Detection and Dynamic Changes of \textit{EGFR} Mutations from Circulating Tumor DNA as a Predictor of Survival Outcomes in NSCLC Patients Treated with First-line Intercalated Erlotinib and Chemotherapy

Tony Mok\textsuperscript{1}, Yi-Long Wu\textsuperscript{2}, Jin Soo Lee\textsuperscript{3}, Chong-Jen Yu\textsuperscript{4}, Virote Sriuranpong\textsuperscript{5}, Jennifer Sandoval-Tan\textsuperscript{6}, Guia Ladrera\textsuperscript{7}, Sumitra Thongprasert\textsuperscript{8}, Vichien Srimuninnimit\textsuperscript{9}, Meilin Liao\textsuperscript{10}, Yunzhong Zhu\textsuperscript{11}, Caicun Zhou\textsuperscript{12}, Fatima Fuerte\textsuperscript{13}, Benjamin Margono\textsuperscript{14}, Wei Wen\textsuperscript{15}, Julie Tsai\textsuperscript{15}, Matt Truman\textsuperscript{16}, Barbara Klughammer\textsuperscript{17}, David S. Shames\textsuperscript{18}, and Lin Wu\textsuperscript{15}

Abstract

\textbf{Purpose:} Blood-based circulating-free (cf) tumor DNA may be an alternative to tissue-based \textit{EGFR} mutation testing in NSCLC. This exploratory analysis compares matched tumor and blood samples from the FASTACT-2 study.

\textbf{Experimental Design:} Patients were randomized to receive six cycles of gemcitabine/platinum plus sequential erlotinib or placebo. \textit{EGFR} mutation testing was performed using the cobas tissue test and the cobas blood test (in development). Blood samples at baseline, cycle 3, and progression were assessed for blood test detection rate, sensitivity, and specificity; concordance with matched tumor analysis (\(n = 238\)), and correlation with progression-free survival (PFS) and overall survival (OS).

\textbf{Results:} Concordance between tissue and blood tests was 88\%, with blood test sensitivity of 75\% and a specificity of 96\%. Median PFS was 13.1 versus 6.0 months for erlotinib and placebo, respectively, for those with baseline \textit{EGFR} mut\(^+\) cfDNA [HR, 0.22; 95\% confidence intervals (CI), 0.14–0.33, \(P < 0.0001\)] and 6.2 versus 6.1 months, respectively, for the \textit{EGFR} mut\(^+\) cfDNA subgroup (HR, 0.83; 95\% CI, 0.65–1.04, \(P = 0.1076\)). For patients with \textit{EGFR} mut\(^+\) cfDNA at baseline, median PFS was 7.2 versus 12.0 months for cycle 3 \textit{EGFR} mut\(^+\) cfDNA versus cycle 3 \textit{EGFR} mut\(^-\) patients, respectively (HR, 0.32; 95\% CI, 0.21–0.48, \(P < 0.0001\)); median OS by cycle 3 status was 18.2 and 31.9 months, respectively (HR, 0.51; 95\% CI, 0.31–0.84, \(P = 0.0066\)).

\textbf{Conclusions:} Blood-based \textit{EGFR} mutation analysis is relatively sensitive and highly specific. Dynamic changes in cfDNA \textit{EGFR} mutation status relative to baseline may predict clinical outcomes.

Translational Relevance

Several studies have retrospectively assessed blood-based analysis of EGFR mutation status; however, a prospective analysis of blood-based EGFR mutation assessment was needed. The prospective analysis of blood-based assessment of EGFR mutation status in the FASTACT-2 trial showed that this was a relatively sensitive and highly specific method for mutation detection. In those with blood-based EGFR mutation–positive results at baseline, the dynamic change in EGFR status in blood samples was linked with efficacy outcomes. Those with EGFR mutation–negative assessment at cycle 3 had better efficacy outcomes in terms of PFS and OS than those whose samples were still EGFR mutation positive at cycle 3. This suggests that the dynamic change in blood-based EGFR status could be used to predict benefit of further treatment with erlotinib. These current results show that plasma cfDNA is a potential source material for EGFR mutation analysis in clinical practice for those unable to provide tissue-based samples.

colleagues showed a detection rate of 16.7% and a concordance with tumor mutation status of 92.9% using Scorpion-ARMS in patients treated with gefitinib (14). This method had a sensitivity of 78.9% and a specificity of 97.0%. Patients with EGFR mutations in both tumor and blood samples had significantly longer median PFS than patients without blood-based mutations (P = 0.044; ref. 14). A detection rate of 23.7% and concordance of 66.3% were reported by Goto and colleagues, as assessed by Scorpion-ARMS in a Japanese subgroup of patients from the IPASS study (15). Sensitivity and specificity were 43.1% and 100%, respectively. Recently, a Scorpion-ARMS methodology was used in a first-line, single-arm study of gefitinib, resulting in a concordance rate of 94.3%, with sensitivity of 65.7% and specificity of 99.8% (16). Kim and colleagues used peptide nucleic acid-mediated PCR clamping to analyze EGFR mutations in blood samples, resulting in a detection rate of 16.7%, concordance of 27.5%, and sensitivity of 20.7% (17). Couraud and colleagues have developed multiplex PCR-based assays to identify EGFR mutations from plasma, resulting in concordance with tumor samples of 81% for exon 19 mutations and 97% for exon 21 mutations (18). Recent studies have also shown the feasibility of using digital droplet PCR for plasma-based assessment of mutations (19). The variable outcomes of these studies are explained by the different technologies used, lack of standardization, and the absence of prospective clinical and biomarker data. In addition, these studies did not evaluate the utility of measuring pharmacodynamic changes in cfDNA EGFR mutation levels during treatment, which could better inform clinical decision-making and improve outcomes.

FASTACT-2 (First-line Asian Sequential Tarceva And Chemotherapy Trial), a randomized, phase III study, was designed to improve informed consent before any study-related procedure, including provision of samples for biomarker testing.

Materials and Methods

Study design

FASTACT-2 was a multicenter, randomized, placebo-controlled, double-blind, phase III study of intercalated erlotinib or placebo with gemcitabine plus platinum (carboplatin or cisplatin) followed by maintenance erlotinib or placebo as first-line treatment in patients with stage IIIB/JV NSCLC (21). All patients provided written informed consent. In addition, these studies did not evaluate the utility of measuring pharmacodynamic changes in cfDNA EGFR mutation levels during treatment, which could better inform clinical decision-making and improve outcomes.

Procedures

Full methodology has been previously described (21). Briefly, patients were randomized 1:1 by interactive internet response system to receive six cycles of gemcitabine (1,250 mg/m² intravenously on days 1 and 8 of a 4-week cycle) plus platinum (carboplatin 5 × AUC, or cisplatin 75 mg/m² intravenously on day 1 of a 4-week cycle), plus either sequential erlotinib (150 mg/day orally; erlotinib arm) or placebo (placebo arm) on days 15 to 28 of each cycle. Those who did not progress during the six cycles of sequential treatment continued to receive erlotinib or placebo until disease progression (PD), unacceptable toxicity, or death. At PD, treatment was unblinded and patients in the placebo group could receive open-label erlotinib; patients in the erlotinib group could receive further treatment at the discretion of the investigator.

Biomarker analysis

Tumor tissue samples from either initial diagnosis, diagnosis of advanced/metastatic disease, or resection or biopsy 14 days before first study dose, were required. Blood for plasma and serum isolation was collected according to standard procedures at baseline (within 7 days before first study dose), at day 1 of cycle 3 (C3; before C3 study treatment) and at the time of PD. Samples will be stored for up to 5 years after the final study database lock, at which time they will be destroyed.

Retrospective EGFR mutation testing of formalin-fixed paraffin-embedded tissue (FFPET) and plasma/serum was performed with two allele-specific PCR assays: the cobas 4800 FFPET test utilized as per manufacturer’s instructions (Roche Molecular Systems Inc.) and the cobas 4800 blood test (in development, provided by Roche Molecular Systems Inc.). The EGFR blood test was performed using 2 mL of blood. Each patient’s blood samples from all three time points were tested. For approximately 80% of the baseline samples, it was necessary to combine one aliquot of serum with the plasma sample to achieve the required 2 mL (the blood assay is highly concordant between serum and plasma samples, data on file). A total of 2 mL of blood was used for cfDNA extraction using the cobas cell-free DNA Purification Kit (in development; ref. 22). The cfDNA was eluted in 100 µL, 75 µL.
of which was used for EGFR mutation detection. Both the tissue and blood tests detect 41 EGFR mutations (including G719A/S/C in exon 18, deletions and complex mutations in exon 19, S768I, T790M, and exon 20 insertions, and L858R in exon 21).

The detection limit of copy number for each mutation was determined using minigenes carrying each mutation titrated into samples. ECOG PS, Eastern Cooperative Oncology Group performance status.

Table 1. Baseline characteristics stratified by tumor or cfDNA EGFR mutation status at baseline

<table>
<thead>
<tr>
<th>Tumor EGFR mut</th>
<th>cDNA EGFR mut</th>
<th>cDNA EGFR mut</th>
<th>cDNA EGFR mut</th>
<th>cDNA EGFR mut</th>
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<tr>
<td>(N = 97)</td>
<td>(N = 136)</td>
<td>(N = 144)</td>
<td>(N = 149)</td>
<td>(N = 154)</td>
</tr>
<tr>
<td>GC: E</td>
<td>GC: P</td>
<td>GC: E</td>
<td>GC: P</td>
<td>GC: E</td>
</tr>
<tr>
<td>(N = 49)</td>
<td>(N = 48)</td>
<td>(N = 69)</td>
<td>(N = 67)</td>
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<tr>
<td>Male</td>
<td>21 (43.0)</td>
<td>23 (48.0)</td>
<td>21 (48.0)</td>
<td>21 (50.0)</td>
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<tr>
<td>Female</td>
<td>28 (57.0)</td>
<td>25 (52.0)</td>
<td>28 (41.0)</td>
<td>25 (50.0)</td>
</tr>
<tr>
<td>Median age, ye</td>
<td>57.0</td>
<td>56.0</td>
<td>55.0</td>
<td>58.0</td>
</tr>
<tr>
<td>ECOG PS</td>
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<tr>
<td>0</td>
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<td>12 (26.0)</td>
<td>21 (30.0)</td>
<td>17 (25.0)</td>
</tr>
<tr>
<td>1</td>
<td>36 (75.0)</td>
<td>35 (74.0)</td>
<td>48 (70.0)</td>
<td>50 (75.0)</td>
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<tr>
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<td>Smoking status</td>
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<tr>
<td>Current</td>
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<td>7 (15.0)</td>
<td>22 (32.0)</td>
<td>26 (39.0)</td>
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<tr>
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<td>8 (17.0)</td>
<td>17 (25.0)</td>
<td>20 (30.0)</td>
</tr>
<tr>
<td>Never</td>
<td>35 (71.0)</td>
<td>33 (69.0)</td>
<td>30 (45.0)</td>
<td>21 (31.0)</td>
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<tr>
<td>Disease stage</td>
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<td></td>
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<tr>
<td>IIB</td>
<td>1 (2.0)</td>
<td>2 (4.0)</td>
<td>11 (16.0)</td>
<td>8 (12.0)</td>
</tr>
<tr>
<td>IV</td>
<td>48 (98.0)</td>
<td>46 (96.0)</td>
<td>58 (84.0)</td>
<td>59 (88.0)</td>
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<td>Histology</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Adenocarcinoma</td>
<td>45 (92.0)</td>
<td>44 (92.0)</td>
<td>48 (70.0)</td>
<td>45 (67.0)</td>
</tr>
<tr>
<td>Other</td>
<td>4 (8.0)</td>
<td>4 (8.0)</td>
<td>21 (30.0)</td>
<td>22 (33.0)</td>
</tr>
</tbody>
</table>

NOTE: Of the 241 tumor samples available at baseline, eight had single resistance mutations, which were not counted as EGFR mutation positive or negative. Abbreviation: ECOG PS, Eastern Cooperative Oncology Group performance status.

Statistical analysis
The primary objective of this exploratory analysis was to assess the diagnostic utility of the blood test for sensitivity, specificity, positive predictive value, negative predictive value, concordance rate, and comparison with tumor tissue EGFR mutation status, as assessed by the FFPET assay. Using matched tissue and plasma samples, concordance rate was calculated as the number of samples positive in both tissue and plasma, plus the number of samples negative in both tissue and plasma, out of the total number of matched samples. Sensitivity was calculated as the number of samples positive in both tissue and plasma out of the positive tissue samples, whereas specificity was calculated from the number of plasma- and tissue-negative samples out of the total negative tissue samples. Positive predictive value was the tissue- and plasma-positive rate in positive plasma samples and negative predictive value was the tissue- and plasma-negative rate in the negative plasma samples. Secondary objectives included: assessing the predictive value of baseline cfDNA EGFR mutations on treatment outcomes in FASTACT-2, including PFS, OS, and objective response rate (ORR); evaluating the clinical utility of measuring dynamic changes in cfDNA EGFR mutation allele copy number at baseline, C3 and PD; and exploring the predictive value of C3 cfDNA EGFR mutation status in terms of treatment outcomes. The positive predictive value of C3 cfDNA EGFR mutation status was explored using a subset of patients who were both cfDNA EGFR positive at baseline and had valid C3 cfDNA EGFR results. The requirement of C3 cfDNA results meant that patients withdrawing before C3 were not included in this analysis. The clinical outcomes for PFS and OS with all patients combined (erlotinib and placebo pooled) and erlotinib-treated patients only were evaluated for the C3 analysis.

RESULTS
At baseline, 241 tissue samples (from 53.4% of patients) and 447 blood samples (99.1% of patients) were available for analysis. Baseline clinical characteristics stratified by mutation status and therapy were well balanced between treatment groups (Table 1).

Of the tissue and blood samples, there were a total of 238 matched samples. At C3, 362 blood samples were available for analysis and at PD, 376 blood samples were available. In all, 305 patients (67.6%) had blood-based results at all three time points (Fig. 1). Overall concordance between blood and tissue samples at baseline was 88% (209/238; Table 2). Sensitivity and specificity were 75% (72/96) and 96% (137/142), respectively. The positive predictive value was 94% (72/77) and the negative predictive value was 85% (137/161). In total, five cases were EGFR mutation positive in blood samples but EGFR mutation negative in the corresponding tissue sample, whereas 24 cases were mutation positive in tissue and mutation negative in blood samples. Mutation-specific concordance was 94.5% for exon 19 deletions, 93.3% for L858R mutations, 99.6% for G719x, and 100% for L861Q.
L861Q (Supplementary Table S1). Sensitivity was 82.5% for exon 19 deletions, 62.2% for L858R, 50% for G719x, and 100% for L861Q; specificity was 98.3%, 99%, 100%, and 100%, respectively. The number of tissue-positive G719x and L861Q mutations were very low (n = 2 and n = 1, respectively) which makes the sensitivity estimates highly variable.

Of the 241 tissue samples at baseline, 105 (43.5%) were confirmed to harbor EGFR mutations and the most common mutations identified were exon 19 deletions only (56/105; 53.3%) and L858R mutations only (33/105; 31.4%; Supplementary Table S2). The frequency of EGFR mutation detection was similar in the baseline blood samples. EGFR mutation types in the 238 matched samples are shown in Supplementary Table S3.

Predictive power of cfDNA EGFR mutations

The cfDNA EGFR mutation–positive (mut+) subgroup (n = 144) had a median PFS of 13.1 months versus 6.0 months for erlotinib and placebo arms, respectively [HR, 0.22; 95% confidence interval (CI), 0.14–0.33, P < 0.0001; Supplementary Fig. S1A], with median OS of 29.3 versus 21.4 months, respectively (HR, 0.59; 95% CI, 0.30–1.15, P = 0.1202; Supplementary Fig. S1B). Similar results were obtained from the analysis of tissue EGFR mutation–positive status (21). In the cfDNA EGFR mutation–negative (mut−) group (n = 303), median PFS was reported as 6.2 months for erlotinib versus 6.1 months for placebo (HR, 0.83; 95% CI, 0.65–1.04, P = 0.1076; Supplementary Fig. S1E), with median OS of 15.3 months and 13.6 months for erlotinib and placebo, respectively (HR, 0.94; 95% CI, 0.72–1.22, P = 0.6449; Supplementary Fig. S1F). Again, tissue-based analysis resulted in similar outcomes (21). Therefore, EGFR mutation status defined by blood-based cfDNA analysis appears to produce similar results to tissue-based assessment in terms of predicting outcomes. In those with only blood-based samples available, cfDNA mut− status resulted in median PFS of 5.5 months for erlotinib and 5.9 months for placebo (HR, 0.85; 95% CI, 0.60–1.19, P = 0.3398; Supplementary Fig. S1G) and median OS of 13.0 months and 13.6 months, respectively (HR, 1.07; 95% CI, 0.73–1.56, P = 0.7387; Supplementary Fig. S1H).

Dynamic changes in cfDNA EGFR mutations

Dynamic changes in EGFR mut− cfDNA levels at baseline, C3, and PD are shown in Fig. 2. Total EGFR mutation–specific cfDNA levels decreased at C3 and returned at time of PD. There were fewer mutant EGFR alleles at both the C3 and PD time points in samples derived from patients in the erlotinib arm compared with the
placebo arm (C3 medians: 0 copy/mL for erlotinib, 5 for placebo; PD medians: 6 copy/mL for erlotinib, 83 for placebo).

Although the small sample size should be noted, in patients with cfDNA EGFR mut status at baseline, ORR was lower in patients whose cfDNA samples remained EGFR mut at C3 (33%, 14/42) compared with patients whose cfDNA samples registered as EGFR mut (66%, 53/80) at C3. When assessed in patients from the erlotinib and chemotherapy combination arm only, ORR was 67% (6/9) for those with cfDNA EGFR mut+ samples at C3 and 83% (47/57) for those with cfDNA EGFR mut- samples at C3 (Table 3).

Treatment outcomes were also assessed in all patients (erlotinib and placebo arms combined) who were cfDNA mut+ at baseline, according to C3 cfDNA EGFR mutation status. Median PFS for patients who continued to have detectable mutant EGFR alleles at C3 was 7.2 months versus 12.0 months for patients with no detectable mutant alleles (HR, 0.32; 95% CI, 0.21–0.48, P < 0.0001; Fig. 3A). Similarly, median OS for patients who continued to have detectable EGFR mutations at C3 was 18.2 months, whereas for patients without detectable mutations median OS was 31.9 months (HR, 0.51; 95% CI, 0.31–0.84, P = 0.0066). Patients in the erlotinib arm only were further analyzed: cfDNA EGFR mut+ status at C3 was associated with significantly improved PFS (HR, 0.38; P = 0.0083) and numerically longer OS (HR, 0.45; P = 0.0831) compared with patients whose cfDNA was EGFR mut+ at C3 (Fig. 3B). In the placebo arm, cfDNA EGFR mut- status at C3 resulted in numerically longer PFS (HR, 0.64; P = 0.1112) and OS (HR, 0.71; P = 0.3325) versus patients with cfDNA EGFR mut+ status at C3 (Table 3).

**Table 3.** Efficacy outcomes for baseline cfDNA mut+ patients by C3 cfDNA mutation status

<table>
<thead>
<tr>
<th>C3</th>
<th>ORR, %</th>
<th>Median PFS, mo</th>
<th>Median OS, mo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EGFR mut+</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC+P (n = 33)</td>
<td>24.2</td>
<td>6.8</td>
<td>18.8</td>
</tr>
<tr>
<td>GC+E (n = 9)</td>
<td>46.7</td>
<td>7.8</td>
<td>17.7</td>
</tr>
<tr>
<td>OR, 6.25 (95% CI, 1.26–30.90)</td>
<td>HR, 0.38 (95% CI, 0.17–0.90)</td>
<td>HR, 0.98 (95% CI, 0.40–2.42)</td>
<td></td>
</tr>
<tr>
<td><strong>EGFR mut-</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC+P (n = 23)</td>
<td>26.1</td>
<td>7.8</td>
<td>26.3</td>
</tr>
<tr>
<td>GC+E (n = 57)</td>
<td>82.5</td>
<td>16.6</td>
<td>32.4</td>
</tr>
<tr>
<td>OR, 13.32 (95% CI, 4.20–42.23)</td>
<td>HR, 0.23 (95% CI, 0.13–0.41)</td>
<td>HR, 0.61 (95% CI, 0.31–1.21)</td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

To our knowledge, this is the first study to demonstrate the predictive value of baseline and C3 cfDNA EGFR mutation status in blood in a phase III, randomized, controlled study. In this study, blood-based testing for EGFR activating mutations was relatively sensitive (75%) and highly specific (96%), with high concordance between matched blood-based and tumor tissue samples (88%), suggesting that a blood-based assay may have utility in clinical practice. Concordance was >90% for specific mutations when analyzed separately; however, sample size for the G719X and L861Q subsets was too small for adequate individual analysis. Reasons for the relatively low sensitivity in detection of L858R are unclear. This should be further investigated in a larger trial and possibly with an alternative technology such as Digital PCR.

A number of prior studies have investigated the use of cfDNA for the assessment of EGFR mutation status with varying results. The variation in concordance and detection rates of these different methods highlights the need for a sensitive, standardized method for blood-based testing. Results of the current study suggest that cfDNA EGFR mutation analysis is a potential alternative testing method for those patients from whom a tumor tissue sample cannot be obtained. This approach may also enable faster turnaround for molecular diagnosis in the first-line setting, and could be used as an initial screening tool for earlier diagnosis alongside current tissue-based approaches. Patients usually present with a radiologic image suggestive of primary bronchogenic carcinoma. The standard course of action with the suspicion of lung cancer...
include bronchoscopy and/or needle biopsy and pathologic evaluation before sending out for molecular testing. With the blood test concordance of 88%, molecular analysis could be performed much earlier and be accurate in three out of four patients.

This analysis reported 24 false-negative cases and five potentially false-positive cases. This could lead to the potential risk of inappropriate selection of patients for first-line treatment, although it is also possible that the tissue result was misleading due to selection bias of the biopsied lesion. Of the five false-positive cases, three received erlotinib plus chemotherapy and had best overall responses of stable disease (n=2) and partial response (n=1); PFS in these 3 patients was 7.2, 12.7, and 5.5 months, respectively, and OS was 9.1, 21.6, and 18.1 months, respectively. Of the false-negative cases, 11 received erlotinib and had median PFS of 19.1 months, and median OS of 31.4 months. Because of the nature of the study design, it cannot be confirmed whether these were true false-positive or false-negative cases. The observation that the blood result differed from the tissue result may be explained by the heterogeneous nature of NSCLC tumor biology (23). Presuming the presence of both EGFR mut⁺ and mut⁻ tumor tissue in a patient, their cfDNA could test positive, while a biopsy of primarily EGFR mut⁺ tissue would show the contrary result. This question might be resolved in the future by multiple tumor sampling and/or clinical correlation of tumor response to single-agent EGFR TKI.

Total EGFR mutation–specific cfDNA levels decreased at C3 and returned at time of PD, which may be due to changes in tumor volume or increased metastases (24). Larger tumor volume or more metastatic tumors may provide more DNA to “leak” from the necrotic tumor into the bloodstream, resulting in higher DNA levels. Finding fewer EGFR-mutant alleles in the erlotinib arm at the C3 timepoint is consistent with the mode of action of erlotinib, in inhibiting EGFR-mutant tumor cells. Serial quantitative measurement of EGFR mut⁺ cfDNA could therefore be an alternative method to assess tumor progression. Limited by infrequent sampling, small sample size, and use of combination therapy, our current study results may only establish the feasibility for future prospective studies.

Median PFS with first-line EGFR TKIs in patients with EGFR mutations ranges from 9.2 to 14.0 months, but not all patients benefit equally (1, 3, 4, 5). Genomic markers such as BIM...
polymorphisms are predictive of shorter PFS in patients treated with first-line EGFR TKIs (25). cfDNA EGFR mutation status at C3 could offer another simple predictive biomarker of outcomes before eventual radiologic progression. The complete disappearance of EGFR mutations in cfDNA is reminiscent of the molecular remission of Philadelphia chromosome in patients with chronic myeloid leukemia (26). cfDNA EGFR mut⁺ status at C3 was predictive of worse PFS and OS. Again, this could be linked to the change in tumor burden or increased metastases, as this may be associated with worse survival outcomes. Leduc and colleagues reported significant reductions in PFS with increasing tumor volume in patients with EGFR mut⁺ disease receiving EGFR TKIs (27). Median PFS for tumor volume <35cc, 35 to 74cc, and >74cc was 9 months, 8 months, and 7.3 months, respectively (P = 0.04). A future study correlating serial blood-based EGFR mutation status with tumor volume is warranted.

Key questions to address in any future trials would include what a treatment algorithm would look like based on dynamic change in cfDNA EGFR status; for example, whether baseline cfDNA mut⁺ and C3 cfDNA mut⁺ status without radiologic progression should result in a change of treatment, such as the addition of another agent or a switch to another regimen.

The limitations of the study must be noted when interpreting this analysis, including the exploratory nature of these results and the small sample size, particularly in the C3 analysis (n = 66 for the erlotinib arm). In addition, although the control arm of this study was standard chemotherapy, a total of 85% of patients in the erlotinib arm received TKI therapy as second-line treatment. In addition, although the control arm of this study was standard chemotherapy, a total of 85% of patients in the erlotinib arm received TKI therapy as second-line treatment.

Conclusions

Use of blood-based cfDNA for EGFR mutation analysis is feasible and the PCR-based assay offers a sensitive and highly specific test with potential clinical application. C3 cfDNA EGFR mutation status is potentially predictive of clinical outcomes and warrants further investigation in a prospective study.

Disclosure of Potential Conflicts of Interest

T.S.K. Mok reports receiving speakers bureau honoraria from Amgen, AstraZeneca, Boehringer Ingelheim, Eli Lilly, Merck Serono, Pfizer, and Roche/Genentech; and is a consultant/advisory board member for AstraZeneca, BioMarin, Boehringer Ingelheim, Clovis Oncology, Eli Lilly, Janssen, Merck Serono, Novartis, Pfizer, and Roche/Genentech. Y.-L. Wu is a consultant/advisory board member for MSD. J. Tsai is an inventor on a patent application for blood mutation detection. M. Truman has ownership interest (including patents) and is a consultant/advisory board member for Roche Products Ltd. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: T. Mok, Y.-L. Wu, J.S. Lee, S. Thongprasert, M. Truman, B. Klughammer


Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T. Mok, Y.-L. Wu, J.–J. Yu, V. Sriuranpong, J. Sandoval-Tan, G. Ladefoge, S. Thongprasert, V. Sirimunninimit, M. Liao, Y. Zhu, C. Zhou, F. Fuerte, B. Margono, M. Truman, B. Klughammer

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T. Mok, Y.-L. Wu, J.S. Lee, S. Thongprasert, C. Zhou, W. Wen, J. Tsai, M. Truman, B. Klughammer, D.S. Shames


Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): W. Wen, M. Truman, B. Klughammer

Study supervision: T. Mok, Y.-L. Wu, S. Thongprasert, C. Zhou, B. Klughammer

Other (final approval of manuscript): J. Tsai

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