Management and Future Directions in Non-Small Cell Lung Cancer with Known Activating Mutations

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OVERVIEW

Lung cancer accounts for a quarter of all cancer deaths. Non-small cell lung cancer (NSCLC) is currently segregated by the presence of actionable driver oncogenes. This review will provide an overview of molecular subsets of lung cancer, including descriptions of the defining oncogenes (EGFR, ALK, KRAS, ROS1, RET, BRAF, ERBB2, NTRK1, FGFR, among others) and how these predict for response to small molecule tyrosine kinase inhibitors (TKIs) that are either clinically available or in clinical trial development for advanced NSCLC. Particular focus will be placed on subsets with EGFR mutated and ALK rearranged NSCLC. Somatic TKI-sensitizing EGFR mutations (such as exon 19 deletions and L858R substitutions) are the most robust predictive biomarker for symptom improvement, radiographic response, and increment in progression-free survival (PFS) when EGFR TKIs (gefitinib, erlotinib, and afatinib) are used for patients with advanced NSCLC. However, the palliative benefits that EGFR TKIs afford are limited by multiple biologic mechanisms of tumor adaptation/resistance (such as the EGFR-T790M mutation and oncogene bypass tracks), and future efforts toward delaying, preventing, and treating resistance are underway. Similar to EGFR mutations, ALK rearrangements exemplify an oncogene-driven NSCLC that can be effectively palliated with a precision TKI therapy (the multitargeted ALK/MET/ROS1 TKI crizotinib). When resistance to first-line crizotinib therapy occurs, multiple second generation ALK TKIs have demonstrated impressive rates of disease control in clinical trials, and these may modify long-term outcomes for patients with ALK-positive NSCLC. The development of TKIs for other oncogene-driven NSCLCs may expand the portfolio of precision therapies for this recalcitrant cancer.

Although lung cancer remains the leading cause of cancer-related mortality for both men and women worldwide, accounting alone for a quarter of all cancer deaths,1 recent years have seen substantial advances in the understanding and treatment of this recalcitrant malignancy. Principal among these has been the emergence of molecular subsets of lung cancer, defined by specific oncogenic aberrations. This evolving division of NSCLC into distinct actionable subtypes with mutually exclusive driver oncogenes has spurred the development of small molecule TKIs that are now either clinically available [for example, gefitinib, erlotinib, and afatinib for epidermal growth factor receptor (EGFR)–mutated NSCLC or crizotinib for anaplastic lymphoma kinase (ALK)–rearranged NSCLC] or in early- to late-stage development as therapies for advanced NSCLC.

With these advances have also come numerous challenges. First, from the deluge of data emerging from large-scale genomic studies, the subset of genetic alterations that drive and maintain tumorigenesis (i.e., driver mutations) must be elucidated. As an example, in a study involving whole-genome sequencing of 188 lung adenocarcinomas, more than 1,000 nonsynonymous somatic mutations were identified.2 To identify the drivers among these mutations, a number of bioinformatics methods have been developed.3 These include sequence-based approaches (assessing the functional effect mutation has on candidate driver gene and protein product), machine learning-based approaches (using algorithms to model existing knowledge of driver and passenger mutations to classify driver mutations), frequency-based approaches (differentiating driver and passenger mutations according to the number of mutations observed in candidate driver gene compared with expected number of functionally neutral passenger mutations), and pathway-based approaches (identifying driver mutations according to the projected effect a mutated gene would have on biologic pathways). Second, although lung cancer remains a common malignancy, a number of recently recognized molecularly defined subsets are rare (some representing ≤ 1% of cases). To identify these patients and connect them with targeted clinical trials requires a centralized effort. In the United States, the Lung Cancer Mutation Consortium (LCMC) has provided a platform for (1) the testing of multiple driver mutations in advanced lung adenocarcinoma, and (2) the conduct of clinical trials directed toward these disease subsets.4
Finally, more than ever, clinicians caring for patients with lung cancer require rapidly changing and expanding knowledge. Until recently, lung cancer treatment selection was based solely on the distinction between non-small cell and small cell histology. Now lung cancer comprises more than 10 different molecularly defined diseases, and the landscape is constantly changing. Maintaining awareness and understanding of the numerous driver mutations and associated treatment options requires ready access to a frequently updated source. Vanderbilt University’s MyCancerGenome (www.mycancergenome.org) is one example of a freely available online personalized cancer medicine resource for physicians, patients, and researchers.

To date, the majority of driver mutations in lung cancer have been disproportionately associated with clinicopathologic characteristics such as adenocarcinoma histology, female sex, and never- or oligo-smoking status. More recently, molecular profiling of lung squamous cell carcinomas has revealed targetable oncogenes in that molecular subset, among them PIK3CA mutations, FGFR amplification, and DDR2 mutations. Additionally, comprehensive genomic analyses of small cell lung cancer have revealed this malignancy to have one of the highest mutational rates of any cancer (7.4 mutations per million base pairs, compared with 6.3 for melanoma and 0.4 – 1.5 for various other solid and liquid tumors).

This article will provide an overview of molecular subsets of lung cancer (Fig. 1 and Table 1), including descriptions of the defining oncogenes, clinicopathologic features, and current and potential therapies. Particular focus will be placed on those subsets for which we currently have the greatest understanding, such as EGFR-mutated and ALK-rearranged NSCLC.

**EGFR Mutations in NSCLC**

**Frequencies and Types of EGFR Mutations in NSCLC**

EGFR mutations were initially reported in 2004 and currently define the most prevalent actionable genomically classified subgroup of NSCLC. The most frequent EGFR mutations are depicted in Fig. 2A. EGFR mutations are more frequent in tumors with adenocarcinoma histology, in never smokers with NSCLC, in women, and in East-Asian patients. However, the College of American Pathologists, International Association for the Study of Lung Cancer and Association for Molecular Pathology recommend rapid testing for EGFR mutations and ALK rearrangements in all patients with advanced-stage adenocarcinoma, regardless of sex, race, smoking history, or other clinical risk factors. The etiology (environmental or inherited) underlying the initial genomic insult that either leads to or selects for EGFR mutations in lung tissues remain elusive.

**EGFR Biology and How It Applies to EGFR Mutations in NSCLC**

EGFR is a member of the ErbB family of transmembrane receptor tyrosine kinases involved in signal transduction pathways that regulate proliferation and apoptosis. Activating EGFR mutations pertinent to NSCLC (such as exon 19 deletions and exon 21 L858R substitution) are spatially related to the adenosine triphosphate (ATP)–binding site of the kinase (Fig. 2B), resulting in mutated proteins that favor the active kinase and generate a wide “therapeutic window” in relation...
to wild-type EGFR that transform EGFR TKIs into resourceful compounds. In contrast, most exon 20 insertions result in EGFR TKI binding mode and affinity similar to that of wild-type EGFR and, therefore, do not sensitize to available EGFR TKIs.

EGFR mutations induce prosurvival and antiapoptotic signals through downstream targets, including the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT), mitogen-activated protein kinase (MAPK)/extracellular-signal-regulated kinase (ERK), and janus kinase (JAK)/signal transducer and activator of transcription (STAT) cascades; whose knotted networks make cells with EGFR mutations inheritably dependent on a functional EGFR for their survival (Fig. 2C). Inhibition of the mutated EGFR with TKIs leads to upregulation/activation of proapoptotic molecules that initiate the intrinsic mitochondrial apoptotic pathway (Fig. 2C). The most enhanced apoptotic signal is that of BCL2-like 11 (BIM). BIM expression or inherited BIM polymorphisms dictate—to a certain extent—the degree of cellular apoptosis induced by inhibition of EGFR. This oncogene-addicted model likely explains the initial profound clinical and radiographic results seen with EGFR TKIs in the majority of EGFR-mutated NSCLCs.

**TABLE 1. Driver Oncogenes and Their Inhibitors in Lung Adenocarcinomas and Squamous Cell Carcinomas**

<table>
<thead>
<tr>
<th>Molecular target/driver oncogene</th>
<th>Prevalence (%)</th>
<th>Approved therapies</th>
<th>Potential treatments in clinical trials</th>
<th>Potential treatments in preclinical development</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adenocarcinoma predominant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KRA$ mutations</td>
<td>25-30</td>
<td>None</td>
<td>Cytoxic chemotherapy + MEK inhibitors (selumetinib, trametinib and others), PI3K inhibitors, FAK inhibitors</td>
<td>KRA$ G12C inhibitors, KRA$ inhibitors, MEK and PI3K inhibitors, JAK/TKB1/IKK inhibitors</td>
</tr>
<tr>
<td>EGFR mutations</td>
<td>15-20</td>
<td>Gefitinib, erlotinib, icotinib, afatinib</td>
<td>CO1686, AZD9291, cetuximab + afatinib, Hsp90 inhibitors (AUY922 and others)</td>
<td>Mutation-specific EGFR inhibitors</td>
</tr>
<tr>
<td>ALK rearrangements</td>
<td>3-7</td>
<td>Crizotinib</td>
<td>Ceritinib, alectinib, PF-06463922, TSR-011, AP26113, ASP3026.X-396, Hsp90 inhibitors (AUY922 and others)</td>
<td>ALK inhibitors (more potent)</td>
</tr>
<tr>
<td>ROS1 rearrangements</td>
<td>1-3</td>
<td>None</td>
<td>Crizotinib, ceritinib, PF-06463922</td>
<td>ROS inhibitors, Hsp90 inhibitors</td>
</tr>
<tr>
<td>HER2 mutations</td>
<td>1-3</td>
<td>None</td>
<td>ERBB/HER2 inhibitors (neratinib and others), mTOR/PI3K inhibitors (temsirolimus and others)</td>
<td>HER2 inhibitors, Hsp90 inhibitors</td>
</tr>
<tr>
<td>BRAF mutations</td>
<td>1-3</td>
<td>None</td>
<td>Vemurafenib, dabrafenib, MEK inhibitors (selumetinib, trametinib and others), dasatinib</td>
<td>RAF inhibitors</td>
</tr>
<tr>
<td>RET rearrangements</td>
<td>1</td>
<td>None</td>
<td>Cabozantinib, vandetanib, sunitinib, ponatinib</td>
<td>RET inhibitors, Hsp90 inhibitors</td>
</tr>
<tr>
<td>MET amplification</td>
<td>1</td>
<td>None</td>
<td>Crizotinib, tivantinib, onartuzumab and other MET inhibitors</td>
<td>MET inhibitors</td>
</tr>
<tr>
<td>NRAS mutations</td>
<td>1</td>
<td>None</td>
<td>None</td>
<td>MEK inhibitors</td>
</tr>
<tr>
<td>PIK3CA mutations</td>
<td>1</td>
<td>None</td>
<td>PI3K inhibitors (buparlisib and others)</td>
<td>PI3K inhibitors, mTOR inhibitors</td>
</tr>
<tr>
<td>NTRK1 rearrangements</td>
<td>&lt;1</td>
<td>None</td>
<td>Crizotinib</td>
<td>TRKA inhibitors</td>
</tr>
<tr>
<td>AKT mutations</td>
<td>&lt;1</td>
<td>None</td>
<td>None</td>
<td>Pan-AKT inhibitors</td>
</tr>
<tr>
<td>MEK mutations</td>
<td>&lt;1</td>
<td>None</td>
<td>None</td>
<td>MEK inhibitors</td>
</tr>
<tr>
<td><strong>Squamous cell carcinoma predominant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGFR amplifications</td>
<td>20</td>
<td>None</td>
<td>FGFR inhibitors (AZD4547, JNJ-42756493, BGJ398, ponatinib and others)</td>
<td>FGFR inhibitors (more potent)</td>
</tr>
<tr>
<td>FGFR2/3/4 mutations and rearrangements</td>
<td>5-10</td>
<td>None</td>
<td>FGFR inhibitors (AZD4547, JNJ-42756493, BGJ398, ponatinib and others)</td>
<td>FGFR inhibitors (more potent)</td>
</tr>
<tr>
<td>PIK3CA mutations</td>
<td>5-10</td>
<td>None</td>
<td>PI3K inhibitors (buparlisib and others)</td>
<td>PI3K inhibitors, mTOR inhibitors</td>
</tr>
<tr>
<td>DDR2 mutations</td>
<td>3-5</td>
<td>None</td>
<td>Dasatinib</td>
<td>DDR2 inhibitors (more potent)</td>
</tr>
</tbody>
</table>

**CLINICAL DATA ON THE USE OF EGFR TKIS FOR EGFR-MUTATED NSCLC AND APPROVAL OF Gefitinib, Erlotinib, and Afatinib FOR TUMORS HARBOURING EGFR EXON 19 DELETIONS OR L858R**

Two oral reversible EGFR TKIs, gefitinib and erlotinib, reached regulatory approval for the treatment of unselected NSCLC in the early 2000s before the knowledge of EGFR mutations as a predictive biomarker. In EGFR wild-type tumors, radiographic response to EGFR inhibitors is generally below 5%, and clinical outcomes with EGFR inhibitors appears in-
In contrast to their limited activity in EGFR wild-type NSCLC, EGFR TKIs (gefitinib, erlotinib, icotinib, dacomitinib, and afatinib) lead to clinical/radiographic responses in the majority of patients with tumors harboring sensitizing EGFR-activating mutations when given as first, second, or subsequent lines of therapy. Numerous randomized trials have demonstrated that, in these cases, EGFR TKIs result in substantially higher response rates and longer PFS than does chemotherapy. Furthermore, quality of life parameters were substantially superior with EGFR TKIs. Table 2 summarizes the major assessments from these trials in relation to primary trial outcome (in all of them PFS) and key secondary outcomes (response rate [RR] and overall survival [OS]). Based on these data, gefitinib (since 2009 in Europe and many Asian countries), erlotinib (since 2009 in Europe and many Asian countries), erlotinib, dacomitinib, and afatinib can also lead to durable responses in tumors with EGFR-G719X (G719A, C, or S), exon 19 insertions, exon 20 A763_Y764insFQEA and afatinib (since 2013 in the United States) and afatinib (since 2013 in the United States) have approval labels that indicate that, in the first-line setting, their use should be restricted to the treatment of NSCLC harboring classic activating EGFR mutations. One well-conducted trial has demonstrated that the addition of platinum-doublet chemotherapy to erlotinib was not superior to erlotinib alone in the management of never/light smokers with EGFR-mutated NSCLC. Therefore the current clinical strategy is to use EGFR TKI as monotherapy in the front-line setting.

The use of EGFR TKIs in clinical practice for NSCLCs that harbor less-frequent EGFR TKI-sensitizing mutations is guided by retrospective series that have confirmed that gefitinib, erlotinib, dacomitinib, and afatinib can also lead to durable responses in tumors with EGFR-G719X (G719A, C, or S), exon 19 insertions, exon 20 A763_Y764insFQEA and...
TABLE 2. Representative Phase III Clinical Trials of Gefitinib (250 mg daily), Erlotinib (150 mg daily), and Afatinib (40 mg daily) versus Platinum-Doublet Chemotherapy as First-Line Therapy for Advanced EGFR-Mutated NSCLC

<table>
<thead>
<tr>
<th>Trial</th>
<th>Patients</th>
<th>Treatment</th>
<th>ORR</th>
<th>P</th>
<th>PFS (mos)</th>
<th>HR (P)</th>
<th>OS (mos)</th>
<th>HR (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPASS14</td>
<td>437</td>
<td>Gefitinib</td>
<td>94/132 (71.2%)</td>
<td>&lt;0.001</td>
<td>9.5</td>
<td>0.48 (&lt;0.001)</td>
<td>21.6</td>
<td>1 (0.99)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carboplatin-paclitaxel</td>
<td>61/129 (47.3%)</td>
<td></td>
<td>6.3</td>
<td></td>
<td>21.9</td>
<td></td>
</tr>
<tr>
<td>WJTOG 340599</td>
<td>177</td>
<td>Gefitinib</td>
<td>36/58 (62.1%)</td>
<td>&lt;0.001</td>
<td>9.2</td>
<td>0.49 (&lt;0.001)</td>
<td>30.9</td>
<td>1.64 (0.21)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carboplatin-docetaxel</td>
<td>19/59 (32.2%)</td>
<td></td>
<td>6.3</td>
<td></td>
<td>NR (0.31)</td>
<td></td>
</tr>
<tr>
<td>NEJ 00216</td>
<td>230</td>
<td>Gefitinib</td>
<td>84/114 (73.7%)</td>
<td>&lt;0.001</td>
<td>10.8</td>
<td>0.30 (&lt;0.001)</td>
<td>30.5</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carboplatin-paclitaxel</td>
<td>35/114 (30.7%)</td>
<td></td>
<td>5.4</td>
<td></td>
<td>23.6</td>
<td></td>
</tr>
<tr>
<td>OPTIMAL15</td>
<td>154</td>
<td>Erlotinib</td>
<td>68/82 (83%)</td>
<td>&lt;0.001</td>
<td>13.1</td>
<td>0.16 (&lt;0.001)</td>
<td>22.6</td>
<td>1.06 (0.68)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carboplatin-gemcitabine</td>
<td>26/72 (36%)</td>
<td></td>
<td>4.6</td>
<td></td>
<td>28.8</td>
<td></td>
</tr>
<tr>
<td>EURTAC16</td>
<td>173</td>
<td>Erlotinib</td>
<td>50/86 (58%)</td>
<td>&lt;0.001</td>
<td>9.7</td>
<td>0.37 (&lt;0.001)</td>
<td>19.3</td>
<td>1.04 (0.87)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Platinum-doublet</td>
<td>13/87 (15%)</td>
<td></td>
<td>5.2</td>
<td></td>
<td>19.5</td>
<td></td>
</tr>
<tr>
<td>LUX-Lung37</td>
<td>345</td>
<td>Afatinib</td>
<td>NR/NR (56%)</td>
<td>0.001</td>
<td>11.1</td>
<td>0.58 (&lt;0.001)</td>
<td>NR (25th percentile)</td>
<td>11.2 (0.60)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carboplatin-pemetrexed</td>
<td>NR/NR (23%)</td>
<td></td>
<td>6.9</td>
<td></td>
<td>NR (25th percentile)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: EGFR, epidermal growth factor receptor; NSCLC, non-small-cell lung cancer; ORR, overall response rate; PFS, progression-free survival; OS, overall survival; RECIST, response evaluation criteria in solid tumors v1.0; ref, reference; HR, hazard ratio; p, p-value; IPASS, Iressa Pan-Asia Study; WJTOG, West Japan Thoracic Oncology Group; EJ, North East Japan; EURTAC, European Tarceva versus Chemotherapy; NR, not reported; NRea, not reached.

In aggregate, these EGFR-mutated NSCLCs have been described to have RRs that exceed 50% and PFS greater than 5 months with gefitinib or erlotinib treatment.19 Exon 20 in-frame insertion mutations (the most common are D770_N771insSVD, V769_D770insASV, and H773_V774insX) are associated with primary resistance to EGFR TKIs.13,20 Patients with tumors harboring these nonsensitizing mutations should be offered cytotoxic chemotherapy or a clinical trial instead of approved EGFR TKIs.13

Dermatologic, mucosal, and gastrointestinal adverse events are the most common toxicities of EGFR TKIs. Most patients will develop a cutaneous rash that can vary from mild to severe.14,16,17 The irreversible EGFR inhibitor afatinib appears to be associated with higher incidence of cutaneous and oral (mucositis) adverse events than are reversible EGFR TKIs. The management of EGFR TKI–induced rash with systemic and topical agents, of diarrhea, of mucositis, and of other toxicities have been reviewed elsewhere.14,16,1721

ACQUIRED RESISTANCE TO EGFR INHIBITORS AND DEVELOPMENT OF NOVEL TREATMENT STRATEGIES

Acquired resistance to EGFR TKI therapy in patients with EGFR-mutated NSCLC is the main limitation of the prolonged palliative benefit of these drugs. The topic of acquired resistance to EGFR TKIs is too vast to be covered completely in this article, and the authors recommend recent in-depth reviews that address the issue.22-24

The most common (and first identified) mechanism of resistance is the gatekeeper EGFR-T790M mutation, which accounts for 50% to 65% of cases with acquired resistance to gefitinib or erlotinib.23,26 It is still unclear if EGFR-T790M–bearing clones already exist in the heterogeneous bulk of an EGFR-mutated TKI–sensitive NSCLC (and then are selected for during TKI therapy) or if this genomic change is acquired (and then selected for) during TKI therapy. The mutated EGFR-T790M confers resistance by reversing the sensitization conferred by primary activating mutations through alteration of the gatekeeper T790 residue in the ATP-binding site and subsequent reactivation of the PI3K/AKT, MAPK/ERK, and JAK/STAT pathways (Fig. 2D).12

Tumors with EGFR-T790M and acquired resistance to gefitinib and erlotinib are minimally responsive to the irreversible EGFR TKI afatinib (as a result of the inability to achieve clinical levels that can inhibit T790M and spare WT EGFR).27,28 Dual inhibition of EGFR with a monoclonal antibody and an irreversible EGFR TKI (the best-studied scheme is with cetuximab and afatinib) has demonstrated activity in preclinical models of EGFR-T790M and is the first treatment strategy to demonstrate responses in NSCLC patients with acquired resistance to EGFR TKIs (a RR of ~38% has been reported).24,27 However, this strategy is associated with significant—and at times intolerable—cutaneous adverse events. Clinical development of this strategy is ongoing and awaits randomized trials.

Direct inhibitors of EGFR-T790M have been developed using the scaffold of the EGFR-T790M alteration to identify compounds that selectively inhibit T790M-bearing mutations, when in association with L858R or exon 19 deletions, relative to wild-type EGFR.29-31 The covalent pyrimidine EGFR inhibitors initially identified are up to 100-fold more potent against EGFR-T790M and 100-fold less potent against wild-type EGFR than gefitinib or erlotinib, while maintaining activity against classic EGFR mutations. These data support an inhibitor that would have less cutaneous adverse effects through covalent targeting of T790M.
events of EGFR TKIs and be effective in most tumors with acquired resistance to gefitinib, erlotinib, and afatinib. The clinical candidates that are undergoing clinical development as covalent pyrimidine EGFR inhibitors include CO-1686 and AZD9291.29-31 Although both are only in phase I first-in-human clinical trial development (trial NCT01526928 for CO-1686 and NCT01802632 for AZD9291), early results have demonstrated responses (RR >50%) in the population of patients with recalcitrant tumors with EGFR-T790M and acquired resistance to EGFR TKIs.32 If these results are con-

FIG 3. Management of acquired resistance to tyrosine kinase inhibitor (TKI) therapy. A. Proposed algorithm for clinical management of acquired resistance to the EGFR TKIs gefitinib, erlotinib and afatinib in EGFR-mutated NSCLC. B. Proposed algorithm for clinical management of acquired resistance to the ALK/MET/ROS1 TKI crizotinib in ALK-rearranged NSCLC.
firmed in additional well-designed trials, this class of drugs may further catapult the beneficial effects obtained in the clinic with inhibition of EGFR in EGFR-mutated NSCLC.

Another mechanism of acquired resistance to EGFR TKIs is the development of bypass tracks through reactivation of signal transduction pathways such as the PI3K/AKT, MAPK/ERK, and JAK/STAT pathways (Fig. 2D). Validated oncogenes that participate in these bypass tracks include MET, ERBB2 (HER2), BRAF, and AXL, among others. In clinical specimens, the frequency of any one of these alterations seems to be below 10% to 15%. These alterations can be co-identified with EGFR-T790M. The complexity of this resistance circuitry, the overt toxicities of combination therapies, and the difficulties in diagnosing these alterations in clinical specimens have hindered the development of successful treatment strategies for these resistance cases. Another less well-understood alteration observed in clinical specimens with acquired resistance to gefitinib or erlotinib is histologic transformation to a poorly differentiated neuroendocrine phenotype (i.e., small-cell lung cancer), which may be mediated in part by the epithelial-to-mesenchymal transition (EMT) phenomenon. Some of these cases appear to respond to small-cell-type regimens such as platinum-etoposide.

In cases where EGFR TKI resistance manifests as worsening of limited sites of disease (Fig. 3A), local treatment options such as surgery or stereotactic radiation to those sites may allow for continuation of the EGFR TKI for a prolonged period (up to several months) before more widespread systemic progression. In many instances of asymptomatic radiographic progression, the use of continued EGFR TKI alone may continue to provide palliative disease control for substantial periods of time and may also prevent a “disease flare” phenomenon that has been described on discontinuation of EGFR TKI therapy. When symptomatic widespread progression occurs during EGFR TKI treatment, options include clinical trials (including those directed against any known resistance mechanism) as well as conventional cytotoxic chemotherapy. After progression, the effect of continued EGFR TKI therapy in combination with chemotherapy is not clear. One retrospective series comparing this strategy to the use of chemotherapy alone found an improvement in RR but not PFS or OS. Clinical trials of continuation of EGFR TKI therapy or not during initiation of chemotherapy after progression on an EGFR TKI are ongoing (one example is NCT01928160 of platinum-pemetrexed + erlotinib at time of acquired resistance to erlotinib).

### ALK REARRANGEMENTS IN NSCLC
#### Frequencies and Types of ALK Rearrangements in NSCLC

ALK rearrangements were first identified in NSCLC in 2007, primarily as fusions to echinoderm microtubule-like protein 4 (EML4) and other partners. These fusion proteins are found in approximately 3% to 7% of adenocarcinomas and 2% to 5% of all NSCLCs overall but have been identified primarily in lung adenocarcinomas, more frequently in younger patients, and in never or light smokers. Like EGFR, KRAS, ERBB2, and BRAF, they are generally found exclusive of other commonly known activating mutations.

Among EML4-ALK fusion proteins, several EML4 breakpoints have been described. In rare cases, other fusion partners have been described, including TFG and KIF5B. Most fusions link to ALK in frame with exon 20. The resulting protein carries a coiled-coil basic domain from the upstream fusion partner, which may facilitate activation of the ALK tyrosine kinase by promoting dimerization. Most of the identified EML4-ALK fusion proteins have been demonstrated to be oncogenic both in vitro and in vivo. EML4-ALK variant 1 expressed in mouse fibroblasts generates tumors in nude mice and results in multiple adenocarcinomas when expressed in the lungs of transgenic mice. Overexpression in cell lines results in both STAT3 and ERK activation. ALK inhibition results in downregulation of both AKT and ERK phosphorylation, resulting in apoptosis mediated by ERK-dependent BIM upregulation and STAT3-dependent survivin downregulation.

### PRECISION THERAPY FOR ALK REARRANGED NSCLC

Given increased awareness of the benefit of targeted therapy in oncogene-driven cancers such as EGFR-mutated NSCLC, the finding of ALK rearrangements in NSCLC prompted immediate screening of patients and enrollment onto a phase I trial of crizotinib, a MET, ROS1, and ALK inhibitor (and the only drug targeting ALK in development at the time). Following responses seen in two ALK-positive patients during dose-escalation, the study was amended to include an expanded cohort for ALK-positive lung cancer, onto which nearly 150 patients have been enrolled at the currently U.S. Food and Drug Administration (FDA)–approved dose of 250 mg twice daily. The overall response rate among these patients is 61%, with a disease control rate of 71% at 16 weeks and median PFS of 9.7 months. A dedicated phase II trial showed very similar results (Table 3). Overall, these findings are strikingly similar to the efficacy of EGFR TKIs against tumors harboring EGFR mutations, leading to the rapid approval of crizotinib for ALK-rearranged NSCLC in August 2011, only 3 years after the first patients were enrolled onto the phase I study. While a study comparing crizotinib to first-line chemotherapy is still ongoing, crizotinib showed clear superiority to second-line chemotherapy (docetaxel or pemetrexed) in a randomized trial (Table 3). Notably, the FDA approval of crizotinib did not restrict its use to a particular line of therapy.

### ACQUIRED RESISTANCE TO CRIZOTINIB

Unfortunately, as with EGFR TKI–targeted therapy, the duration of clinical benefit of crizotinib in ALK-positive NSCLC is limited. Progression can frequently occur in the central nervous system (CNS) and may not necessarily reflect the emergence of resistant intracellular pathways (Fig. 3B). CNS metastases were found at baseline assessment in more than 20% to 30% of patients entered into the PROFILE 1001, above.
1005, and 1007 trials; these findings may explain why this is a common site of subsequent progression. For such patients the combination of local CNS therapy and continued crizotinib may extend the duration of benefit.33

Systemic resistance mechanisms are variable. Unlike EGFR-mutated NSCLCs treated with EGFR TKIs, in which the majority of resistance occurs via the emergence of the gatekeeper EGFR-T790M mutation, multiple secondary ALK mutations have been identified.41,42,47,50 Whereas some of the identified resistance mutations, such as ALK-L1196M and G1269A, are found within the active site and likely interfere with crizotinib binding, other mutations (i.e., L1152 and C1156Y) do not have a clear mechanism for causing resistance to crizotinib.51 Studies of acquired resistance in vitro have identified these clinically identified mutations as well as other resistance mutations52,53; however, the identified mutations have variable resistance to crizotinib in vitro.

In many resistant cases, no ALK secondary mutation is identified. Other mechanisms of resistance include HER family pathway activation (including EGFR), ALK copy number gain, KIT amplification, and KARS mutations.47,50,51,53,54 The largest series published to date suggests that less than 25% of resistance occurs via acquisition of a secondary point mutation and up to 50% of resistance may occur via alternate tyrosine kinase activation.47 In both series of published interrogations of clinical samples, a substantial proportion of patients had no identified molecular mechanism of resistance.47,50,52

### SECOND-GENERATION ALK INHIBITORS

Although crizotinib is the first approved agent to target ALK-positive NSCLC, it is a relatively weak ALK TKI in NSCLC. The IC$_{50}$ of crizotinib in blocking cell proliferation and apoptosis in ALK-rearranged lymphoma cell lines is 25–50 nmol/L,55,56 but the IC$_{50}$ of crizotinib in NSCLC lines carrying an EML4-ALK fusion is considerably higher, ranging from 250–340 nmol/L.46 Several second-generation ALK TKIs are in clinical development including ceritinib (formerly LDK378; Novartis), alectinib (CH5424802/RO5424802; Chugai/Roche), AP26113 (Ariad), ASP3026 (Astellas), PF-06463922 (Pfizer), TSR-011 (Tesaro) and X-396 (Xcovery), all of which have more than 10-fold higher potency against ALK than does crizotinib. Of these, the first three have already shown promising activity in both patients who are crizotinib-naive and for whom crizotinib has failed (Table 3). Notably, response rates for all exceed the expected rate of ALK-dependent resistance mechanisms (secondary mutation or copy number gain). This suggests that ALK dependence may still exist to some degree in a majority of patients with ALK-positive NSCLC who develop resistance and that heightened potency may be a critical piece of the efficacy of these second-line agents (Fig. 3B). Importantly, secondary mutations convey variable resistance not only to crizotinib but also to all ALK inhibitors.

Beyond ALK inhibition, heat shock protein 90 (Hsp90) inhibition has also shown unique clinical efficacy in ALK-positive NSCLC. ALK is a client protein of Hsp90, which is effectively downregulated by Hsp90 inhibition to result in blockade of growth signaling, cell death, and tumor shrinkage in crizotinib-naïve and crizotinib-resistant settings.44,57-60 The various ALK rearrangements have varying degrees of protein stability, resulting in a spectrum of sensitivity to Hsp90 inhibition.61 Combination studies of Hsp90 inhibition and ALK inhibition, which may provide synergistic effects, are also underway.

Another key consideration for all ALK-directed therapies is control of CNS disease. Despite reports of crizotinib providing disease control within the CNS and prolonging duration of response, the brain remains a major site of disease progression. Ceritinib, alectinib, and AP26113 have all shown significant CNS activity both in the brain and cerebrospinal fluid (Table 3).

In summary, similar to EGFR mutations, ALK rearrangement define another example of an oncogene-driven NSCLC that can be effectively treated with targeted therapy (crizotinib and second-generation ALK TKIs). Several of these second-generation drugs have demonstrated impressive systemic plus CNS disease control and may modify long-term outcomes well beyond the effects of crizotinib for patients who are ALK-positive.

### TABLE 3. Representative Clinical Trials of Crizotinib (250 mg Twice Daily) and Other ALK Inhibitors for Advanced ALK Rearranged NSCLC

<table>
<thead>
<tr>
<th>Trial</th>
<th>Patients</th>
<th>Treatment</th>
<th>ORR (%)</th>
<th>PFS (months)</th>
<th>OS (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROFILE100146</td>
<td>143</td>
<td>Crizotinib</td>
<td>61</td>
<td>9.7</td>
<td>NR</td>
</tr>
<tr>
<td>PROFILE100542</td>
<td>259</td>
<td>Crizotinib</td>
<td>60</td>
<td>8</td>
<td>NR</td>
</tr>
<tr>
<td>Seto et al93</td>
<td>46</td>
<td>Alectinib</td>
<td>93.5</td>
<td>NYR</td>
<td>NYR</td>
</tr>
<tr>
<td>PROFILE100749</td>
<td>173</td>
<td>Crizotinib</td>
<td>65</td>
<td>7.7</td>
<td>20.3</td>
</tr>
<tr>
<td>PROFILE1007</td>
<td>99</td>
<td>Pemetrexed</td>
<td>29</td>
<td>4.2</td>
<td>22.8</td>
</tr>
<tr>
<td>PROFILE1007</td>
<td>74</td>
<td>Docetaxel</td>
<td>7</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Gadgeel et al94</td>
<td>47</td>
<td>Alectinib</td>
<td>59.5</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Shaw et al95</td>
<td>78</td>
<td>Ceritinib</td>
<td>60</td>
<td>8.6</td>
<td>NR</td>
</tr>
<tr>
<td>Camidge et al96</td>
<td>24</td>
<td>AP26113</td>
<td>62.5</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

Abbreviations: ALK, anaplastic lymphoma kinase; NR, not reported; NYR, not yet reached; a, reflects combined OS of docetaxel and pemetrexed arms; b, phase I trial, patients are those treated at > 400 mg twice daily; c, phase I/II trial, patients treated at 750 mg twice daily; d, phase I trial, patients treated at multiple doses.
OVERVIEW OF OTHER LUNG CANCER ONCOGENES

KRAS and NRAS

Kirsten rat sarcoma virus (KRAS) is a member of the family of Ras family of GTPases that promote cell growth and division through Ras/Raf signaling. These enzymes are activated by the binding of guanosine triphosphate (GTP) and are able to phosphorylate proteins downstream in the signaling cascade until GTP is converted to guanosine diphosphate (GDP) through a GTPase activity intrinsic to the Ras family enzymes. KRAS acquires oncogenic characteristics when mutations (primarily in exons 12 [80%), 13, and 61) arise that decrease its intrinsic GTPase activity. Consequently, an increased fraction of GTP- versus GDP-bound KRAS exists, causing marked upregulation of kinase activity and downstream growth and mitotic signaling. KRAS mutations occur in approximately 15% to 20% of NSCLC (25% to 30% of adenocarcinomas) and are associated with smoking and adenocarcinoma histology.62

To date, KRAS has been considered a non-druggable target that predicts poor response to standard and targeted therapies. In contrast to colorectal cancer, in NSCLC, KRAS mutations are not clearly associated with resistance to the anti-EGFR antibody cetuximab.63 High affinity binding to the GTP substrate has hindered the development of therapeutic agents that inhibit KRAS directly. In late 2013, initial reports of KRAS G12C inhibitors that bind to an allosteric site specific to the mutated molecule were published,64,65 but such drugs are likely years away from clinical use. Therapeutic strategies currently under clinical investigation focus primarily on interfering with signal transduction of downstream pathways such as PI3K, MEK, and focal adhesion kinase (FAK).66-68 The most clinically advanced strategy with some inkling of activity is the use of cytotoxic chemotherapies with MEK inhibitors. Docetaxel and selumetinib (formerly AZD6244, an oral MEK inhibitor) have been tested in preclinical models of KRAS-mutated NSCLC and in a phase II randomized trial that disclosed improved RR and PFS with docetaxel and selumetinib when compared to docetaxel alone.66-68 This combination is now undergoing evaluation in a registration clinical trial (SELECT-1, NCT01933932).

Other members of the RAS family include HRAS (formerly AZD6244, an oral MEK inhibitor) have been tested in preclinical models of KRAS-mutated NSCLC and in a phase II randomized trial that disclosed improved RR and PFS with docetaxel and selumetinib when compared to docetaxel alone.66-68 This combination is now undergoing evaluation in a registration clinical trial (SELECT-1, NCT01933932).

ROS1

Rearrangements of the receptor tyrosine kinase c-ros oncogene 1 (ROS1) appear to occur in approximately 1% to 2% of NSCLC. ROS1 is located on chromosome 6 and has a high degree of amino acid homology with ALK (49% within the kinase domain and 77% within the ATP-binding site).70 ROS1 fusions may be detected by fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR), and immunohistochemistry (IHC). Clinicopathologic features of ROS1-positive cases resemble those of ALK-rearranged NSCLC and include younger age, never smokers, and adenocarcinoma histology.71 Multietargeted ALK/MET/ROS1 inhibitors, such as crizotinib, have demonstrated efficacy in this population, with preliminary response rate of 57% and disease-control rate of 79% within an expansion of the crizotinib (at a dose of 250 mg twice daily) PROFILE 1001 study.72

AKT

The serine-threonine kinase AKT is a downstream mediator of PI3K signaling and is inhibited by the tumor suppressor PTEN. Mutations in AKT1 (E17K) occur in approximately 1% of NSCLC (predominantly squamous cases) and appear non-overlapping with other driver mutations.73 An oral pan-AKT inhibitor has completed phase I testing.74 The role of AKT1 mutations in the selection of therapy is not established.

BRAF

BRAF is a serine-threonine kinase in the MAP kinase pathway. BRAF mutations are found in 1% to 3% of NSCLC. These cases are predominantly adenocarcinoma and occur in former/current smokers and appear mutually exclusive to other driver mutations in TKI-naive cohorts of NSCLC. In contrast to melanoma (in which almost all BRAF mutations are V600E), a number of BRAF mutations have been identified in NSCLC: V600E (50%), G469A (40%), D594G (11%).75 A number of BRAF inhibitors, including sorafenib, vemurafenib, and dabrafenib (formerly GSK2118436, which has received fast track development by the FDA for lung cancer), are under clinical development. For tumors harboring non-V600E BRAF mutations, for which V600E-specific mutations are unlikely to be effective, inhibitors of downstream targets such as MEK are being explored.

DDR2

Discoidin death receptor 2 (DDR2) is a receptor tyrosine kinase activated by collagen that may signal via the SRC and signal transducer and activator of transcription (STAT) pathways. DDR2 mutations occur in 4% of squamous NSCLC and in less than 1% of nonsquamous cases.76 The multitargeted kinase inhibitor dasatinib inhibits DDR2 in preclinical models and cases of clinical response have been reported.77

FGFR

The fibroblast growth factor receptor (FGFR) family includes four receptor tyrosine kinases (FGFR1, FGFR2, FGFR3, FGFR4). FGFR1 amplifications occur in approximately 20% of squamous NSCLC but in less than 2% of adenocarcinomas.78 These amplifications, as well as mutations and rearrangement of other FGFR members, appear to confer in different degrees “addiction” to FGFR signaling, which regulates proliferation via the MAPK and PI3K pathways. Studies of FGFR inhibitors (such as AZD4547, INJ-42756493, BGJ398, and ponatinib) for NSCLC therapy are underway.
**HER2 (ERBB2)**

Human epidermal growth factor receptor 2 (HER2) is a tyrosine kinase in the HER (ErbB) family. It has no known ligand but is a dimerization partner for all other HER family members. In contrast to breast cancer and gastric cancer, in lung cancer HER2 amplification does not have a prognostic or predictive role. However, HER2 mutations (predominantly in-frame insertions in exon 20: the most common is A775_G776insYVMA) have been associated with some degree of disease control (unfortunately not as robust as observed in other oncogene-driven tumors treated with TKIs) to therapies with HER2 activity, such as the dual EGFR/HER2 inhibitors neratinib, dacomitinib, and afatinib.79 HER2 mutations are detected in 2% to 4% of NSCLC, most commonly in never smokers and adenocarcinoma histology.80 These mutations are non-overlapping with other common oncogenic mutations. Attempts to improve the effects of dual EGFR/HER2 TKIs by combining them to mTOR/PI3K (other downstream inhibitors) are underway (such as clinical trial NCT01118125 of neratinib + temsirolimus). Other HER2-directed therapies, such as trastuzumab, have also been reported to have some activity in case series but not directly tested in clinical trials.81

**MEK1**

MEK1 (also called MAP2K1) is a serine-threonine kinase involved in transduction of proliferation signals downstream of BRAF. MEK1 mutations (K57N, Q56P, D67N) occur in approximately 1% of NSCLC (predominantly adenocarcinoma).82 In preclinical models, MEK1 mutations result in constitutive signaling and sensitivity to MEK inhibitors, but clinical effects are not yet known.

**MET**

MET is a receptor tyrosine kinase activated by binding of its ligand hepatocyte growth factor (HGF), which is also known as scatter factor (SF). MET protein overexpression occurs in more than 25% of NSCLC and is associated with poor prognosis. Amplification of the MET gene occurs in 2% to 4% of both squamous and nonsquamous NSCLC (up to 20% of EGFR-mutated NSCLC with acquired resistance to EGFR inhibitors), is associated with poor prognosis, and may confer sensitivity to MET inhibitors (including the ALK/ROS1/MET inhibitor crizotinib).83 Rare MET point mutations have been described in the juxtamembrane and kinase domains.2 Ongoing clinical trials of MET TKIs (crizotinib, tivantinib, and others) or monoclonal antibodies (onartuzumab and others) are ongoing in both unselected and MET overexpressing/amplified tumors.

**NTRK1**

In 2013, oncogenic rearrangements of NTRK1, which encodes the high-affinity nerve growth factor (TRKA protein), were identified in tumor samples from 3/91 (3%) patients (never-smokers) with lung cancer without known oncogenic alterations.84 In preclinical models, kinase inhibitors with activity against TKRA—including ARRY-470, lestaurtinib (CEP-701), and crizotinib—induced cell-cycle arrest and inhibited proliferation. A single patient with an NTRK1 rearrangement has been treated with crizotinib (a relatively weak TKRA inhibitor), which resulted in a minor radiographic response lasting 3 months.

**PIK3CA**

The phosphoinositide-3-kinase, catalytic, α polypeptide (PIK3CA) gene encodes a catalytic subunit of PI3K, a lipid kinase involved in cell growth and proliferation via AKT-mTOR signaling. PIK3CA mutations (principally exon 9 and 20) occur in 1% to 3% of NSCLC. They have similar frequency in squamous and adenocarcinoma histologies, are seen in smokers and never smokers, and—in contrast to most driver mutations—may coexist with EGFR mutations. PIK3CA mutations account for up to 5% of EGFR-mutated NSCLC acquired resistance to EGFR inhibitors.85 The clinical effect of PI3K, AKT, and mTOR inhibitors in these cases is not known. Clinical trials of PI3K inhibitors, such as buparlisib, are ongoing in NSCLC.

**PTEN**

Phosphatase and TENsin homolog deleted on chromosome ten (PTEN) is a lipid/protein phosphatase that acts as a tumor suppressor by negatively regulating PI3K/AKT signaling. PTEN mutations occur in various exons in 4% to 8% of NSCLC, more commonly in smokers and squamous histology.86 Clinical trials of PI3K inhibitors are being planned for tumors with PTEN deficiency.

**RET**

An estimated 1% of lung cancers have rearrangements in the rearranged during transfection (RET) gene, which encodes the RET receptor tyrosine kinase.87 Two specific gene fusions (CCDC6-RET, KIF5B-RET) have been described. RET rearrangements appear limited to adenocarcinoma histology and are not seen concurrently with EGFR, ALK, or KRAS mutations. A number of commercially available multitargeted kinase inhibitors have RET activity (vandetanib, sorafenib, sunitinib, cabozantinib), but the efficacy of these agents in RET rearranged lung cancer has not been completely established. Cabozantinib has led to radiographic responses in an ongoing clinical trial.88

**CONCLUSION**

The initial division of some lung adenocarcinomas into distinct actionable subtypes with either EGFR mutation or ALK rearrangements has ushered the development of oral precision therapies that improve outcomes of patients with advanced NSCLC. The development of TKIs for other oncogene-driven NSCLCs may expand the portfolio of precision therapies for this recalcitrant cancer as we enter a new paradigm of molecular therapies in oncology. The narrative of how EGFR (gefitinib, erlotinib, and afatinib) and ALK TKIs (crizotinib) were developed and how tumors become
resistant to their effects have catapulted our understanding of the benefits and limitations of single-agent precision targeted therapies in NSCLC.

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