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Assessment of Resistance Mechanisms and Clinical Implications in Patients With *EGFR* T790M–Positive Lung Cancer and Acquired Resistance to Osimertinib

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➕ **Supplemental content**

IMPORTANCE Osimertinib mesylate is used globally to treat *EGFR*-mutant non–small cell lung cancer (NSCLC) with tyrosine kinase inhibitor resistance mediated by the *EGFR* T790M mutation. Acquired resistance to osimertinib is a growing clinical challenge that is poorly understood.

OBJECTIVE To understand the molecular mechanisms of acquired resistance to osimertinib and their clinical behavior.

DESIGN, SETTING, AND PARTICIPANTS Patients with advanced NSCLC who received osimertinib for T790M-positive acquired resistance to prior EGFR tyrosine kinase inhibitor were identified from a multi-institutional cohort (n = 143) and a confirmatory trial cohort (NCT01802632) (n = 110). Next-generation sequencing of tumor biopsies after osimertinib resistance was performed. Genotyping of plasma cell-free DNA was studied as an orthogonal approach, including serial plasma samples when available. The study and analysis were finalized on November 9, 2017.

MAIN OUTCOMES AND MEASURES Mechanisms of resistance and their association with time to treatment discontinuation on osimertinib.

RESULTS Of the 143 patients evaluated, 41 (28 [68%] women) had tumor next-generation sequencing after acquired resistance to osimertinib. Among 13 patients (32%) with maintained T790M at the time of resistance, *EGFR* C797S was seen in 9 patients (22%). Among 28 individuals (68%) with loss of T790M, a range of competing resistance mechanisms was detected, including novel mechanisms such as acquired *KRAS* mutations and targetable gene fusions. Time to treatment discontinuation was shorter in patients with T790M loss (6.1 vs 15.2 months), suggesting emergence of pre-existing resistant clones; this finding was confirmed in a validation cohort of 110 patients with plasma cell-free DNA genotyping performed after osimertinib resistance. In studies of serial plasma levels of mutant *EGFR*, loss of T790M at resistance was associated with a smaller decrease in levels of the *EGFR* driver mutation after 1 to 3 weeks of therapy (100% vs 83% decrease; P = .01).

CONCLUSIONS AND RELEVANCE Acquired resistance to osimertinib mediated by loss of the T790M mutation is associated with early resistance and a range of competing resistance mechanisms. These data provide clinical evidence of the heterogeneity of resistance in advanced NSCLC and a need for clinical trial strategies that can overcome multiple concomitant resistance mechanisms or strategies for preventing such resistance.

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Osimertinib mesylate is a third-generation tyrosine kinase inhibitor (TKI) targeting mutant epidermal growth factor receptor (EGFR) now available worldwide for the management of non–small cell lung cancer (NSCLC) carrying the *EGFR* (NCBI 1956) T790M resistance mutation after acquired resistance to prior EGFR TKI therapy. Patients treated in this setting experience a high response rate and median progression-free survival (PFS) of approximately 10 months.[1,2] The clinical activity and favorable toxicity profile of osimertinib has led this drug to be studied broadly as a strategy for controlling and preventing drug resistance in *EGFR*-mutant NSCLC.[3,4]

With widespread clinical use of osimertinib, acquired resistance has become a clinical problem. A range of mechanisms of resistance has been described, including *EGFR* C797S mutations, *MET* amplification, and small cell transformation.[5-11] The standard therapy after EGFR TKI options are exhausted is chemotherapy, such as the regimen of platinum/pemetrexed established in the IMPRESS trial, with a 34% response rate and 5.4-month median PFS.[10] To improve on this outcome, a range of targeted treatment approaches is now in development to overcome resistance to osimertinib, an approach that requires an improved molecular and clinical understanding of what drives drug resistance. Genomic analysis of plasma cell-free DNA has been used to describe 2 new resistance mechanisms: acquired *EGFR* C797S mutations and loss of the T790M mutation.[12] Herein, we report our study of resistance in a larger cohort to understand clinical and molecular indicators that might suggest a specific molecular mechanism of resistance and treatment approach.

Methods

Patients with advanced NSCLC treated with osimertinib were identified from institutional databases of 4 contributing cancer centers. Patients who received single-agent osimertinib for acquired resistance to prior EGFR TKI and were *EGFR* T790M-positive in either tumor or plasma were included. Patients from the first-in-human AURA trial[13] were studied with the same inclusion criteria as a validation cohort; all patients signed informed consent forms for treatment in this protocol. Each site's institutional review board gave approval with waiver of consent for the present analysis. The study and analysis were finalized on November 9, 2017.

Patients from the institutional cohort were eligible for resistance analysis based on the availability of tumor genotyping from a biopsy performed after development of osimertinib resistance. Patients who discontinued osimertinib due to toxic effects before progression were excluded. Genotyping was performed as part of clinical care or as a research test (with patient consent) using a Clinical Laboratory Improvement Amendments–approved next-generation sequencing (NGS) assay[14]; *MET* fluorescence in situ hybridization was also performed, when possible.

Plasma was collected in the AURA trial with patient consent. Patients from the AURA cohort were eligible for resistance analysis based on the availability of plasma genotyping results after development of osimertinib resistance. Plasma

Key Points

Question What molecular and clinical biomarkers can be used to better understand osimertinib mesylate resistance and develop treatment strategies?

Findings In this cohort study of 143 patients who underwent tumor next-generation sequencing, loss of the *EGFR* T790M mutation was common on resistance to osimertinib and was associated with early treatment failure and development of a range of competing resistance mechanisms, some expected (*MET* amplification, small cell transformation) and some novel (acquired *KRAS* mutations, targetable gene fusions). Early changes in plasma *EGFR* mutation levels may indicate probable resistance patterns.

Meaning Strategies to detect and target multiple coexistent resistance mechanisms will be needed to achieve more durable control of drug resistance in *EGFR*-mutant lung cancer.

cell-free DNA was studied for *EGFR* driver (L858R and exon 19 del) and resistance mutations (T790M and C797S) using droplet digital polymerase chain reaction (PCR) or beads, emulsion, amplification, magnetics (BEAMing), as previously described.[15,16] Patients with no detectable *EGFR* driver mutation in resistance plasma were excluded because there was no evidence of tumor DNA for analysis. In addition, some patients from the institutional cohort consented to serial plasma collection during treatment with osimertinib.
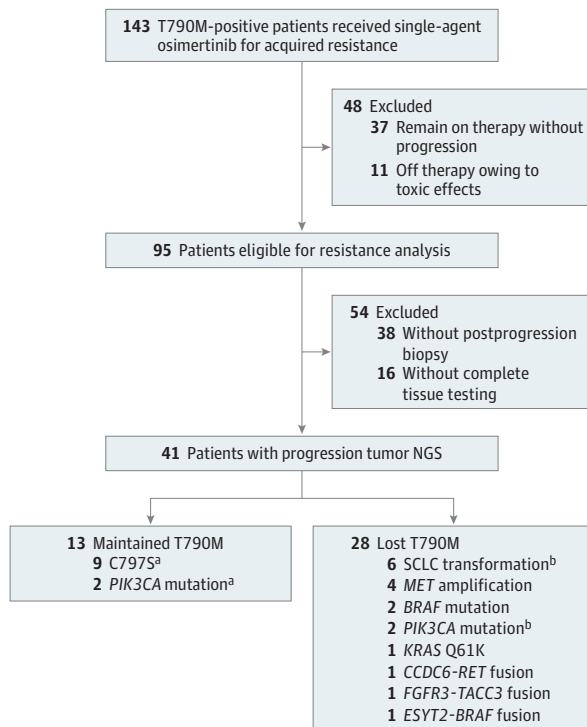
To study clinical outcomes of osimertinib, time to treatment discontinuation (TTD) was measured,[17] defined as the time until the end of therapy for any reason. Patients were censored at the date that they were last known to have received therapy. Time to treatment discontinuation was studied rather than PFS because many patients received therapy as part of their standard clinical care, making it difficult to perform an objective analysis of radiographic progression. Furthermore, many patients can continue therapy beyond objective progression,[18] such that TTD may more accurately capture the clinical benefit of therapy. A PFS analysis was included for the AURA cohort.

Statistical Analysis

The Kaplan-Meier method was used to estimate the TTD and PFS distributions. Fisher exact test was used to compare the distribution of categorical variables and Wilcoxon rank sum test was used to compare the distribution of continuous variables. Logistic regression modeling was used to estimate the association of time receiving therapy with the odds of T790M loss at the time of resistance. All *P* values are 2-sided and considered significant at the .05 level.

Results

We first studied a cohort of 143 patients from 4 institutions with *EGFR*-mutant NSCLC who were *EGFR* T790M-positive based on either a tumor or plasma genotyping assay before starting treatment with single-agent osimertinib. Forty-one patients (28 [68%] women) experienced progression and underwent a resistance biopsy adequate for genomic analysis (**Figure 1**). The

Figure 1. Patient Flowchart of Multi-institutional Cohort With Acquired Resistance to Osimertinib
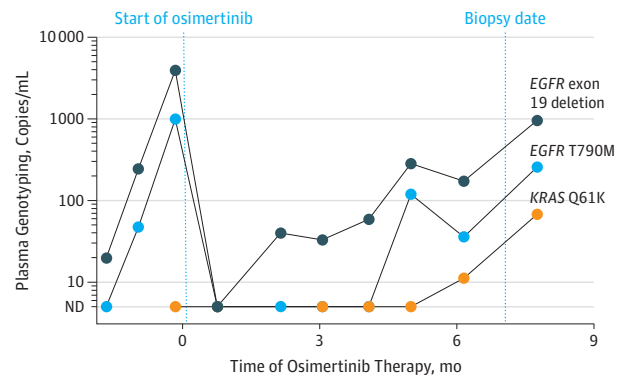
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┌─────────────────────────────────────────┐
│ 143 T790M-positive patients received     │
│     single-agent osimertinib for         │
│     acquired resistance                  │
└─────────────────────────────────────────┘
             │
             │      ┌─────────────────────────────────┐
             │      │ 48 Excluded                     │
             │      │    37 Remain on therapy without │
             ├─────▶│       progression               │
             │      │    11 Off therapy owing to      │
             │      │       toxic effects             │
             │      └─────────────────────────────────┘
             ▼
┌─────────────────────────────────────────┐
│ 95 Patients eligible for resistance      │
│    analysis                              │
└─────────────────────────────────────────┘
             │
             │      ┌─────────────────────────────────┐
             │      │ 54 Excluded                     │
             │      │    38 Without postprogression   │
             ├─────▶│       biopsy                    │
             │      │    16 Without complete          │
             │      │       tissue testing            │
             │      └─────────────────────────────────┘
             ▼
┌─────────────────────────────────────────┐
│ 41 Patients with progression tumor NGS   │
└─────────────────────────────────────────┘
       │                    │
       ▼                    ▼
┌──────────────────┐  ┌──────────────────────────────┐
│ 13 Maintained    │  │ 28 Lost T790M                │
│    T790M         │  │    6 SCLC transformationᵇ    │
│    9 C797Sᵃ      │  │    4 MET amplification       │
│    2 PIK3CA      │  │    2 BRAF mutation           │
│      mutationᵃ   │  │    2 PIK3CA mutationᵇ        │
│                  │  │    1 KRAS Q61K               │
│                  │  │    1 CCDC6-RET fusion        │
│                  │  │    1 FGFR3-TACC3 fusion      │
│                  │  │    1 ESYT2-BRAF fusion       │
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A total of 143 T790M-positive patients received single-agent osimertinib for *EGFR*-acquired resistance. Of these patients, 41 developed disease progression while receiving osimertinib and tumor genotyping during therapy, and tumor genotyping was completed on a resistance biopsy, revealing a range of competing resistance mechanisms in patients with T790M loss. NGS indicates next-generation sequencing.

ᵃ Patient with both *EGFR* C797S and *PIK3CA*.

ᵇ Patient with both *PIK3CA* and small-cell lung cancer transformation.

## Figure 2. Acquired *KRAS* Mutation During Osimertinib Therapy

In 1 patient with osimertinib resistance, next-generation sequencing of a tumor biopsy showed loss of T790M and an acquired *KRAS* Q61K mutation. Serial plasma genotyping confirmed the acquired *KRAS* mutation but also detected reemergence of the *EGFR* T790M mutation. ND indicates not detected.

on tumor NGS (allelic fraction [AF], 30%) in addition to the known *EGFR* 19 deletion mutation (AF, 31%) (**Figure 2**).

In several cases, serial plasma genotyping performed retrospectively offered insight into the kinetics of osimertinib response and resistance heterogeneity (eFigure 1 in the Supplement). For example, in a patient who developed small cell transformation, plasma T790M levels dropped during osimertinib therapy while *EGFR* driver levels increased (eFigure 1A, top left in the Supplement), consistent with early progression after 6 weeks. In a patient with acquired *EGFR* C797S, an initial complete response in plasma was followed by emergence of the T790M plus 2 *EGFR* C797S variants (eFigure 1B, top left in the Supplement). Finally, in the patient with an acquired *KRAS* mutation, a new droplet digital PCR assay was developed to confirm the presence of the *KRAS* Q61K mutation, which was absent before therapy but gradually emerged with resistance (Figure 2); the T790M mutation reemerged in plasma but was not detected on tumor NGS. In several cases, plasma genotyping detected resistance mechanisms not detected on tumor NGS: 1 patient had a *KRAS* mutation (G12V) detected on plasma NGS at resistance that was not detected in the tumor, 1 patient with *MET* amplification detected in the tumor had T790M and an *ALK* rearrangement detected on plasma NGS, and a third patient with SCLC on biopsy had T790M and *EGFR* G724S detected in plasma (eTable 1 in the Supplement).

Patients developing loss of T790M were clinically similar to patients with maintained T790M aside from a predominance of women with T790M maintained (eTable 2A in the Supplement). We then explored the timing of osimertinib resistance, dividing patients based on maintained or lost *EGFR* T790M mutation in the resistance biopsy. Patients with T790M loss had a median TTD of 6.1 months, which was shorter than the median TTD of 15.2 months in patients with maintained T790M (log rank *P* = .01) (**Figure 3**). In 1 patient with a prolonged TTD of 23.4 months, NGS of a resistance biopsy showed an exon 19 deletion at a low AF (5%) and no detectable T790M; however, plasma droplet digital PCR detected the driver (AF, 47%) and both T790M (AF, 44%) and C797S (AF, 28%) mutations.

median TTD for these patients was 8.0 months, which was lower than the expected PFS during osimertinib therapy.[1] This TTD may represent an enrichment for patients with earlier acquired resistance—a common bias when studying patients developing therapy resistance.

For all patients, tumor NGS was completed at resistance and an *EGFR* driver mutation was again detected (eTable 1 in the Supplement). Maintained *EGFR* T790M was detected in 13 patients (32%) and loss of T790M was seen in 28 patients (68%). Mutations of *EGFR* C797S were detected in 9 patients (22% overall; 69% of those with retained T790M), all in *cis* with a maintained T790M. Non-*EGFR* resistance mechanisms were identified in 19 patients (46%), with 17 (41%) occurring with T790M loss (Figure 1). Of the 28 patients with T790M loss, 13 (46%) developed resistance mechanisms that are well described after first-generation EGFR TKIs: 6 small-cell lung cancer transformation, 4 *MET* amplifications, 2 *PIK3CA* mutations (1 occurring with small cell transformation), and 2 *BR*AF mutations. Other patients with T790M loss developed unexpected resistance mechanisms, including *RET*, *FGFR3*, and *BRAF* fusions (1 each). Finally, 1 patient had a *KRAS* Q61K mutation detected

Figure 3. Early Time to Treatment Discontinuation (TTD) in 41 Patients With T790M Loss at Osimertinib Resistance
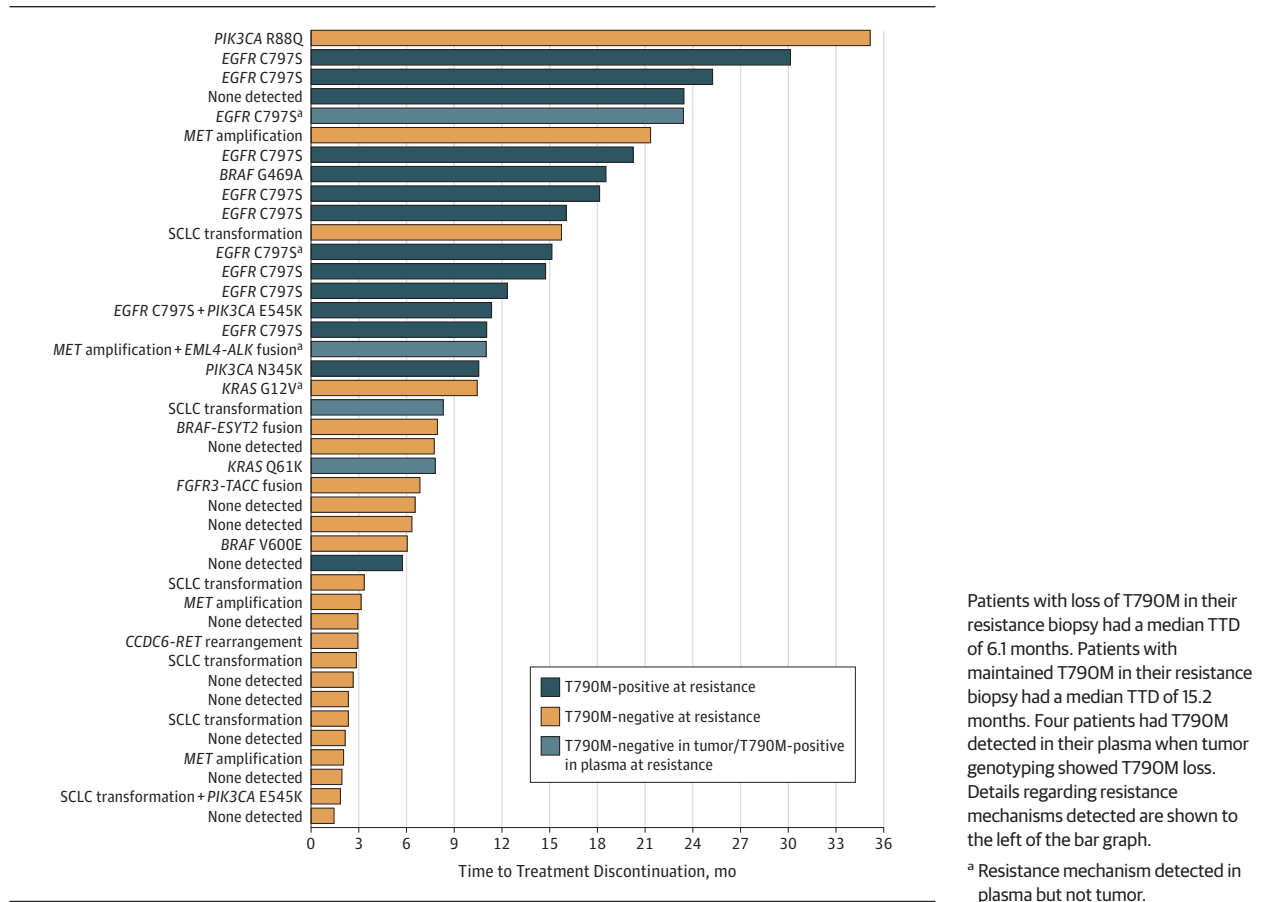
Patients with loss of T790M in their resistance biopsy had a median TTD of 6.1 months. Patients with maintained T790M in their resistance biopsy had a median TTD of 15.2 months. Four patients had T790M detected in their plasma when tumor genotyping showed T790M loss. Details regarding resistance mechanisms detected are shown to the left of the bar graph.

a Resistance mechanism detected in plasma but not tumor.

To validate our finding of early resistance in patients developing loss of T790M, we performed a retrospective analysis of patients in the AURA trial (eFigure 2 in the Supplement).[1,19] Of 157 patients with plasma samples available after development of resistance, 110 had a driver *EGFR* mutation detected in plasma cell-free DNA and were deemed adequate for resistance analysis. Of these patients, 52 (47%) had loss of T790M and 58 (53%) maintained the T790M mutation (eTable 2B in the Supplement); 24 patients (22%) acquired *EGFR* C797S, all with maintained T790M. Patients with loss of T790M had a shorter median TTD (5.5 months) (Figure 4A) than patients with maintained T790M either without C797S (12.6 months) or with C797S (12.4 months) (log rank *P* = .006). Patients with loss of T790M similarly had a shorter PFS. With the cohort divided into tertiles, loss of T790M was seen in 26 of 38 patients (68%) with 5.5-months or less TTD; maintained T790M was seen in 26 of 36 patients (72%) with 13 months more TTD (Figure 4B).

We studied strategies for anticipating loss of T790M using pretreatment features. Of the 19 patients with non-*EGFR* resistance mechanisms detected on biopsies after progression, 16 had pre-osimertinib resistance biopsies (eTable 1 in the Supplement). Among 6 individuals with small cell transformation, 1 had small cell differentiation detected on a biopsy that preceded osimertinib therapy by 8.5 months, although only adenocarcinoma was seen on a biopsy immediately preceding osimertinib therapy; 2 other patients showed *RB1* loss on tumor NGS before osimertinib.[20,21] Among 4 patients with *MET* amplification after osimertinib, 2 had pre-osimertinib *MET* fluorescence in situ hybridization, 1 of which showed focal *MET* amplification (6% of cells with 10 *MET* copies) in addition to the T790M mutation (AF, 7%); after treatment with osimertinib for 9 weeks, T790M was no longer detectable on rebiopsy, but *MET* amplification was seen in 94% of the cells (eFigure 3 in the Supplement). Pre-osimertinib NGS for the patient with the *BRAF* fusion (eFigure 4 in the Supplement) after osimertinib showed this fusion before therapy, coexistent with a T790M mutation (AF, 10%). Separately, 2 patients with early osimertinib failure and T790M loss in tumor had T790M detected in plasma but not in tumor before osimertinib treatment. We also studied the biologic outcome of genomic *TP53* loss, which has been associated with earlier resistance on first-generation EGFR TKI.[22,23] The *TP53* mutational status was available for all 41 patients with resistance biopsies, and 26 individuals had a pathogenic *TP53* mutation or 2-copy loss of *TP53*. Although these mutations were more common in patients with T790M loss (71% vs 46%), the difference was not statistically significant (*P* = .17).

Given the limited availability of matched pre- and post-osimertinib biopsies, we studied plasma genotyping to estimate the eventual pattern of resistance. Fifty patients from the

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Figure 4. Acquired Resistance to Osimertinib in a Validation Cohort (n = 110) From the AURA Trial
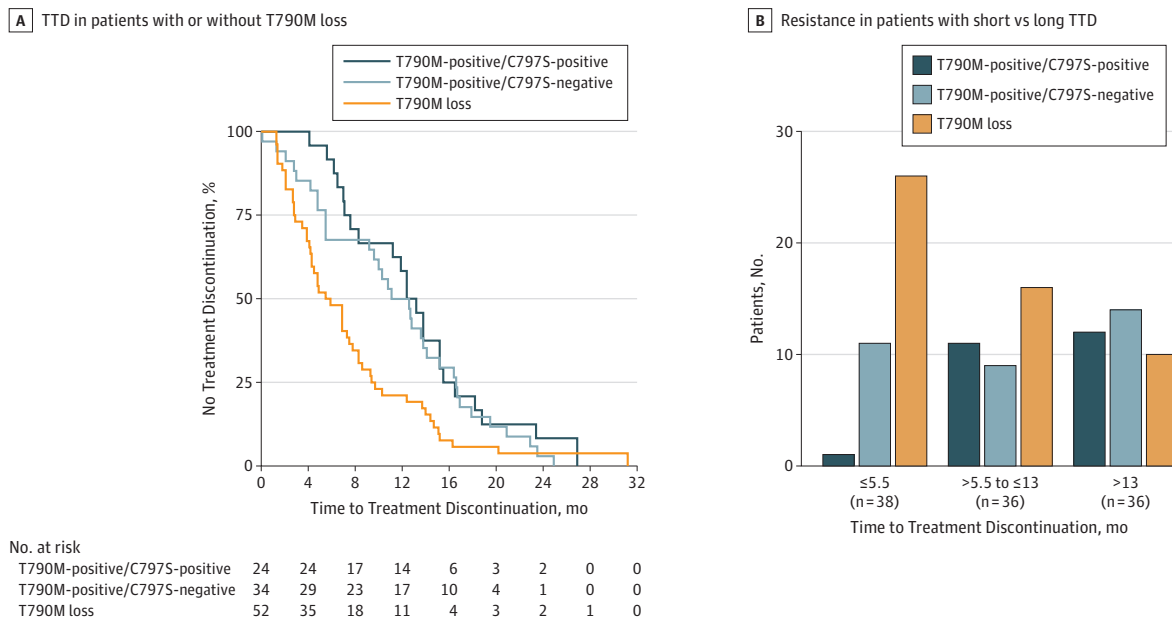
A, This Kaplan-Meier curve shows that, in 110 patients, those with T790M loss had a median time to treatment discontinuation (TTD) of 5.5 months, which was lower than patients who were T790M-positive and C797S-negative (median, 12.6 months) or T790M-positive and C797S-positive (median, 12.4 months). B, A higher percentage of patients with short TTD (68%) had T790M loss; a higher percentage of patients with long TTD (72%) maintained T790M.

AURA cohort had plasma genotyping performed before osimertinib administration and after development of resistance. The relative AF of T790M compared with the *EGFR* driver was calculated, as described previously,[24] as a potential measure of T790M allelic prevalence. Pre- and postosimertinib relative T790M AF were correlated (eFigure 5A in the Supplement) (Pearson correlation coefficient = 0.70; *P* < .001). However, when this association is studied in a dichotomous fashion, patients who develop T790M loss have only a slightly lower relative T790M AF before osimertinib (29% vs 38% median, *P* = .06) (**Figure** 5A). Generation of a receiver operating curve (eFigure 5B in the Supplement) revealed an area under the curve of 0.66, suggesting poor performance as a diagnostic test for estimating the probable loss of T790M.

We then studied serial plasma genotyping to assess whether early response in plasma could indicate the eventual pattern of resistance. Nineteen patients from the institutional cohort had an early response plasma specimen (after 1-3 weeks) and detectable tumor DNA before treatment and at development of resistance.[25] In the plasma samples drawn at resistance, 8 patients had maintained T790M and 11 had T790M loss. Study of the relative change in plasma *EGFR* mutation concentration after 1 to 3 weeks of osimertinib therapy showed a decrease in plasma T790M levels (ΔT790M) both in patients with maintained T790M and T790M loss (median 100% decrease for both) (Figure 5B). Evaluation of the relative change in *EGFR* driver levels (ΔDriver) demonstrated a larger decrease for patients with maintained T790M vs those who lost T790M (median, 100% vs 83% decrease, *P* = .01). We then analyzed the differential plasma response between the *EGFR* driver and T790M (ΔDriver – ΔT790M) and found that this difference was significant

between patients who went on to have T790M loss or T790M maintained at resistance (median difference, 16% vs 0%; *P* = .003) (Figure 5C). Eight of 9 patients with a greater decrease in T790M than in driver *EGFR* mutation (ΔDriver – ΔT790M>1%) went on to develop loss of T790M at resistance.

Discussion

Osimertinib has become the first targeted therapy to receive regulatory approval for the treatment of solid tumors harboring a specific resistance mechanism. Although osimertinib is expected to move into the first-line setting for treatment of *EGFR*-mutant NSCLC,[4] the challenge of detecting and targeting specific resistance mechanisms has relevance across a range of targetable genotypes where treatment of drug resistance is under active investigation.[26,27]

Our data provide clinical evidence of the influence of heterogeneity on treatment outcomes with osimertinib and suggest that there may be 2 types of T790M-positive resistance. In some patients, the T790M mutation represents the dominant resistance mechanism, and these patients will have a more durable response to treatment. In patients with heterogeneous mechanisms of resistance, T790M-mutant subclones coexist with subclones harboring distinct resistance mechanisms; targeting T790M alone is likely to result in transient benefit. What is currently unclear is how to determine whether there is a dominant or minor T790M-positive population. Some prior studies have found that quantification of the relative T790M AF is associated with the degree of response to third-generation EGFR TKIs, although this associa-

Figure 5. Estimating the Probability of T790M Loss From Baseline and Serial Plasma Samples
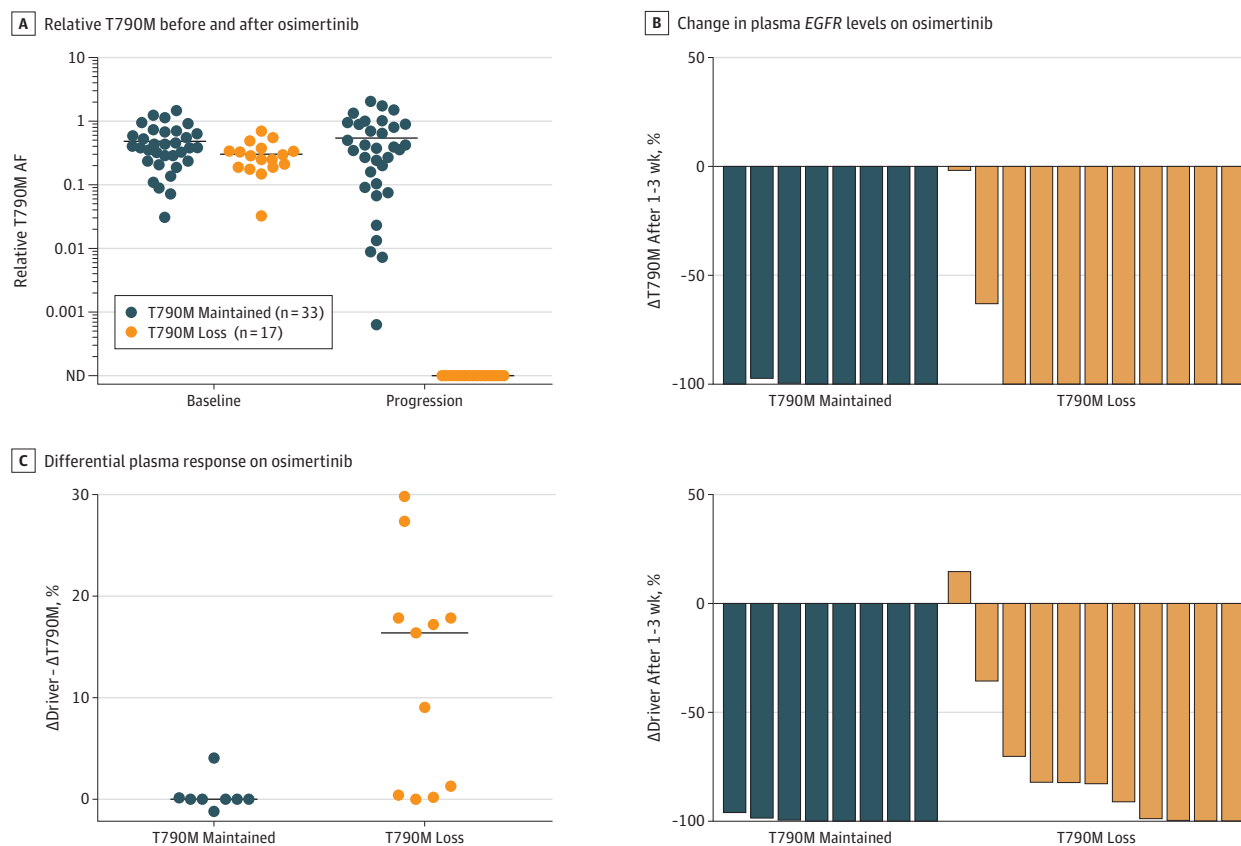
A, Fifty patients from the AURA cohort had plasma genotyping performed before and after osimertinib treatment. Relative T790M allelic fraction (AF) was calculated as T790M AF/*EGFR* driver AF. Patients with T790M loss tended to have a lower relative T790M AF at baseline (29% vs 38% median, *P* = .06) than patients with T790M maintained, although the difference was modest. B, Measuring the relative change in plasma *EGFR* levels after 1 to 3 weeks of osimertinib therapy, both T790M loss and T790M maintained patients had

decreases in T790M levels (ΔT790M). Patients with T790M maintained had a larger decrease in *EGFR* driver levels (ΔDriver, *P* = .01). C, In analysis of the differential plasma response between the *EGFR* driver and the T790M, patients had a greater T790M response than *EGFR* driver response compared with those who maintained T790M (median difference, 16% vs 0%; *P* = .003).
ND indicates not detectable.

tion has not been consistent across published reports.[24,28,29] In our analysis, we studied whether such a calculation of relative T790M AF in plasma could indicate probable subsequent loss of T790M, and our data did not reveal a clear predictive ability. Instead, serial plasma monitoring may offer insight into subsequent resistance patterns.

The overgrowth of non-T790M resistance mechanisms highlights that coexistence of multiple resistance mechanisms is a reality with clinical implications—some resistance mechanisms are not acquired during osimertinib therapy but instead coexist with T790M. For example, in 1 case positive for T790M prior to osimertinib, *MET* was amplified in just 6% of cells; with osimertinib treatment, this subclone became the dominant resistance mechanism, detectable in 94% of cells (eFigure 3 in the Supplement). The finding of multiple resistance mechanisms has been described previously. Yu et al[30] identified multiple resistance mutations in 4% of 155 patients with *EGFR*-mutant lung cancer and acquired resistance. Yet, the increased use of PCR assays for T790M detection means that coexistent resistance mechanisms may go undetected, although emerging data sug-

gest that plasma NGS approaches can detect, in a subset of cases, resistance mechanisms that coexist with T790M.[31,32] Further prospective study is needed to determine whether multigene analyses of tumor or plasma could detect competing resistance mechanisms reliably enough to allow early delivery of appropriate osimertinib-based combination therapies.

Loss of T790M is a potentially confusing clinical state that could be misinterpreted as resensitization to first-line EGFR TKIs. Our data do not support this hypothesis; most of the time, loss of T790M is associated with development of alternative competing resistance mechanisms (eFigure 6 in the Supplement). Many of these mechanisms, such as small cell transformation and *MET* amplification, have been reported in T790M-negative acquired resistance.[33,34] However, we also have identified a novel finding of an acquired *KRAS* mutation, which was confirmed using tumor NGS and plasma droplet digital PCR. The complex variety of resistance mutations seen in patients with loss of T790M, including several acquired fusion genes, highlights the need for better strategies to prevent or delay the emergence of resistance. Because loss

of T790M is associated with early resistance, this phenomenon could be more evident in usual clinical care than prospective cohorts would suggest; indeed, it was seen in 68% of patients in our post hoc institutional cohort, but 47% of patients in the prospective AURA cohort (P = .03).

Our study has potentially important implications in the care of patients who develop resistance to osimertinib. There is a wide range of osimertinib combination therapies now in clinical trials, but selecting the right approach will require insight into disease biology. Patients who develop early resistance to osimertinib likely have competing resistance mechanisms in other tumor subclones, and patients who develop late resistance are more likely to have maintained T790M and acquired C797S; different targeted therapies could be considered for these biologically varying populations. New clinical trials aiming to target patients with T790M-positive resistance and acquired resistance to osimertinib must consider which type of resistance is being targeted. Repeated testing for T790M would be an intuitive approach to stratify these 2 biologically distinct types of osimertinib resistance. Another strategy would be to improve the efficacy of osimertinib by developing combination approaches that prevent the development of resistance.[35,36] However, the complex range of resistance mechanisms seen after osimertinib treatment make it difficult for any single combination approach to significantly delay osimertinib resistance overall.

Limitations

The limitations of our study must be acknowledged. Genomic analysis of resistance biopsies was performed using available clinical NGS assays rather than a single central assay, and pre-osimertinib NGS was available on only a subset of patients. To compensate for the limited numbers of patients receiving resistance biopsies, we performed plasma genotyping on a clinical trial cohort to validate our findings of different clinical behaviors in different types of osimertinib resistance. Loss of T790M was more common in our institutional cohort compared with the trial cohort—we suspect that this is because a retrospective institutional cohort will enrich data for patients presenting with early resistance, which is more often loss of T790M. Even the trial cohort may be falsely enriched for T790M loss because patients who are still receiving therapy without resistance may be enriched for C797S, which is seen more in late resistance. The development of therapies targeting the *EGFR* C797S resistance mutation will need to consider the possibility that C797S will be a late resistance mechanism on osimertinib, and extra time may be needed to identify these patients.[37]

Conclusions

Our data indicate that *EGFR* T790M is a key biomarker not only for indicating the probability of osimertinib sensitivity but also for understanding the biology of osimertinib resistance. Retesting for T790M after osimertinib failure is an important step for guiding patients to appropriate subsequent treatment strategies and relevant clinical trials. Our data indicate that drug resistance in some patients is genetically heterogeneous, impairing the effectiveness of targeted therapies. These findings must be considered as strategies are developed to treat resistance to newer first-line targeted therapies (eg, osimertinib, alectinib)[4,38] with their unique resistance mechanisms. If we wish to achieve more durable outcomes, early interventions may be needed, such as combination targeted therapies, to prevent the development of drug resistance.

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