

# Association of *ERBB* Mutations With Clinical Outcomes of Afatinib- or Erlotinib-Treated Patients With Lung Squamous Cell Carcinoma

## Secondary Analysis of the LUX-Lung 8 Randomized Clinical Trial

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**IMPORTANCE** Treatment choice for lung squamous cell carcinoma could be aided by identifying predictive biomarkers.

**OBJECTIVE** To assess whether patient outcomes in the LUX-Lung 8 trial were associated with *ERBB* gene family member aberrations in tumor specimens.

**DESIGN, SETTING, AND PARTICIPANTS** Ad hoc secondary analysis of the LUX-Lung 8 trial conducted at 183 centers in 23 countries from March 30, 2012, to January 30, 2014. Eligible patients had stage IIIB or IV lung squamous cell carcinoma with progressive disease after 4 or more cycles of platinum-based chemotherapy. Tumor genetic analysis (TGA) was performed using next-generation sequencing in a cohort enriched for patients with progression-free survival (PFS) of more than 2 months. Epidermal growth factor receptor (EGFR) expression levels were assessed by immunohistochemistry in a separate cohort of patients from the LUX-Lung 8 population. Associations of PFS and overall survival (OS) with *ERBB* gene alterations and EGFR expression levels were assessed. This analysis was conducted from February 26, 2015, to June 12, 2017.

**INTERVENTIONS** Patients were randomized 1:1 to treatment with afatinib dimaleate (40 mg/d; n = 398) or erlotinib hydrochloride (150 mg/d; n = 397).

**MAIN OUTCOMES AND MEASURES** Overall survival, PFS, pooled and individual *ERBB* gene mutations, *ERBB* copy number alterations, and EGFR expression.

**RESULTS** Tumor specimens from 245 patients were eligible for next-generation sequencing (TGA subset: 132 patients treated with afatinib; 113 patients treated with erlotinib). In this population, outcomes were improved with afatinib vs erlotinib treatment (PFS: median, 3.5 vs 2.5 months; hazard ratio [HR], 0.69; 95% CI, 0.51-0.92;  $P = .01$ ; OS: median, 8.4 vs 6.6 months; HR, 0.81; 95% CI, 0.62-1.05;  $P = .12$ ). Of 245 patients in the TGA subset, 53 (21.6%) had tumors with 1 or more *ERBB* mutations. Among afatinib-treated patients, PFS (median, 4.9 vs 3.0 months; HR, 0.62; 95% CI, 0.37-1.02;  $P = .06$ ) and OS (median, 10.6 vs 8.1 months; HR, 0.75; 95% CI, 0.47-1.17;  $P = .21$ ) were longer among those with *ERBB* mutation-positive disease than among those without. The presence of *HER2* mutations was associated with favorable PFS and OS following afatinib vs erlotinib treatment. There was no apparent association between copy number alteration or EGFR expression level and outcome.

**CONCLUSIONS AND RELEVANCE** Next-generation sequencing may help identify patients with lung squamous cell carcinoma who would derive additional benefit from treatment with afatinib. The role of *ERBB* mutations, particularly *HER2* mutations, as predictive biomarkers for afatinib treatment in this setting warrants further evaluation.

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Squamous cell carcinoma (SqCC) of the lung is one of the most genetically complex and difficult-to-treat cancers.<sup>1</sup> Until recently, platinum doublet chemotherapy was the first-line treatment of choice for most patients with lung SqCC, and second-line treatment options were also limited, with erlotinib hydrochloride (an epidermal growth factor receptor [EGFR] tyrosine kinase inhibitor) and docetaxel as the only approved options.<sup>2</sup> Recently, several new agents have been approved for patients with lung SqCC, including the EGFR monoclonal antibody necitumumab (combined with standard first-line chemotherapy)<sup>3</sup> and the immune checkpoint inhibitor pembrolizumab (in tumors with high expression levels of programmed cell death ligand 1 [PD-L1])<sup>4</sup> as first-line treatments. The immune checkpoint inhibitors nivolumab,<sup>5</sup> pembrolizumab,<sup>6</sup> and atezolizumab<sup>7</sup>; the anti-vascular endothelial growth factor receptor 2 antibody ramucirumab (combined with docetaxel)<sup>8</sup>; and the ERBB family blocker afatinib have been approved as second-line treatments.<sup>9</sup> This expansion of the armamentarium invites questions regarding optimal treatment of lung SqCC, which could be addressed by identifying biomarkers that may predict outcomes.

Afatinib dimaleate was approved as a second-line treatment of lung SqCC based on the phase 3 LUX-Lung 8 randomized clinical trial, which compared treatment with afatinib vs erlotinib following progression during and after chemotherapy.<sup>9</sup> Compared with erlotinib therapy, treatment with afatinib significantly improved overall survival (OS; median, 7.9 vs 6.8 months; hazard ratio [HR]; 0.81; 95% CI, 0.69-0.95;  $P = .008$ ) and progression-free survival (PFS; median, 2.6 vs 1.9 months; HR, 0.81; 95% CI, 0.69-0.96;  $P = .01$ ). The tolerability profile was similar in both treatment arms and was consistent with prior experience.<sup>9</sup>

To investigate whether the efficacy of afatinib in lung SqCC varied depending on the molecular characteristics of tumors, we conducted a comprehensive genetic analysis using Foundation Medicine FoundationOne next-generation sequencing (NGS) in a cohort of 245 patients who were included in the LUX-Lung 8 trial. The aims were to (1) assess the genetic characteristics of SqCC tumors in this cohort; (2) identify individual genes that are commonly mutated in this cohort and assess outcomes with respect to these genes; and (3) characterize and calculate the cumulative mutation frequency in the ERBB family of genes, assessing outcomes among patients with tumors with or without such mutations. Previous studies have reported mutation frequencies in EGFR, HER2, HER3, and HER4 of approximately 1% to 3%, 4%, 1% to 2%, and 8%, respectively.<sup>10-13</sup> Given that afatinib irreversibly inhibits signaling from all homodimers and heterodimers of the ERBB family and that these receptors cooperate via interconnected intracellular pathways to regulate cellular proliferation,<sup>14</sup> it is possible that genetic aberrations within the family might identify a subgroup of patients who could particularly benefit from afatinib treatment. Therefore, analysis of ERBB/HER family aberrations was prespecified in the present analysis. Furthermore, given that up to 80% of lung SqCC tumors overexpress EGFR<sup>15</sup> and that EGFR, HER2, and HER3 amplifications

## Key Points

**Question** What is the association between ERBB family mutations and outcomes of patients with lung squamous cell carcinoma who received treatment with afatinib dimaleate or erlotinib hydrochloride?

**Findings** In this secondary analysis of the LUX-Lung 8 trial, of 245 clinically selected patients, 21.6% had tumors with at least 1 ERBB mutation. Although progression-free survival and overall survival were improved with afatinib vs erlotinib treatment among patients with ERBB wild-type tumors, this was more pronounced among patients with tumors having at least 1 ERBB family mutation, with the largest benefits observed among those with HER2 mutations.

**Meaning** Mutations of ERBB, particularly HER2 mutations, may be used as a biomarker to identify patients with lung squamous cell carcinoma who would derive additional benefit from afatinib.

have been reported,<sup>10,13</sup> we assessed outcomes based on EGFR expression levels and ERBB family copy number alterations (CNAs).

## Methods

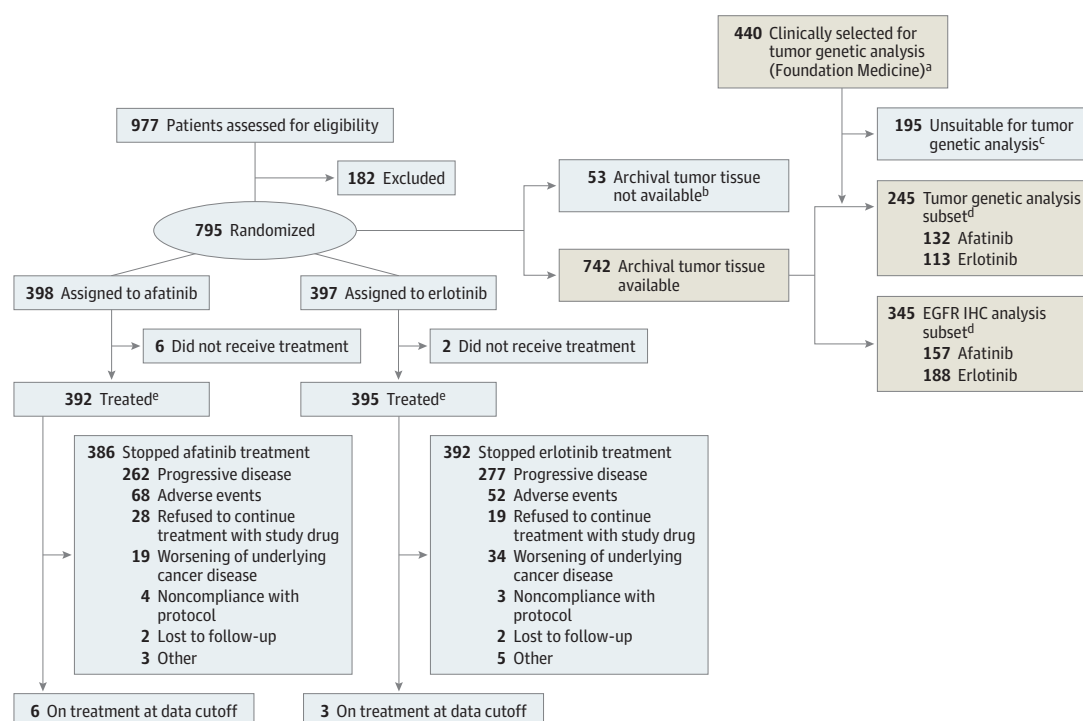
### Study, Design, and Participants

The design of the LUX-Lung 8 trial has been previously described.<sup>9</sup> In brief, patients had stage IIIB or IV non-small cell lung cancer (NSCLC) of squamous (including mixed) histology and had progressed during or after 4 or more cycles of platinum-based doublet chemotherapy as a first-line treatment. The trial included 795 patients were randomized (1:1) to treatment with afatinib (40 mg/d; 398 patients) or erlotinib (150 mg/d; 397 patients), and randomization was stratified by race/ethnicity (East Asian vs non-East Asian origin) to eliminate any potential bias in EGFR mutation frequency. The primary end point was PFS (independent review). The key secondary end point was OS. Tumor specimens and blood samples for exploratory biomarker analysis were required at study entry. The study was conducted in accordance with the Declaration of Helsinki,<sup>16</sup> Good Clinical Practice guidelines, and applicable region-specific regulatory requirements and was approved by independent ethics committees at each center. All patients provided written informed consent. The present study is an ad hoc, retrospective secondary analysis of the LUX-Lung 8 trial. Patients included in LUX-Lung 8 were required to have archival tissue available for investigational assessment of tumor biomarkers. Therefore, collection of tumor samples was preplanned.

### Tumor Genetic Analysis

Formalin-fixed, paraffin-embedded tumor specimens were submitted to Foundation Medicine for genetic analysis. The DNA was extracted from sections (40- $\mu$ m thick) of these specimens obtained from 245 patients. Next-generation sequencing was performed on hybridization-captured, adaptor ligation-based libraries for 4557 coding exons of 287 cancer-related genes, plus 47 introns from 19 genes frequently rearranged in

Figure 1. Patient Disposition



EGFR indicates epidermal growth factor receptor; IHC, immunohistochemistry.

<sup>a</sup> Some specimens requested back by the sites; some patients randomized prior to discovery of inadequate tissue availability.

<sup>b</sup> Specimens enriched for those with progression-free survival of 2 or more months.

<sup>c</sup> Pathology laboratories unable to provide all tissue requested; specimens contained insufficient tumor content.

<sup>d</sup> Eleven patients included in both the tumor genetic analysis and the EGFR IHC subsets.

<sup>e</sup> Received at least 1 dose of study drug.

cancer, and 3549 polymorphisms located throughout the genome to detect mutations (single-nucleotide variants), CNAs, and rearrangements. The NGS-based clinical cancer gene assay has been published previously, and the assay performance has been validated.<sup>17</sup> The Foundation Medicine T7 assay used in this study is able to detect mutations in all 4 ERBB receptors (EGFR, HER2, HER3, and HER4), unlike more commonly used circulating tumor DNA platforms, such as Foundation ACT (which detects EGFR alterations only) or Guardant360 (which measures EGFR and HER2 alterations).

The specimens for tumor genetic analysis (TGA) were retrospectively selected and enriched for patients with PFS of more than 2 months (149 of 245 patients [60.8%] with specimens used for TGA had PFS of more than 2 months vs 341 of 795 patients [42.9%] in the overall LUX-Lung 8 population) to ensure that the data set reflected a range of responsiveness to EGFR-targeted tyrosine kinase inhibitors (eAppendix in the Supplement).

### EGFR Immunohistochemistry

The expression of EGFR was assessed using immunohistochemistry (IHC) with an EGFR pharmDx kit (Dako). Two separate criteria for EGFR positivity, based on definitions from previous studies,<sup>15,18</sup> were used. For the first criterion, a tumor was considered positive for EGFR if at least 10% of the tumor cells showed staining of any intensity. For the second criterion,

a tumor was considered positive for EGFR if the H-score was 200 or greater when using the H-score method with magnification rule, which assigns a score from 0 to 300 to tumor specimens depending on the percentage of positive cells and the intensity of staining.<sup>15</sup>

### Statistical Analysis

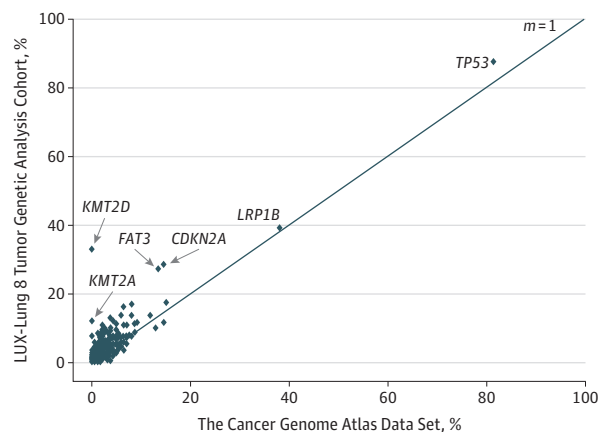
The OS and PFS were calculated using the Kaplan-Meier method. A Cox proportional hazards regression model was used to calculate HRs and 95% CIs. All analyses were conducted with SAS, version 9.2 (SAS Institute Inc). Two-sided  $P < .05$  was considered statistically significant.

## Results

### Patients

The LUX-Lung 8 trial was conducted at 183 centers in 23 countries from March 30, 2012, to January 30, 2014. In total, 977 patients were enrolled and 795 patients were randomized, with 398 in the afatinib arm and 397 in the erlotinib arm (Figure 1). Among the 742 patients with archival tumor tissue specimens available, 440 specimens were selected for TGA (PFS >2 months, 320 specimens; PFS ≤2 months, 120 specimens). Of these specimens, 195 were ineligible for NGS; thus, the TGA

Figure 2. Comparison of Mutation Allele Frequencies in the LUX-Lung 8 Tumor Genetic Analysis Cohort and in The Cancer Genome Atlas Data Set



The  $m = 1$  line shows the theoretical line on which all points would lie if the frequencies in both studies were identical.

subset consisted of 245 patients. Tumor specimens for IHC analysis were available for 345 patients from the LUX-Lung 8 population. Apart from 11 patients, the TGA subset and the IHC subset represented mutually exclusive populations. Blood samples from 675 patients were assessed using VeriStrat; the details for this analysis are published elsewhere.<sup>19</sup> Tumor genetic analysis, IHC, and VeriStrat analysis were conducted from February 26, 2015, to June 12, 2017.

Baseline demographic and clinical characteristics were similar in the TGA and IHC cohorts and the overall LUX-Lung 8 population (eTable 1 in the Supplement). Current or ex-smokers made up 224 (91.4%) of the TGA cohort and 318 (92.2%) of the IHC cohort; 197 (80.4%) of the TGA cohort and 246 (71.3%) of the IHC cohort were of non-East Asian origin.

### Tumor Genetic Analysis

Figure 2 correlates the distribution of genetic aberrations detected in the TGA cohort with the findings previously reported by The Cancer Genome Atlas (TCGA) Research Network, which analyzed tumor specimens from 178 patients with lung SqCC.<sup>12</sup> Overall, the genetic characteristics of SqCC tumors in the TGA cohort were similar to those in the TCGA analysis, although the mutation frequency of certain genes, including *KMT2A*, *KMT2D*, *FAT3*, and *CDKN2A*, was higher than that in the TCGA study. The most frequently observed genetic aberrations in the TGA cohort (245 patients) were somatic mutations in *TP53* (214 patients [87.3%]), *LRP1B* (96 patients [39.2%]), *KMT2D* (81 patients [33.1%]), *CDKN2A* (70 patients [28.6%]), and *FAT3* (67 patients [27.3%]) and CNAs (245 patients) in *SOX2* (105 patients [42.9%]), *KLHL6* (97 patients [39.6%]), *PIK3CA* (89 patients [36.3%]), *MAP3K13* (79 patients [32.2%]), *BCL6* (75 patients [30.6%]), and *FGF12* (69 patients [28.2%]). All aberrations detected are given in eTable 2 in the Supplement.

In the TGA cohort, both PFS (median, 3.5 vs 2.5 months; HR, 0.69; 95% CI, 0.51-0.92;  $P = .01$ ) and OS (median, 8.4 vs 6.6 months; HR, 0.81; 95% CI, 0.62-1.05;  $P = .12$ ) favored treatment

Table. Frequency of *ERBB* Family Mutations in the Overall TGA Cohort and in Patients Who Were LTRs to Treatment With Afatinib<sup>a</sup>

Gene	TGA subset, % (n = 245)	Afatinib LTRs, % (n = 10)
<i>ERBB</i> wild type	78.4	50.0
<i>ERBB</i> mutation	21.6	50.0
<i>EGFR</i>	6.5	20.0
<i>HER2</i>	4.9	20.0
<i>HER3</i>	6.1	0
<i>HER4</i>	5.7	10.0

Abbreviations: EGFR, epidermal growth factor receptor; LTR, long-term responder; TGA, tumor genetic analysis.

<sup>a</sup> Three patients were long-term responders to erlotinib: 1 patient had a tumor with an EGFR mutation, and 2 patients had tumors that were *ERBB* wild type.

with afatinib over erlotinib, with systematically lower HRs and longer median PFS and OS than those observed in the overall LUX-Lung 8 population (eFigure 1 in the Supplement). This finding reflects the selection of a high proportion of specimens from patients with PFS of more than 2 months. Therefore, HRs in subgroups should be interpreted relative to the HR in the TGA cohort and not the overall LUX-Lung 8 population (eAppendix in the Supplement). None of the most frequently observed aberrations identified by TGA predicted PFS or OS benefit with afatinib over erlotinib treatment (eFigure 2 in the Supplement).

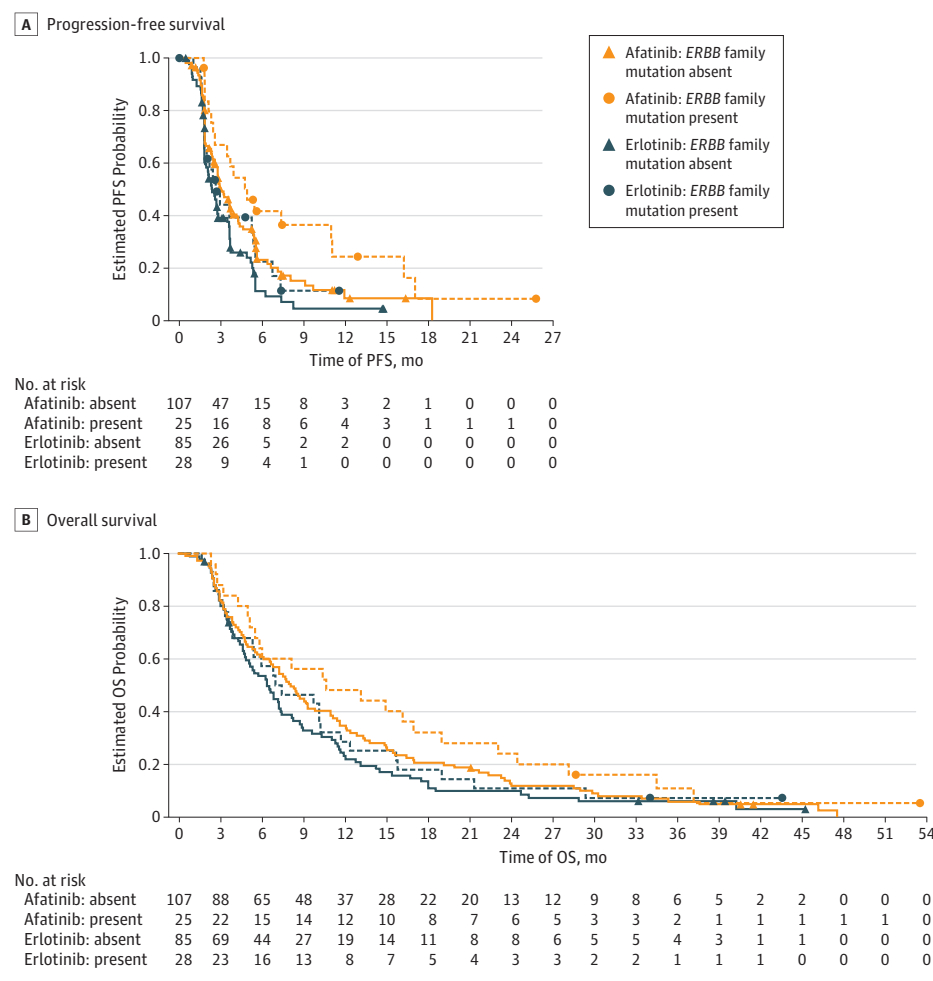
### Outcomes Among Patients With *ERBB* Mutation-Positive Tumors

Overall, 53 of 245 patients (21.6%) had tumors with at least 1 *ERBB* family mutation (Table). Of 245 patients, 1 patient (0.4%) had tumors with *EGFR*, *HER2*, and *HER3* mutations, and 2 patients (0.8%) had tumors with *HER3* and *HER4* mutations. Sixteen patients (6.5%) had tumors with at least 1 *EGFR* mutation; only 7 of the 17 *EGFR* mutations detected had previously been described (L861Q, E114K, Q102I, E746, A750del, V843I, and L858R). No tumor tested positive for EGFR variant III mutations, although such mutations are detectable using the Foundation Medicine NGS assay. The *HER2* mutations were detected in tumors from 12 patients (4.9%), *HER3* mutations in 15 patients (6.1%), and *HER4* mutations in 14 patients (5.7%). Two patients (0.8%) had both a *HER3* and a *HER4* mutation. One patient (0.4%) had an EGFR, a *HER3*, and a *HER4* mutation. Four of the 16 *HER3* mutations (P1212S, R103H, and V104L in 2 patients) and 1 of the *HER4* mutations (D931Y) have been previously described. Details of the *ERBB* mutation-positive tumors and the location of the *HER2* mutations (detected in both the intracellular and extracellular domains) are shown in eFigure 3 in the Supplement.

In the overall LUX-Lung 8 data set of 398 patients, 21 patients (5.3%) were long-term responders to treatment with afatinib (received  $\geq 12$  months of treatment). Ten of these patients were in the TGA cohort, among whom 5 (50.0%) had *ERBB* mutation-positive tumors (Table). Therefore, the frequency of *ERBB* mutation-positive tumors was nominally higher among long-term responders than among the overall afatinib-treated population.

For patients receiving afatinib, both PFS and OS were longer among patients with *ERBB* mutation-positive tumors than

**Figure 3. Comparison of Progression-Free Survival (PFS) and Overall Survival (OS) Among Patients With Tumors Treated With Afatinib or Erlotinib in the Presence or Absence of *ERBB* Gene Family Mutations**



among those without (PFS: median, 4.9 vs 3.0 months; HR, 0.62; 95% CI, 0.37-1.02;  $P = .06$ ; OS: median, 10.6 vs 8.1 months; HR, 0.75; 95% CI, 0.47-1.17;  $P = .21$ ) (Figure 3). By contrast, in the erlotinib arm, PFS and OS were similar among patients with tumors with or without *ERBB* mutations (PFS: median, 2.7 vs 2.4 months; HR, 0.76; 95% CI, 0.46-1.26;  $P = .29$ ; OS: median, 7.2 vs 6.4 months; HR, 0.84; 95% CI, 0.54-1.32;  $P = .46$ ).

Outcomes assessed with respect to individual *ERBB* gene family members (Figure 4) indicated that *EGFR* mutations, in isolation, did not predict PFS or OS benefit of treatment with afatinib over erlotinib; indeed, the accentuated benefit of treatment with afatinib over erlotinib among patients with *ERBB* mutation-positive tumors appeared to be driven by *HER3*, *HER4*, and, in particular, *HER2*. Among the 12 patients with tumors having a *HER2* mutation, PFS (HR, 0.06; 95% CI, 0.01-0.59;  $P = .02$ ) and OS (HR, 0.06; 95% CI, 0.01-0.57;  $P = .02$ ) strongly favored treatment with afatinib vs erlotinib. This observation was further underpinned by standardized effect plots (eFigure 4 in the Supplement). Despite the small sample size, the interaction  $P$  values were significant for PFS ( $P = .006$ ) and OS ( $P = .003$ ), indicating that the presence of a *HER2* mutation may predict better outcomes with afatinib vs erlotinib treatment.

### Outcomes According to *ERBB* CNAs

Seventeen (6.9%) and 9 (3.7%) of 245 patients had tumors with CNAs in *EGFR* and *HER2*, respectively. No CNA was detected in *HER3* or *HER4*. There was no apparent correlation between CNAs and outcomes among patients treated with afatinib or erlotinib (data not shown).

### Outcomes According to *EGFR* Overexpression

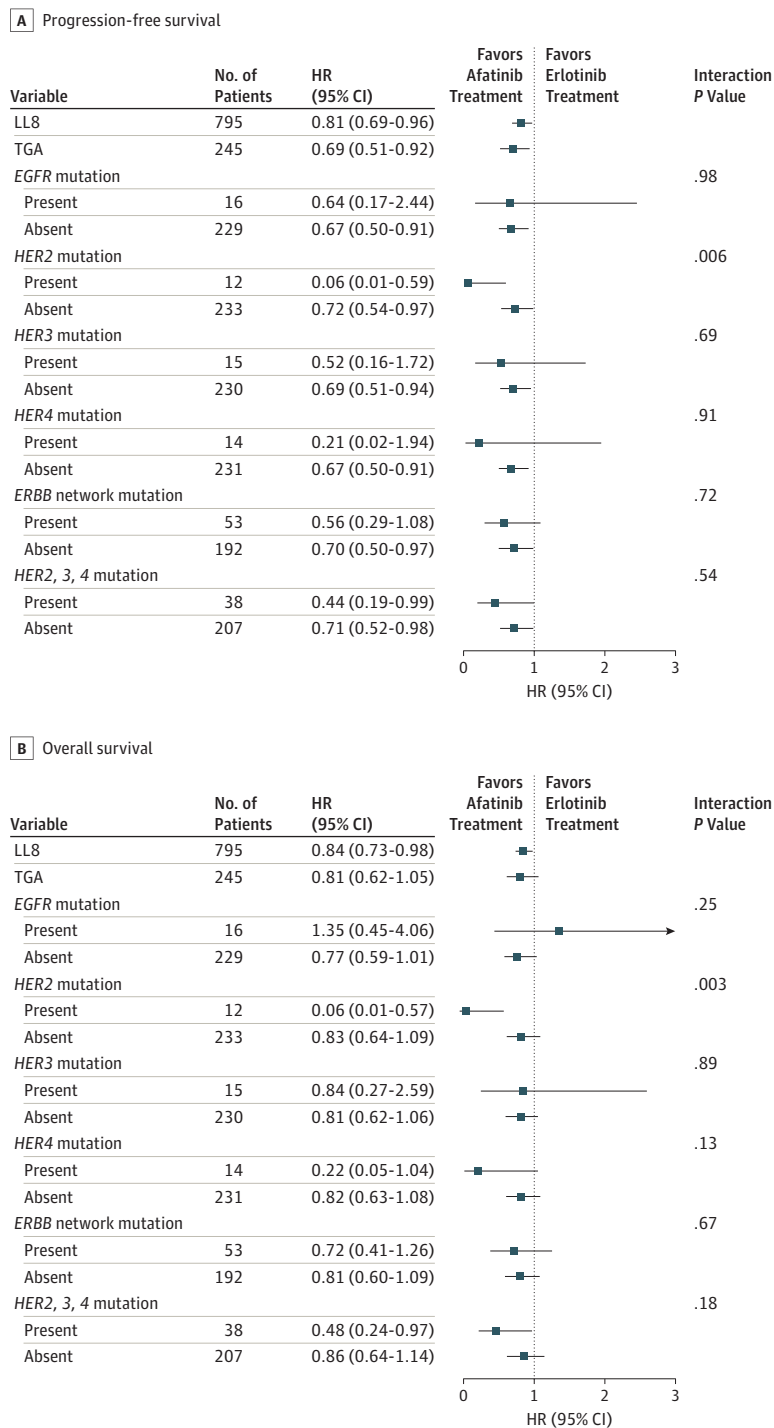
Outcomes in the IHC cohort ( $n = 345$ ) were similar to those in the overall LUX-Lung 8 data set (PFS: median, 2.0 vs 1.9 months; HR, 0.74; 95% CI, 0.58-0.95;  $P = .14$ ; OS: median, 7.4 vs 6.6 months; HR, 0.79; 95% CI, 0.63-0.99;  $P = .04$ ). *EGFR* overexpression (by either method) did not predict PFS or OS benefit with afatinib vs erlotinib treatment (eTable 3 in the Supplement).

## Discussion

To our knowledge, this secondary analysis of the LUX-Lung 8 trial represents the largest and most detailed evaluation of genetic mutations among patients with lung SqCC. Tumors were selected for analysis with Foundation Medicine NGS<sup>17</sup>



Figure 4. Comparison of Progression-Free Survival (PFS) and Overall Survival (OS) Among Patients in the Presence or Absence of *EGFR*, *HER2*, *HER3*, or *HER4* Mutations



from 245 patients whose baseline demographic and clinical characteristics were similar to those in the overall LUX-Lung 8 population. Because afatinib is an *ERBB* family blocker and because other *ERBB* family members have been implicated in the pathogenesis of lung SqCC,<sup>12,20</sup> the focus of this study was to assess outcomes among patients with *ERBB* mutation-positive tumors vs those with *ERBB* wild-type

tumors. Although aberrations among individual *ERBB* family members were rare, cumulatively, 53 of the 245 patients (21.6%) had tumors with mutations in at least 1 *ERBB* family member. Although PFS and OS benefit with afatinib over erlotinib treatment was apparent among patients with *ERBB* wild-type tumors, the effects were more pronounced among patients with tumors that had at least 1 *ERBB* family

mutation. Accentuated benefit with afatinib treatment did not appear to be driven by *EGFR* mutations; indeed, the largest benefits were observed among patients with tumors having *HER2* or *HER4* mutations.

Given the molecular heterogeneity of lung SqCC and the expanding armamentarium of available agents in this setting along with the ability of afatinib to irreversibly inhibit signaling via all ERBB heterodimers and homodimers, the present hypothesis-generating results suggest that identification of mutations in any *ERBB* family member might identify a subgroup of patients who may particularly benefit from treatment with afatinib following the failure of chemotherapy. Therefore, the role of *HER2* as a potential biomarker warrants further investigation. Although *HER2* mutations are rare, afatinib has previously demonstrated activity in heavily pretreated patients with adenocarcinoma NSCLC whose tumors had such a mutation.<sup>21</sup> Notably, the pattern of *HER2* mutations in the present study was different from that observed in adenocarcinoma NSCLC, in which the most frequently occurring mutations are in-frame insertion alleles in exon 20.<sup>22</sup> In squamous disease, *HER2* mutations are individually rarer and more heterogeneously distributed. In the present study, only 2 of the 9 mutations (Q57R and P489L) identified in tumors derived from afatinib-treated patients occurred in more than 1 patient, with 7 of the mutations being detected in the extracellular domain of *HER2* and only 2 mutations occurring in the intracellular domain (G815R and P1037L). Overall, *HER2* mutations were associated with better outcomes, suggesting that these mutations are important for tumor growth or survival, although more research is needed to characterize the individual aberrations and their likely variable role in receptor activation and response to afatinib. Mutations occurring outside the *HER2* kinase domain are reported to be transforming in several cancers. Rare mutations occurring in the *HER2* transmembrane domain<sup>23</sup> and in the *HER2* extracellular domain<sup>24</sup> have been shown to be oncogenic in adenocarcinoma NSCLC. The *HER2* extracellular domain mutations are also activating in colorectal<sup>25</sup> and breast cancer<sup>26</sup> and have been associated with response to afatinib treatment in urothelial cancer.<sup>27</sup>

The present study assessed the broad genetic characteristics of SqCC tumors in a large number of patients, potentially providing insights into the molecular pathogenesis of the disease. We found that the prevalence of the molecular aberrations in the LUX-Lung 8 population was consistent with data reported by the TCGA.<sup>12</sup> The higher mutation frequencies observed in certain genes in the LUX-Lung 8 population (*KMT2A*, *KMT2D*, *FAT3*, and *CDKN2A*) may be due to the high read coverage obtained, which enabled the detection of mutations with lower variant allele frequency than in the TCGA analysis. In the cases of *KMT2A* and *KMT2D*, the mutation frequencies were reported as zero by the TCGA but were found to be 12.2% and 33.1%, respectively, in this study. The *KMT2A* and *KMT2D* mutations have been reported in squamous NSCLC in the COSMIC database,<sup>28</sup> albeit at a lower frequency, suggesting that mutation detection in these genes is challenging and should be interpreted

cautiously. None of the commonly mutated genes predicted superior OS or PFS outcomes with afatinib over erlotinib treatment.

In line with other studies,<sup>13,15</sup> we identified high levels of *EGFR* overexpression in the IHC cohort ( $n = 345$ ) of the LUX-Lung 8 population. Previous analyses have indicated that *EGFR* overexpression can predict outcomes with first-generation *EGFR*-targeted tyrosine kinase inhibitors in *EGFR* wild-type NSCLC.<sup>18</sup> However, we found that the benefits of treatment with afatinib over erlotinib in the LUX-Lung 8 population were apparent regardless of *EGFR* expression levels or *EGFR* gene copy number. It seems, therefore, that the efficacy of afatinib in the LUX-Lung 8 population is not driven by *EGFR* overexpression.

In addition to the emergence of afatinib as a second-line treatment option for lung SqCC, the immune checkpoint inhibitors nivolumab, pembrolizumab, and atezolizumab have been approved on the basis of the phase 3 studies CheckMate 017 (in lung SqCC), Keynote 010, and OAK (the latter 2 in NSCLC of any histology), respectively.<sup>5-7</sup> There is some evidence for PD-L1 as a predictive biomarker for these agents. For example, pembrolizumab is more active in patients with NSCLC tumors expressing high PD-L1 levels,<sup>6</sup> whereas nivolumab efficacy in CheckMate 017 appears to be independent of PD-L1 expression.<sup>5</sup> In other settings, nivolumab has demonstrated differential activity according to PD-L1 expression levels across different tumor types.<sup>29</sup> Based on available data, checkpoint inhibitors are a second-line treatment option for lung SqCC.<sup>30</sup> However, treatment with afatinib may be a viable alternative among those patients for whom checkpoint inhibitors are unsuitable. Furthermore, given our findings, it will be interesting to assess checkpoint inhibitors in tumors with *ERBB* family mutations. It may be that afatinib represents a preferable treatment option for these patients.

### Limitations

Although the biomarker data presented herein are extensive, the present study has a number of limitations. First, because of the retrospective nature of the analysis and the lack of statistically significant findings (with the exception of outcomes among patients with *HER2* mutations), the results should be considered hypothesis generating rather than hypothesis testing. Second, TGA is limited in the ability to detect functional aberrations (eg, hypermethylation and phosphorylation), which may be key to differential efficacy. Third, only a minority of patients from the overall LUX-Lung 8 population could be included in tumor genetic and IHC analyses. As such, it is difficult to extrapolate the results to the overall LUX-Lung 8 population given that the subgroups were relatively small and subject to selection bias. Indeed, the TGA cohort was enriched for patients with PFS of 2 or more months; consequently, the OS and PFS benefit with afatinib over erlotinib treatment was greater than that observed in the overall LUX-Lung 8 data set. Finally, we do not present independent evidence for the activating role of the individual *ERBB* mutations or for their sensitization of the tumors to afatinib.

## Conclusions

Treatment with afatinib showed better outcomes than that with erlotinib across patient subgroups in this secondary analysis of the LUX-Lung 8 trial. The PFS and OS benefit with afatinib over erlotinib treatment was more pronounced among patients with

ERBB mutation-positive tumors than among those without, especially among patients with tumors having *HER2* or *HER4* mutations. We hypothesize that the additional benefit with afatinib is attributable to its broad, irreversible inhibition of the entire ERBB signaling network. Finally, afatinib is a second-line treatment option for lung SqCC and may be particularly suitable for patients whose tumors carry at least 1 *ERBB* mutation.

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## Invited Commentary

## In Search of an Oncogene Driver for Squamous Lung Cancer

David R. Gandara, MD; Jonathan W. Riess, MD, MS; Primo N. Lara Jr, MD

**Checkpoint inhibitor immunotherapies** are playing an increasing role in the therapeutic armamentarium of advanced stage non-small cell lung cancer, regardless of histologic subtype. By contrast, advances in targeted therapy have largely



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bypassed patients with squamous cell cancers. Identification of new treatable oncogenic drivers has predominately been confined to the nonsquamous subset, or more specifically, adenocarcinoma. The favorable results of tyrosine kinase inhibitor (TKI) therapy among patients whose cancers exhibit these anomalies (eg, alterations in *EGFR*, *ALK*, *ROS1*, *BRAF*, *MET*, or *RET*) have resulted in their inclusion in current guidelines for molecular testing and regulatory approval for associated therapies in most of these genomic subsets.<sup>1</sup> By comparison, no substantial progress has been made in the clinical application of targeted therapy for patients with squamous cancers during this same time, despite the discovery of a number of potential molecular targets and the initiation of associated clinical trials.<sup>2</sup> For example, potentially actionable abnormalities in phosphoinositide 3-kinase (PI3K), cyclin-dependent kinases (CDK) 4/6, and fibroblast growth factor receptor (FGFR) were present in 8%, 19%, and 16% of squamous lung cancers, respectively, in patients screened for the Lung-MAP (S1400) master protocol in advanced squamous lung cancer. Unfortunately, the recently completed Lung-MAP genotype-directed substudies on

each of these 3 targets, S1400B (PI3K-directed TKI), S1400C (CDK 4/6-directed TKI), and S1400D (FGFR-directed TKI), failed to reach predetermined efficacy endpoints, with response rates of only 4%, 6%, and 7% and median overall survival of 5.9, 7.2, and 7.5 months, respectively.<sup>3-5</sup>

Thus, the study by Goss et al<sup>6</sup> in this issue of *JAMA Oncology* identifying *ERBB* family mutations as potential oncogenic drivers in squamous lung cancer is timely. The study design of the LUX-Lung 8 trial is particularly applicable to this analysis because it directly compares treatment with the pan-*ERBB* inhibitor afatinib dimaleate to that with the EGFR-only inhibitor erlotinib hydrochloride among patients with squamous cancers, in whom EGFR-sensitizing mutations are rare.<sup>7</sup> As assessed by next-generation sequencing, *ERBB* family mutations were detected in tumors from 53 of 245 patients (21.6%), an unexpectedly high prevalence in squamous lung cancers. Only 16 of these tumors (6.5%) were EGFR-sensitizing mutations. Although the benefits of treatment with afatinib in the previously reported intent-to-treat population were modest for both progression-free survival (PFS) and overall survival (OS), the analysis by Goss et al<sup>6</sup> reveals that efficacy was magnified among afatinib-treated patients with underlying *ERBB* mutations vs those without, an effect not observed among the corresponding erlotinib-treated cohorts. The differential efficacy in favor of treatment with afatinib was particularly notable among those patients with *HER2*