Understanding Mechanisms of Resistance in the Epithelial Growth Factor Receptor in Non-Small Cell Lung Cancer and the Role of Biopsy at Progression

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Gefitinib • Erlotinib • Afatinib • Osimertinib • Non-small cell lung cancer • Treatment

ABSTRACT

Molecular profiling and the discovery of drugs that target specific activating mutations have allowed the personalization of treatment for non-small cell lung cancer (NSCLC). The epithelial growth factor receptor (EGFR) is frequently over-expressed and/or aberrantly activated in different cancers, including NSCLC. The most common activating mutations of EGFR in NSCLC fall within the tyrosine kinase-binding domain. Three oral EGFR tyrosine kinase inhibitors (TKIs) have been approved by the U.S. Food and Drug Administration (FDA) for first-line use in patients with EGFR mutation-positive NSCLC (exon 19 deletions or exon 21 [L858R] substitution mutations), as detected by an FDA-approved test. However, disease progression is common and is often the result of secondary mutations, of which the EGFR T790M mutation is the most prevalent. Few options were available upon progression until the introduction of osimertinib, a kinase inhibitor that targets the T790M mutation, which was recently approved for use in patients with metastatic EGFR T790M mutation-positive NSCLC, as detected by an FDA-approved test, who progressed on or after EGFR TKI therapy. With the introduction of osimertinib, outcomes can now be improved in select patients. Therefore, performing a biopsy at progression to determine the underlying molecular cause of the acquired resistance is important for the enabling of individualized options that may provide the greatest opportunity for improved outcomes. This review discusses the latest updates in molecular testing at progression and outlines treatment options for this difficult-to-treat population.

The Oncologist 2017;22:3–11

Implications for Practice: Although the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs)—gefitinib, erlotinib, and afatinib—have changed the treatment paradigm for non-small cell lung cancer among those with EGFR mutation positive disease, most patients experience progression after approximately 12 months of treatment. Until recently, options were limited for patients who progressed, but improvements in molecular profiling and the approval of osimertinib, which targets the resistance mutation T790M, afford the opportunity for improved outcomes in many patients with this mutation. This article explains the options available after progression on initial EGFR TKI therapy and the importance of molecular testing at progression in making treatment decisions.

INTRODUCTION

The clinical utility of using a single gene-based biomarker as a therapeutic focus for non-small cell lung cancer (NSCLC) was first realized with the 2004 discovery of mutations in the tyrosine kinase domain of the epithelial growth factor receptor (EGFR); this allowed identification of patients with greater sensitivity to small-molecule tyrosine kinase inhibitors (TKIs) [1–4]. This discovery, in turn, led to the subsequent regulatory approval in the United States of three oral EGFR TKIs: gefitinib, erlotinib, and afatinib. These agents are the National Comprehensive Cancer Network (NCCN)-recommended first-line therapies in patients with advanced or metastatic NSCLC harboring EGFR mutations (exon 19 deletions [ex19del] or exon 21 [L858R] substitution, as detected by a U.S. Food and Drug Administration [FDA]-approved test), based on the success of clinical trials in EGFR mutation-positive selected populations (Table 1) [1, 5–13]
Table 1. Overview of pivotal studies of EGFR TKI as first-line therapy in patients with EGFR-positive NSCLC

<table>
<thead>
<tr>
<th>Trial</th>
<th>Drug</th>
<th>Population</th>
<th>Patients, n</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPASSa [1, 6]</td>
<td>Gefitinib Carboplatin + paclitaxel</td>
<td>East Asian patients with advanced pulmonary adenocarcinoma</td>
<td>88 98</td>
<td>BICR assessed: Median PFS: 11 mo vs. 7 mo PFS HR: 0.54 (95% CI, 0.38–0.79) ORR: 67% (95% CI, 56%–77%) Median DoR: 10 mo vs. 6 mo</td>
</tr>
<tr>
<td>IFUM [10]</td>
<td>Gefitinib</td>
<td>White patients with EGFR-positive locally advanced or metastatic NSCLC</td>
<td>106</td>
<td>Median PFS: 9.7 mo (95% CI, 9–11 mo) ORR: 70% (95% CI, 61%–78%) Median OS: 19 mo (95% CI, 17%–NC; 27% maturity)</td>
</tr>
<tr>
<td>OPTIMAL [7]</td>
<td>Erlotinib Gemcitabine + carboplatin</td>
<td>Chinese patients with advanced or metastatic EGFR-positive NSCLC</td>
<td>82 72</td>
<td>Median PFS: 13 mo (95% CI, 11–17 mo) vs. 4.6 mo (95% CI, 4–5 mo) PFS HR: 0.16 (95% CI, 0.10–0.26; p &lt; .0001) ORR: no difference</td>
</tr>
<tr>
<td>EURTACb [11]</td>
<td>Erlotinib Cisplatin + docetaxel or cisplatin + gemcitabine</td>
<td>European patients with stage IIIB NSCLC with pleural effusion or stage IV NSCLC</td>
<td>86 87</td>
<td>Median PFS: 10 mo (95% CI, 8–12 mo) vs. 5 mo (95% CI, 5–6 mo) PFS HR: 0.37 (95% CI, 0.25–0.54; p &lt; .0001) OS: no difference</td>
</tr>
<tr>
<td>LUX-Lung 3 [12, 90]</td>
<td>Afatinib Cisplatin + pemetrexed</td>
<td>Global population of patients with advanced EGFR-positive NSCLC</td>
<td>229 111</td>
<td>Median PFS: 11 mo vs. 7 mo PFS HR: 0.58 (95% CI, 0.43–0.78; p = .001) OS: no difference</td>
</tr>
<tr>
<td>LUX-Lung 6 [13, 90]</td>
<td>Afatinib Cisplatin + pemetrexed</td>
<td>Asian patients with stage IIIB NSCLC with pleural effusion or stage IV NSCLC</td>
<td>242 122</td>
<td>Median PFS: 11 mo (95% CI, 10–14 mo) vs. 6 mo (95% CI, 5–7 mo) PFS HR: 0.28 (95% CI, 0.20–0.39; p &lt; .001) OS: no difference</td>
</tr>
</tbody>
</table>

Data derived from clinical trials included in the prescribing information for each product.
a Subset of EGFR mutation-positive patients.
b This trial was halted early because the primary endpoint was met at the preplanned interim analysis.
c No difference was shown in OS for the overall populations, but for patients with exon 19 deletions, ORR differed between afatinib and chemotherapy.

However, despite the notable efficacy achieved with EGFR TKIs, a majority of patients develop resistance after a median progression-free survival (PFS) of approximately 1 year (range, 8–14 months) [1, 7, 10–14].

MECHANISMS OF ACQUIRED RESISTANCE TO EGFR TKI THERAPY

There are several mechanisms of acquired resistance to EGFR TKIs (Fig. 1). The most common, encompassing approximately 60% of cases, is the result of a secondary point mutation in exon 20 of the EGFR gene, referred to as T790M [15–20]. The T790M mutation is thought to convey resistance to EGFR TKIs through different mechanisms, including steric hindrance, which is a reduction in binding of reversible TKIs; increased binding affinity for adenosine triphosphate; and increased phosphorylation levels, which reduce the potency of the EGFR TKIs [21–23]. A less common (5%–11%) cause of acquired resistance is amplification of the mesenchymal-epithelial transition (MET) gene, and tumors may harbor both the EGFR T790M mutation and MET amplification [15, 18, 24–27]. Another mechanism, occurring in approximately 3%–20% of NSCLC cases, is transformation to small cell undifferentiated carcinoma histology (small cell lung cancer [SCLC]) [15, 27, 28]. Amplification of the ERBB2 (HER2) gene has been found in 12%–13% of patients, and mutations in the PI3KCA gene have been seen in 0%–5% of patients [15, 23]. In anywhere from 18% to 30% of patients, the cause of resistance is unknown [15, 27].

EGFR TKI resistance mutations have been hypothesized to evolve through one of two avenues [29, 30]. The selection model suggests that a very small fraction of EGFR TKI-resistant clones is present before EGFR TKI therapy and that these clones proliferate while the sensitive clones are eradicated during treatment. The acquisition model proposes that tumor cells acquire new genetic or epigenetic defects as a result of EGFR TKI treatment by inducing a state of increased genetic and epigenetic instability. Evidence for the former includes the presence of both EGFR T790M clones may proliferate more slowly than in pretreatment tumor samples [29, 31, 32]. In addition, EGFR T790M clones may proliferate more slowly than EGFR clones with other mutations, which may allow them to escape the effects of EGFR TKI therapy [23, 33]. However, cell culture studies and clinical trials have found that mutations can develop from selective pressure with long term repeated exposure to EGFR TKIs, thereby overcoming the effects of the drugs; this is the more widely accepted mechanism of resistance [23, 27, 29]. Furthermore, acquired resistance may result from target gene modification or activation of alternative pathways, or “bypass tracks,” that provide a compensatory signaling pathway from that of the driver mutation, allowing for continued cell growth and proliferation [30]. MET amplification in
EGFR-mutant lung cancer was the first example of the bypass track resistance mechanism identified [30].

In contrast, transformation to SCLC is less clear but is thought to involve histologic changes that result in molecular and phenotypic characteristics of SCLC, such as loss of retinoblastoma that is universally observed [34]. In one study that examined this issue, all tumors that transformed to SCLC retained the original EGFR mutation, suggesting direct development from the primary cancer [27]. Thus, this transformation may involve a pluripotent population that becomes activated upon exposure to EGFR TKIs [34, 35]. Alternatively, the same cancer stem cells could differentiate into both NSCLC and SCLC, with adenocarcinoma initially predominating, or the presence of SCLC may have been overlooked in the original testing at diagnosis [35].

Progression can also result from inadequate drug exposure against the target protein despite tumor cells remaining sensitive to drug; this is termed “pharmacologic resistance” [30]. Pharmacologic resistance generally evolves from low adherence to the treatment regimen, drug-drug interactions, and/or pharmacokinetics that fail to evenly deliver the drug to all tumor-infiltrating body compartments [30].

**Molecular Testing upon Progression**

Current guidelines state that performing a local recurrence or metastasis biopsy at progression after EGFR TKI therapy is a reasonable course of action to determine the mechanism of acquired resistance [5], and molecular profiling at this stage is increasing in importance as new targeted therapies become available. Several studies have evaluated the clinical feasibility of biopsy upon progression [15, 18, 27, 36–39]. In a real-world study of 100 patients with NSCLC who progressed after first-line treatment (targeted therapy or chemotherapy), biopsy at progression was possible in 82% of patients; among those who underwent rebiopsy, the sample could be histologically analyzed in 94% of cases [36]. In a second study of 39 patients who underwent biopsy at progression, samples were sufficient for histopathologic or cytologic examination in almost 90% of cases [37]. Because both tumor and plasma EGFR mutation load decrease after chemotherapy, EGFR mutation testing should not be conducted too soon after chemotherapy in order to decrease the risk for inaccurate results [40].

The College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology (AMP) developed guidelines for molecular testing at diagnosis and progression, and they currently have open for comment a revised draft that incorporates the most recent data on sensitizing and resistance mutations in NSCLC, as well as new testing methods [41, 42]. The primary methods for acquiring tumor tissue samples in most patients who undergo biopsy at disease progression have historically been fine-needle aspiration and core biopsy with image guidance; surgical excision has generally not been needed [15, 18, 27, 36–39]. However, tumor specimens suitable for testing can also be procured by surgical resection, open biopsy, endoscopy, transthoracic needle biopsy, or thoracentesis [41]. Molecular testing is generally done on tissue samples that have been preserved as formalin-fixed, paraffin-embedded specimens, but testing can also be done on fresh, frozen, or alcohol-fixed specimens [41, 43]. The most commonly used method for determining mutation status of EGFR used to be direct sequencing (Sanger sequencing); however, this method has lower sensitivity, with a risk for contamination of the post-polymerase chain reaction (PCR) products; in addition, the method is not standardized among testing centers [44]. A more sensitive method than direct PCR sequencing is quantitative PCR or real-time PCR (RT-PCR; e.g., cobas EGFR Mutation Test v2; Roche Molecular Diagnostics, Pleasanton, CA, https://molecular.roche.com) that uses specific probes to amplify the DNA section to be sequenced. Other methods include commercial mutation screening assays that use multiplex PCR (e.g., MassARRAY system, Sequenom Inc., San Diego, CA, https://www.sequenom.com; SNAPSHOT Multiplex System, Thermo Fisher Scientific Life Sciences, Waltham, MA, http://www.thermofisher.com), which detect more than 50 point mutations, including EGFR T790M [5]. Next-generation sequencing (NGS), which allows massive parallel analysis of a very large number of DNA fragments, is also a valid method of detecting a variety of mutations and is increasingly being used [5].

Although a greater quantity of specimen is preferred, the laboratory performing the EGFR mutation testing ultimately determines the requirements for tumor content, fixation, and quality and may have validated methods for testing smaller specimens [41]. Nevertheless, small specimens increase the risk for false-negative results, as do samples with low tumor cell content. Immunohistochemistry (IHC) for EGFR protein overexpression and EGFR copy number analysis (by fluorescent in situ hybridization) should not be used because these are not predictive markers of susceptibility to EGFR TKIs [41].

Testing for means of acquired resistance is warranted when patients progress after EGFR TKI therapy. Pathologist review of biopsy specimens using hematoxylin and eosin staining is performed, along with neuroendocrine IHC with synaptophysin, chromogranin, and/or CD56, to determine whether transformation to SCLC has occurred [27]. In a study of 37 patients with drug-resistant NSCLC, of the 14% (n = 5) of patients who transformed to SCLC, all still harbored the original EGFR mutation, and 1 patient additionally acquired a PIK3CA mutation [27]. MET amplification can be identified through dual-color in situ...
hybridization assays and can also be detected by NGS [26, 45–47]. Both in situ hybridization and NGS are also used to detect HER2 amplification [48]. Commercial mutation screening tests that identify multiple point mutations are generally designed to detect PIK3CA alterations. NGS can also detect PIK3CA mutations, along with PIK3CA amplifications and alterations in other PIK3CA pathway genes [49, 50].

As with the initial biopsy and molecular testing at diagnosis, several factors should be considered in weighing the risks and benefits of testing at progression. Because lung biopsy is a fairly invasive procedure, there is a risk for complications. In a study of NSCLC patients who progressed while receiving chemotherapy or an EGFR TKI, 14% experienced a post-procedural complication, the most common being intrapulmonary hemorrhage (7%) and pneumothorax (6%); most cases of pneumothorax resolved spontaneously [38]. In the real-world study by Chouaid et al. (2014), of 82 patients, there were 1 case of pneumothorax that required chest drainage and 2 cases of hemoptysis that required minor prolongation of the hospital stay [36].

Misinterpretation of the biopsy results is also a concern because of intratumor (different areas of the same tumor with different genetic profiles) and intermetastatic (differences in genetic profiles between the primary tumor and metastases) heterogeneity of mutations [19, 51, 52]. The clinical implications of this heterogeneity are profound because the risk for false-negative or false-positive results regarding a given genomic marker may affect treatment decisions and therefore outcomes [51]. EGFR mutation heterogeneity may also reflect the use of an insensitive assay, which itself can produce erroneous results. Careful sampling and handling of specimens are critical to assist in maximizing tumor content and improving molecular testing results.

Less invasive methods for detecting mutations have been introduced to overcome the limitations of tissue biopsy. Such substitute sample types include circulating tumor cells (CTCs) and circulating cell-free tumor DNA (ctDNA), which are both isolated from blood. Although the term “liquid biopsy” is used for both tests, CTCs are cells released from the primary tumor as viable or apoptotic cells, whereas ctDNA is cell-free material released from CTCs or the primary tumor as DNA fragments [53, 54]. There is insufficient evidence to support the prognostic value of CTCs, and this method is not recommended in the revised CAP/JASLC/AMP guidelines in the setting of NSCLC [42]. However, many studies have shown the utility of using ctDNA to assess EGFR mutation status, and it is a clinically and analytically validated method approved by the FDA (e.g., cobas EGFR Mutation Test v2) [55–59]. The most recent study of ctDNA analysis at diagnosis found 94.3% concordance in identifying EGFR mutations between 1,033 tissue samples and 803 plasma samples from the same patients, with 65.7% sensitivity and 99.8% specificity [55]. A meta-analysis found that the overall sensitivity and specificity for blood (pooled plasma and serum) versus tissue testing in detecting EGFR mutations in all exons were 61% and 90%, respectively, and the concordance rate was 79% [60]. In addition, several recent studies have shown that the T790M mutation could effectively be detected by using plasma DNA in patients with NSCLC who progressed after EGFR TKI therapy; in one study, the T790M mutation was detected as early as 2–12 months before radiologic progression [61–63]. Additionally, a urine-based test is under development, and the positive percentage agreement for T790M status between urine and tissue was 83% in a preliminary study [64].

Although these tests hold great promise and are gaining strong use in the clinical trial and community settings, there are a few limitations. Perhaps the most critical is that few standard tests are approved for use in NSCLC [5, 53]. Tumor heterogeneity is another issue, with differences between different ctDNA samples taken from circulation and between a ctDNA sample and a sample from a biopsy of the primary tumor. It is difficult to control the various steps that occur between blood sampling and ctDNA evaluation, such as DNA extraction from the sample, quantification of amount recovered, and contamination from genomic DNA, and this variability can affect the quality and accuracy of the test [53]. Although false-positive results are rare, they are possible, given the small amounts of DNA normally available in blood samples as compared with tissue [63]. False negatives are also possible, and it has been suggested that one way to improve identification of true false negatives is to assess the presence of other EGFR-sensitizing mutations as an internal control for circulating tumor DNA. If they are present but T790M is not, then the result is likely a true negative; if none are present, then it is likely a false negative. In general, when there is a negative result, reflex testing of a tissue biopsy specimen to confirm T790M status may be warranted. Ongoing investigations are studying new methods, including more sensitive RT-PCR and digital PCR assays [63, 65]. In addition, plasma-based molecular testing is being investigated to monitor disease status and clinical response to therapy, which may assist in making more rapid and targeted treatment decisions [66]. Overall, liquid biopsies are a substantial advancement in managing patients with NSCLC.

**Treatment Options at Disease Progression**

Historically, few effective options were available for patients who progressed after EGFR TKI therapy. Thus, there was a significant unmet clinical need for agents that could provide benefit in the setting of progression after first-line TKI therapy. The fact that the T790M mutation is responsible for most cases of progression made it an ideal target to study [15–20]. The 2015 introduction to the worldwide market of osimertinib, a third-generation TKI that specifically targets the EGFR T790M mutation, represented a treatment breakthrough that offers an effective option for a difficult-to-treat population. The NCCN provides guidelines regarding progression after EGFR TKI and has incorporated osimertinib into their most current recommendations [5]. Treatment options are based on the mechanism of resistance to the initial EGFR TKI treatment and whether the patient is symptomatic or asymptomatic [5]. An overview of current recommendations for different progression

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**EGFR mutation heterogeneity may also reflect the use of an insensitive assay, which itself can produce erroneous results. Careful sampling and handling of specimens are critical to assist in maximizing tumor content and improving molecular testing results.**
types is presented in Figure 2, and participation in a clinical trial should always be considered.

**Focus on T790M**

Osimertinib is approved in the U.S., Europe, and elsewhere for the treatment of patients with metastatic EGFR T790M mutation-positive NSCLC, as detected by an FDA-approved test, who progressed on or after EGFR TKI therapy. It is a once-daily, oral, potent, irreversible inhibitor that is selective for both EGFR TKI-sensitizing mutations and the T790M resistance mutation [67, 68]. Because osimertinib is approved for use in patients with metastatic EGFR T790M mutation-positive NSCLC, a companion diagnostic test was developed. The cobas EGFR Mutation Test v2 uses tumor tissue or plasma to detect 42 mutations in exons 18–21 of the EGFR gene, including T790M.

Approval of osimertinib was based on two multicenter, single-arm, open-label trials in patients whose disease progressed after previous treatment with an EGFR TKI [67]. In a pooled analysis of patients (n = 411) who received the recommended dose of 80 mg/day, the blinded independent central review objective response rate (ORR) was 59% (95% confidence interval, 54%–64%), and 96% of patients with confirmed ORR had ongoing responses ranging from 1.1 to 5.6 months after a median follow-up duration of 4.2 months for the first study and 4.0 months for the second study [67].

The most common adverse events in patients treated with osimertinib 80 mg/day (n = 411) were diarrhea (42% overall; 1.0% grade ≥3) and rash (41% overall; 0.5% grade ≥3) [67]. The incidences of diarrhea and rash with osimertinib were not dissimilar, and were arguably improved, compared with those observed with gefitinib (diarrhea, 27%–47% overall and 3%–4% grade ≥3; rash, 37%–66% overall and 2%–3% grade ≥3), erlotinib (diarrhea, 26%–57% overall and 1%–5% grade ≥3; rash, 75%–80% overall and 2%–13% grade ≥3), and afatinib (diarrhea, 88%–95% overall and 5%–14% grade ≥3; rash, 81%–89% overall and 15%–16% grade ≥3) [1, 7, 10–13, 69]. Although further investigation is needed before osimertinib can be universally recommended for all patients who express the T790M mutation, these results suggest that osimertinib should be considered for these patients unless there are contraindications, including weighing the risks for adverse events.

**Other Alternatives**

The current NCCN guidelines (version 4.2016) recommend that for asymptomatic patients who are not candidates for osimertinib, first-line EGFR TKI therapy can be continued upon progression [5]. This is supported by a study in patients with EGFR TKI failure that stratified progression as dramatic (rapid progression of multiple target lesions, progressive involvement of nontarget lesions, symptom score of 2), gradual (no significant increase in tumor burden, symptom score ≤1), or local (progression due to solitary extracranial lesion or limitation in intracranial lesions, symptom score ≤1) [70]. In the gradual progression group, continuation with EGFR TKI therapy resulted in a longer overall survival (39.4 months) compared with switching to chemotherapy (17.8 months; p = .02) [70]. In a phase II study (ASPIRATION [Asian-Pacific trial of Tarceva as first-line therapy in EGFR mutation]) investigating continuation of erlotinib after progression with first-line erlotinib, an additional 3 months of PFS was observed, but results suggested that outcomes may have been better for patients who had better initial responses [71]. However, in the phase III gefitinib plus chemotherapy versus
placebo plus chemotherapy in EGFR mutation-positive non-small cell lung cancer after progression on first-line gefitinib (IMPRESS) trial of patients treated with first-line gefitinib plus cisplatin/pemetrexed, no benefit was observed with continuation of gefitinib plus the doublet chemotherapy regimen at progression versus continuing on chemotherapy alone [72]. Nevertheless, cessation of EGFR TKI therapy is associated with a “disease flare” that may develop in some (9%–23%) patients within a median of 7–8 days of discontinuation [73, 74]. Patients who experience a disease flare have a poorer prognosis than those who do not [73]. Although continuing targeted therapy beyond progression is common practice in other molecularly defined tumors (e.g., trastuzumab in HER2-positive breast cancer), the decision to continue EGFR TKI after acquired resistance in NSCLC should be made on an individual basis considering the nature of the progression, the tolerability of the current treatment regimen, and the patient’s preferences.

For patients who exhibit symptomatic brain metastases after first-line EGFR TKI therapy, the NCCN (version 4.2016) recommends considering local treatment while continuing EGFR TKI therapy for an isolated lesion or whole-brain radiotherapy plus continuation of EGFR TKI therapy for multiple lesions. It is thought that central nervous system relapse may be the result of poor penetration of the EGFR TKI into the brain as opposed to the emergence of resistant clones; thus, continuing EGFR TKI therapy could potentially maintain the systemic remission [75].

For symptomatic extracranial lesions, local therapy should be added to the EGFR TKI for an isolated lesion, whereas chemotherapy with or without an EGFR TKI should be considered for multiple systemic lesions [5]. Because systemic progression after EGFR TKI is thought to result from the emergence of EGFR TKI-resistant clones, which may be confirmed upon molecular testing at progression, switching to chemotherapy after progression is common [75, 76]. A reasonable approach for oligometastases is stereotactic body radiation or surgical resection with continuation of the EGFR TKI, provided the systemic remission is maintained [75].

Despite the risk for a disease flare with discontinuation of EGFR TKI therapy, some patients have experienced benefits with reintroduction of the same EGFR TKI after a drug holiday. In a study of 23 patients who took a median 7-month break from gefitinib, during which time they received cytotoxic anticancer therapy, retreatment with gefitinib resulted in a partial response in 22% of patients, with a disease control rate of 65% [77]. A similar study (n = 14) with erlotinib showed that after reintroduction of erlotinib following a median 9.5-month holiday, 36% of patients experienced a partial response and 50% had stable disease [78]. These were small studies, and further investigation in this area is needed.

Areas Under Investigation
Other agents that target T790M are under investigation. A recent study of olmutinib (HM61713), another third-generation EGFR TKI for patients who harbor the T790M mutation and who progressed on prior EGFR TKI therapy, demonstrated a confirmed ORR of 44% and a median duration of response of 8.3 months in this population [79]. Olmutinib is approved in South Korea for this indication. Interim results of a phase I trial of ASP8273, another agent that targets the T790M mutation, showed a disease control rate of 65% in patients with the T790M mutation and previous treatment with an EGFR TKI (n = 40) [80]. Nazartinib (EGF816) binds and inhibits the most common mutant forms of EGFR, including L858R and ex19del, as well as T790M, with minimal inhibition of wild-type EGFR [81]. Phase I study results in patients with T790M mutation positive NSCLC (n = 132) showed that nazartinib was well tolerated, and the confirmed ORR was 44% for a disease control rate of 91%; phase II and III studies are ongoing [82]. Another third-generation TKI, rociletinib, showed early promise in a phase I/II trial [83], but further analyses showed that efficacies were not as great as in initial reports, and almost half of patients experienced a serious adverse event [84]; therefore, development was subsequently halted.

For patients who progress through a pathway other than T790M mutation, research has been aimed at adding agents to the first-line EGFR TKI. MET amplification is the second most common cause of acquired resistance (approximately 5%–11% of cases), and investigations into adding a MET inhibitor with an EGFR TKI inhibitor are ongoing [15, 18, 26, 27]. A phase II study of capmatinib, a dual MET/vascular endothelial growth factor receptor 2 inhibitor, plus erlotinib in patients who progressed on EGFR TKI therapy demonstrated significant tumor growth rate reduction [85]. Studies (NCT01610336 and NCT01911507) of capmatinib plus either gefitinib or erlotinib in patients with acquired resistance to gefitinib or erlotinib and with MET amplification are underway, with results expected in 2017.

Immune checkpoint inhibitors (e.g., nivolumab and pembrolizumab) that target the programmed cell death protein-1 (PD-1) receptor to restore antitumor immunity have been approved for NSCLC, with response rates up to 20% in heavily pretreated patients [86, 87]. However, results have been less impressive in EGFR mutation-positive patients, with outcomes potentially favoring chemotherapy over PD-1 inhibitors [86–89]. This may be because EGFR-driven NSCLC tends to have a lower total mutational burden, and investigations have suggested that sensitivity to immune checkpoint inhibitors may be greater in tumors with high levels of somatic mutations [88]. In addition, the majority of EGFR mutation-positive tumors lack concurrent programmed death-ligand 1 (PD-L1) expression and have low levels of CD8 tumor infiltrating lymphocytes, which is not conducive to creating an inflammatory microenvironment and thereby may limit the effectiveness of PD-1 and PD-L1 inhibitors [89]. Further characterization of these agents in patients with EGFR mutation-positive disease is ongoing.

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CONCLUSION
Progression of EGFR-driven NSCLC after EGFR TKI therapy presents a significant challenge for clinicians. With more than half of cases of progression attributed to acquired resistance with the T790M mutation, osimertinib, a new agent that targets the EGFR T790M mutation, represents a significant advancement for this difficult-to-treat population and should be considered for patients who progress on first-line EGFR TKI therapy and who are found to harbor the T790M mutation.

Thus, performing molecular testing at progression is critically important to identify patients whom osimertinib would benefit. Furthermore, plasma-based testing for the T790M mutation is a viable option that may prevent the need for a metastasis biopsy in a significant subset of patients, making testing at progression easier to accomplish. As understanding of the underlying genomics of acquired resistance increases, further treatments that target these mechanisms of progression will be developed, coming closer to the promise of personalized medicine for optimal outcomes.

ACKNOWLEDGMENTS
We thank Meredith Rogers, M.S., C.M.P.P., of The Lockwood Group, for providing writing and editorial assistance funded by AstraZeneca.

AUTHOR CONTRIBUTIONS
Conception/Design: Mark A. Socinski, Liza C. Villaruz, Jeffrey Ross
Manuscript writing: Mark A. Socinski, Liza C. Villaruz, Jeffrey Ross
Final approval of manuscript: Mark A. Socinski, Liza C. Villaruz, Jeffrey Ross

DISCLOSURES
Mark A. Socinski: Celgene, Genentech, Novartis (H), Celgene, Genentech, Novartis, GlaxoSmithKline, Pfizer, Synta, Boehringer Ingelheim, Clovis (RF); Jeffrey Ross: AstraZeneca (C/A, H), Foundation Medicine, Inc. (E). The other author indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (O) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

REFERENCES
14. McClellan JD, Paez JG, Jänne PA et al. MET amplification leads to gefitinib resistance in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proc Natl Acad Sci USA 2007;104:20932–20937.


75. Oh J, Ban HJ, Kim KS et al. Retreatment of gefitinib in patients with non-small-cell lung cancer who previously controlled to gefitinib: A single-arm,


For Further Reading:

Implications for Practice:
Anti-epidermal growth factor receptor (EGFR) therapies remain controversial in unselected/wild-type EGFR squamous non-small cell lung cancer (NSCLC). Recent meta-analyses and squamous-only NSCLC EGFR-inhibition trials have overcome the power limitations of early trials and can now inform the management of squamous NSCLC with anti-EGFR therapies. With the approval of immunotherapeutics in the second-line management of squamous NSCLC, there exists an opportunity for novel combination therapies to improve efficacy and durable tumor control. The optimal timing and sequencing of available second-line targeted therapies, however, have yet to be defined. This review analyzes randomized clinical trials of EGFR inhibition in NSCLC and meta-analyses of these trials, with a focus on patients with squamous histology.