuperscript{r}R mutation. Confirmed objective RR and DCR were 60\% and 86\%, respectively, for all patients (n = 129). RR and DCR of patients with tumors harboring a deletion in exon 19 of EGF\textsuperscript{r}R (n = 52) were similar to those of patients harboring L858R mutations (n = 54).\textsuperscript{88} Median PFS was 14 months for patients who received at least 1 dose of afatinib.\textsuperscript{89} The most common drug-related adverse events (AEs) were diarrhea (95\%; grade 3, 19\%) and rash/acne (91\%; grade 3, 21\%).\textsuperscript{88}

Afatinib has also shown preliminary clinical activity in patients with NSCLC harboring a HER2 mutation, present in approximately 2\% to 4\% of adenocarcinomas.\textsuperscript{89} In an exploratory phase II study, PRs were achieved in 3 of 3 evaluable patients with HER2 mutations in exon 20 in whom chemotherapy had failed.

The preliminary activity witnessed in early studies led to the initiation of several larger trials with afatinib. A randomized double-blind multicenter phase IIb/III trial (LUX-Lung 1) has evaluated afatinib plus best supportive care (BSC) in patients with advanced NSCLC.\textsuperscript{90} Eligibility criteria included advanced pulmonary adenocarcinoma (stage IIB or stage IV; Eastern Cooperative Oncology Group performance status 0-2), failure of 1 or 2 lines of chemotherapy (including platinum-based regimens), and disease progression after \(\geq 12\) weeks of erlotinib or gefitinib treatment. Patients (N = 585) were randomly assigned in a 2:1 ratio to afatinib 50 mg/day plus BSC or placebo plus BSC until disease progression or unacceptable toxicity. The primary endpoint was OS; this analysis (n = 358) revealed a median OS of 10.8 months with afatinib plus BSC vs. 12.0 months with placebo plus BSC (HR, 1.08; 95\% CI, 0.86-1.35). An independent review determined that median PFS was 3.3 months with afatinib plus BSC vs. 1.1 with placebo plus BSC (HR, 0.38, p < .0001). DCR at 8 weeks was 58\% with afatinib plus BSC vs. 19\% with placebo plus BSC (p < .0001), and confirmed objective RR was 7.4\% with afatinib plus BSC vs. 0.5\% with placebo plus BSC by independent review. Differential use of postprogression treatments was suggested as an explanation for the apparent discrepancy between the PFS and OS results. The 2 most common AEs observed in the afatinib arm were diarrhea (87\%; grade 3, 17\%) and rash/acne (78\%; grade 3, 14\%).\textsuperscript{90}

Several additional phase III randomized trials investigating afatinib are ongoing. LUX-Lung 3 (NCT00949650) is comparing afatinib 40 mg/day with cisplatin/pemetrexed for first-line treatment of patients with EGFR mutation–positive adenocarcinoma and began accrual in August 2009. LUX-Lung 6 (NCT01121393) is comparing first-line afatinib with cisplatin/gemcitabine chemotherapy in patients with EGFR mutations in China, Korea, and India. The

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**Table 3 Currently/Recently Evaluated Irreversible Inhibitors of the HER Receptor Family**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Structure</th>
<th>Target (IC\textsubscript{50}, nM)a</th>
<th>Phase of Clinical Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afatinib</td>
<td><img src="image1.png" alt="Afatinib Structure" /></td>
<td>EGFR (0.5) HER2 (14) HER4 (1)</td>
<td>Phase III: NSCLC, breast Phase II: head and neck, glioma, prostate</td>
</tr>
<tr>
<td>HKI-272</td>
<td><img src="image2.png" alt="HKI-272 Structure" /></td>
<td>EGFR (92) HER2 (50)</td>
<td>Phase II: NSCLC Phase III: breast</td>
</tr>
<tr>
<td>PF-00299804</td>
<td>Unavailable</td>
<td>EGFR (6.0) HER2 (45.7) HER4 (73.7)</td>
<td>Phase II: NSCLC, head and neck</td>
</tr>
</tbody>
</table>

Abbreviations: EGFR = epidermal growth factor receptor; HER = human epidermal growth factor receptor; IC\textsubscript{50} = half-maximal inhibitory concentration; NA = not applicable; NSCLC = non–small-cell lung cancer.

*a The IC\textsubscript{50} values shown are from cell-free in vitro kinase assays of afatinib, HKI-272, and PF-00299804 against wild-type HER receptors.
LUX-Lung 5 trial (NCT01085136) is comparing afatinib plus weekly paclitaxel with single-agent chemotherapy of the investigator’s choice for patients in whom previous chemotherapy and erlotinib or gefitinib failed and who initially benefited (> 12 weeks) from afatinib monotherapy.

A phase Ib trial testing the combination of afatinib with cetuximab for patients with clinically defined acquired resistance to EGFR TKIs (according to Jackman et al) was recently reported.71-79 Disease control was observed in all 22 patients who were treated with the recommended phase II dose, with 8/22 confirmed PRs; 4/13 (29%) confirmed PRs were reported in T790M mutation-positive patients.79 An expansion cohort is now accruing (NCT01090011).

**HKI-272 (neratinib).** This agent is an irreversible inhibitor with activity against both EGFR and HER2. Kwak et al used the AIS cell line NCI-H1650, which has an in-frame deletion of EGFR (de/E746-A750), to create gefitinib-resistant cell lines that were approximately 50-fold less sensitive to gefitinib than were the parental cells.83 Conversely, the gefitinib-resistant cell lines showed persistent sensitivity to HKI-272.83 HKI-272 was also more effective than gefitinib at suppressing ligand-induced EGFR autophosphorylation and its downstream signaling in the NCI-H1975 AIS cell line, which possesses L858R and T790M mutations in **EGFR.**80 HKI-272 also suppressed proliferation in this cell line under gefitinib-resistant conditions.83 In another study, Ba/F3 cells transformed by the **EGFRvIII** mutant were relatively resistant to gefitinib and erlotinib but were sensitive to HKI-272, suggesting that this agent may be an alternative therapeutic strategy for **EGFRvIII**-mutant tumors that are resistant to reversible EGFR TKIs.85 In preclinical xenograft studies, HKI-272 significantly impeded the growth of HER2- and ERG-dependent tumors.

In a phase II trial of HKI-272 in patients with advanced NSCLC in whom previous chemotherapy had failed, patients (N = 167) were composed of 3 groups: those who experienced failure of gefitinib or erlotinib and had tumors with an **EGFR** mutation (arm A; n = 91) or without an **EGFR** mutation (arm B; n = 48) and those who did not undergo previous **EGFR** TKI treatment (arm C; n = 28).92 Of 158 patients evaluable for efficacy, 3 (1.9%) had PRs, 81 (51%) exhibited SD, and 14 (9%) had SD for ≥ 6 cycles (24 ± 2 weeks). All PRs were in arm A (3.4% of arm A patients), whereas no responses were seen in arms B and C. The most common AE observed was diarrhea (91%; grade ≥ 3, 28%), necessitating a reduction of the given dose from 320 mg to 240 mg after the first 39 patients.92 At present, there are no ongoing trials of HKI-272 in patients with NSCLC.

**PF-00299804.** This agent is an irreversible pan-ErbB inhibitor. It has shown preclinical activity in both cellular assays and tumor xenograft models against **EGFR** T790M.93 In a phase I study of patients with refractory NSCLC, PF-00299804 induced PR in 2 of 29 evaluable patients and SD in 8 patients.94 An ongoing phase II trial is evaluating PF-00299804 in patients with advanced NSCLC and wild-type **KRAS** who have experienced failure of 1 or 2 previous chemotherapy regimens and in whom disease has progressed after erlotinib treatment.95 Patients with both adenocarcinoma (arm A) and nonadenocarcinoma histologic types (arm B) were enrolled. Three confirmed PRs were reported, with response durations of ≥ 127, ≥ 44, and ≥ 82 days. DCR beyond 2 cycles (eg, 6 weeks) of 67% (n = 24) among 36 evaluable patients in arm A and of 60% (n = 2) among 5 evaluable patients in arm B were reported. Importantly, prolonged SD was reported in patients with a T790M mutation and exon 20 insertion. Grade 3 toxicities included skin toxicity (14%), diarrhea (10%), fatigue (10%), and vomiting (3%).95 A meaningful improvement of lung cancer symptoms was also reported from this trial.96 A similar study was conducted in Asia, recruiting 42 patients, 59% of whom had received more than 3 previous treatment regimens.97 The objective RR was 15%, and clinical benefit was seen in 25% of patients. Of the 7 patients with a known **EGFR**-activating mutation, 2 had a PR, 2 had SD, and 2 had PD (1 undetermined). The 2 patients with a T790M mutation had SD. The probabilities of 6-month PFS and OS were estimated at 32% and 90.2%, respectively. A randomized phase II study (188 patients) compared erlotinib with PF-00299804 in patients whose disease progressed on chemotherapy and who were never exposed to **EGFR** TKIs.98 **EGFR** mutation status was not well balanced, with 11.7% of the erlotinib-treated group harboring an **EGFR** mutation, whereas 20.2% had such a mutation in the PF-00299804 group. The objective RR and DCR > 24 weeks in the PF-00299804 group were 17.0% and 27.7%, respectively, compared with 4.3% and 13.8%, respectively, in the erlotinib group. Patients receiving PF-00299804 had a PFS of 12.4 weeks compared with 8.3 weeks for patients receiving erlotinib (HR, 0.681; 95% CI, 0.490-0.945; p = .019). A benefit was demonstrated in all subgroups, including patients with wild-type **EGFR** and nonadenocarcinomas but was more apparent in patients with wild-type **KRAS** and in younger patients. Most common AEs were diarrhea and dermatitis aceneiform/rash, with grade 3 incidences for 14.8% and 6.5% for these AEs, respectively, and for erlotinib of 3.2% and 5.3%/2.1%, respectively. Four grade 5 treatment-related AEs were reported (2 in each treatment group): pneumonia (n = 2 [n = 1 per group]), pulmonary embolism (n = 1 with erlotinib), and pneumonitis (n = 1 with PF-00299804). Another phase II study evaluated PF-00299804 as first-line treatment for NSCLC with a sensitizing **EGFR** mutation or clinical characteristics correlated with a mutated **EGFR.**99 Of 29 patients, CR, PR, and SD were seen in 1, 6, and 16 patients, respectively. All tumors with a known **EGFR** mutation showed tumor shrinkage.

PF-00299804 is currently being evaluated in a phase III trial as a single-agent treatment for patients with NSCLC whose disease has progressed on standard treatments (NCT01000025) and in a phase II study as a single-agent treatment for patients in whom chemotherapy and erlotinib treatment failed (NCT00548093).

**Simultaneous Inhibition of Multiple Signal Transduction Pathways.**

**BMS-690514.** This agent is a TKI targeting both **EGFR** and **VEGFR** that has shown interesting phase II data with patients with NSCLC.100 A phase III trial (not yet recruiting) is evaluating this agent in patients with NSCLC whose disease has progressed on erlotinib or gefitinib and were either shown to harbor an **EGFR** mutation or have responded to previous **EGFR** TKI therapy (NCT01167244).

**XL647.** This agent is an inhibitor of several receptor TKs (**EGFR**, HER2, VEGFR-2, and ephrin type-B receptor 4 [**EphB4**]) that has been shown to inhibit the growth of cell lines such as H1975 that contain the **EGFR** T790M resistance mutation.101 Preliminary re-
Overcoming Resistance to EGFR Inhibitors

sults from a phase II trial (N = 23) in patients with relapsed or recurrent NSCLC (stage IIIb or stage IV) who had experienced progression after responding to gefitinib or erlotinib revealed that of 22 evaluable patients, 7 (32%) had SD and 1 (5%) achieved a PR.104 Fourteen patients were not yet evaluable. In addition, patients commonly reported symptom improvement. When tumor biopsy specimens from 6 patients whose disease progressed after an initial response to XL647 were analyzed for secondary mutations in EGFR, only 1 was found to harbor the T790M mutation; 2 of these patients received subsequent benefit from erlotinib therapy. This observation was investigated further using pulmonary adenocarcinoma PC-9 cells, which contain a deletion in the EGFR gene exon 19 from E746-A750. Cells were grown with increasing concentrations of XL647, erlotinib, or afatinib until cells proliferated at drug concentrations approximately 50-fold higher than the IC50 observed in the parental cells. Resulting erlotinib-resistant and afatinib-resistant cells harbored second-site T790M mutations, whereas XL647-resistant cells did not have secondary EGFR mutations and were immediately sensitive to erlotinib, suggesting that selective pressure for T790M mutations may differ between XL647 and erlotinib or afatinib.105 Further clinical studies are required to determine the effectiveness of XL647 in NSCLC. Currently there are no ongoing trials with this agent.

Other Agents

**MET Inhibitors.** Given the role of MET amplification in resistance to EGFR TKIs, it is reasonable to speculate that blocking this pathway could offset such resistance. Dual EGFR-MET inhibition has been proposed as a strategy for overcoming resistance to EGFR TKIs. In addition, because both MET amplification and the EGFR T790M mutation can occur in the same tumor,63,103 the combination of an irreversible EGFR TKI and a MET inhibitor may be a rational approach to overcoming resistance. In preclinical studies, this combination was shown to be effective in suppressing the growth of gefitinib-resistant NSCLC cell lines.63 Numerous MET inhibitors are currently under clinical development for NSCLC, including TKIs such as XL184, tivantinib (ARQ 197), and crizotinib (PF-03441066).106 and recombinant antibodies directed at the receptor (MetMaB) or ligand (ficlatuzumab, AV-299; formerly SCH 900105). Tivantinib was tested in a randomized phase II study of 167 patients, in combination with erlotinib, compared against placebo plus erlotinib, with PFS as the primary endpoint.107 Patients treated with tivantinib plus erlotinib had a better PFS (3.8 vs. 2.3 months), with similar AE rates between the arms. Interestingly, tivantinib seemed to confer the most benefit in patients with tumor characteristics of nonsquamous histologic type, wild-type EGFR, and mutant KRAS. Two of 26 (8%) confirmed PRs were noted in patients in the erlotinib-placebo arm who crossed over to erlotinib-tivantinib after disease progression.108 Several additional phase II studies are under way to evaluate the combination of MET inhibitors and EGFR TKIs (NCT00854308, NCT01039948, and NCT01068587). MetMaB was also evaluated recently as an addition to erlotinib treatment in a randomized phase II trial.109 For all the treated patients (N = 128), no significant differences in outcome were observed. However tumor sample analysis (available from 121 patients) allowed separation of the patients expressing high MET levels (about half of the patients based on the study’s criteria), and results demonstrated OS benefit (HR, 0.55; p = .11) from treatment with MetMaB in these patients. Conversely, in the patients with low MET levels, MetMaB-treated patients had a reduced OS (HR, 3.26; p = .01). The cause of the poorer outcome in the patients with low MET levels was not reported but might be related to a higher rate of grade 3 or worse toxicity reported in this group. No differences in outcome were seen in other subgroups, including different histologic types or EGFR/KRAS mutation bearers.

A phase Ib study of XL184 with erlotinib reported some responses in EGFR TKI–resistant NSCLC, including patients with T790M mutations and MET amplification.107 Crizotinib is also an ALK inhibitor, and activity was reported in NSCLC tumors with an EML4-ALK translocation.109 Crizotinib is currently being tested in a phase I/II trial in combination with erlotinib vs. erlotinib alone (NCT00965731), and a phase I trial will evaluate it in combination with PF-00299804 (NCT01121575). Ficlatuzumab is currently being tested in a phase I/II trial in combination with gefitinib vs. gefitinib alone (NCT01039948).

**IGF1R Inhibitors.** Similar to MET, IGF1R has been implicated in EGFR TKI resistance; thus, this receptor may be an appropriate target for therapeutic exploitation. Agents aimed at blocking the IGF pathway, including monoclonal antibodies and TKIs, are currently in development for NSCLC.108-110 One strategy that may be effective for overcoming IGF1R-mediated resistance to EGFR TKIs is the combination of an IGF1R inhibitor and an EGFR-targeted agent. However a phase II trial of figitumumab (CP-751,871), an anti-IGF1R antibody, in combination with erlotinib in patients with NSCLC was recently discontinued because of futility (NCT00673049). A trial testing figitumumab in combination with carboplatin and paclitaxel was also recently discontinued because of futility, with serious AEs noted in the experimental arm, including dehydration, hyperglycemia, and hemoptysis.111 Targeting both EGFR and IGF1R is currently being evaluated in an ongoing phase II clinical trial of erlotinib and another anti-IGF1R antibody, IMC-A12 (cixutumumab), in patients with advanced NSCLC in whom platinum-based chemotherapy failed (NCT00778167). Possible resistance to IGF1R inhibition might arise from feedback regulation of IGF-1 levels and activation of the insulin receptor substrate 1/2 through the insulin receptor. OSI-906 is a dual kinase inhibitor targeting both the IGF1R and the insulin receptor that might prevent such resistance.112 Randomized phase II studies are currently recruiting and comparing erlotinib combined with OSI-906 or placebo for chemonaive patients with NSCLC with EGFR-activating mutations (NCT01212077) and as maintenance treatment (NCT01186861). MK-0646 (dalotuzumab) is another IGF1R antibody being studied in patients with NSCLC, as is AMG-479. A trial evaluating AMG-479 in combination with chemotherapy for patients with squamous NSCLC was recently terminated (based on the negative results of the figitumumab trial). It is unknown at this time whether results of the figitumumab trial can be generalized to all of the IGF1R antibodies or TKIs.

**Heat Shock Protein 90 (HSP90) Inhibitors.** Several kinases implicated in dysregulated intracellular signaling and proliferation in human cancers rely on HSP90 for conformational maturation.113 Preclinical data generated in NSCLC cell lines containing EGFR mutations (NCI-H1650 [del E746-A750], NCI-H3255 [L858R],...
and NCI-H1975 (L858R plus T790M) revealed that HSP90 inhibition resulted in the rapid depletion of mutant EGFR, accompanied by enhanced apoptosis and a marked reduction in levels of cyclin D and phosphorylated Akt.11,12 These data suggest that mutational activation of EGFR creates a dependence on HSP90 for conformational maturity and EGFR stability. Inhibition of HSP90 in cancer cells bearing these mutations may be a viable therapeutic option. STA-9090 (ganetespib), IPI-504, and AU922 are HSP90 inhibitors that are currently being evaluated in patients with NSCLC (NCT01031225, NCT00431015, and NCT01259089).

**mTOR/PI3K Inhibitors.** Activation of PI3K/Akt signaling pathways by MET amplification or other mechanisms is a potential mechanism of EGFR TKI resistance. Mammalian target of rapamycin (mTOR) is a key mediator of PI3K/Akt downstream signaling and is commonly activated in NSCLC. Concurrent mTOR and EGFR inhibition was found to be effective in a mouse model of pulmonary carcinoma bearing the EGFR T790M mutation.11,13 mTOR, PI3K, and dual PI3k/mTOR inhibitors are being evaluated in early-stage clinical trials of lung cancer, either alone or in combination with EGFR inhibitors. A randomized phase II trial found improved RR and DCR for patients treated with erlotinib and erlotinib in comparison with erlotinib alone. However the difference did not reach the predefined threshold to support a phase III trial of this combination.11,15 The combination of everolimus and gefitinib was also evaluated in a phase II trial, with a 13% PR, mostly in **EGFR or KRAS** (G12F) mutation–bearing tumors.11,15

**Mevalonate Pathway Inhibitors.** Several mevalonate metabolites play a role in transducing EGFR signaling and therefore impact cellular proliferation and survival signals.116 Inhibition of the mevalonate pathway by a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase such as lovastatin has been shown to inhibit EGFR dimerization and autophosphorylation.117 Combining EGFR TKIs with lovastatin is synergistic in inducing apoptosis in cancer cell lines,116 including cells that harbor the constitutively active EGFRvIII.118 However both lovastatin and erlotinib/gefitinib are metabolized through the cytochrome P450 enzyme, and their combination may result in increased toxicity. As a result, combination of EGFR TKIs with another HMG-CoA reductase inhibitor, rosvastatin, which is not metabolized by cytochrome P450, is under investigation in NSCLC. A phase I clinical trial evaluating erlotinib and rosvastatin in squamous cell carcinomas and NSCLC is currently recruiting patients (NCT00966472).

**Discussion**

Only a small subset of patients with NSCLC responds to the EGFR TKIs gefitinib and erlotinib. In addition, almost all patients who initially experience a response to gefitinib or erlotinib will acquire resistance. Primary and secondary resistance to reversible EGFR inhibitors limits the clinical benefit of these agents. Several underlying mechanisms of resistance have been identified, in particular the T790M resistance mutation in **EGFR** and **MET** amplification. The role of NF-κB activation was recently highlighted and requires further investigation.107 Biopsies of resistant tumors would identify additional mechanisms, such as the recently reported emergence of SCLC from EGFR TKI–resistant NSCLC.69 Clearly, more effective anti-EGFR therapies are needed that can overcome resistance to currently available agents. Emerging data support a potential role for irreversible EGFR inhibitors in preventing and overcoming resistance to reversible EGFR TKIs. Agents targeting additional pathways are also in clinical development and may be evaluated alone or in combination with EGFR inhibitors in NSCLC. After the recent reports of OS improvements in patients treated with agents targeting the MET pathway in combination with erlotinib, much interest has been drawn to this pathway. Further understanding of the underlying biological mechanisms is needed, and molecular stratification of patients would probably be required.

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