ORIGINAL ARTICLE

Serial Plasma Cell-Free Circulating Tumor DNA Tests Identify Genomic Alterations for Early Prediction of Osimertinib Treatment Outcome in *EGFR* T790M–Positive NSCLC

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ABSTRACT

Introduction: Recent advances in the detection of genomic DNA from plasma samples allow us to follow tumor DNA shedding in plasma during systemic treatment. Osimertinib is the standard of care for patients with NSCLC with acquired *EGFR* T790M mutations. We assessed changes in serial plasma cell-free circulating tumor DNA (ctDNA) genomic alterations to predict osimertinib efficacy.

Methods: We prospectively collected plasma from patients having *EGFR*-mutated advanced NSCLC previously treated with EGFR tyrosine kinase inhibitor therapy and with acquired *EGFR* T790M mutation detected by standard

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methods. Plasma samples were collected before starting osimertinib treatment, 4 weeks after osimertinib treatment, and on progression. ctDNA was analyzed using the Guardant360 assay.

Results: A total of 15 eligible patients received osimertinib. Before starting treatment, *EGFR*-activating mutations were detected in the ctDNA of all patients, and *EGFR* T790M was detected in 93% of the cases. Osimertinib treatment was associated with an objective response rate of 53% and a median progression-free survival of 7.3 months. A total of 12 of the 15 patients had undetectable plasma T790M and decreased activating mutation allelic frequency (AF) at week 4. None of the 12 patients had disease progression within 16 weeks. For the remaining three patients, with detectable plasma T790M (n = 2) or increased activating mutation AF (n = 1) at week 4, two had progressive disease within 16 weeks ($p = 0.03$).

Conclusions: In patients with *EGFR*-mutated advanced NSCLC, persistent *EGFR* T790M or increasing activating mutation AF as detected in ctDNA 4 weeks after the start of osimertinib treatment may predict disease progression within 16 weeks.

Keywords: Non–small cell lung cancer; *EGFR* mutations; ctDNA; Osimertinib; Outcome prediction

Introduction

First- and second-generation EGFR tyrosine kinase inhibitors (TKIs), such as gefitinib, erlotinib, and afatinib, are effective as first-line treatment of advanced NSCLC harboring *EGFR*-activating mutations (e.g., deletions in exon 19 and exon 21 L858R mutation).[1–7] The *EGFR* T790M mutation develops in 55% of tumors after EGFR TKI therapy and is the most common mechanism of acquired resistance.[8–11] Osimertinib monotherapy currently is the recommended second-line treatment for *EGFR* T790M mutation–positive NSCLC.[12–14] In contrast, resistance to osimertinib inevitably develops, with *EGFR* C797S being one example of a mutation associated with acquired resistance.[15]

In some patients with advanced NSCLC, it may be challenging to acquire tumor tissue to assess for *EGFR* T790M. Plasma cell-free circulating tumor DNA (ctDNA) analysis is an alternative method for detecting genomic alterations in tumor DNA. The ability to collect blood at multiple time points makes ctDNA analysis a convenient approach for following changes in tumor DNA during systemic therapy and at the time of disease progression. In recent years, ctDNA analysis (liquid biopsy) has been widely used to detect *EGFR* T790M and to identify mechanisms of resistance; however, the role of ctDNA analysis in early prediction of treatment efficacy is unknown.[15–17]

We used serial plasma ctDNA genomic alterations to predict osimertinib efficacy and searched for possible resistance mechanisms to osimertinib.

Materials and Methods

Patients

This study was approved by the Research Ethics Committee of the National Taiwan University Hospital (NTUH REC No. 201705042RIPC). All patients provided signed informed consent for this study. We prospectively collected plasma from patients with *EGFR*-mutated advanced NSCLC who harbored acquired *EGFR* T790M mutation detected by various standard hotspot tissue- or plasma-based methods (e.g., cobas *EGFR* mutation Test version 2 [Roche Molecular Systems, Pleasanton, CA]) after previous EGFR TKI therapy and planned to start osimertinib therapy. We collected the clinical data prospectively, including patient and tumor characteristics, previous anticancer treatments, methods to detect *EGFR* T790M–positive disease, and time of disease progression.

Plasma ctDNA Analysis

Plasma samples were collected before starting osimertinib treatment, 4 weeks after the initiation of osimertinib treatment, and on disease progression. ctDNA was extracted from the plasma samples, and, after bar coding and hybrid capture, subjected to digital next-generation sequencing (NGS) and analysis using Guardant360 (Guardant Health, Redwood City, CA). Detailed protocols of blood sample processing, ctDNA isolation, sequencing, data analysis, and detection limits have been described elsewhere.[18]

Statistical Analysis

Objective responses were evaluated on the basis of the Response Evaluation Criteria in Solid Tumors version 1.1 by the treating physicians. Progression-free survival (PFS) was defined as the time from the first dose of osimertinib to the date of radiological or clinical disease progression or death and was estimated using the Kaplan-Meier method. The data cutoff date was July 31, 2019. Differences in treatment outcomes between the compared groups were determined using Fisher's exact test.

Table 1. Patient Characteristics

Variable	N	%
Total	15	100
Sex		
Male	8	53
Female	7	47
Age (y)		
Median (range)	62 (48-77)	
Smoking		
Never-smoker	12	80
Histology		
Adenocarcinoma	15	100
EGFR mutation		
Deletion 19	8	53
L858R	7	47
Metastasis		
Brain	5	33
Bone	10	67
Liver	5	33
Pleural effusion	5	33
Prior systemic therapy		
Platinum[a]	3	20
Pemetrexed	3	20
Gefitinib	3	20
Erlotinib	7	47
Afatinib	5	33

[a]Cisplatin or carboplatin.

Results

A total of 15 patients were enrolled. *EGFR*-activating mutations consisted of *EGFR* exon 19 deletions (eight patients) and L858R mutation (seven patients). Erlotinib, afatinib, and gefitinib were administered in seven (47%), five (33%), and three (20%) patients, respectively. A total of 10 (66.7%) and five (33.3%) patients presented bone metastases and liver metastases before the commencement of osimertinib therapy, respectively. Other patient characteristics are illustrated in Table 1. All patients received osimertinib at a standard dose of 80 mg/day. A total of 12 patients concurrently participated in the single-arm PLASMA study (ClinicalTrials.gov Identifier: NCT02811354), which treated plasma *EGFR* T790M–positive patients detected by droplet digital polymerase chain reaction (PCR)–based technology (Sanomics, Hong Kong, People's Republic of China) with osimertinib. Acquired T790M mutation was diagnosed by using plasma samples alone (cobas *EGFR* mutation test version 2 or droplet digital PCR) (n = 11), tissue or pleural effusion alone (n = 2), or a combination of both tissue and plasma samples (n = 2) (Fig. 1). Treatment decisions were based on the aforementioned T790M mutation test results. Guardant360 detected *EGFR*-activating mutations in all patients before starting osimertinib treatment; T790M was detected by Guardant360 in 93% (n = 14) of the patients. *TP53* mutations, *EGFR* amplification, and *MET* amplification were detected in eight, eight, and one patient, respectively (Fig. 2).

After osimertinib treatment, the objective response rate was 53%, and the median PFS was 7.3 months (Fig. 1). After 4 weeks of osimertinib, in six of the 15 patients, ctDNA was undetectable (Fig. 2). Among the 14 patients with *EGFR* T790M detected by Guardant360 at baseline, 12 had no detectable plasma *EGFR* T790M at week 4. Among these, eight had undetectable, three had decreased, and one (patient 15) had increased *EGFR*-activating mutations allelic frequency (AF). Two patients (patients 13 and 14) had persistent *EGFR* T790M at week 4 (Fig. 2).

Among patients with persistent plasma *EGFR* T790M mutations at week 4, one presented disease progression at week 15 (patient 14) and one at week 18 (patient 13) (Figs. 1 and 2). Patient 15 had rapid progression on osimertinib treatment, with clinical progression at week 5. The initial *EGFR* T790M AF was low (0.2%) and was not detectable at week 4, but the *EGFR*-activating mutation AF increased at week 4 and furthermore on progression (48.5% at baseline, 66.5% at week 4, and 66.9% on progression). Alterations in *TP53* and *PTEN* were also detected at baseline in this patient and increased at week 4 (Fig. 3*C*, panel 15). Patient 14 developed a new liver metastasis after stable disease as best response. This patient presented plasma *TP53* and *CDKN2A* mutations detected at baseline, with AF of those mutations having decreased at week 4 (Fig. 3*C*, panel 14). Patient 13 presented an initial tumor response but later progressed with a resistant *EGFR* C797S mutation (Fig. 3*C*, panel 13).

Among the eight patients harboring *TP53* mutations at baseline, three (38%) with undetectable plasma *TP53* mutation at week 4 achieved disease control (stable disease or partial response by Response Evaluation Criteria in Solid Tumors 1.1), whereas two of the three with detectable *TP53* and *EGFR*-activating mutations at week 4 presented disease progression within 16 weeks (Figs. 1 and 2).

On the basis of the Guardant360 tumor response map, we classified the patterns into the following three categories: (1) undetectable mutations of any kind at week 4 (Fig. 3*A*); (2) no detectable *EGFR* T790M and no increase in *EGFR*-activating mutation AF at week 4, with or without mutations in other genes (Fig. 3*B*); and (3) detectable *EGFR* T790M or increasing *EGFR*-activating mutation AF at week 4 (Fig. 3*C*). Patients in category 3 had the worst osimertinib treatment outcome (Fig. 3), which, compared with the combined categories 1 and 2, was associated with early disease progression within 16 weeks ($p = 0.03$). Presence of liver metastases was not associated with early disease progression within 16 weeks ($p = 0.07$).

Among the nine patients with disease progression during the observation period, four presented

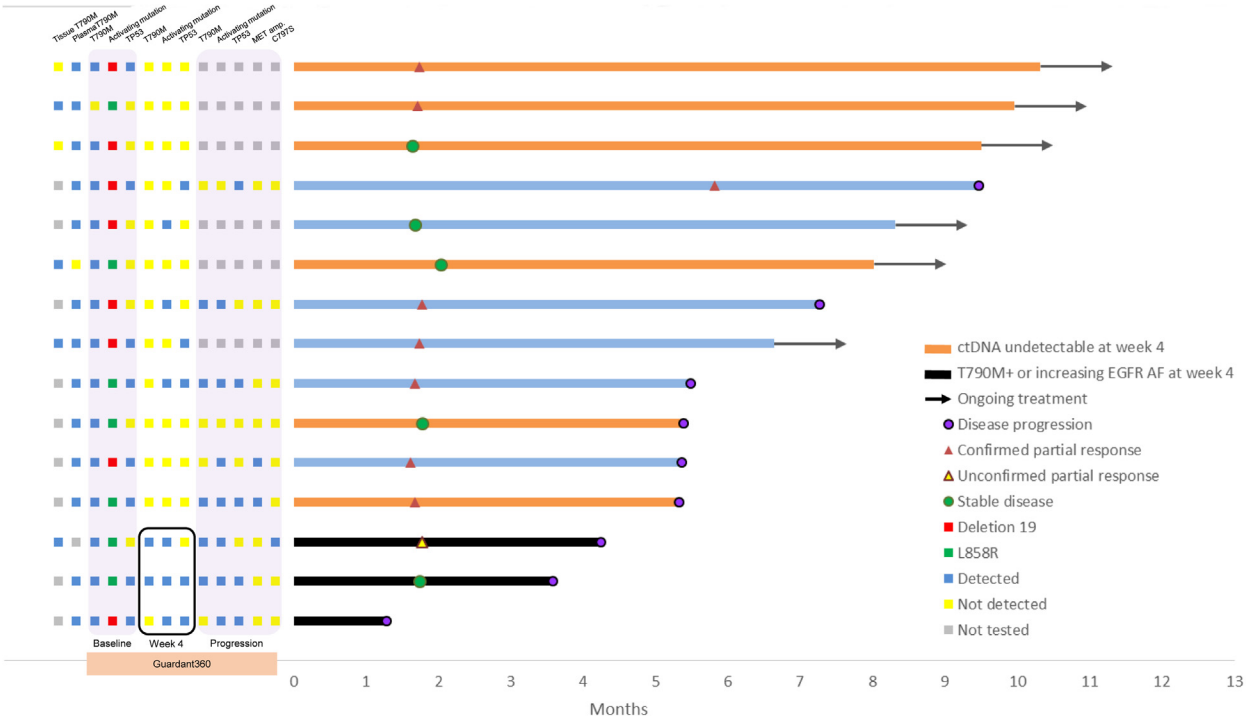

Figure 1. PFS of patients receiving osimertinib for *EGFR* T790M-positive disease, with major Guardant360 results at baseline, week 4, and progression. AF, allelic frequency; ctDNA, circulating tumor DNA; PFS, progression-free survival.

documented resistance alterations, as follows: *EGFR* C797S (one), *EGFR* G724S (one), and *MET* amplification (two) (Fig. 2).

Discussion

In this study, we reported that, in patients with *EGFR* T790M mutation–positive NSCLC treated with

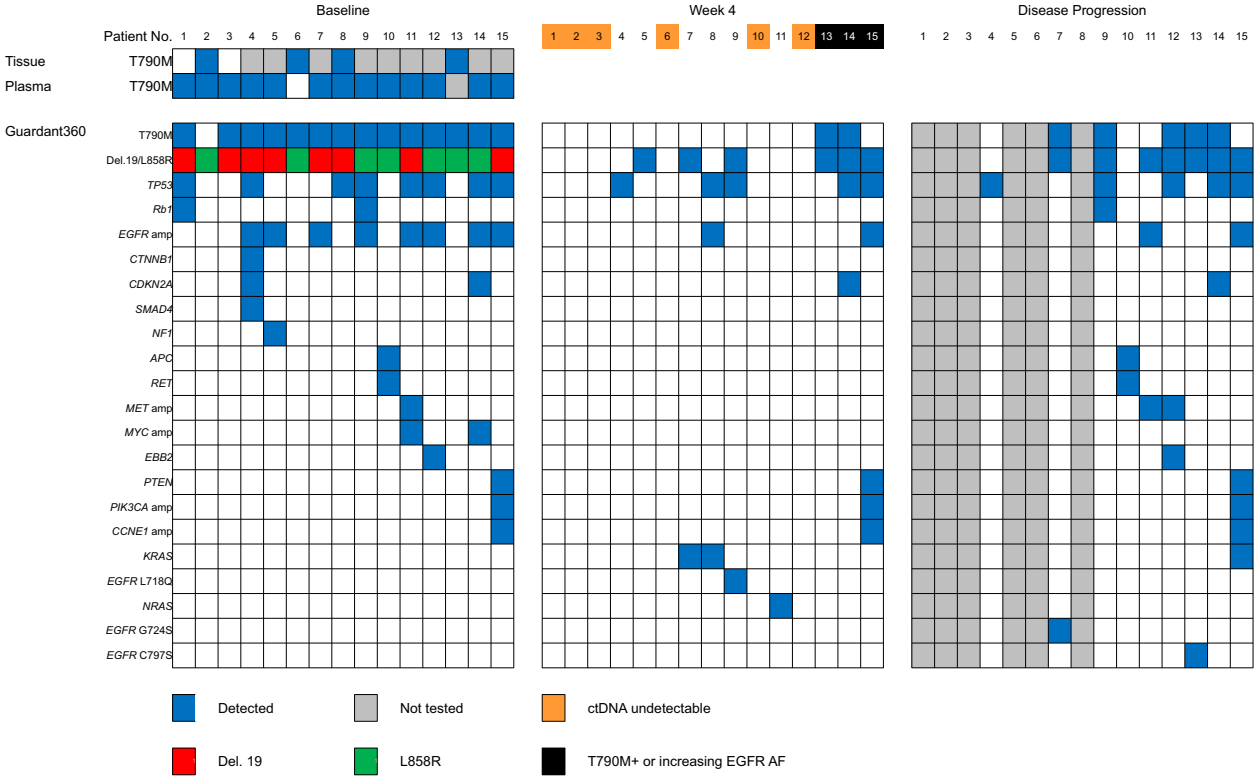

Figure 2. Heatmap summarizing the findings from tissue and plasma before starting osimertinib therapy and detailed Guardant360 results at three time points. AF, allelic frequency; ctDNA, circulating tumor DNA.

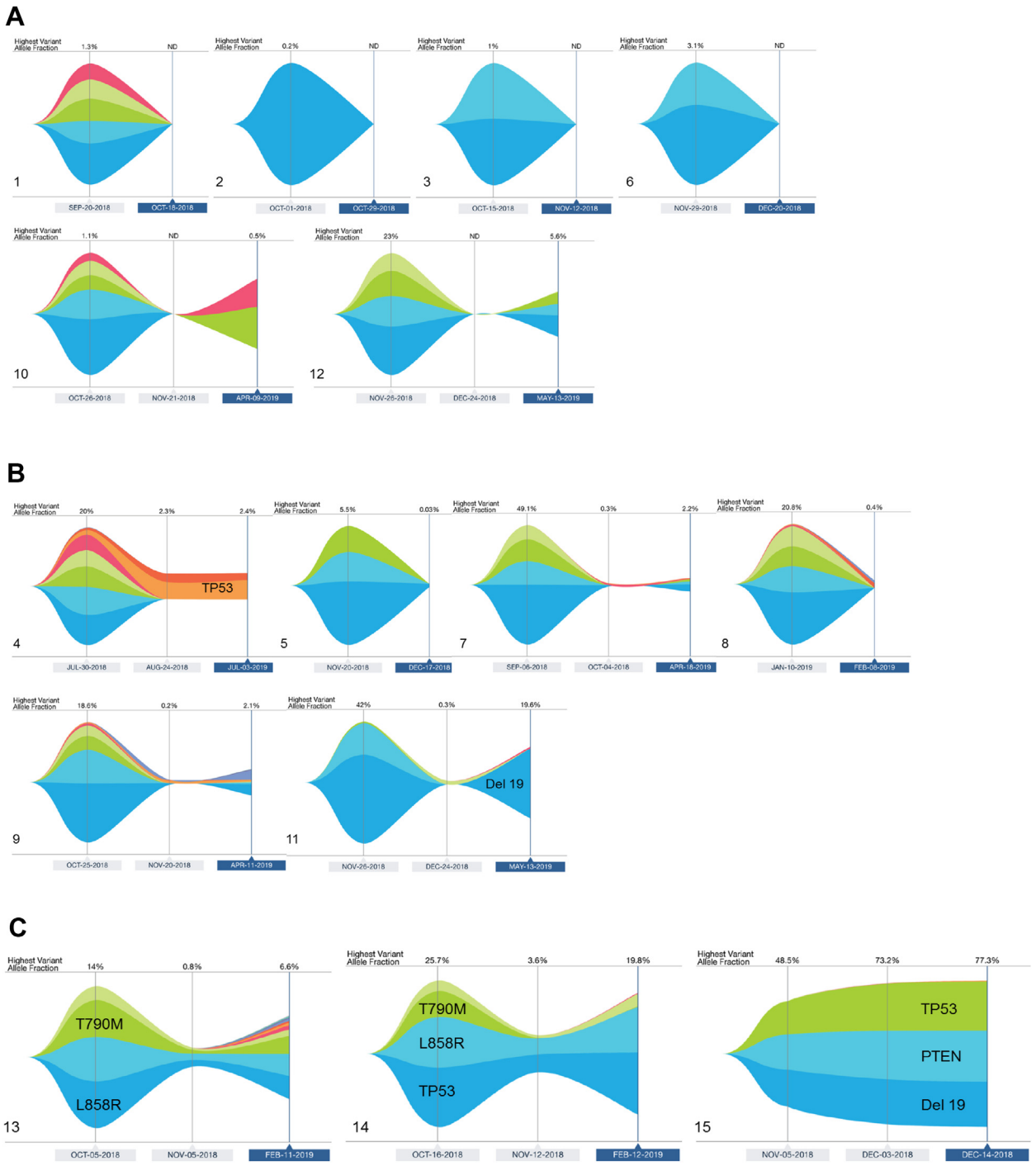

Figure 3. Patterns of Guardant360 tumor response maps. (*A*) Undetectable mutations of any kind at week 4. (*B*) No detectable *EGFR* T790M and no increase in *EGFR*-activating mutation AF at week 4. (*C*) Detectable *EGFR* T790M or increasing *EGFR*-activating mutation AF at week 4. AF, allelic frequency; APR, April; AUG, August; ctDNA, circulating tumor DNA; DEC, December; FEB, February; JAN, January; JUL, July; ND, not determined; NOV, November; OCT, October; SEP, September.

osimertinib, undetectable *EFGR* T790M in ctDNA at 4 weeks after treatment initiation was associated with a favorable clinical outcome. In contrast, patients with detectable *EGFR* T790M or increasing AF for *EGFR*-activating mutations in ctDNA at the same time point had a less favorable prognosis.

The Guardant360 assay is a hybrid capture-based NGS technology, with high concordance with tissue-based genotyping in previously untreated patients with metastatic NSCLC.[19] When used in combination with tissue-based NGS, this assay increases the detection rate of targetable mutations in patients with NSCLC.[20] In

patients previously treated with EGFR TKI therapy, this technology can detect resistance mutations[15] and favorably affects clinical decision making at the time of disease progression in one-third of patients with NSCLC, including those with *EGFR*-mutated tumors.[21] To the best of our knowledge, this is the first study to use this test during osimertinib therapy (at week 4) to identify possible genomic alterations or changes in AF to predict osimertinib treatment outcomes.

Plasma-based detection (liquid biopsy) of *EGFR* mutations is an alternative to tissue-based diagnosis.[22] One example, the cobas assay, which uses PCR technology to evaluate mutations in *EGFR*, is approved by the U.S. Food and Drug Administration to identify appropriate patients for EGFR TKI treatment.[16] A retrospective analysis of plasma samples from patients from the AURA3 study revealed that the plasma *EGFR* T790M–positive percentage agreement was higher using the Guardant360 NGS assay than with cobas plasma, using cobas tissue test results as a reference.[23] Liquid biopsy can also be used in identifying resistance mechanisms to osimertinib.[15] A recent report revealed that clearance of all *EGFR* mutations as assessed by cobas plasma analysis after second-line osimertinib treatment was a positive predictor of clinical outcome.[24] An exploratory study of AURA3 study using droplet digital PCR technology also revealed that clearance of plasma *EGFR* mutations may predict response to osimertinib.[25] In this study, we highlighted the role of plasma ctDNA analysis by using NGS technology in monitoring treatment efficacy. Most patients presented decreased and even undetectable *EGFR* ctDNA after 4 weeks of osimertinib treatment; however, the persistence of *EGFR* T790M mutation in ctDNA may predict a worse outcome. Nevertheless, in one patient in whom *EGFR* T790M was cleared from the ctDNA (patient 15), the original *EGFR* driver mutation remained and the AF increased at week 4, and the patient experienced rapid tumor progression. Hence, undetectable *EGFR* T790M alone may not be the optimal predictor of treatment outcome.

The presence of concurrent mutations in *TP53* and *EGFR* before treatment was reported in 30% to 50% of patients with lung cancer, which may be a prognostic marker for diminished clinical benefit from first-generation EGFR TKIs.[26–28] However, in our study, with 53% of cases having both *EGFR* and *TP53* mutations, this did not seem to be a clinical disadvantage, consistent with previous findings for second-line osimertinib treatment.[23,29] The increments and decrements of *TP53* AF were in line with *EGFR* T790M changes at week 4 in all patients except for one (patient 4) who had undetectable T790M AF (20% to undetectable) and slightly increased *TP53* AF (1.8%–2.3%) at week 4. This patient had a relatively longer PFS of 9.5 months on osimertinib treatment. Hence, the predictive role of *TP53* remained elusive.

One patient (patient 15) in this study cohort, whose tumor presented a *PTEN* mutation (*PTEN* T319 frameshift) at baseline did not have a clinical response to osimertinib. The AFs of *EGFR* and *PTEN* mutations increased 4 weeks after osimertinib treatment and on disease progression. Concurrent mutations in *EGFR* and *PTEN* have been reported in 5% of patients with *EGFR*-mutated NSCLC at diagnosis.[26] Alterations in *PTEN,* as detected in tissue samples, were reported as resistance mechanisms to erlotinib in *EGFR* mutation–positive NSCLC.[30] It has also been reported as a mechanism of acquired and primary resistance to osimertinib therapy by analyzing osimertinib-resistant tumor tissues.[31–34] Primary resistance to osimertinib developed in 7.7% of patients with *EGFR* T790M–positive NSCLC. Mechanisms of primary resistance, such as small cell transformation, *ERBB2* exon 16 skipping or amplification, *MET* amplification, BIM deletion polymorphism, *EZH2* mutation, and *KRAS* G12D mutation, have been reported.[33–37] To the best of our knowledge, this is the first report of inferring *PTEN* alteration as a potential mechanism of primary resistance by using plasma ctDNA analysis.

Regarding the plasma shedding status of tumor DNA, in one retrospective study, detection of *EGFR* T790M in plasma was more likely in patients with bone metastases or more than three metastatic sites by using various non-NGS techniques after progression on first- or second-generation EGFR TKIs.[38] Furthermore, the retrospective analysis of plasma samples from patients from the AURA3 study revealed that plasma *EGFR* T790M detection was associated with the presence of extrathoracic disease and a larger tumor size.[23] In the AURA3 study, osimertinib-treated patients with cobas plasma *EGFR* T790M–positive disease had shorter PFS as compared with patients with plasma *EGFR* T790M–negative disease (median, 8.3 versus 12.5 mo).[23] In a real-world study, ctDNA was detectable by the Guardant360 assay in 65% of first- or second-generation EGFR TKI–pretreated patients with *EGFR*-mutated NSCLC who developed resistance.[29] Among these shedders, 45% of them had *EGFR* T790M. In this study, most of the patients (66.7%) presented bone metastases. In this study, the two patients with disease limited to the lungs had the lowest plasma ctDNA AF. Of note, most of these selected patients had had positive plasma *EGFR* T790M detected by standard methods before participating this study; hence, these patients are not representative for the general patients with *EGFR*-mutated NSCLC.

This study has some limitations. First, the sample size was relatively small, and the results are more likely hypothesis-generating rather than definitive. Nevertheless, our findings support addressing these questions in

a larger prospective study designed to identify the role of ctDNA monitoring during EGFR TKI treatment for advanced-stage NSCLC. Such a study could also address the role of earlier interventions for targetable resistance mutations. Second, standard testing for *EGFR* T790M in most patients was performed by a hotspot plasma-based test; only a few patients (n = 4) had tissue-based diagnosis of *EGFR* T790M–positive disease. This likely improved the sensitivity of Guardant360, as such cases were known to have DNA shedding before liquid-based NGS testing. As mentioned previously, shedders of ctDNA usually have extrathoracic disease and a larger tumor size.[23] Patients with *EGFR* T790M–negative plasma still need a tumor rebiopsy to determine the presence and absence of *EGFR* T790M.[22]

In conclusion, in patients with *EGFR*-mutated advanced NSCLC, persistent *EGFR* T790M or increasing *EGFR*-activating mutation AF in ctDNA at week 4 after osimertinib treatment may predict early disease progression, defined in our study as within 16 weeks. On the basis of these findings, future studies could incorporate the evaluation of ctDNA at week 4 to assess the role of early treatment interventions for emerging resistance to osimertinib.

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