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Summary

Background Most patients with non-small-cell lung cancer tumours that have EGFR mutations have deletion mutations in exon 19 or the Leu858Arg point mutation in exon 21, or both (ie, common mutations). However, a subset of patients (10%) with mutations in EGFR have tumours that harbour uncommon mutations. There is a paucity of data regarding the sensitivity of these tumours to EGFR inhibitors. Here we present data for the activity of afatinib in patients with advanced non-small-cell lung cancer that have tumours harbouring uncommon EGFR mutations.

Methods In this post-hoc analysis, we used prospectively collected data from tyrosine kinase inhibitor-naive patients with EGFR mutation-positive advanced (stage IIIb–IV) lung adenocarcinomas who were given afatinib in a single group phase 2 trial (LUX-Lung 2), and randomised phase 3 trials (LUX-Lung 3 and LUX-Lung 6). Analyses were done in the intention-to-treat population, including all randomly assigned patients with uncommon EGFR mutations. The type of EGFR mutation (exon 19 deletion [del19], Leu858Arg point mutation in exon 21, or other) and ethnic origin (LUX-Lung 3 only; Asian vs non-Asian) were pre-specified stratification factors in the randomised trials. We categorised all uncommon mutations as: point mutations or duplications in exons 18–21 (group 1); de-novo Thr790Met mutations in exon 20 alone or in combination with other mutations (group 2); or exon 20 insertions (group 3). We also assessed outcomes in patients with the most frequent uncommon mutations, Gly719Xaa, Leu861Gln, and Ser768Ile, alone or in combination with other mutations. Response was established by independent radiological review. These trials are registered with ClinicalTrials.gov, numbers NCT00525148, NCT00949650, and NCT01121393.

Findings Of 600 patients given afatinib across the three trials, 75 (12%) patients had uncommon EGFR mutations (38 in group 1, 14 in group 2, 23 in group 3). 27 (71·1%, 95% CI 54·1–84·6) patients in group 1 had objective responses, as did two (14·3%, 1·8–42·8) in group 2 and two (8·7%, 1·2–28·0) in group 3. Median progression-free survival was 10·7 months (95% CI 5·6–14·7) in group 1, 2·9 months (1·2–8·3) in group 2; and 2·7 months (1·8–4·2) in group 3. Median overall survival was 19·4 months (95% CI 16·4–26·9) in group 1, 14·9 months (8·1–24·9) in group 2, and 10·7 months (4·1–14·2) in group 3. For the most frequent uncommon mutations, 14 (77·8%, 95% CI 52·4–93·6) patients with Gly719Xaa had an objective response, as did nine (56·3%, 29·9–80·2) with Leu861Gln, and eight (100·0%, 63·1–100·0) with Ser768Ile.

Interpretation Afatinib was active in non-small-cell lung cancer tumours that harboured certain types of uncommon EGFR mutations, especially Gly719Xaa, Leu861Gln, and Ser768Ile, but less active in other mutations types. Clinical benefit was lower in patients with de-novo Thr790Met and exon 20 insertion mutations. These data could help inform clinical decisions for patients with non-small-cell lung cancer harbouring uncommon EGFR mutations.

Funding Boehringer Ingelheim.

Introduction Drugs that target the EGFR or the wider ERBB tyrosine kinase receptor family are the standard of care for patients with EGFR-mutant, non-small-cell lung cancer tumours.1–3 The clinical benefit of first-line therapy with erlotinib and gefitinib compared with platinum-based combination chemotherapy in this patient population has been noted in several phase 3 trials.1–3 Afatinib, an irreversible ERBB family blocker, was recently approved in the USA, Europe, and several other countries worldwide for first-line treatment of non-small-cell lung cancer tumours with EGFR mutations following results of two large randomised controlled trials4,5 that showed that afatinib treatment resulted in improved objective responses and progression-free survival compared with platinum-based chemotherapy. Additionally, in pre-specified analyses of two independent phase 3 trials, compared with chemotherapy, afatinib significantly improved overall survival in patients with exon 19 deletions but did not improve...
Panel: Research in context

Evidence before the study
We searched PubMed for articles published between Jan 1, 2004, and Dec 31, 2014, using the search string “uncommon EGFR mutations OR rare EGFR mutations AND NSCLC”. Of 150 articles, 19 examined the sensitivity of uncommon EGFR mutations to EGFR tyrosine kinase inhibitors, either in vitro or in vivo. These studies reported that Gly719Xaa and Leu861Gln mutations confer sensitivity to first-generation, reversible EGFR tyrosine kinase inhibitors in some but not all cases, while Thr790Met and exon 20 insertion mutations generally conferred resistance to EGFR tyrosine kinase inhibitors. However, most of these studies did not prospectively analyse treatment effects in a clinical trial setting, and were restricted to analysis of a few patients.

Added value of this study
To the best of our knowledge, this is the largest analysis of prospective clinical trial data of patients with advanced non-small-cell lung cancer harbouring uncommon EGFR mutations after treatment with an irreversible ERBB family blocker or chemotherapy. Afatinib showed activity in patients with point mutations or duplications in exons 18–21, suggesting that this group of uncommon mutations can be categorised as sensitising EGFR mutations. However, similar to first-generation, reversible EGFR tyrosine kinase inhibitors, afatinib did not provide clinical benefit to patients harbouring de-novo Thr790Met or exon 20 insertion mutations, and results of an exploratory analysis showed that chemotherapy might be a preferable first-line option for patients with these mutations.

Implications of all the available evidence
Data from our study suggest that afatinib is a treatment option for patients with some uncommon EGFR mutations. Performing larger randomised trials in patients with uncommon EGFR mutations is unlikely in view of the small population size and genetic diversity of these tumours; although limitations exist (eg, inclusion of patients treated beyond progression), a global registry might be more feasible to obtain additional data for these particular patients.

Methods

Study design and participants
We combined individual data from adult patients (aged ≥18 years) with advanced (stage IIIb–IV) lung adenocarcinomas with uncommon EGFR mutations in LL2, LL3, and LL6. The study design, eligibility criteria, and primary results of these three clinical trials of afatinib in EGFR mutation-positive patients with non-small-cell lung cancer have been published elsewhere.5,7,9 All patients in this series were prospectively diagnosed and treated, and tumour responses were independently reviewed. Briefly, LL2 was a single-group phase 2 trial done in Taiwan and the USA, which included patients with EGFR mutation-positive lung adenocarcinoma with at least one measurable tumour lesion (by CT or MRI), Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, life expectancy of 3 months or longer, and who had received no previous treatment for advanced disease or progressed after one previous chemotherapy regimen.10 Both LL3 and LL6 were phase 3 trials in treatment-naive patients with advanced adenocarcinoma of the lung harbouring EGFR mutations who had ECOG performance status of 0 or 1, life expectancy of 3 months or longer, and at least one measurable lesion according to Response Evaluation Criteria in Solid Tumors (RECIST [version 1.1]). LL3 was a worldwide study, whereas LL6 was done in China, South Korea, and Thailand. Exclusion criteria for all three trials included diagnosis of any other cancers at screening or within the past 5 years (excluding non-melanoma skin cancer and in-situ cervical cancer); pre-existing interstitial lung disease; active brain metastases; clinically relevant (past or present) cardiovascular abnormalities (eg, poorly controlled arrhythmia, unstable angina, congestive heart failure of New York Heart Association classification of 3) or myocardial infarction less than 6 months before randomisation (LL3 and LL6 only); significant or recent acute gastrointestinal disorders (with diarrhoea as a major symptom); cardiac left ventricular function with resting ejection fraction of less than 50%; known HIV carrier or presence of active hepatitis B or C infection. All trials were done in accordance with the Declaration of Helsinki and Good Clinical Practice, and all study protocols were approved by the ethics committees of all
Baseline characteristics of patients with uncommon exon 20 insertions.

<table>
<thead>
<tr>
<th>Ethnic origin</th>
<th>CAucasian</th>
<th>Asian</th>
<th>Other</th>
<th>Stage (AJCC 6.0)</th>
<th>ILIB (wt)</th>
<th>IV</th>
<th>ECOG performance status</th>
<th>0</th>
<th>1</th>
<th>Line of therapy</th>
<th>First-line</th>
<th>Second-line</th>
<th>Mutation status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 (13%)</td>
<td>20 (87%)</td>
<td>0 (0%)</td>
<td>14 (61%)</td>
<td>1 (4%)</td>
<td>22 (96%)</td>
<td>11 (57%)</td>
<td>13 (57%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>10 (43%)</td>
<td>13 (57%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>8 (31%)</td>
<td>17 (65%)</td>
<td>0 (0%)</td>
<td>26 (100%)</td>
<td>1 (4%)</td>
<td>25 (96%)</td>
<td>5 (19%)</td>
<td>21 (81%)</td>
<td>14 (44%)</td>
<td>11 (44%)</td>
<td>26 (100%)</td>
<td>26 (100%)</td>
<td>25 (100%)</td>
</tr>
</tbody>
</table>

Data are n (%) or median (range). AJCC=American Joint Committee on Cancer. ECOG=Eastern Cooperative Oncology Group. *Four patients received 40 mg afatinib per day as the starting dose; the remaining 19 patients received 50 mg afatinib per day as the starting dose. †Consists of patients with all point mutations or duplications in exons 18–21 (Leu858Arg, Gly719Ser, Gly719Ala, Gly719Cys, Thr790Met, Ser768Ile, and rare others). ‡Consists of patients with de novo Thr790Met mutations. C Consists of patients with exon 20 insertions.

Table 1: Baseline characteristics of patients with uncommon EGFR mutations

Procedures

Patients in the LL2 trial originally received 50 mg afatinib once a day as the recommended starting dose (the first 99 patients [77%] of the 129 treated); however, the starting dose was subsequently reduced for newly enrolled patients to 40 mg once a day because durable responses and improved tolerability were reported in patients who underwent dose reductions to 40 mg in the first part of the study. Treatment continued until disease progression, intolerable adverse events, or withdrawal of consent. Tumours in LL2 were assessed by CT scan or MRI at baseline, week 4, week 8, week 12, and every 8 weeks thereafter.

Patients in the LL3 and LL6 trials were randomly assigned (2:1) to receive first-line 40 mg afatinib once a day (starting dose per protocol) or up to six cycles of intravenous pemetrexed (500 mg/m²) plus cisplatin (75 mg/m²) once every 21 days in LL3 or gemcitabine (1000 mg/m²; days 1 and 8) plus cisplatin (75 mg/m²; day 1) once every 21 days in LL6. Patients receiving afatinib in the two randomised trials were allowed to increase their dose to 50 mg after the first course of treatment if it was well tolerated, or reduce to lower doses (by 10 mg decrements) if treatment-related grade 3 or prolonged grade 2 adverse events occurred. Treatment continued until disease progression, intolerable adverse events, or withdrawal of consent. Tumours were assessed by CT or MRI in 6-week intervals for the first 48 weeks, and in 12-week intervals thereafter until disease progression or initiation of new anticancer treatment.

EGFR mutations were identified prospectively by direct sequencing in LL2 and by central testing with a validated test kit (TheraScreen EGFR 29 [TheraScreen29]; Qiagen, Manchester, UK) in LL3 and LL6. In LL2, exons 18–21 amplified by PCR and analysed bidirectionally by direct sequencing for the presence of somatic mutations according to previously described methods. All mutations were confirmed by several independent PCR analyses. Allele-specific quantitative real-time PCR for the two most common activating mutations (del19 and Leu858Arg) was undertaken in cases that were deemed inadequate for direct sequencing (ie, insufficient sample or quality), based on review by a molecular pathologist or for specimens with a high percentage of normal cells (<50% tumour cells). TheraScreen29, used in LL3 and LL6, is designed to detect 29 EGFR mutations against a background of wild-type genomic DNA; the mutations detectable include 19 different deletions in exon 19, Leu858Arg substitution in exon 21, three specific insertions in exon 20 (2307_2308ins9, 2319_2320insCAC, 2310_2311insGAC), and several point mutations (Leu861Gln [exon 21], Gly719Ser [exon 18], Gly719Ala [exon 18], Gly719Cys [exon 18], Thr790Met [exon 20], and Ser768Ile [exon 20]). Direct sequencing was used in LL2 allowed for the detection of additional uncommon EGFR mutations (ie, not limited to the 29 identifiable with the TheraScreen29 test). If both Leu858Arg and del19 were detected in the same sample, the patient was allocated to the stratification category Leu858Arg. In other instances participating centres. All patients provided written informed consent.

Randomisation

For the randomised trials, eligible patients were randomly assigned (2:1) to receive afatinib or chemotherapy, stratified by EGFR mutation type (ie, del19 mutations, Leu858Arg mutations, or other [all other single or complex] mutations) and ethnic origin (Asian vs non-Asian; LL3 only). Within each strata, a block size of three was used. Randomisation was done at Boehringer Ingelheim with a validated random-number generating system, and was verified by a trial-independent statistician. Randomisation was implemented centrally with an interactive voice-web response system. Access to the randomisation schedule was prohibited for individuals directly involved in trial conduct and analysis. There was no randomisation between doses in any of the three studies.
when more than one mutation was detected, the patient was allocated to the stratification category “other”.

Outcomes

The primary endpoint in LL2 was objective response (independent review); secondary endpoints included disease control, time to and duration of objective response, tumour shrinkage, progression-free survival, and overall survival. The primary endpoint of both LL3 and LL6 was progression-free survival (by independent review), with objective response, disease control, and overall survival as secondary endpoints. Other secondary endpoints included patient-reported outcomes and safety.

This post-hoc analysis focused on the activity of afatinib in uncommon mutations that were categorised into three cohorts: point mutations and duplications in exons 18–21 (Leu861Gln, Gly719Ser, Gly719Ala, Gly719Cys, Ser768Ile, and other point mutations alone or in combination with each other; group 1), de-novo Thr790Met mutation in exon 20 (alone or in combination with other mutations; group 2), or exon 20 insertions (group 3). Thr790Met and exon 20 insertion mutations were divided into separate groups because they have been previously shown to confer resistance and decreased sensitivity to EGFR tyrosine kinase inhibitors. An additional analysis looked at outcomes in a subgroup of patients, mainly from group 1, with the mutation types Gly719Xaa (exon 18), Leu861Gln (exon 21), and Ser768Ile (exon 20), alone or in combination with other mutations as this group of point mutations represents about half of the uncommon mutations in EGFR that are associated with non-small-cell lung cancer.

Tumour response was assessed with RECIST by a central independent radiology review prospectively as part of the original study design.

Statistical analysis

Detailed statistical analyses of the individual trials have been previously published. This post-hoc, intention-to-treat analysis included all randomly assigned and entered patients with an uncommon EGFR mutation. For each mutation group, the proportions of patients that achieved objective response (complete response or partial response) and disease control (complete response, partial response, or stable disease) were calculated along with exact Clopper-Pearson 95% CIs. Median duration of response, progression-free survival, and overall survival were calculated with corresponding 95% CIs, estimated with Kaplan-Meier methods. No formal statistical comparisons were done because of the small patient numbers. Median duration of follow-up for progression-free survival and overall survival were calculated with the reverse Kaplan-Meier method. Statistical analyses were done with SAS (version 9.4).

The ongoing LL2, LL3, and LL6 trials are registered with ClinicalTrials.gov, numbers NCT00525148, NCT00949650, and NCT01121393.

Role of the funding source

JC-HY and Boehringer Ingelheim designed the LL2 and LL3 studies; LVS designed LL3 and YLW designed LL6, together with Boehringer Ingelheim. JC-HY and Boehringer Ingelheim designed and analysed this post-hoc study. Boehringer Ingelheim managed the clinical trial database and coordinated the development of the report. JC-HY, LVS, and Y-LW retained full access to the data.
Table 3: Activity of afatinib in specific uncommon mutations

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Objective response</th>
<th>Progression-free survival (months)</th>
<th>Overall survival (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly719Xaa (n=18)</td>
<td>14 (77.8%, 52.4–93.6)</td>
<td>13 (6.8–NE)</td>
<td>26 (16.4–NE)</td>
</tr>
<tr>
<td>Gly719Xaa-Thr790Met (n=1)</td>
<td>8 (4.5–16.6)</td>
<td>17 (15.3–21.6)</td>
<td>NE</td>
</tr>
<tr>
<td>Gly719Xaa+Ser768Ile (n=5)</td>
<td>9 (56.3%, 29.9–80.2)</td>
<td>8 (4.5–16.6)</td>
<td>17 (15.3–21.6)</td>
</tr>
<tr>
<td>Gly719Xaa+Leu861Gln (n=3)</td>
<td>8 (100.0%, 63–100.0)</td>
<td>14 (7.2–NE)</td>
<td>NE</td>
</tr>
<tr>
<td>Gly719Xaa-Thr790Met+Leu953Arg (n=1)</td>
<td>8 (100.0%, 63–100.0)</td>
<td>14 (7.2–NE)</td>
<td>NE</td>
</tr>
<tr>
<td>Leu861Gln (n=16)</td>
<td>9 (9–NE)</td>
<td>7 (4.1–12.5)</td>
<td>12 (5.6–NE)</td>
</tr>
<tr>
<td>Leu861Gln+Gly719Xaa (n=3)</td>
<td>7 (20–NE)</td>
<td>1.7 (0.5–4.2)</td>
<td>NE</td>
</tr>
<tr>
<td>Leu861Gln+del19 (n=1)</td>
<td>7 (20–NE)</td>
<td>1.7 (0.5–4.2)</td>
<td>NE</td>
</tr>
<tr>
<td>Ser768Ile (n=8)</td>
<td>8 (100.0%, 63–100.0)</td>
<td>14 (7.2–NE)</td>
<td>NE</td>
</tr>
<tr>
<td>Ser768Ile+Thr790Met (n=1)</td>
<td>8 (100.0%, 63–100.0)</td>
<td>14 (7.2–NE)</td>
<td>NE</td>
</tr>
<tr>
<td>Ser768Ile+Leu953Arg (n=2)</td>
<td>8 (100.0%, 63–100.0)</td>
<td>14 (7.2–NE)</td>
<td>NE</td>
</tr>
</tbody>
</table>

Data are n (%), 95% CI or median (95% CI). NE=not estimable. Uncommon mutation categories overlap for those with compound mutations, so individual patients might appear in more than one category.

Table 2: Response to treatment with afatinib in patients with uncommon mutations

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Objective response</th>
<th>Progression-free survival (months)</th>
<th>Overall survival (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n=25)*</td>
<td>27 (71.1%, 54–1–84.6)</td>
<td>11 (4.1–15.2)</td>
<td>32 (84.2%, 68.7–94.0)</td>
</tr>
<tr>
<td>Group 2 (n=14)!</td>
<td>2 (14.3%, 1.8–42.8)</td>
<td>8 (4.1–12.4)</td>
<td>9 (64.3%, 35.1–87.2)</td>
</tr>
<tr>
<td>Group 3 (n=23)!!</td>
<td>2 (8.7%, 1.1–28.0)</td>
<td>7 (4.2–10.1)</td>
<td>15 (65.2%, 42.7–83.6)</td>
</tr>
</tbody>
</table>

Data are n (%). *Consists of patients with all point mutations or duplications in exons 18–21 (Leu861Gln, Gly719Ser, Gly719Ala, Gly719Cys, Ser768Ile, and rare others). †Consists of patients with de-novo Thr790Met mutations. ‡Consists of patients with exon 20 insertions.

Table 1 shows baseline patient demographic and study data and were involved in preparation of the manuscript draft. All authors were involved in the development of the manuscript.

Results

Patients were screened between Aug 21, 2007, and June 4, 2009, for LL2; between Aug 17, 2009, and Feb 28, 2011, for LL3; and between Apr 27, 2010, and Nov 16, 2011, for LL6. The overall frequency of patients with uncommon EGFR mutations was 18% (23/129) for LL2, 11% (37/345) for LL3, and 11% (40/364) for LL6. 75 (13%) of 600 patients (129 in LL2, 229 in LL3, 242 in LL6) given afatinib were divided into three cohorts: group 1 (n=38), group 2 (n=14), and group 3 (n=23; figure 1). Of those with group 1 mutations, the most common mutations were Leu861Gln alone (n=12), Gly719Xaa alone (n=8), and Gly719Xaa plus either Ser768Ile (n=5) or Leu861Gln (n=3).

Afatinib was associated with decreases in tumour size, and this decrease was associated with an increase in progression-free survival (figure 2). One patient with a Gly719Xaa mutation (group 1) had a complete response. The other two patients with 100% tumour shrinkage had K739del19 and Leu858Arg mutations (appendix). Across the three trials, patients with uncommon mutations given afatinib were divided into three cohorts: group 1 (n=38), group 2 (n=14), and group 3 (n=23; figure 1). Of those with group 1 mutations, the most common mutations were Leu861Gln alone (n=12), Gly719Xaa alone (n=8), and Gly719Xaa plus either Ser768Ile (n=5) or Leu861Gln (n=3).

For all patients with uncommon mutations (n=100), the median duration of follow-up was 19·2 months (IQR 8·2–19·4) for progression-free survival and 34·7 months (32·4–39·2) for overall survival. For all patients with rare mutations who received afatinib, median progression-free survival and median overall survival was substantially longer for patients in group 1 than it was for those in groups 2 and 3 (table 2 and figure 3). Patients with Gly719Xaa mutations had a substantially longer median progression-free survival and median overall survival than did those with Leu861Gln mutations; median progression-free survival was longest for patients with Ser768Ile mutations, for whom median overall survival was not estimable (table 3).
In group 2, six patients had a Thr790Met mutation in addition to a Leu858Arg mutation. These patients had a longer median progression-free survival (7·5 months [95% CI 0·8–11·0]) and median overall survival (22·9 months [95% CI 8·7–NE]) than the overall group 2 (Thr790Met) cohort. By contrast, for the three patients with a Thr790Met mutation in addition to a del19 mutation, median progression-free survival (1·2 months [95% CI 0·3–3·0]) and median overall survival (8·1 months [95% CI 0·8–11·0]) were shorter than in the overall group 2 cohort.

Of all 25 patients with uncommon EGFR mutations receiving chemotherapy, objective responses were noted in six (24·0%, 95% CI 9·4–45·1) patients, median progression-free survival was 8·2 months (95% CI 5·2–10·8), and median overall survival was 30·2 months (95% CI 13·0–42·3; figure 3). A further exploratory analysis of patients given chemotherapy who had mutations that fell into the same mutation groups 1 (n=18), 2 (n=3), and 3 (n=4) as defined for those treated with afatinib was also done (appendix). Median progression-free survival and median overall survival was longer for group 1 patients treated with chemotherapy than for patients in the other two groups (appendix).

Of the patients with the most frequent uncommon mutations treated with chemotherapy, four (30·8%, 95% CI 9·1–61·4) of 13 with with Gly719Xaa mutations, none (0–0%, 0·0–52·2) of five with Leu861Gln mutations, and two (33·3%, 4·3–77·7) of six with Ser768Ile had objective responses. The longest median progression-free survival was noted for five patients with Leu861Gln mutations (11·9 months [95% CI 8·4–NE]), whose median overall survival was not estimable at the time of the analysis. For patients with tumours harbouring Gly719Xaa or Ser768Ile mutations, median progression-free survival was 5·6 months (95% CI 3·0–11·1) and median overall survival was 3·8 months (0·5–NE) months, respectively, while median overall survival was 30·2 months (3·8–40·8) and 18·9 months (0·5–NE), respectively.

At the time of analysis, 71 (95%) of 75 patients given afatinib had stopped treatment and 48 of these patients had started other systemic anti-cancer treatments. Across all subsequent lines of treatment, 42 (59%) of 71 patients received chemotherapy and 14 (20%) patients received an EGFR tyrosine kinase inhibitor. Of the 17 patients who received subsequent treatments in the chemotherapy group, 12 (48%) of 25 were given an EGFR tyrosine kinase inhibitor and eight (32%) were given additional chemotherapy.

Discussion

In this study, we examined the clinical response to afatinib in patients with uncommon EGFR mutations, defined as any mutation other than del19 or Leu858Arg. We found a high degree of molecular heterogeneity in this uncommon EGFR mutation population and, for the purposes of this analysis, we grouped them into three distinct cohorts based on the previously described differences in sensitivity to EGFR tyrosine kinase inhibitors: point mutations or duplications in exons 18–21, de-novo Thr790Met mutations; group 3=exon 20 insertions.


Figure 3: Kaplan-Meier plot of progression-free survival (A) and overall survival (B).

Group 1=point mutations or duplications in exons 18–21, Group 2=de-novo Thr790Met mutations; group 3=exon 20 insertions.
support the use of afatinib in these patients but not in those patients with group 2 or 3 mutations.

A set of pre-specified analyses of LL3 and LL6, which compared afatinib with chemotherapy in patients with the del19 mutation or Leu858Arg mutation, showed that afatinib significantly improved overall survival compared with chemotherapy in patients with the del19 mutation, but not in those with Leu858Arg mutations. Our data suggest that in patients with uncommon EGFR mutations who were given afatinib, overall survival varied between different mutation subgroups (table 2). However, no formal statistical comparisons between patients with common versus uncommon EGFR mutations were done due to high molecular heterogeneity and the relatively small size of the uncommon mutation subgroups.

Objective responses to afatinib were least common in patients with exon 20 insertions (group 3), similar to findings with gefitinib and erlotinib,12–14 and consistent with preclinical data.22,23 This suggests that EGFR family inhibitors as a whole may be an ineffective treatment option for this patient population. Mechanistic studies of exon 20 insertion mutations show important differences compared with common EGFR mutations. Unlike common EGFR mutations, exon 20 insertion mutations do not affect the ATP-binding pocket required for kinase activity but instead form a wedge at the end of the C-helix that promotes active kinase conformation but does not increase the affinity for EGFR tyrosine kinase inhibitors.22,23 Because patients with exon 20 insertions treated with afatinib have an objective response of less than 10% and a median progression-free survival of 2.7 months, chemotherapy might be a better first-line treatment option for this cohort. These findings confirm that a large unmet need exists for patients with exon 20 insertions.

We noted a wide range of responses within the subset of patients with de-novo Thr790Met mutations (group 2), which might reflect variation in Thr790Met allelic frequency. Although data for variations in Thr790Met allelic frequency for the three patients with de-novo Thr790Met mutation alone (rather than in combination with other mutations) were not collected, previous reports27,28 have shown that patients with tumours with even small allelic frequencies of Thr790Met mutations (detected by techniques such as next generation sequencing), respond to EGFR inhibitors, but have a shorter progression-free survival than patients without detectable Thr790Met.27,28 Conversely, patients with high allelic frequencies of Thr790Met mutations rarely respond to EGFR tyrosine kinase inhibitors. Additionally, the presence or absence of another mutation affects the response to EGFR inhibitors. For example, both progression-free survival and overall survival were longer in patients with both Thr790Met and Leu858Arg mutations than in patients with the Thr790Met and del19 mutations in our analysis, representing a juxtaposition of the typical results recorded in cancers with these mutations that are negative for Thr790Met.25–26 However, patient numbers in each subgroup were small, and results of these subanalyses should be interpreted with caution; overall, afatinib was not highly active in patients with de-novo Thr790Met mutations, and chemotherapy might be a preferable first-line treatment option for these patients. This is an active area of clinical investigation.

High afatinib activity was recorded in patients with the uncommon EGFR mutations Gly719Xaa, Leu861Gln, and Ser768Ile; these are the three most frequently detected types of uncommon EGFR mutations in lung adenocarcinoma.29–31 However, the scarce clinical data available regarding the activity of reversible EGFR inhibitors in tumours that harbour these three mutations are inconclusive, anecdotal, and mostly retrospective; findings of gefitinib from case reports are mixed.25,33 Although findings of an earlier study had originally shown that several of these EGFR point mutations (Gly719Cys and Leu861Gln) conferred sensitivity to gefitinib,34 gefitinib was recently characterised in the NEJ002 trial as being ineffective against both Gly719Xaa and Leu861Gln.35 By contrast with the NEJ002 findings, investigators of a previous retrospective analysis that sought to characterise the effectiveness of gefitinib and erlotinib in tumours with uncommon EGFR mutations noted that objective responses associated with Gly719 and Leu861 mutations were more frequent than for tumours with other uncommon mutations.14 A limitation of this study is the small size of the uncommon EGFR mutation cohort, which required us to combine patients from three trials. Furthermore, the molecular heterogeneity of the uncommon mutations population and numeric imbalances within genetic subgroups limits the ability to formally compare the 25 patients who received chemotherapy and the 75 patients who received afatinib, especially because different outcomes were associated with each mutation group, and different chemotherapy regimens were used in each trial. Moreover, any statistical comparisons between patients given afatinib and those given chemotherapy for each mutation group are unlikely to be informative in view of the small number of patients within each group, especially in the subgroups given chemotherapy. Therefore, data were summarised on an individual patient basis, categorising the mutations or double-mutant cases into post-hoc groups. For completeness, patients receiving chemotherapy were also categorised into the same post-hoc mutation groups as patients given afatinib; however, no biological differences were expected to underpin response to chemotherapy in view of its cytotoxic mode of action. Our findings show that it is important to assess uncommon EGFR mutations independently or appropriately grouped, and not as a whole group (ie, uncommon) because mutation-specific responses may not be identified and activity in certain mutations might be masked by a lack of response in others. Finally, although 84% of the patient population in this study were Asian, results of LL3 (in which 28% of the...
population were non-Asian) suggest that the presence of an EGFR mutation is the main biological determinant of response to afatinib, which takes precedence over demographic or baseline characteristics (eg, ethnic origin, age, and sex). The higher percentage of Asian patients in this study reflects the higher incidence of EGFR mutations in Asians than in non-Asians. In summary, afatinib showed activity in patients with non-small-cell lung cancer tumours that contained the more frequently reported types of uncommon EGFR mutations. However, we noted poor activity for patients with de-novo Thr790Met and exon 20 insertion mutations, for whom treatment with chemotherapy might be considered rather than EGFR inhibitors. Although these data should be interpreted with caution because of the small number of patients assessed in this study, these data might inform therapeutic options for patients with non-small-cell lung cancer with uncommon EGFR mutations.

**Contributors**
JC-HY contributed to the scientific literature search, accrual and treatment of patients, study design, oversight, and supervision, and data acquisition, analysis, and interpretation. LVS contributed to accrual and treatment of patients, study design, oversight, and supervision, and data interpretation. SLG contributed to data acquisition. C-MT and NY contributed to accrual and treatment of patients and data acquisition and interpretation. TKSM contributed to study design and data interpretation. MS contributed to provision of study material, patient accrual, and data acquisition, analysis, and interpretation. C-JY contributed to data acquisition and interpretation. S-HIO contributed to patient accrual and data interpretation. CZ contributed to data acquisition, analysis, and interpretation. DM contributed to study design and data analysis and interpretation. VZ contributed to the literature search and data acquisition, analysis, and interpretation. Y-LW contributed to study conception and design, data analysis and interpretation, and study oversight and supervision. All authors were involved in the drafting and reviewing of the manuscript, and provided approval for submission.

**Declaration of interests**
JC-HY received honoraria from Astellas, AstraZeneca, Bayer, Boehringer Ingelheim, Clovis Oncology, Eli Lilly, Innopharma, Merck Serono, MSD, Novartis, Ono Pharmaceuticals, Pfizer, and Roche/Genentech. He also reports receiving an institutional fee for advisory board participation from Takeda and a grant from Boehringer Ingelheim. LVS reports non-compensated consulting for AstraZeneca, Boehringer Ingelheim, Eli Lilly, Pfizer, and Roche. TKSM received honoraria from Amgen, AstraZeneca, AVEO, Bayer, BioMarin, BMS, Boehringer Ingelheim, Clovis Oncology, Eisai, Eli Lilly, Janssen, Merck Serono, Novartis, Pfizer, Roche, and Taiho, and received a grant from AstraZeneca. MS received honoraria from AstraZeneca, Eli Lilly, GlaxoSmithKline, Novartis, and Pfizer, and reports that his institution received grants from Boehringer Ingelheim and Novartis. DM is an employee of Boehringer Ingelheim. VZ is an employee of Boehringer Ingelheim. Y-LW received honoraria from Roche, AstraZeneca, Eli Lilly, Sanofi, and Pfizer. All other authors declare no competing interests.

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