EGFR Mutations in Non-Small-Cell Lung Cancer
Find, Divide, and Conquer
Daniel Morgensztern, MD; Katerina Politi, PhD; Roy S. Herbst, MD, PhD

Mutations in the epidermal growth factor receptor (EGFR), most commonly deletions in exon 19 affecting the amino acid motif LREA (delE746-750) or substitution of arginine for leucine at position 858 (L858R) in exon 21, are present in approximately 17% of tumors in patients with pulmonary adenocarcinoma and lead to constitutive activation of the EGFR tyrosine kinase. These activating EGFR mutations are associated with high response rates to EGFR tyrosine kinase inhibitor (TKI) therapy, with combined results from 21 studies from 2004 to 2006 showing responses in 210 of 268 patients (78%). These response rates observed in patients with EGFR-mutant tumors receiving TKIs were much higher than what had been described in patients treated with standard platinum-doublet chemotherapy. Follow-up studies directly comparing the 2 treatment strategies in patients with advanced-stage non–small-cell lung cancer harboring activating EGFR mutations confirmed the superiority of EGFR TKI therapy to chemotherapy in this patient population as described herein.

In the Iressa Pan-Asia Study (IPASS), patients with previously untreated advanced-stage pulmonary adenocarcinoma who were never or light smokers were randomized to receive the EGFR TKI gefitinib or standard chemotherapy with carboplatin plus paclitaxel. Progression-free survival (PFS), the primary end point of the study, was significantly improved with gefitinib therapy among patients with EGFR-mutant tumors (hazard ratio [HR], 0.48; P < .001) but worse in those without EGFR mutations (HR, 2.85; P < .001). An updated analysis of the trial defined EGFR mutation as the strongest predictive biomarker for response to EGFR TKI therapy and median PFS. Subsequent studies in Asia comparing the first-generation EGFR TKIs gefitinib or erlotinib to chemotherapy in previously untreated patients with EGFR-mutant lung cancer showed similar results, with increased response rates and PFS for patients treated with targeted therapy. The European Tarceva vs Chemotherapy (EURTAC) trial was the first study comparing targeted therapy with an EGFR TKI to chemotherapy in a non-Asian population. In this multicenter phase 3 trial, 173 patients with activating EGFR mutations were randomized to receive erlotinib or a platinum-based chemotherapy doublet. Erlotinib therapy was associated with improved median PFS, the primary end point of the study, compared with chemotherapy (9.7 vs 5.2 months; HR, 0.37; P < .001). There were no significant differences in the median overall survival (OS) in these studies, most likely as a result of crossover at progression.

Circulating free DNA (cfDNA) consists of small fragments of DNA released into the bloodstream through several possible mechanisms including release of DNA from macrophages after they engulf apoptotic and necrotic cells, or spontaneous release of DNA fragments by cells from the primary cancer site, metastases, or circulating tumor cells. In patients with cancer, cfDNA carries tumor-related genetic and epigenetic alterations involved in cancer development, progression, and resistance to therapy. The EURTAC investigators had the foresight to envision the potential utility of detecting EGFR mutations in blood and included analysis of EGFR mutations in serum as a secondary end point for the study. In their first publication, the EURTAC investigators demonstrated that EGFR mutations could be detected in serum and, indeed, found that these were detected in specimens from 58 (53%) of 109 patients whose baseline blood samples were available for testing. In this issue of JAMA Oncology, the EURTAC authors provide an in-depth analysis of the feasibility of using cfDNA as a surrogate for tumor biopsy in the determination of EGFR mutation status and its effect on outcomes. Using multiplexed TaqMan assays performed in the presence of a peptide nucleic acid clamp designed to mask wild-type EGFR (Eurogentec), EGFR mutations were detected in 76 (78%) of the 97 samples tested. The higher mutation detection frequency in this updated study compared with what was originally reported by the authors (58%) is possibly due to differences in the sequencing assays used between the studies.

Karachaliou et al then went on to establish the relationship between EGFR mutations in the blood and outcomes. Importantly, similar to findings in the entire cohort of 173 patients, the 97 patients tested for cfDNA showed increased median PFS in patients treated with erlotinib compared with chemotherapy, whereas there were no significant differences in median OS. Among the 49 patients tested for cfDNA and treated with erlotinib, the median PFS was longer for patients harboring exon 19 deletions compared with those with L858R mutations either in tissue (14.7 vs 8.4 months; P = .07) or in cfDNA (15.5 vs 6.9 months; P = .02). Similarly, median OS was significantly higher in patients with exon 19 deletion mutations detected in tissue (24.9 vs 17.7 months; P = .006) and cfDNA (30.0 vs 13.7 months; P < .001) compared with those with L858R mutations.

Overall, these data from cfDNA are consistent with studies in tissue specimens. Indeed, although both exon 19 deletions and L858R mutations predict benefit from EGFR TKI therapy, early single-arm studies showed increased median PFS and OS for patients with tumors harboring exon 19 deletion mutations. In the EURTAC trial, for example, direct comparison of patients treated with erlotinib showed a signifi-
cant difference in median PFS and OS for patients with mutations found in both tissue and cfDNA, all favoring exon 19 deletions. The data are not as clear-cut with gefitinib, in which randomized trials comparing EGFR TKIs to chemotherapy showed improved overall response rates but no significant differences in median PFS according to EGFR mutation subtype among patients treated with gefitinib. However, updated results from the IPASS trial showed a nonsignificant increased benefit from gefitinib therapy compared with chemotherapy in patients with exon 19 deletions (Table).

More recent studies have further cemented the data indicating that exon 19 deletion mutations are associated with better outcomes. In the LUX-3 trial, the magnitude of the PFS benefit from afatinib therapy compared with cisplatin plus pemetrexed was significantly higher in patients with tumors harboring exon 19 deletions. Similar findings were observed in the LUX-6 trial comparing afatinib to chemotherapy with cisplatin plus gemcitabine. In the pooled analysis of the 2 LUX studies, the median OS was identical for afatinib and chemotherapy (28.2 months). Nevertheless, in a preplanned analysis, the median OS for patients with exon 19 deletions was significantly increased in patients treated with afatinib compared with chemotherapy (31.7 vs 20.7 months; HR, 0.59; P < .001). In contrast, afatinib was not associated with improvement in median OS for patients with L858R mutations (22.1 vs 26.9 months; HR, 1.25; P = .16). Moreover, the J025567 trial, comparing erlotinib alone or in combination with bevacizumab in patients with activating EGFR mutations, showed increased response rates and median PFS for the combination compared with erlotinib alone. The median PFS was numerically superior in patients with exon 19 deletions for both single-agent erlotinib and the combination therapy compared with L858R mutations. Furthermore, the benefit on the combination arm was statistically significant only in exon 19 deletion mutant cases. The mechanisms that underlie the observed differences between the 2 classes of EGFR mutations and outcome remain unclear.

In the updated EURTAC study, intriguingly, Karachaliou et al also found that for patients treated with erlotinib, the presence of an EGFR mutation in cfDNA was associated with decreased median OS (13.7 vs 27.7 months; P = .03) for L858R mutations and increased median OS for exon 19 deletions (30.0 vs 14.2 months; P = .02). The presence of EGFR mutations in cfDNA was not associated with differences in response rates. The negative prognostic impact of L858R mutations in cfDNA and tissue suggests that more aggressive treatment strategies should be evaluated in patients in which this mutation is detected at baseline. Such treatment strategies could include dual targeting of EGFR using afatinib plus cetuximab or higher doses of TKIs, possibly with third-generation drugs, which have less toxicity.

The current standard for detection of EGFR mutations is through biopsy and analysis of the tissue sample. Mutation detection in cfDNA has several potential advantages over analysis of tissue biopsies. First, tumor samples from patients with lung cancer are often limited, particularly when obtained from bronchial washings or fine-needle aspiration. These samples are used mainly for pathological diagnosis, frequently leaving insufficient numbers of cancer cells to be tested for an increasing number of targetable genomic abnormalities in addition to EGFR mutations, particularly ALK and ROS1 fusions, for which targeted therapy is associated with high response rates and prolonged benefit. Because of the risks of complications from procedures used to obtain a tissue diagnosis and the frequent requirement for prompt initiation of therapy in patients with metastatic lung disease, some patients with targetable gene abnormalities may not be exposed to highly effective drugs. One advantage of detecting cfDNA is that this “liquid biopsy” provides a noninvasive method for detection of genetic abnormalities without the risks from additional diagnostic procedures, particularly in patients for whom the pathological diagnosis has already been established. Second, with analysis of cfDNA, the serial evaluation of specimens becomes possible, which is rarely feasible through repeated biopsies. The detection of mutations in blood samples can serve as a surrogate for response to therapy and be used to monitor the emergence of drug resistance. In the case of EGFR-mutant lung cancer, the most common mechanism of acquired resistance to EGFR TKI therapy is the development of a second site mutation at position T790M in EGFR. In a study evaluating 6 patients with advanced cancer, serial whole-exome sequencing of plasma DNA revealed multiple changes in the genomic profile of treated tumors including increased mutation frequency and the development of new mutations. In this study, a patient with EGFR-mutant lung cancer was found to have the T790M mutation in cfDNA at the time of progression. Third, analysis of cfDNA allows the evaluation of the entire tumor landscape, including primary and metastatic lesions, whereas tissue biopsies provide a biased and limited evaluation of t tumor site. Because emerging data highlight substantial heterogeneity in lung cancers, the detection of mutations in cfDNA may allow for the evaluation of such heterogeneity and a determination of optimal treatment strategies based on the cadre of mutations identified. For example, in a study evaluating multiregional whole-exome or whole-genome sequencing of 25 tumor regions from 7 patients with resected lung cancer, all patients had spatial intratumor heterogeneity, defined as the presence of mutations in at least 1 but not

### Table. Differences in Outcomes According to Subtype of EGFR Mutation

<table>
<thead>
<tr>
<th>Study</th>
<th>Drug</th>
<th>Key Findings in Patients Treated With EGFR TKIs (Exon 19 Deletion vs L858R)</th>
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</thead>
<tbody>
<tr>
<td>IPASS</td>
<td>Gefitinib</td>
<td>ORR, 84.8% vs 60.9%; PFS HR for gefitinib vs chemotherapy, 0.38 (95% CI, 0.26-0.56) vs 0.55 (95% CI, 0.35-0.87)</td>
</tr>
<tr>
<td>NEJSSG</td>
<td>Gefitinib</td>
<td>ORR, 82.8% vs 67.3%; median PFS, 11.5 vs 10.5 mo (P = .90)</td>
</tr>
<tr>
<td>WJTOG3405</td>
<td>Gefitinib</td>
<td>Median PFS, 9.0 vs 9.6 mo (P = .68)</td>
</tr>
<tr>
<td>LUX-3</td>
<td>Afatinib</td>
<td>PFS HR for afatinib vs chemotherapy, 0.28 (0.18-0.44) vs 0.73 (0.46-117) (P = .01)</td>
</tr>
<tr>
<td>JO25567</td>
<td>Erlotinib with or without bevacizumab</td>
<td>PFS for erlotinib alone, 10.3 vs 7.1 mo; erlotinib + bevacizumab, 18 vs 13.9 mo; PFS was significantly prolonged for erlotinib + bevacizumab vs erlotinib alone for exon 19 deletions (HR, 0.41; P = .001) but not for L858R (HR, 0.67; P = .16)</td>
</tr>
<tr>
<td>EURTAC</td>
<td>Erlotinib</td>
<td>Median PFS, 14.7 vs 8.4 mo (P = .07); median OS, 24.9 vs 17.7 mo (P = .006)</td>
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</table>

Abbreviations: EGFR, epidermal growth factor receptor; EURTAC, European Tarceva vs Chemotherapy; HR, hazard ratio; IPASS, Iressa Pan-Asia Study; NEJSSG, North-East Japan Study Group; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; TKI, tyrosine kinase inhibitor; WJTOG, West Japan Thoracic Oncology Group.
all regions sequenced.22 In 1 of the patients, all biopsy sites showed an activating BRAF mutation whereas a single region also showed an activating PIK3CA mutation. It is possible that treatment with a BRAF inhibitor would induce tumor regression in all sites except for the one harboring the PIK3CA mutation, for which a combined therapy with an inhibitor of the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin (PI3K-Akt-mTOR) pathway could potentially be more effective. Tumor heterogeneity was also found in a similar study in which multi-region whole-exome sequencing was performed on 48 regions from 11 resected pulmonary adenocarcinomas.73

In conclusion, the updated EURTAC study10 demonstrates that mutations detected in cfDNA are prognostic and consistent with data obtained from tumor biopsies. Because treatment with EGFR TKIs has been associated with better outcomes for patients with tumors harboring exon 19 deletions compared with L858R mutations, future studies may need to address each of the 2 main mutations individually. More broadly, the potential benefits of liquid biopsies include a better evaluation of the tumor genome landscape with the identification of a potential set of targetable mutations and the serial noninvasive monitoring, which may allow the detection of additional mutations from emerging subclones, including those involved in the development of acquired resistance. Finally, the presence of specific mutations in cfDNA may help identify populations of patients who are likely to have worse (or better) outcomes and who may require alternative treatments.

**ARTICLE INFORMATION**

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**REFERENCES**


