New Strategies in Overcoming Acquired Resistance to Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors in Lung Cancer

Geoffrey R. Oxnard¹, Maria E. Arcila², Juliann Chmielecki², Marc Ladanyi², Vincent A. Miller², and William Pao³

Abstract

The management of non–small cell lung carcinoma (NSCLC) has been transformed by the observation that lung adenocarcinomas harboring mutations in epidermal growth factor receptor (EGFR) are uniquely sensitive to EGFR tyrosine kinase inhibitors (TKI). In these patients, acquired resistance to EGFR-TKI develops after a median of 10 to 14 months, at which time the current standard practice is to switch to conventional cytotoxic chemotherapy. Several possible mechanisms for acquired resistance have been identified, the most common being the development of an EGFR T790M gatekeeper mutation in more than 50% of cases. In this review, we discuss recent advances in the understanding of acquired TKI resistance in EGFR-mutant lung cancer and review therapeutic progress with second generation TKIs and combinations of targeted therapies. Clin Cancer Res; 17(17); 5530–7. ©2011 AACR.

Background

Epidermal growth factor receptor (EGFR)–mutant lung cancer is a well-described molecular subgroup of lung adenocarcinoma characterized by a high prevalence in females, never-smokers, and Asians, and a unique sensitivity to rationally targeted therapy with EGFR–tyrosine kinase inhibitor (TKI; refs. 1, 2). Patients with EGFR-mutant lung cancer develop disease progression after a median of 10 to 14 months on TKI (2, 3); no optimal therapy thereafter has yet been established.

Importantly, what defines acquired resistance is progression while receiving EGFR-TKI, irrespective of “line of therapy.” Rather, “TKI resistance” in EGFR-mutant lung cancer is a therapy-dependent clinical state, much like “castrate resistance” in prostate cancer (4). As in prostate cancer, one should ensure that patients are truly resistant before altering therapy. For example, patients who develop progression after having stopped TKI can still be sensitive to retreatment (5, 6), a phenomenon also described in gastrointestinal stromal tumors (GIST; ref. 7). Also, some patients can develop central nervous system (CNS)–only progression, likely because of inadequate TKI penetration through the blood-brain barrier, and may still be sensitive to higher doses of TKI (8, 9); this “pharmacokinetic failure” has also been seen in anaplastic lymphoma kinase–mutant lung cancers treated with crizotinib, and such patients can continue to do well on TKI after receiving local therapy to the CNS (10). Including cancers that remain sensitive to erlotinib or gefitinib in clinical trials for acquired resistance could confound the interpretation of results.

As in castrate-resistant prostate cancer, indications show that EGFR-mutant lung cancers maintain a degree of sensitivity to TKI despite development of acquired resistance. In a pilot study, Riely and colleagues did serial computed tomography (CT) and positron emission tomography (PET) imaging of patients with acquired resistance at 3 time points: before stopping TKI, after 3 weeks off TKI, and after 3 weeks of restarted TKI (11). Tumor volume and fluorodeoxyglucose (¹⁸F) avidity increased significantly after stopping TKI and plateaued when TKI was restarted. In a separate series of patients discontinuing EGFR-TKI prior to enrolling in a clinical trial for acquired resistance, 22% developed accelerated progression (or “flare”) leading to hospitalization (12), occurring after a median of 8 days. Because of these clinical observations, our current practice is to continue erlotinib in patients with acquired resistance in addition to subsequent chemotherapies. This strategy is not yet the standard of care, but has led to a median survival of 33 months from start of TKI in 1 institutional cohort (13). A randomized trial studying postprogression erlotinib plus pemetrexed is ongoing, but eligibility is not limited to EGFR-mutant cancers, so the study may not accurately gauge the added value of continuing erlotinib with chemotherapy. A new randomized trial studying...
chemotherapy with or without erlotinib in EGFR-mutant lung cancers with acquired resistance is under development.

One challenge for trials studying acquired resistance to EGFR-TKI is that tumor tissue is not always available to confirm the presence of a sensitizing EGFR mutation. To ensure that these patients are not considered ineligible for trials in this setting, consensus clinical criteria for eligibility were recently published (14). These criteria note that patients who have had an objective response or durable stable disease (>6 months) to single-agent EGFR-TKI have a relatively high likelihood of harboring sensitizing mutations and could be considered eligible, in addition to cases with known EGFR mutations. However, the Jackman clinical criteria for acquired resistance have only a 66% positive predictive value for presence of an EGFR-sensitizing mutation, so molecular results should trump clinical criteria for eligibility at centers where mutation results are commonly available.

Although multiple clinical trials have studied therapies for acquired TKI resistance, no published results have been practice changing (Table 1). One limitation of these studies is varying definitions of acquired resistance and limited genotype data. Trials of single-agent, second-generation TKIs (discussed below) have been disappointing. Trials combining erlotinib or gefitinib with targeted agents, such as cetuximab, everolimus, and dasatinib, have not shown any objective responses (11, 15, 16). Lastly, though HSP90 inhibition showed some preclinical activity against xenograft models of TKI resistance (17), clinical trial results were discouraging (18). In the remainder of this review, we discuss emerging treatment strategies, focusing on those that could have the greatest promise in the future management of acquired resistance to EGFR-TKIs.

On the Horizon

T790M-mediated acquired resistance

At least half of EGFR-mutant tumors that develop TKI resistance will harbor a second mutation in cis with the primary EGFR mutation (Fig. 1; refs. 19–21). The most common resistance mutation results from a threonine-methionine substitution at position 790 (T790M). T790M is analogous to the ABL T315I and KIT T670I "gatekeeper" mutations observed in imatinib-resistant chronic myelogenous leukemia and GIST, respectively (22, 23). Whereas most EGFR mutations are vulnerable to TKI because they decrease the receptor’s affinity for its natural substrate ATP, the acquisition of T790M restores its affinity for ATP to wild-type levels, reducing the effect of TKI (24). Biochemical assays showed that T790M confers synergistic kinase activity and transformation potential when expressed concurrently with a TKI-sensitive mutation (25, 26). However, despite this enhanced oncogenicity, T790M-harboring tumors in patients can display surprisingly slow rates of growth (27).

Multiple groups have modeled acquired resistance in vitro using EGFR-mutant non–small cell lung carcinoma (NSCLC) cell lines and increasing levels of TKI exposure. The resultant TKI-resistant cells harbor T790M and/or MET amplification, validating this approach as a useful in vitro tool for the study of clinically relevant acquired resistance mechanisms (25, 28–30). We have used a similar approach with erlotinib and the irreversible EGFR inhibitor BIBW2992 to derive T790M-harboring PC9 cells (carrying an EGFR exon 19 deletion). We observed a distinct growth disadvantage in T790M-containing cells versus their TKI-sensitive parental counterparts (Fig. 2; ref. 27). These differential growth kinetics may be partly responsible for the "flare" and "re-response" phenomenon (discussed above) observed in some patients with acquired resistance, and they allow us to predict that resistant tumors are likely a mixed population of TKI-sensitive and TKI-resistant cells. Upon withdrawal of the selective pressure (TKI), previously arrested TKI-sensitive cells can now repopulate more quickly than resistant cells, and tumors may regain sensitivity to TKI. Through evolutionary modeling based on these growth kinetics (27), we predict clinical benefit to the continuation of TKI with chemotherapy in acquired resistance as discussed above.

In patients with acquired resistance, T790M has also been found to be associated with a more indolent phenotype. We did T790M testing on rebiopsy specimens from 93 patients with EGFR-mutant lung cancer and acquired resistance to TKI and found that those with T790M-mediated resistance had a better prognosis (31). Lack of T790M on rebiopsy was associated with a poorer performance status at progression, earlier development of new sites of metastatic disease, and shorter postprogression survival. The indolent nature of T790M-mediated resistance means that these patients can sometimes do well for months on continued single-agent TKI despite progression (32); the eventual development of more aggressive growth suggests a molecular "third hit," the biology of which requires further characterization. The favorable prognosis associated with presence of T790M on rebiopsy suggests a valuable clinical role for rebiopsies in the management of these patients.

Detecting T790M in resistant tumors can be challenging because of limited tissue availability. Most lung cancers progress in areas that are not easily amenable to tissue sampling. Ideally, sampling procedures should be minimally invasive to reduce the risk of biopsy-related complications, but they must still provide sufficient material for both morphologic and mutational analysis. In the Memorial Sloan-Kettering Cancer Center (MSKCC) experience (20), small tumor samples obtained through minimally invasive procedures provided sufficient tissue when adequately handled in the majority of cases. Cell blocks prepared from malignant effusions can be a useful alternative to core biopsy in some patients. Prospective histologic and/or cytologic assessment is imperative in all cases to ensure adequate tumor content above the sensitivity level of testing method. For bone metastases, aspirates are preferable to core biopsies, as these may provide higher tumor content and bypass the need for decalcification, which compromises DNA quality. Finally, the T790M mutation can also
### Table 1. Trials studying the efficacy of new therapies for acquired resistance to EGFR tyrosine kinase inhibitors

<table>
<thead>
<tr>
<th>Study published or in press</th>
<th>Treatment</th>
<th>Phase</th>
<th>Key eligibility criteria</th>
<th>Number of patients (% EGFRm)</th>
<th>Response rate, %</th>
<th>Efficacy, months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riely et al (11)</td>
<td>Gefitinib or erlotinib and everolimus (mTORi)</td>
<td>II</td>
<td>EGFRm and SD × 6 mo on TKI, or PR on TKI</td>
<td>13 (62)</td>
<td>0</td>
<td>3 (TTP)</td>
</tr>
<tr>
<td>Soria et al (59)</td>
<td>Everolimus (mTORi)</td>
<td>II</td>
<td>Prior TKI × 1 mo</td>
<td>43 (6)</td>
<td>2</td>
<td>2.7 (TTP)</td>
</tr>
<tr>
<td>Sequist et al (40)</td>
<td>Neratinib (2gen TKI)</td>
<td>II</td>
<td>EGFRm and PR/SD × 3 mo on TKI</td>
<td>91 (100)</td>
<td>3</td>
<td>3.6 (PFS)</td>
</tr>
<tr>
<td>Sequist et al (18)</td>
<td>IPI-504 (HSP90i)</td>
<td>II</td>
<td>EGFRm and progressed on TKI</td>
<td>28 (100)</td>
<td>4</td>
<td>NR</td>
</tr>
<tr>
<td>Janjigian et al (15)</td>
<td>Erlotinib and cetuximab (anti-EGFR mAb)</td>
<td>II</td>
<td>EGFRm, or PR/SD × 3 mo on TKI</td>
<td>19 (84)</td>
<td>0</td>
<td>3 (PFS)</td>
</tr>
<tr>
<td>Johnson et al (16)</td>
<td>Erlotinib and cetuximab (anti-EGFR mAb)</td>
<td>II</td>
<td>EGFRm, or PR/SD × 6 mo on TKI</td>
<td>12 (100)</td>
<td>Dasatinib: 0</td>
<td>0.5 (PFS)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Results presented</th>
<th>Treatment</th>
<th>Phase</th>
<th>Key eligibility criteria</th>
<th>Number of patients (% EGFRm)</th>
<th>Response rate, %</th>
<th>Efficacy, months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miller et al (60)</td>
<td>XL647 (2gen TKI)</td>
<td>II</td>
<td>EGFR T790M, or PR/SD × 3 mo on TKI</td>
<td>23 (NR)</td>
<td>One PR in 8 evaluable pts</td>
<td>NR</td>
</tr>
<tr>
<td>Campbell et al (41)</td>
<td>PF00299804 (2gen TKI)</td>
<td>II</td>
<td>KRAS wt and any prior TKI</td>
<td>66 (50)</td>
<td>5</td>
<td>4.5 (PFS) in EGFRm pts</td>
</tr>
<tr>
<td>Park et al (61)</td>
<td>PF00299804 (2gen TKI)</td>
<td>II</td>
<td>KRAS wt and any prior TKI</td>
<td>42 (21)</td>
<td>15; 2 PR in pts with EGFRm</td>
<td>3.6 (PFS)</td>
</tr>
<tr>
<td>Wakelee et al (49)</td>
<td>Erlotinib and XL184 (METi)</td>
<td>I and II</td>
<td>Unselected (most had prior TKI exposure)</td>
<td>54 (37)</td>
<td>8; 1 PR in an EGFRm pt</td>
<td>NR</td>
</tr>
<tr>
<td>Miller et al (42)</td>
<td>Afatinib (2gen TKI) vs. placebo</td>
<td>III</td>
<td>PR/SD × 3 mo on TKI</td>
<td>390 (NR)</td>
<td>Afatinib: 7</td>
<td>3.3 (PFS)</td>
</tr>
<tr>
<td>Janjigian et al (46)</td>
<td>Afatinib (2gen TKI) and cetuximab (anti-EGFR mAb)</td>
<td>I and II</td>
<td>EGFRm, or PR/SD × 6 mo on TKI</td>
<td>47 (96)</td>
<td>Placebo: 0.5</td>
<td>1.1 (PFS)</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Trials in progress or results pending</th>
<th>Treatment</th>
<th>Phase</th>
<th>Key eligibility criteria</th>
<th>Number of patients</th>
<th>Response rate</th>
<th>Efficacy, months</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT00503971</td>
<td>Erlotinib and vorinostat (HDACi)</td>
<td>I and II</td>
<td>EGFRm and prior TKI × 3 mo</td>
<td>~50 planned</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT00660816</td>
<td>Erlotinib and pemetrexed vs. pemetrexed alone</td>
<td>II</td>
<td>Prior TKI × 3 mo</td>
<td>~78 planned</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT00994123</td>
<td>Erlotinib and MM-121 (anti-ERBB3 mAb)</td>
<td>I and II</td>
<td>EGFRm, or PR × 3 mo on TKI</td>
<td>~43 planned</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT01121575</td>
<td>PF00299804 (2gen TKI) and PF02341066 (METi)</td>
<td>I and II</td>
<td>PR/SD × 6 mo on TKI</td>
<td>~70 planned</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT01259089</td>
<td>Erlotinib and AUY922 (HSP90i)</td>
<td>II</td>
<td>EGFRm, or PR/SD × 6 mo on TKI</td>
<td>~43 planned</td>
<td></td>
<td></td>
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</tbody>
</table>

Abbreviations: 2gen, second-generation; EGFRm, EGFR mutation positive; HDACi, histone deacetylase inhibitor; i, inhibitor; mAb, monoclonal antibody; NR, not reported; PR, partial response; pt, patient; SD, stable disease; TTP, time to progression; vs, versus; wt, wild-type.
be detected in blood, either in DNA from circulating tumor cells or free plasma DNA (33, 34), but this remains a largely investigational approach at this time.

The establishment of high-sensitivity testing methods is essential for accurate identification of the T790M mutation. Small tissue samples often preclude microdissection to enrich for tumor cells and, thus, may be vulnerable to false-negative results. It has also been found that T790M can be present in a very low proportion of EGFR alleles (30), and this allelic dilution can obscure detection of the T790M mutation. These factors mean that conventional Sanger sequencing is suboptimal for T790M testing in the acquired resistance setting. Higher sensitivity assays, such as high-performance liquid chromatography, mass spectrometry, and locked nucleic acid PCR techniques, have been proposed as alternative methods (20, 35, 36). With high-sensitivity assays, clinical laboratories must be unusually vigilant to guard against false-positive results. For example, the DxS real-time quantitative PCR assay for T790M was associated with false-positive results, leading the manufacturer to remove this mutation from its panel (37).

Given that T790M-mediated resistance is the most prevalent mechanism of acquired resistance, multiple trials in this setting have attempted to target T790M (Table 1). In preclinical studies, irreversible EGFR-TKIs have been shown to effectively inhibit T790M-mutant cancers (38, 39). However, a phase II trial of neratinib showed little activity against EGFR-mutant lung cancers with acquired resistance to TKI, likely because toxicity limited administration of necessary dosing to attain therapeutic drug concentrations (40). In a phase II trial of PF00299810 after failure of erlotinib, no responses were seen in 7 patients with T790M-mutant NSCLC (41). In a phase III study of afatinib (BIBW2992) versus placebo given to 585 patients who progressed after ≥12 weeks of TKI (42), overall survival (the primary endpoint) was equivalent in the 2 arms ($P = 0.74$), although progression-free survival (PFS) was significantly prolonged with afatinib (median 3.3 months versus 1.1 months, $P < 0.001$), with a 7% response rate. A new class of selective EGFR kinase inhibitors has recently shown potent activity against T790M-mutant cell lines and mouse models; however, these agents have not yet been tried in patients (43).

Another strategy for overcoming T790M-mediated resistance has been the addition of EGFR-targeted antibodies such as cetuximab, which may target EGFR in a different way than TKIs (44). No objective responses were seen when cetuximab was added to erlotinib in 13 patients with acquired resistance, but minor responses were seen in several patients (15). Mouse studies have examined what combination of EGFR-targeted therapies had maximal efficacy against T790M-harboring transgenic tumors and found that the combination of an irreversible TKI plus cetuximab effectively reduced tumor burden, whereas neither agent was effective alone (45). A phase Ib trial of afatinib plus cetuximab has now confirmed this activity in patients with acquired resistance, with a 40% objective response rate in 47 patients treated at the recommended phase II dose (46). These results validate preclinical data suggesting that many tumors are still addicted to the EGFR-signaling pathway, despite development of acquired resistance. To date, this has been the most promising approach in the treatment of patients with acquired resistance to EGFR-TKIs; PFS and overall survival results are eagerly awaited.
Other mechanisms of resistance

Another well-described mechanism of acquired TKI resistance relies upon increased signaling through MET, also a transmembrane receptor tyrosine kinase (29, 47). Coupling of MET to ERBB3 leads to sustained activation of the phosphoinositide 3-kinase (PI3K)/AKT signaling pathway, bypassing the inhibited EGFR (29). In the initial studies, MET amplification was reported in up to 22% of acquired resistance cases, independent of T790M status. Two more recent studies each tested 37 patients for MET amplification by FISH (Fig. 1). One study found high amplification (MET to CEP7 ratio > 3) in 1 case (3%) and lower level amplification (MET to CEP7 ratio 2–3) in 3 cases (in total, 11% amplified; ref. 20); the second study reported 2 cases (5%) of high amplification (21). The lower prevalence of MET amplification in these recent studies may be due to difficulty in identifying this genetic alteration in clinical specimens. The original studies used several methods to assess for amplification (29, 47), including array comparative genomic hybridization (aCGH), quantitative real-time PCR, and FISH. FISH is most widely available in clinical laboratories and requires only a single paraffin section. Importantly, both MET and EGFR are on chromosome 7, and polysomy of chromosome 7 is common in NSCLC, particularly in those samples harboring EGFR mutations (48). Studies are needed to determine how best to distinguish clinically meaningful MET amplification and copy number gain from underlying polysomy, both in EGFR-mutant and wild-type lung cancers.

The efficacy of MET inhibition in the treatment of acquired resistance is not well described. A phase I trial combining the MET inhibitor XL184 with erlotinib has been presented (49), and 1 patient with EGFR-mutant lung cancer had a confirmed partial response after progression on erlotinib alone. Another response was reported with the addition of ARQ197 to erlotinib for a patient who had progressed on the erlotinib arm of the first-line erlotinib with or without ARQ197 study (50); this patient harbored an EGFR mutation and elevated MET copy number. Recent data have suggested that a monoclonal antibody targeting MET (MetMAb), when combined with erlotinib, has activity against lung cancers with high MET protein expression (51), but neither this agent nor this biomarker have been assessed in cancers with acquired EGFR-TKI resistance.

A possible interaction between the EGFR/PI3K axis and DNA repair pathways represents a therapeutic opportunity that has not yet been pursued clinically. Cells with knockdown of the tumor suppressor gene PTEN, with constitutive PI3K activation, have been shown to have deficient homologous DNA repair and heightened sensitivity to PARP inhibition and cisplatin (52). Similar PI3K activation in EGFR-mutant lung cancer could explain the greater platinum sensitivity reported in this population compared
with EGFR wild-type cancers (3). Separately, erlotinib has been found to inhibit DNA repair in studies of breast cancer cell lines (53), an effect that could be potentiated in cells with deficient homologous DNA repair. Recent data have shown that patients with EGFR-mutant tumors and low BRCA1 levels gain a more durable benefit from erlotinib (median PFS = 27 months, P = 0.02; ref. 54). In light of these data, trials are in development to assess the clinical activity of PARP inhibition in EGFR-mutant lung cancers with acquired resistance.

A handful of EGFR-mutant lung cancers have been found to exhibit histology consistent with small cell lung cancer when biopsied after acquired resistance (20, 21, 55, 56). Five such cases were recently identified from 37 studied (14%); all had adenocarcinoma at their baseline biopsy, all maintained a sensitizing EGFR mutation at baseline and rebiopsy, and none of these resistant specimens harbored T790M or MET amplification. A lower prevalence was identified in the MSKCC experience, with 3 of 103 cancers with acquired resistance (3%) exhibiting either small cell or high-grade neuroendocrine carcinoma on rebiopsy (20). Interestingly, pulmonary alveolar cells have also been found to occasionally transform to a small cell morphology when loss of p53 and Rb1 is induced (57). Some have found that this histologic transformation is associated with sensitivity to platinum-etoposide combinations (21). Another morphologic change reported in some tumors with acquired resistance is epithelial-mesenchymal transition (21, 58), the therapeutic implications of which are unclear.

In conclusion, steady progress is being made in understanding the biology of acquired resistance to EGFR-TKI in EGFR-mutant lung cancer and leveraging these understandings into rationally designed targeted and combination therapies. We predict that the relatively favorable prognosis of lung cancers harboring EGFR-mutations means that patients with these cancers will gradually represent a larger proportion of the lung cancer population receiving treatment. As the prevalence of acquired resistance to EGFR-TKI grows, the importance of understanding the behavior and optimal management of these cancers becomes even more pressing. The most important initial steps remain clear: (1) widespread implementation of EGFR genotyping for lung adenocarcinoma; (2) development of a distinct management paradigm for these oncogene-addicted cancers; (3) improved utilization of rebiopsy tissue for molecular typing of resistance; and (4) genotype-driven trials of rationally targeted therapies for patients with acquired TKI resistance.

Disclosure of Potential Conflicts of Interest

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