Title	Plasma EGFR Mutation Detection Associated With Survival Outcomes in Advanced-Stage Lung Cancer
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Original Study

Plasma *EGFR* Mutation Detection Associated With Survival Outcomes in Advanced-Stage Lung Cancer

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Abstract

We confirmed the performance of an array method for plasma epidermal growth factor receptor (*EGFR*) mutation detection and showed the association of plasma *EGFR* mutation with survival outcomes.

Background: Noninvasive detection of epidermal growth factor receptor (*EGFR*) mutation in plasma is feasible and could be adjunct for therapeutic monitoring especially when repeated biopsy of tumor tissue is challenging. The aims of this study were to establish the diagnostic performance of peptide nucleic acid-locked nucleic acid polymerase chain reaction followed by custom array for plasma *EGFR* mutation and to evaluate the association of detection with clinical characteristics and survival outcomes. **Materials and Methods:** Plasma genomic DNA from consecutive advanced lung cancer subjects was tested for *EGFR* mutations before anticancer treatment, and compared with mutation status in tumor tissue. Clinical characteristics were compared between patients who were *EGFR*-mutant and wild type; and within *EGFR* mutants, whether *EGFR* mutations could be detected in plasma. **Results:** In 74 lung cancer patients, the sensitivity, specificity, and positive and negative predictive values of plasma *EGFR* detection were 79.1%, 96.8%, 97.1%, and 76.9%, respectively. *EGFR* mutants with concomitant detection of plasma *EGFR* mutation showed worse survival compared with mutants with no concomitant plasma mutation detected in biopsy specimens. **Conclusion:** Plasma *EGFR* mutation detected using this method demonstrated high diagnostic performance. In *EGFR* mutants, plasma *EGFR* mutation detection correlated not only *EGFR* mutation status in biopsy but was also associated with worse prognosis compared with *EGFR* mutant without plasma *EGFR* mutation detection.

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Keywords: *EGFR* mutation, Lung adenocarcinoma, Plasma DNA, Prognosis, Survival outcomes

Introduction

The detection of epidermal growth factor receptor (*EGFR*) mutations in tissue biopsy has become a routine molecular test with diagnostic and therapeutic implications for advanced stage non−small-cell lung cancer (NSCLC). The presence of activating *EGFR* mutations, namely exon 19 deletion or L858R mutation at exon 21, predicts favorable response to the *EGFR* tyrosine kinase inhibitors (TKIs).[1] In clinical situations in which patients are not medically fit for invasive monitoring procedures, it could be dangerous or technically impossible to repeat a biopsy. Even if repeat biopsy is possible, the amount of tissue obtained could either be inadequate for molecular testing because of its small quantity or very low tumor content or is not readily available.

Plasma DNA might provide a noninvasive means of detecting plasma *EGFR* mutations. Several different methods, including high performance liquid chromatography,[2] allele-specific polymerase chain reaction (PCR) with Scorpion-amplification[3,4] or subsequent sequencing,[5] peptide nucleic acid (PNA)-mediated PCR clamping methods,[6] mass spectrophotometry genotyping,[7] BEAMing,[8] and the droplet digital PCR,[9,10] and next-generation sequencing[11] have been established and reported for the detection of *EGFR* mutations in DNA isolated from plasma[2,10,12] with variable but comparable performance[13] and showed some correlation between mutation status in

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plasma, pleural fluid, and tumor tissue.[14] Furthermore, *EGFR* mutations detected in plasma or serum might, by itself, be predictive of response to EGFR TKIs.[2,12,14] All of these reports described detection sensitivity from 66% to 100% and a specificity from 63% to 100%.

We reported previously, using the same method as in this study, the successful detection of *EGFR* mutation in plasma DNA extracted from patients with advanced-stage NSCLC.[15] The aim of this study was to establish the performance of the PNA-locked nucleic acid (LNA) PCR followed by specific custom array hybridization in the detection of *EGFR* mutations in plasma samples of patients with advanced stage NSCLC and to study for any prognostication or association between plasma *EGFR* mutation detection, clinical characteristics, and survival outcomes.

Materials and Methods

Lung Cancer Patients

We recruited patients with newly diagnosed biopsy or cytology-proven stage III or IV NSCLC under medical care in the Department of Medicine, Queen Mary Hospital, University of Hong Kong. Patients with small-cell lung cancer, with Eastern Cooperative Oncology Group (ECOG) performance status ≥ 3, or who could not give informed consent were excluded from recruitment. The study was approved by the University of Hong Kong/Hong Kong Hospital Authority Institutional Review Board and Ethics Committee (HKU/HA HKW IRB UW 13-488). Tumor biopsy samples were analyzed with routine histopathological and molecular pathology service on formalin-fixed, paraffin-embedded specimens. Recruited patients were followed-up for their subsequent management for treatment response, disease control and progression, lines of treatment, and duration of progression-free survival (PFS) and overall survival (OS).

Venous blood samples (10 mL) were collected from all recruited subjects before they were started on any and on the same day of starting anticancer therapy. The choice of treatment was reached by attending physicians and patients without knowing the plasma status of *EGFR* mutation. At recruitment, subject demographic characteristics were recorded. The recruited subjects were followed-up with usual clinical care at every subsequent 3-4 weeks. Chest x-ray was performed at every visit to check for obvious clinical progression and detail imaging with computed tomography (CT) or positron emission tomography-CT was done only when clinical progression was in doubt. PFS was taken as the duration from the commencement of first-line anticancer therapy to the day with clinical disease progression noted on chest imaging or physical examination that showed new sites of involvement like regional lymph node enlargement. OS was taken from the commencement of first-line anticancer therapy to either the day of death or last clinic follow-up. Four recruited subjects (in the *EGFR* wild type group) who had never received any anticancer treatment were not counted for PFS, but their OS was taken as the duration from the day of venous sampling for this study until the day of death or last clinical follow-up. All patients had been informed that EGFR TKIs are known to be less effective in *EGFR* wild type tumors compared with *EGFR* mutant tumors, and the ultimate choice of therapy was decided by the patient. All clinical information were not disclosed to the research laboratory staff responsible for plasma *EGFR* mutation assay.

Blood samples were collected in citrated tubes, and centrifuged at 1800*g* for 10 minutes to separate the plasma. Aliquots of plasma were immediately subjected to DNA extraction (Qiagen DNA mini-kit, Hilden, Germany) and the plasma DNA thus extracted was stored at −80°C until usage for PCR amplification.

Epidermal Growth Factor Receptor Exon 19 Deletion and Exon 21 L858R Assay in Plasma

Detection of the deletions in exon 19 and L858R mutation in exon 21 of *EGFR* was performed using the PNA-LNA PCR clamp method followed by array hybridization with allele-specific arrayed primer extension as previously described.[15] The primer sequences are listed in Table 1. Tests were performed at least twice for every sample.

Statistical Analyses

The relationships between *EGFR* mutation and factors such as sex, age, tumor, node, metastases (TNM) stage, histologic type, smoking, PFS, and OS were examined using the Student *t* test, χ^2 test, or Fisher exact test where appropriate, and Kaplan−Meier analysis using PASW software (IBM, Armonk, NY) version 19. Mean and SD were used for continuous variables in normal distribution and median and interquartile ranges were used for PFS and OS. Data not in normal distribution were logarithmic-transformed before parametric tests. Pearson and Spearman correlations were applied to tests for the correlations between clinical parameters of age, sex, smoking status, TNM staging, ECOG performance status, tumor biopsy *EGFR* mutation status, plasma *EGFR* mutation, plasma detected and not detected among tumor biopsy *EGFR* mutant samples. Stepwise multiple regression models were built to determine the clinical parameters that independently predicted either PFS or OS. Cox regression and Kaplan−Meier analyses were used to compare cumulative survival between different groups. All P values were 2-sided and $P < .05$ was considered statistically significant. The prevalence, sensitivity, specificity, and positive and negative predictive values of *EGFR* detection were calculated using the free Web statistical tools: *www.vassarstats.net*.

Results

Patients and Tumor Characteristics

Of 102 consecutive subjects admitted for management of newly diagnosed advanced-stage NSCLC, 83 patients met all inclusion

Table 1 A List of the PCR Primers Used for Amplification of *EGFR* Exon 19 and 21 in Plasma DNA

For First Round Amplification from Genomic DNA Templates
Exon 19 (5′>3′)
Forward: CCAGATCACTGGGCAGCATGTGGCACC
Reverse: AGCAGGGTCTAGAGCAGAGCAGCTGCC
Exon 21 (5′>3′)
Forward: TCAGAGCCTGGCATGAACATGACCCTG
Reverse: GGTCCCTGGTGTCAGGAAAATGCTGG
For Second Round Seminested Amplification on PCR Amplicons from First Round PCR
Exon 19 Reverse nested
TGGACCCCCACACAGC
Exon 21 Reverse nested
TGCCTCCTTCTGCATGGTATTC

Abbreviation: PCR = polymerase chain reaction.

criteria and 11 small-cell lung cancer patients, 5 with poor performance status (ECOG performance status > 3), and 3 patients mentally incompetent to give consent were excluded. Eight recruited subjects were found to have inadequate tumor biopsy tissue for *EGFR* mutation analysis (4 of them had either squamous-cell carcinoma or large-cell carcinoma that showed necrotic tumor tissue and quality was deemed not suitable for *EGFR* mutation analysis) and 1 patient sample was excluded because of technical problems. Finally, 74 patients with advanced-stage lung cancer were eligible. Patient characteristics are summarized in Table 2. There were 38 men and 36 women, 72 lung adenocarcinomas (97%), 25 smokers (former or current smokers; 34%), and 49 were never-smokers (66%). Twenty-four tumors (32%) had *EGFR* exon 19 deletion (15-base pair deletion in 27 cases), and 19 tumors (26%) had the *EGFR* L858R mutation. The remaining 31 patients (42%) were *EGFR* wild type (Table 3). Of 43 *EGFR*-mutant patients, 42 received EGFR TKIs and 15 of 31 *EGFR* wild type subjects received chemotherapy as first-line treatment, and the remaining 4 of 31 patients opted for no anticancer treatment and 12 of 31 opted for EGFR TKI treatment (Table 3). All patients had been informed that EGFR TKIs are known to be less effective in *EGFR* wild type tumors compared with *EGFR*-mutant tumors, and the ultimate choice of therapy was decided by the patient. Among biopsy *EGFR*-mutant (n = 43), 33 (77%) had plasma *EGFR* mutation detected and 10 (23%) did not have plasma *EGFR* mutation detected (Table 4).

Table 2 Summary of Clinical Characteristics of All Recruited Subjects

Characteristic	Value
Total Recruited, n	74
Age, Years	64.54 ± 11.96
Sex	
M:F ratio	1:1
M	38 (51%)
F	36 (49%)
Smoking History	
SM:NS ratio	1:2
Ever-smoker	25 (34%)
NS	49 (66%)
Histology	
AD	72 (97%)
NSCLC-NOS	1 (1%)
SCC	1 (1%)
Stage	
IIIA	2 (3%)
IIIB	2 (3%)
IV	70 (95%)
M1a	31 (42%)
M1b	39 (53%)
Median PFS (Range), Days	193 (82-374)
Median OS (Range), Days	347 (193-537)

Abbreviations: AD = adenocarcinoma; F = female; M = male; NOS = not otherwise specified; NS = nonsmoker; NSCLC = non–small-cell lung cancer; OS = overall survival; PFS = progression-free survival; SCC = squamous cell carcinoma; SM = smoker.

Diagnostic Performance of the PNA-LNA Method Followed by Array Hybridization

The prevalence of biopsy *EGFR* mutations was 58.1% (43 of 74; 95% confidence interval [CI], 46.1%-69.3%) and 34 of 43 *EGFR* mutant were identified from plasma (true positive), 30 of 31 *EGFR* wild type were confirmed from plasma (true negative; Table 5). The sensitivity of plasma *EGFR* detection was 79.1% (95% CI, 63.5%-89.4%) and specificity was 96.7% (95% CI, 81.5%-99.8%), with a positive predictive value of 97.1% (95% CI, 83.4%-99.9%) and a negative predictive value of 76.9% (95% CI, 60.3%-88.3%; Table 5).

Survival Analysis

Cox regression analysis showed significant correlations between TNM staging (stage III, IV M1a, IV M1b) and log PFS ($r = -0.253$; $P = .03$) and between plasma mutation detection and log OS ($r = 0.324$; $P = .034$), and no correlation was found between TNM staging and plasma mutation detection ($r = 0.199$; $P = .201$). There were no significant correlations between different clinical parameters and PFS or OS in correlation matrix analysis.

For the biopsy *EGFR*-mutant patients, the median PFS for those with plasma mutation detected was shorter than for those without plasma mutation detected but the difference was not statistically significant ($P = .052$) and the median OS for those with plasma mutation detected was significantly shorter than for those without plasma mutation detected ($P = .028$; Table 4). Kaplan–Meier analysis, however, confirmed significantly better PFS and OS in biopsy *EGFR*-mutant compared with biopsy *EGFR* wild type subjects (Figure 1A [PFS], $P = .02$, and Figure 1B [OS], $P = .04$); and among biopsy *EGFR* mutants, a significantly worse PFS and OS in those with plasma *EGFR* mutation detected than for those with no plasma *EGFR* mutation detection (Figure 1C [PFS], $P = .028$, and Figure 1D [OS], $P = .038$).

Discussion

In the present study, we found that this method of *EGFR* mutation detection in plasma DNA samples showed high concordance with tumor genotyping in 86% of the cases (64 of 74; Table 5). In plasma DNA, we detected 79% (19 of 24) of *EGFR* exon 19 deletion cases, and 74% (14 of 19) of *EGFR* L858R mutation cases, compared with the matched primary tumors. Depending on the technique, the concordance between *EGFR* status in tumor and plasma and/or serum samples varied from 66% to 100%, with the highest correlation being found recently in digital droplet PCR.[2,4,5,9,10,12,14,16] The overall diagnostic sensitivity and specificity of 79.1% and 96.8%, respectively, should make it a useful test for subsequent monitoring along the treatment course, especially when repeat biopsy is not always feasible. The high positive predictive value (97.1%) and true negative predictive value (76.9%) indicated the high diagnostic performance of this test and were comparable with other tests reported in the literature.[2,12,17] However, the application and usefulness of plasma *EGFR* mutation detection in longitudinal follow-up warrants further larger scale prospective study, especially when plasma *EGFR* mutations could be assessed repeatedly at different time points of treatment or disease progression much more readily than repeat of tissue biopsies for *EGFR* mutation status.

There was 1 false positive result from a 75-year-old man with stage IV adenocarcinoma of the lung with contralateral lung

Table 3 Characteristics of *EGFR* Mutant Subjects Compared With *EGFR* Wild Type Subjects

Characteristic	*EGFR* Mutant	*EGFR* Wild Type	*P*
Total, n	43	31	
Age, Years	66.72 ± 9.145	61.52 ± 4.651	.064
Sex			
M:F ratio	1:1.6	1:0.5	.017[a]
M	17 (40%)	21 (68%)	
F	26 (60%)	10 (32%)	
Smoking History			
SM:NS	1:4.4	1:0.9	.001[a]
Ever-smoker	8 (19%)	17 (55%)	
NS	35 (81%)	14 (45%)	
Histology			
AD	41 (96%)	31 (100%)	.506 (NSCLC and SCC combined)
NSCLC	1 (2%)	0 (0%)	
SCC	1 (2%)	0 (0%)	
Stage			
IIIA	2 (4%)	0 (0%)	
IIIB	0 (0%)	2 (6%)	
IV	41 (96%)	29 (94%)	.572
M1a	17 (41%)	14 (49%)	
Contralateral lung	6	8	
PI effusion	11	6	
M1b	24 (59%)	15 (51%)	
Intra-abdominal	0	2	
Adrenal	4	0	
Bone	19	11	
Brain	1	2	
ECOG PS			
0	21 (49%)	12 (39%)	.554
1	20 (47%)	16 (52%)	
2	2 (5%)	3 (10%)	
Treatment			
First line			
No treatment	0 (0%)	4 (14%)	
Gefitinib	33 (77%)	6 (19%)	
Erlotinib	9 (21%)	6 (19%)	
Chemotherapy	1 (2%)	15 (48%)	
Second line			
No treatment	21 (49%)	22 (71%)	
Gefitinib	2 (4%)	2 (6%)	
Erlotinib	7 (17%)	2 (6%)	
Chemotherapy	13 (30%)	5 (17%)	
Third line			
No treatment	31 (72%)	24 (77%)	
Gefitinib	0 (0%)	0 (0%)	
Erlotinib	2 (5%)	1 (3%)	
Chemotherapy	10 (23%)	6 (20%)	
Median PFS (Range), Days	239 (151-408)	104 (46-258)	.001[a]
Median OS (Range), Days	371 (239-580)	336 (84-483)	.017[a]

Abbreviations: AD = adenocarcinoma; ECOG PS = Eastern Cooperative Oncology Group performance status; F = female; M = male; NS = nonsmoker; NSCLC = non–small-cell lung cancer; OS = overall survival; PFS = progression-free survival; SCC = squamous cell carcinoma; SM = smoker.
[a] $P < .05$.

nodules. He presented with superior vena cava obstruction and received local radiation therapy. He quickly deteriorated with development of sepsis and pneumonia and died 2 weeks after radiotherapy. His tumor biopsy sample had tested as *EGFR* wild type but the plasma samples had tested as L858R present. Unfortunately, the patient died shortly after radiotherapy and would not allow the time for further monitoring of plasma *EGFR* status. We can only hypothesize that this discrepancy between tested tumor biopsy and tested plasma could be a real false positive result or it might indeed reflect heterogeneity of the tumor. Similar discordance has been reported occasionally when other plasma detection methods were used.[7,18]

We attempted to prove the false negative results with repeat testing and confirmed negative results in the plasma for biopsy *EGFR* wild type tumors. Negative results for plasma tests probably reflect no or undetectable tumor DNA leakage into the systemic circulation, especially when a tumor is relatively nonactive or not inflamed. Among the known *EGFR* mutants from tumor biopsy, we compared the plasma-detected cases with plasma nondetected cases and Kaplan–Meier analysis showed significantly better PFS and OS for plasma nondetected cases (Figure 1). Unlike most other reports for plasma *EGFR* mutation detection,[2,5,7,10] all of the subjects in this study were prospectively recruited, and the corresponding plasma samples were collected, before the subjects received any anticancer therapy. Thus, any plasma detection might be an indirect reflection of the tumor load or activity before treatment, such that those tumors with active replication were leaking DNA into the systemic circulation and thus conferring a worse prognosis for the respective subjects compared with those with no leakage of tumor DNA into the systemic circulation.[19] Similar survival benefits have been observed in subanalyses of a previous Iressa Pan-Asia Study cohort in which subjects with no *EGFR* mutation detected in serum appeared to fare better in terms of PFS and overall response rate after treatment, compared with *EGFR*-mutant subjects with *EGFR* mutation detected in serum.[20] It is possible that the better survival observed in biopsy *EGFR*-mutant patients without concomitant plasma *EGFR* mutation detected could reflect a lower or less active tumor load or disease with lesser leakage of tumor DNA into the systemic circulation, but this remains to be explored in future prospective larger scale randomized studies.

Kim et al reported a very low detection rate (16.7%) in a cohort of 60 patients with pair pretreatment and 2-months posttreatment

Table 4 Comparison of Biopsy *EGFR* Mutant With Plasma Mutation Detection Versus Those With *EGFR* Mutation in Biopsy but Not Detected in Plasma

Characteristic	Biopsy *EGFR* Mutant (n = 43)		*P*
	Plasma Mutation Detected	Mutation Not Detected in Plasma	
Total	33	10	
Age, Years	66.82 ± 8.766	66.4 ± 10.814	.901
Sex			
M:F	1:1.6	1:1.5	1
M	13 (39%)	4 (40%)	
F	20 (61%)	6 (60%)	
Smoking History			
SM:NS	1:5.6	1:2.4	.362
Ever-smoker	5 (15%)	3 (30%)	
NS	28 (85%)	7 (70%)	
Histology			
AD	31 (94%)	10 (100%)	
NSCLC	1 (3%)	0 (0%)	
SCC	1 (3%)	0 (0%)	
Stage			
IIIA	1 (3%)	1 (10%)	
IIIB	0 (0%)	0 (0%)	
IV	32 (97%)	9 (90%)	.45
M1a	12 (38%)	5 (56%)	
Contralateral lung	2	4	
PI effusion	10	1	
M1b	20 (63%)	4 (44%)	
Intra-abdominal	0	0	
Adrenal	4	0	
Bone	15	4	
Brain	1	0	
ECOG PS			
0	17 (52%)	4 (40%)	.322
1	14 (42%)	6 (60%)	
2	2 (6%)	0 (0%)	
Treatment			
First line			
No treatment	0 (0%)	0 (0%)	
Gefitinib	27 (82%)	6 (60%)	
Erlotinib	6 (18%)	3 (30%)	
Chemotherapy	0 (0%)	1 (10%)	
Second line			
No treatment	16 (48%)	5 (50%)	
Gefitinib	2 (6%)	0 (0%)	
Erlotinib	6 (18%)	1 (10%)	
Chemotherapy	9 (27%)	4 (40%)	
Third line			
No treatment	25 (76%)	6 (60%)	
Gefitinib	0 (0%)	0 (0%)	
Erlotinib	1 (3%)	1 (10%)	
Chemotherapy	7 (21%)	3 (30%)	

Table 4 Continued

Characteristic	Biopsy *EGFR* Mutant (n = 43)		*P*
	Plasma Mutation Detected	Mutation Not Detected in Plasma	
Median PFS (Range), Days	239 (146-368)	420 (233-676)	.051
Median OS (Range), Days	305 (211-512)	572 (346-737)	.028[a]

Abbreviations: AD = adenocarcinoma; ECOG PS = Eastern Cooperative Oncology Group performance status; F = female; M = male; NS = nonsmoker; NSCLC = non–small-cell lung cancer; OS = overall survival; PFS = progression-free survival; SCC = squamous cell carcinoma; SM = smoker.
[a] $P < .05$.

samples, using a similar PNA-LNA PCR method.[6] Although we had a similar experience of poor detection rate when we developed this plasma *EGFR* detection method previously,[15] the detection rate markedly improved with fresh processing of collected blood specimens without refrigeration, and citrated blood samples were found to be much better than blood collected with ethylenediaminetetraacetic acid container tubes.[6]

Our study sample showed a significant imbalance between group distributions (ie, significantly more female nonsmokers in the *EGFR*-mutant group; Table 3), but this also reflected the real life setting of gender and smoking status distribution in the local and most other populations[1] and should not have severely confounded the comparison with other similar studies in the literature.[2,6,7,9,12,16] The regression analysis in this study also showed that TNM staging and plasma *EGFR* mutation detection were the predictors for OS independent of the potential confounding effects of age, sex, smoking status, performance status, and lines of treatment. We did not assess the degree of tumor necrosis or inflammation and tumor

Table 5 The Rate of *EGFR* Mutations Detected in Plasma and Biopsy Samples; and Diagnostic Sensitivity and Specificity, and Positive and Negative Predictive Values of This Method of *EGFR* Detection in Plasma

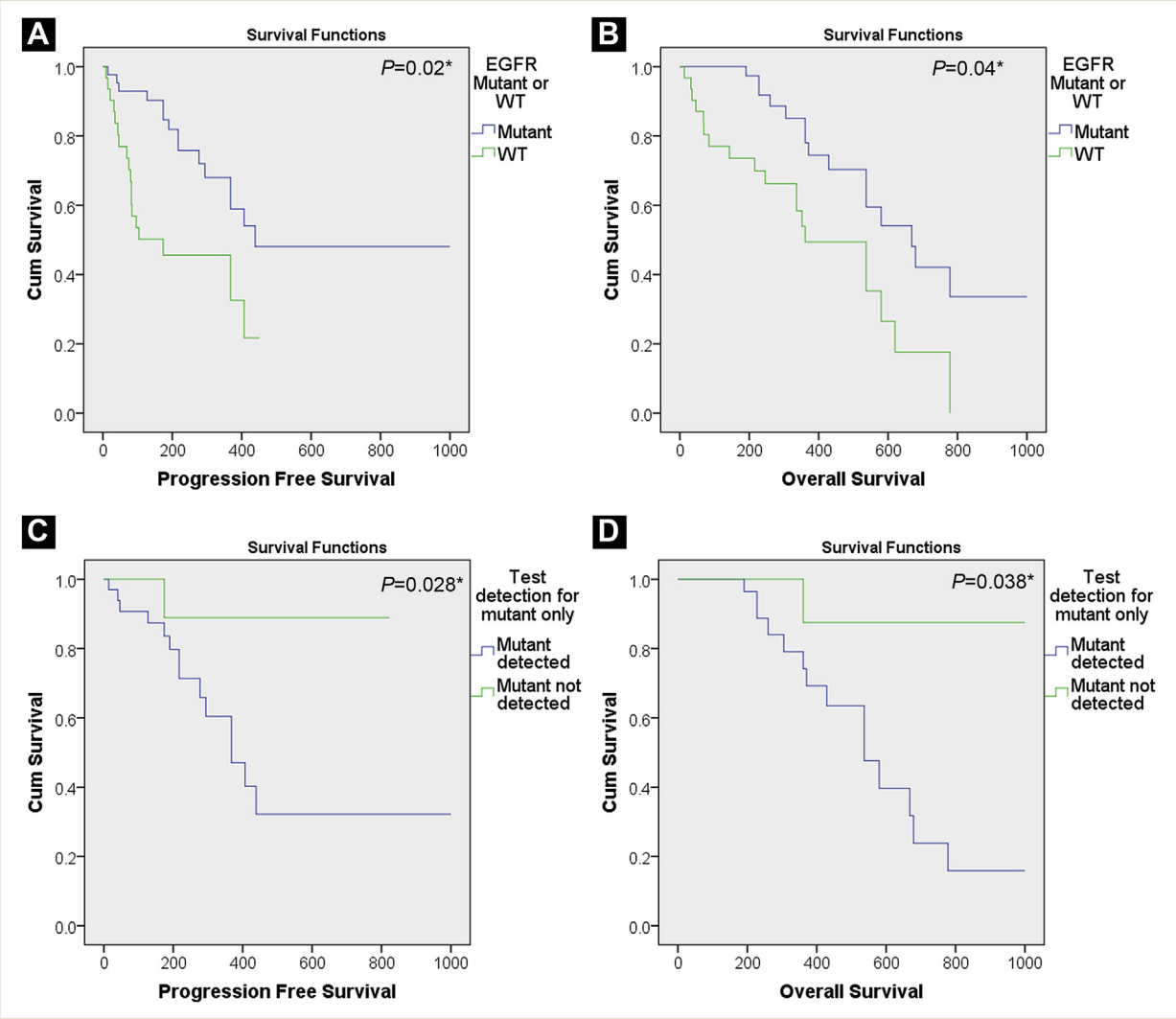
Rate of *EGFR* Mutations Detected in Plasma and Biopsy Samples

	Biopsy *EGFR* Wild Type	Biopsy *EGFR* Mutant	Total
Plasma *EGFR* Mutant	1	34	35
Plasma *EGFR* Wild Type	30	9	39
Total	31	43	74

Diagnostic Sensitivity and Specificity, and Positive and Negative Predictive Values of This Method of *EGFR* Detection in Plasma

	Estimated Value (%)	95% CI	
		Lower Limit	Upper Limit
Sensitivity	79.1	63.5	89.4
Specificity	96.8	81.5	99.8
Positive Predictive Value	97.1	83.4	99.9
Negative Predictive Value	76.9	60.3	88.2

Figure 1 Survival Analysis for (A) PFS and (B) OS for Biopsy *EGFR*-Mutant Versus Wild Type Subjects; (C) PFS and (D) OS for Biopsy *EGFR*-Mutant Subject With Plasma *EGFR* Mutation Detected Versus Plasma *EGFR* Mutation Not Detected



The * Indicates the Statistical Significance of *P* Value.

heterogeneity, factors which might affect the plasma mutation status.[18]

The findings of this study have raised a few interesting issues that warrant further research. In this study, all recruited patients were advanced-stage disease subjects and further studies in early-stage lung cancer subjects are needed to evaluate the possibility of such plasma *EGFR* mutation detection in early-stage disease. Future studies with longitudinal follow-up and serial testing would allow exploration of the clinical usefulness of qualitative and quantitative monitoring of plasma *EGFR* mutation status in advanced-stage lung cancer subjects who receive EGFR-targeted therapy. Finally, it is important to note that, although we and others have demonstrated that the molecular diagnosis of *EGFR* status based on plasma DNA samples is feasible, analysis for *EGFR* mutations in tumor DNA remains the gold standard for diagnosis of *EGFR* mutation status of lung cancer.

Conclusion

The detection method used in this study of EGFR mutation at exon 19 deletions and exon 21 L858R mutations in plasma DNA samples from patients with lung cancer is a reliable, technically feasible method with high diagnostic performance. The better survival observed in biopsy *EGFR*-mutant without concomitant plasma *EGFR* mutation detected could reflect a lower or less active tumor load or disease with lesser leakage of tumor DNA into the systemic circulation, but this remains to be validated in future prospective larger-scale randomized studies.

Clinical Practice Points

- Plasma *EGFR* mutation is detectable in advanced-stage lung cancer patients.

- Plasma *EGFR* mutation detection is not only feasible but the detection of *EGFR* mutation in plasma is also associated with survival outcomes.
- The detection of *EGFR* mutation in plasma could be of prognostic significance in advanced-stage lung cancer patients.

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Disclosure

The authors have stated that they have no conflicts of interest.

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