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ORIGINAL REPORT

# Association Between Plasma Genotyping and Outcomes of Treatment With Osimertinib (AZD9291) in Advanced Non–Small-Cell Lung Cancer

*Geoffrey R. Oxnard, Kenneth S. Thress, Ryan S. Alden, Rachael Lawrance, Cloud P. Paweletz, Mireille Cantarini, James Chih-Hsin Yang, J. Carl Barrett, and Pasi A. Jänne*

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Geoffrey R. Oxnard, Ryan S. Alden, Cloud P. Paweletz, and Pasi A. Jänne, Dana-Farber Cancer Institute, Boston; Kenneth S. Thress and J. Carl Barrett, AstraZeneca, Waltham, MA; Rachael Lawrance and Mireille Cantarini, AstraZeneca, Macclesfield, United Kingdom; and James Chih-Hsin Yang, National Taiwan University and National Taiwan University Hospital, Taipei, Taiwan.

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G.R.O. and K.S.T. contributed equally to this work.

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Corresponding author: Geoffrey R. Oxnard, MD, Dana-Farber Cancer Institute, 450 Brookline Ave, Boston, MA 02215; e-mail: geoffrey_oxnard@dfci.harvard.edu.



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## ABSTRACT

### Purpose
Third-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) have demonstrated potent activity against TKI resistance mediated by *EGFR* T790M. We studied whether noninvasive genotyping of cell-free plasma DNA (cfDNA) is a useful biomarker for prediction of outcome from a third-generation EGFR-TKI, osimertinib.

### Methods
Plasma was collected from all patients in the first-in-man study of osimertinib. Patients who were included had acquired EGFR-TKI resistance and evidence of a common *EGFR*-sensitizing mutation. Genotyping of cell-free plasma DNA was performed by using BEAMing. Plasma genotyping accuracy was assessed by using tumor genotyping from a central laboratory as reference. Objective response rate (ORR) and progression-free survival (PFS) were analyzed in all T790M-positive or T790M-negative patients.

### Results
Sensitivity of plasma genotyping for detection of T790M was 70%. Of 58 patients with T790M-negative tumors, T790M was detected in plasma of 18 (31%). ORR and median PFS were similar in patients with T790M-positive plasma (ORR, 63%; PFS, 9.7 months) or T790M-positive tumor (ORR, 62%; PFS, 9.7 months) results. Although patients with T790M-negative plasma had overall favorable outcomes (ORR, 46%; median PFS, 8.2 months), tumor genotyping distinguished a subset of patients positive for T790M who had better outcomes (ORR, 69%; PFS, 16.5 months) as well as a subset of patients negative for T790M with poor outcomes (ORR, 25%; PFS, 2.8 months).

### Conclusion
In this retrospective analysis, patients positive for T790M in plasma have outcomes with osimertinib that are equivalent to patients positive by a tissue-based assay. This study suggests that, upon availability of validated plasma T790M assays, some patients could avoid a tumor biopsy for T790M genotyping. As a result of the 30% false-negative rate of plasma genotyping, those with T790M-negative plasma results still need a tumor biopsy to determine presence or absence of T790M.

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## INTRODUCTION

Acquired resistance to tyrosine kinase inhibitors (TKIs) that target the epidermal growth factor receptor (EGFR) is a prevalent clinical problem in the management of advanced non–small-cell lung cancer (NSCLC) that harbors TKI-sensitizing mutations in the *EGFR* gene. Resistance to conventional EGFR-TKIs develops after a median of 10 to 12 months and is most commonly mediated by an acquired mutation, T790M[1-5]. With many negative clinical trials in this space,[6] standard therapy for acquired resistance, until recently, remained platinum-based chemotherapy.[7]

Standard of care is rapidly changing with the development of third-generation, mutant-selective EGFR-TKIs that have activity against cells that harbor TKI-sensitive *EGFR* mutations. Unlike conventional EGFR-TKIs, this activity is maintained in the presence of T790M resistance mutation.[8,9] These agents were developed to have reduced activity against wild-type *EGFR* and reduced EGFR-mediated toxicity compared with conventional EGFR-TKIs.[9-11] At least five third-generation EGFR-TKIs are in clinical development

(rociletinib, osimertinib, HM61713/BI1482694, ASP8273, and EGF816) and all have reported objective response rates (ORRs) that exceed 45% in patients with *EGFR*-mutant NSCLC and T790M-positive resistance.[12-16] Clinical activity in patients with tumors negative for T790M has been less favorable, though data are limited. In previously published data from the AURA study, osimertinib exhibited a better response rate (confirmed ORR, 61% *v* 21%) and median progression-free survival (PFS; 9.6 months *v* 2.8 months) in patients with T790M-positive versus T790M-negative tumor genotyping.[12]

With recent regulatory approval of osimertinib in the United States, Europe, Japan, and Korea for treatment of metastatic NSCLC that harbors *EGFR* T790M,[17] this mutation has emerged as a new biomarker for guidance of treatment in patients with NSCLC with acquired resistance to prior EGFR-TKI. This represents a clinical challenge because tumor genotyping for *EGFR* T790M requires a biopsy to be performed after resistance develops, which is a procedure that carries risks, can delay subsequent therapy, and may not always be feasible.[18] Noninvasive genotyping of cell-free plasma DNA (cfDNA) represents an attractive alternative for detection of *EGFR* T790M, noting that plasma genotyping for *EGFR*-sensitizing mutations is an established alternative in Europe when tumor genotyping is not feasible.[19] We hypothesized that cfDNA genotyping for *EGFR* T790M can identify patients who may gain clinical benefit from third-generation EGFR-TKIs, facilitating identification of patients whose tumors have developed resistance via T790M. To address this question, we performed a post hoc analysis of samples from the phase I AURA trial of osimertinib, which was ideally suited for such a biomarker analysis as a result of expansion cohorts that specifically enrolled patients with and without T790M in their tumors.

## METHODS

For this exploratory analysis, we considered all patients who were enrolled in the escalation and expansion cohorts of the phase I AURA study of osimertinib for advanced *EGFR*-mutant NSCLC. This study included enrollment at multiple active doses (20 mg to 240 mg capsule and 80 mg tablet) as well as two first-line cohorts. Analysis was then limited to previously treated patients with NSCLC that harbors a common TKI-sensitive *EGFR* mutation (exon 19 deletion or L858R); as *EGFR* genotyping is clinically routine, this eligibility was based on tumor genotyping—performed either at a central laboratory or locally, if a central test was unavailable—or central plasma genotyping. Those with an uncommon or unknown *EGFR* genotype were excluded. For patients in expansion cohorts, a central tumor genotyping assay (cobas EGFR Mutation Test; Roche, Basel, Switzerland) was used to identify patients whose tumors were T790M positive or negative, as described previously.[12] Data cutoff for this analysis was May 1, 2015.

All patients on study consented to collection of plasma for cfDNA analysis before osimertinib treatment. Blood (10 to 20 mL) was collected in EDTA-containing vacutainers. Within 4 hours of collection, blood was centrifuged for 10 min at 1,200 *g*, and plasma supernatant was then centrifuged for 10 min at 3,000 *g*. Cleared plasma was stored in cryostat tubes at −80°C. cfDNA extraction and digital polymerase chain reaction (PCR) were performed by using BEAMing (Sysmex Inostics, Baltimore, MD) as described elsewhere.[20] Samples were tested for T790M, L858R, and six common exon 19 deletion variants. Results were reported as the relative allelic fraction (AF) of *EGFR*-mutant cfDNA relative to wild-type *EGFR* cfDNA. Plasma was considered positive by BEAMing for a given mutation if the mutation was detected above thresholds used for clinical application (≥ 0.04% AF for exon 19 deletion or L858R, ≥ 0.06% AF for T790M; D. Edelstein, personal communication, November 2015). When plasma T790M genotyping was discordant from tumor T790M genotyping, repeat plasma genotyping was performed by using either the cobas plasma EGFR assay or a validated droplet digital PCR (ddPCR) assay.[21] Relative T790M AF in plasma was calculated as AF of T790M/AF of EGFR-sensitizing mutation.

Clinical efficacy outcomes assessed included confirmed ORR, maximum change in target lesion size, and PFS on the basis of Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 assessed by the investigator. Demographics and PFS are presented for the intent-to-treat analysis set, whereas ORR and change in tumor size are presented for patients with measurable disease at baseline. Subset analyses are performed in the eligible population on the basis of presence or absence of T790M using central tumor or plasma genotyping. For analysis of proportions, the point estimate (percent) and 95% CI, calculated by using Clopper-Pearson exact method for binomial proportions, are presented. Fisher's exact test for each comparison—for differences between biomarker subgroups—or a $\chi^2$ test are presented. For PFS, median and 95% CI within each biomarker group are estimated from a Kaplan-Meier curve and *P* value from a log-rank test of homogeneity is presented.

## RESULTS

### Patient Population

In total, 402 patients were enrolled in the phase I cohorts of AURA (Fig 1). Sixty patients enrolled in the first-line cohort were excluded from this analysis. Of the remaining 342 patients with acquired resistance to EGFR-TKI, 34 were excluded from this

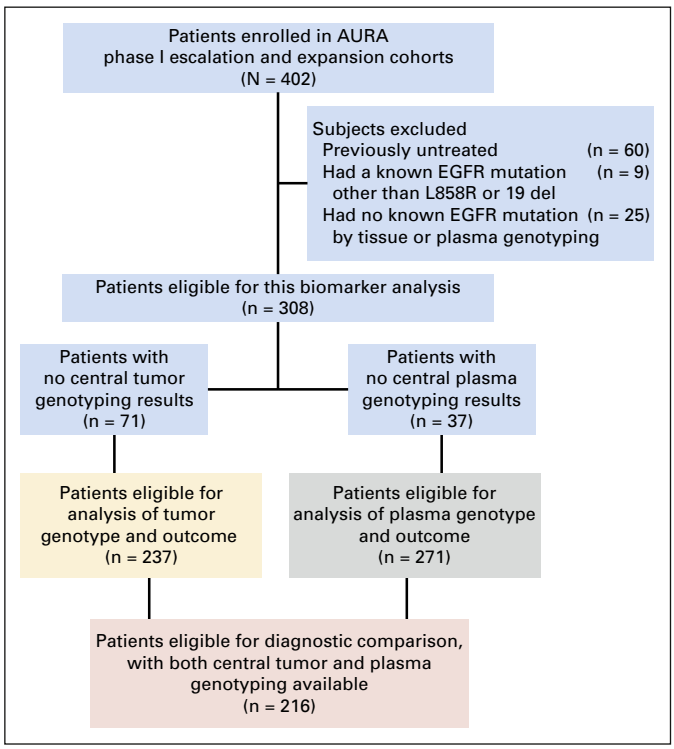


**Fig 1.** Flow diagram of eligible study population. Of 308 eligible patients, 237 had central tumor genotyping results, 271 had central plasma genotyping results, and 216 had both. EGFR, epidermal growth factor receptor.

  

analysis on the basis of absence of a known common *EGFR*-sensitizing mutation (exon 19 deletion, L858R). The remaining 308 patients represent the full cohort for this analysis, noting that the dose expansion cohorts preferentially enrolled patients positive for T790M (Appendix Fig A1, online only); therefore, the full population may not be representative of an all-comers acquired resistance population. These 308 patients were treated at five different active doses, with 126 (41%) treated at 80 mg once per day (Appendix Table A1, online only). Of 308 patients, 237 had central tumor genotyping for T790M and were eligible for outcomes analysis on the basis of tumor genotype, 271 had central plasma genotyping for T790M and were eligible for outcomes analysis on the basis of plasma genotype, and 216 had both central results and were eligible for the diagnostic analysis (Fig 1).

### Diagnostic Analysis

Using the diagnostic analysis set (n = 216) with central tissue genotype as reference, sensitivity of plasma genotyping for the known sensitizing mutation was 82% for exon 19 deletions (112 of 136; 95% CI, 75% to 88%) and 86% for L858R (63 of 73; 95% CI, 76% to 93%; Table 1, Appendix Fig A2A, online only), sensitivities similar to those reported for other plasma genotyping assays.[21-25] Studying the distribution of disease sites in these patients, sensitivity of plasma genotyping for *EGFR*-sensitizing mutations was significantly higher in patients with liver metastases (94% *v* 79%; $P = .008$) and trended lower in patients without any extrathoracic metastases (75% *v* 86%; $P = .06$; Appendix Table A2, online only). Sensitivity of plasma genotyping for T790M was 70% (111 of 158; 95% CI, 63% to 77%) in patients positive for T790M on central tumor genotyping (Table 1, Appendix Fig A2A). Of note, T790M was detected in 80% of patients (110 of 137) with versus only 5% of patients (1 of 21) without a detectable EGFR-sensitizing mutation in plasma ($P < .001$, Fig A2B), which indicates that detection of the resistance mutation in plasma is unlikely when a sensitizing mutation is not detected.

Plasma genotyping resulted in rare false positives for exon 19 deletions and L858R (specificity, 98% and 97%, respectively; Table 1, Appendix Fig A2C, online only); however, of 58 patients negative for T790M on central tumor genotyping, 18 (31%) were positive for T790M in plasma (Table 1, Appendix Fig A2C). To study whether these represent false positives of the assay versus heterogeneous resistance mutations not present in the rebiopsy sample, plasma T790M genotyping was repeated by using an alternative plasma assay for the 18 discordant cases (Appendix Table A3, online only). T790M positivity was confirmed by using an alternative assay in 14 of 18 tested plasmas (78%), which suggests that heterogeneous presence of resistance mutations across disease sites, and not false-positives of the BEAMing assay, account for the majority of T790M tissue–plasma discordance.

### Clinical Outcomes Analysis

Of 237 eligible patients with central tumor genotyping, 231 were evaluable for response to osimertinib. ORR was 62% (108 of 173; 95% CI, 54% to 70%) in patients with T790M-positive tumors, higher than the 26% ORR (15 of 58; 95% CI, 15% to 39%; $P < .001$) in patients with T790M-negative tumors (Figs 2A and 2B). Median PFS was significantly longer in 179 patients with T790M-positive tumors (9.7 months; 95% CI, 8.3 to 12.5 months) than in 58 patients with T790M-negative tumors (3.4 months; 95% CI, 2.1 to 4.3 months; $P < .001$; Fig 3A). Of 271 patients with plasma genotyping, 164 were positive for T790M in plasma and displayed a 63% ORR (103 of 164; 95% CI, 55% to 70%; Fig 2C) and a 9.7-month median PFS (95% CI, 8.3 to 11.1 months; Fig 3B), which is similar to outcomes in patients positive for T790M on tumor biopsy. ORR was surprisingly high at 46% (47 of 102; 95% CI, 36% to 56%) in patients with T790M-negative plasma genotyping; however, this was still lower than in patients with T790M-positive plasma ($P = .011$; Fig 2D), whereas median PFS was not significantly lower in those with T790M-negative plasma genotyping (8.2 months; 95% CI, 5.3 to 10.9 months; $P = .188$; Fig 3B).

As sensitivity of plasma genotyping was only 70% to 86%, tumor genotyping was studied as an additional biomarker for patients with T790M-negative plasma genotyping. Dividing patients with T790M-negative plasma results on the basis of tumor genotyping results, ORR was higher in patients with T790M-positive tumors (31 of 45; 69%; 95% CI, 53% to 82%) than in patients with T790M-negative tumors (10 of 40; 25%; 95% CI, 13% to 41%; $P < .001$), as was median PFS (16.5 months *v* 2.8 months; $P < .001$; Fig 3C). Of interest, patients with T790M-positive plasma could also be divided on the basis of tumor genotyping results, with ORR and median PFS higher in those with T790M-positive tumors (69 of 108; 64%; 95% CI, 54% to 73%; PFS, 9.3 months) than in those with T790M-negative tumors (5 of 18; 28%; 95% CI, 10% to 53%; $P = .004$; PFS, 4.2 months; $P = .0002$; Fig 3D).

We hypothesized that the lower ORR in patients with T790M-positive plasma but with discordant tumor results may be a result of T790M being present as a minor clone (Fig 4A). To study this,

**Table 1.** Sensitivity and Specificity of Plasma Genotyping Assays Compared With Tumor Genotype As a Reference Standard

| Plasma Genotype (BEAMing) | Tumor Genotype (cobas, Central Laboratory) | |
|---|---|---|
| | Exon 19 del+ (n = 136) | Exon 19 del− (n = 80) |
| Exon 19 del+ (n = 114) | 112 (82.3% sensitivity) | 2 |
| Exon 19 del− (n = 102) | 24 | 78 (97.5% specificity) |
| | L858R+ (n = 73) | L858R− (n = 143) |
| L858R+ (n = 68) | 63 (86.3% sensitivity) | 5 |
| L858R− (n = 148) | 10 | 138 (96.5% specificity) |
| | T790M+ (n = 158) | T790M− (n = 58) |
| T790M+ (n = 129) | 111 (70.3% sensitivity) | 18 |
| T790M− (n = 87) | 47 | 40 (69.0% specificity) |

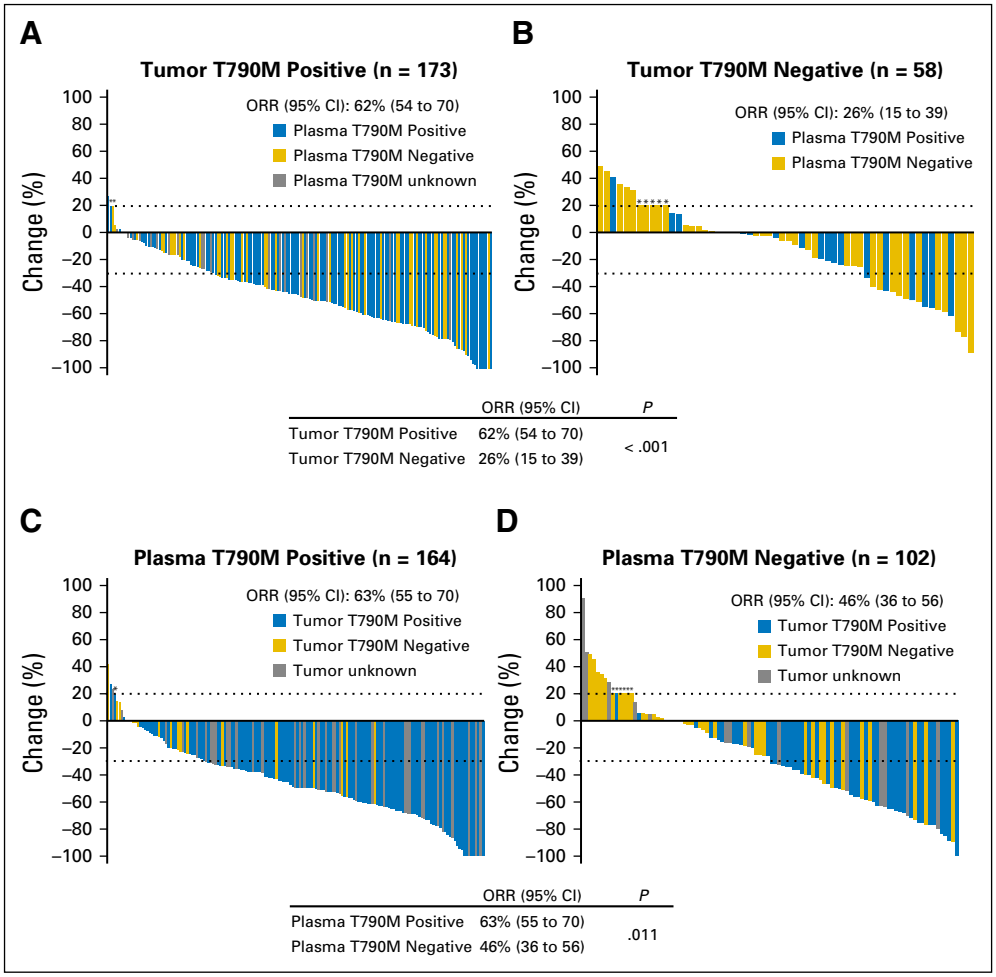


**Fig 2.** Waterfall plots for best percentage change in target lesion diameter for evaluable patients. Results are presented by (A and B) tumor T790M status and by (C and D) plasma T790M status. Colored bars indicate plasma T790M status in panels A and B and tumor T790M status in panels C and D. Asterisks represent imputed values; if it was known that a patient had died within 14 weeks (96 days) after the start of treatment and had no assessments of the target lesion that could be evaluated, the best change was imputed as 20%. ORR, objective response rate.

the relative T790M AF in plasma was calculated for patients with detectable T790M and sensitizing mutation in cfDNA and was higher in 108 patients with T790M-positive tumors than in 16 patients with T790M-negative tumors (median relative T790M AF, 33.6% *v* 16.8%; $P = .0047$; Fig 4B). Across 126 patients with known tumor sensitizing mutation detected in plasma and detectable plasma T790M, increased relative T790M AF was not associated overall with a greater depth of response ($R = -0.183$), but patients with relative T790M AF $> 10\%$ showed a greater depth of response compared with those with relative T790M AF $< 10\%$ ($P = .0407$; Fig 4C).

Lastly, clinical outcomes were explored in 102 patients with T790M-negative plasma using detection of TKI-sensitive *EGFR* mutation (sens) as a control for presence of tumor-derived circulating DNA to determine whether this could help distinguish likelihood of benefit from osimertinib. In 69 patients with TKI-sensitive *EGFR* mutation detected in plasma (T790M negative/sens positive), ORR was 38% (26 of 69; 95% CI, 26% to 50%) and median PFS was 4.4 months (95% CI, 2.8 to 6.8 months; Appendix Fig A3, online only). In contrast, in 33 patients with no *EGFR* mutations detected (T790M negative/sens negative), ORR was higher at 64% (21 of 33; 95% CI, 45% to 79%; $P = .019$) and median PFS was longer at 15.2 months (95% CI, 11.0 to 17.9 months; $P = .002$; Appendix Fig A3), which suggests that the absence of T790M in these cases may be uninformative given the lack of any detectable tumor DNA in the plasma.

## DISCUSSION

Emergence of *EGFR* T790M as a predictive biomarker in lung cancer presents a new challenge, as this is the first time that a biopsy at progression has been needed to guide subsequent care. With recent regulatory approval of osimertinib in the United States, Europe, and Japan, testing for T790M at acquired resistance must now become a standard component of patient care (Fig 5A); however, biopsies involve numerous challenges in terms of logistics, safety, and cost. If ever there were a clinical setting where such a liquid biopsy has an intuitive role, it is for avoidance of repeat biopsies for treatment resistance. The specific clinical role of plasma genotyping in this setting, however, is not yet clear.

Our data suggest that plasma and tumor genotyping can have complementary roles for T790M testing, where plasma genotyping could be the initial step and a biopsy for tumor genotyping could be supplementary (Fig 5B). If plasma genotype is positive for T790M, this may obviate the need for a biopsy—this predicts for

  

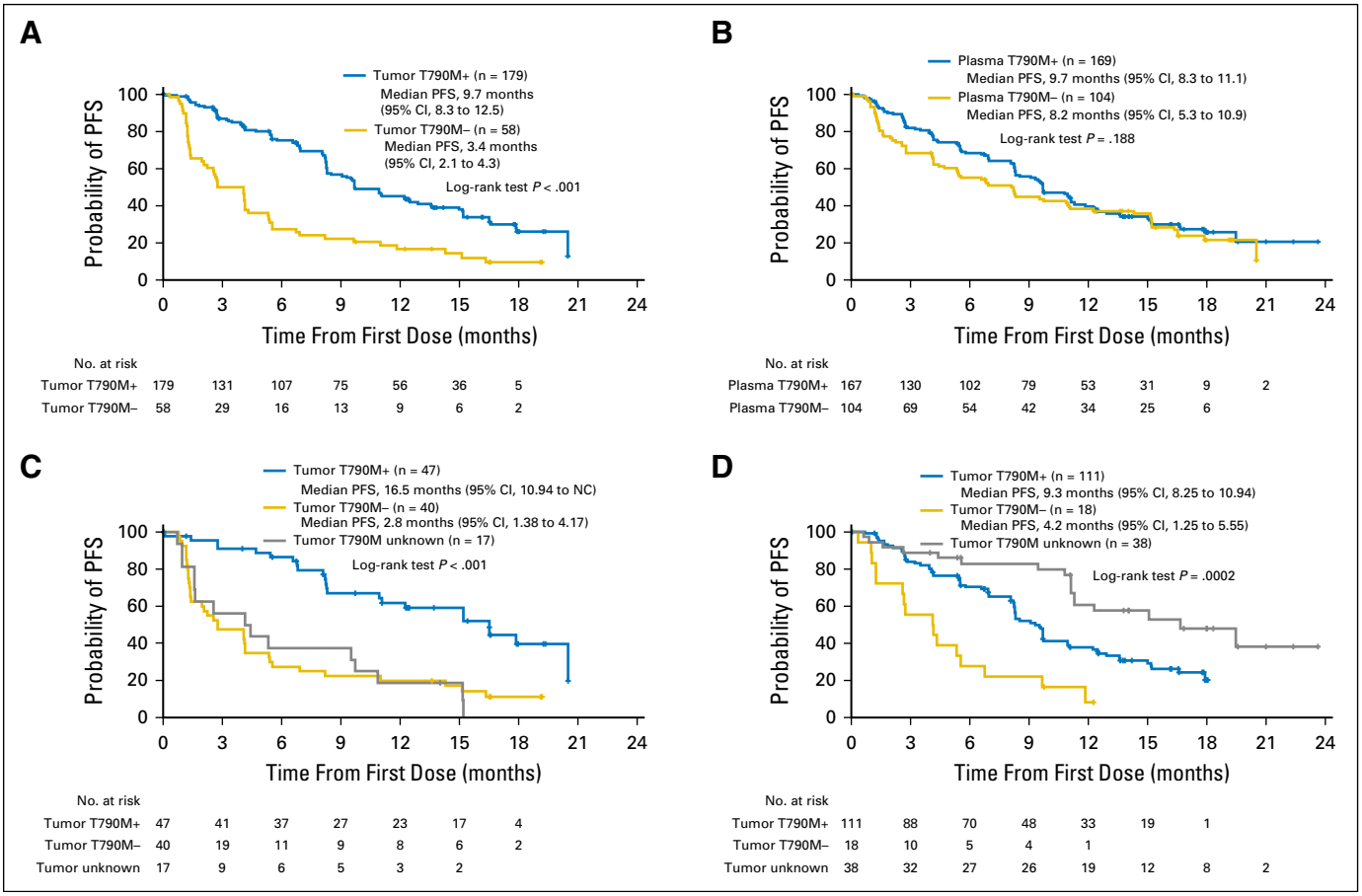


**Fig 3.** Kaplan-Meier curves of progression-free survival (PFS) in T790M-positive (T790M+) and T790M-negative (T790M−) subpopulations treated with osimertinib. (A) Patients with T790M+ tumors have a dramatically longer PFS than do patients with T790M− tumors ($P < .001$). (B) Plasma genotyping for T790M fails to identify two subgroups with different PFS ($P = .188$). (C) Patients with T790M− plasma subdivided into tumor T790M+ (blue) and T790M− (yellow) demonstrate significantly improved PFS in tumor T790M+ patients ($P < .001$). (D) A similar trend is observed in patients with T790M+ plasma when subdivided by tumor genotyping status, though the T790M+ tumor group is limited to only 18 patients.

excellent outcomes on osimertinib (ORR, 63%; median PFS, 9.7 months), similar to that observed when treating with osimertinib on the basis of tumor genotyping results (ORR, 62%; median PFS, 9.7 months). This is consistent with the high positive predictive value seen with plasma genotyping in prior studies.[23,25] For example, consider a real-world distribution of acquired resistance cases with a 60:40 ratio of T790M positive to T790M negative in resistance biopsies,[1] and 70% plasma T790M sensitivity in tumors positive for T790M with 30% plasma positive for T790M in patients with T790M-negative tumors. In this example, approximately 54% of resistant patients would be T790M positive in plasma and, if treated on the basis of that result, could potentially avoid a biopsy.

In contrast, if plasma genotyping for T790M is negative, this result cannot fully obviate need for a tumor biopsy. As the plasma T790M-negative population is a mixture of true and false negatives, biopsy to further investigate the presence of T790M-positive tumor tissue is warranted (Fig 5B). Of 102 patients from this study with T790M-negative plasma, 45 had tumor genotyping that was positive for T790M, and these patients had a median PFS of 16.2 months, whereas 40 patients had tumor genotyping that was negative for T790M and a median PFS of 2.8 months. It is less clear how much value a biopsy for tumor genotyping adds in patients with T790M-positive plasma. Of 164 patients from this study with T790M-positive plasma genotyping, 18 had T790M-negative tumor genotyping, presumably as a result of heterogeneous presence of resistance mutation as a minor clone, and these patients had a response rate of 28% (5 of 18) and median PFS of 4.2 months. As this observation is based on just 18 patients, future research should study whether quantitative plasma genotyping and calculation of relative T790M AF can offer insight into heterogeneity, rather than relying on invasive tumor biopsies.

Of interest, we found that testing for *EGFR* driver mutations in plasma may help identify those patients with T790M-negative plasma who are more or less likely to benefit from osimertinib treatment. In patients with T790M-negative/sens-positive plasma genotyping, ORR to osimertinib was 38% and median PFS was 4.4 months. With such a mixed outcome as this, tumor genotyping of T790M is needed to identify candidates for osimertinib; however, when no sensitizing mutation is detected in patients with known *EGFR*-mutant lung cancer and acquired resistance, plasma genotyping for T790M becomes uninformative. In patients with T790M-negative/sens-negative plasma results, high ORR and prolonged PFS was observed. Favorable clinical outcomes in this patient subset

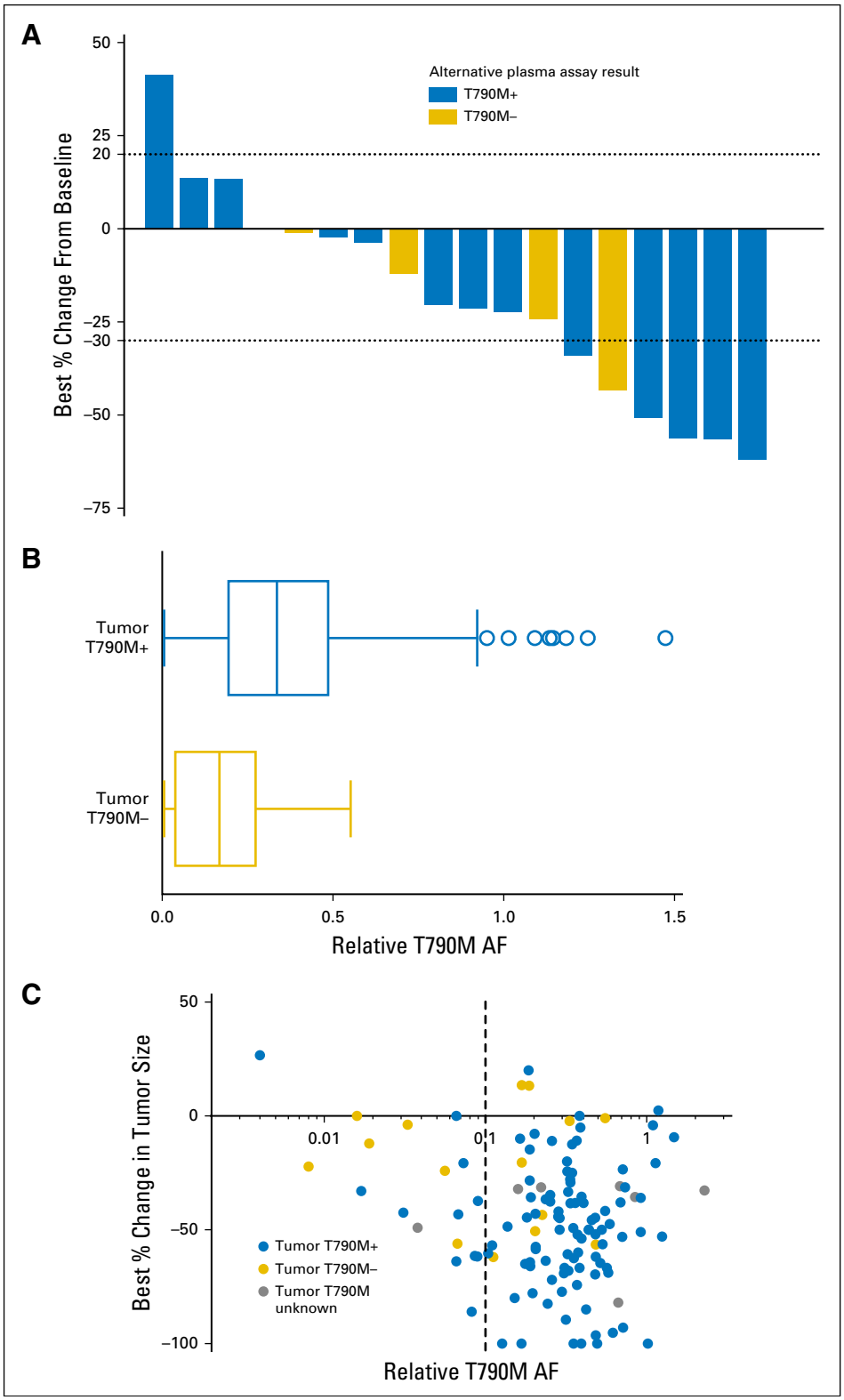


**Fig 4.** Heterogeneity in patients with T790M-positive (T790M+) plasma/T790M-negative (T790M−) tumors. (A) Objective response rate was 28% (5 of 18; 95% CI, 10% to 53%) in patients with T790M+ plasma but with discordant tumor genotyping. (B) Studying the relative T790M allelic fraction (AF; calculated as the ratio of AF of T790M to AF of the sensitizing mutation), this was significantly lower in 16 patients with a T790M− tumor genotype (yellow) than in 118 patients with a T790M+ tumor genotype (blue; $P = .0047$). Circles indicate outliers, solid lines indicate medians, and boxes represent interquartile ranges. (C) Studying relative T790M AF and depth of response, no significant association overall is seen ($R = -0.183$); however, patients with a relative T790M AF $> 10\%$ showed a greater depth of response compared with patients with a relative T790M AF $< 10\%$ ($P = .0407$).

could be related, in part, to lack of detectable tumor DNA in the plasma, which suggests a lower disease burden or less aggressive disease state. As these data are based on a relatively small patient subgroup, future studies are needed to study the best management approach for patients who lack detectable *EGFR* cfDNA.

Our results highlight the challenge of identifying a reference standard for development of new genotyping assays for drug resistance. Resistant cancers are inherently more heterogeneous than treatment-naïve cancers and, therefore, a single tumor biopsy may not be representative of the entire resistant cancer. We found that

  

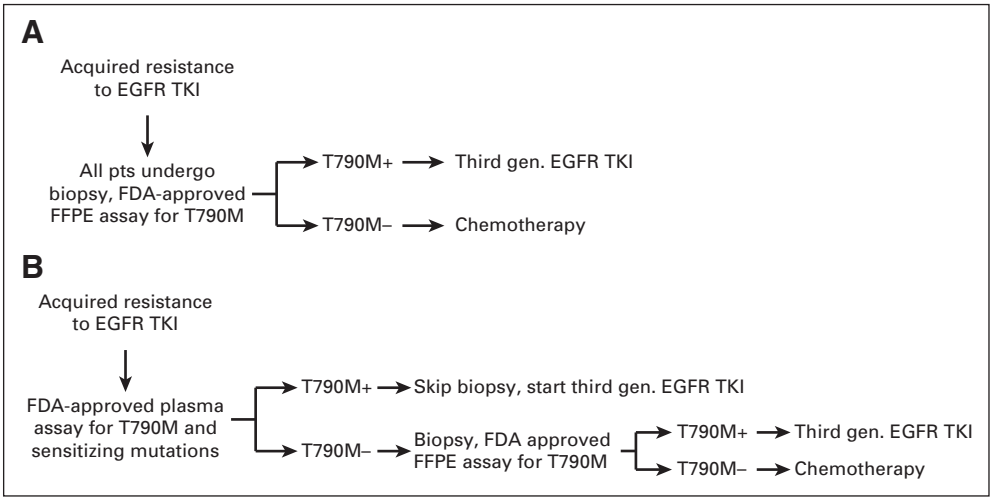


**Fig 5.** A proposed paradigm for use of plasma genotyping for epidermal growth factor receptor (EGFR) T790M. (A) In the conventional paradigm, all patients undergo a resistance biopsy for T790M genotyping, and this result is used to guide treatment decisions. (B) Our data support an alternate paradigm where plasma genotyping for T790M is used as a screening test before biopsy, which would only be needed for patients with no T790M detected in plasma. FDA, US Food and Drug Administration; FFPE, formalin-fixed, paraffin-embedded; pts, patients; TKI, tyrosine kinase inhibitor.

31% of patients who are negative for T790M on central tumor genotyping have detectable T790M in plasma with a BEAMing assay that otherwise has negligible false positives, though the relative AF of plasma T790M is lower in these patients than in patients with T790M-positive plasma and tumors. Despite this discordance, outcomes in patients with T790M-positive plasma are similar to outcomes of patients with T790M-positive tumors, which highlights that a single tumor biopsy may not be representative of the spatial heterogeneity of resistant lung cancer. Other plasma assays have similarly identified unexpected false-positives for T790M in the absence of false-positives for other mutations.[25] In a setting in which it is unclear whether tumor genotyping is representative of the entirety of the disease, clinical outcome will be the best reference standard for development of a noninvasive assay.

This analysis has several practical limitations. Whereas it is a large analysis of a prospective dataset, it was not a preplanned analysis, in part because of the rapid evolution of plasma genotyping technologies. The study population is also not fully representative of all patients with acquired EGFR-TKI resistance given the intentional enrichment for T790M-positive patients. Lastly, BEAMing was not performed under Clinical Laboratory Improvement Amendments conditions; this analysis used an investigational assay that is identical to the commercially available BEAMing assay. Prospective validation is needed to confirm the clinical benefit of osimertinib in patients with T790M-positive plasma genotyping.

In conclusion, these data support the use of both plasma- and tissue-based assays for T790M genotyping. Clinical outcomes on osimertinib in patients with T790M-positive plasma genotyping results are similar to those achieved when treating patients with T790M-positive tumor genotyping results. Given the ease and reduced risk of plasma analysis compared with an invasive biopsy procedure, data support a new paradigm for resistance management, with rapid plasma genotyping as a diagnostic option before undergoing a tumor biopsy. Patients negative for T790M in plasma, however, should undergo a biopsy to determine T790M status because of the risks of false-negative plasma results.

## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

## AUTHOR CONTRIBUTIONS

**Conception and design:** Geoffrey R. Oxnard, Kenneth S. Thress, J. Carl Barrett, Pasi A. Jänne
**Financial support:** Geoffrey R. Oxnard
**Provision of study materials or patients:** Geoffrey R. Oxnard, Kenneth S. Thress, James Chih-Hsin Yang, Pasi A. Jänne
**Collection and assembly of data:** All authors
**Data analysis and interpretation:** Geoffrey R. Oxnard, Kenneth S. Thress, Ryan S. Alden, Rachael Lawrance, Mireille Cantarini, James Chih-Hsin Yang, J. Carl Barrett, Pasi A. Jänne
**Manuscript writing:** All authors
**Final approval of manuscript:** All authors

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## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

**Association Between Plasma Genotyping and Outcomes of Treatment with Osimertinib (AZD9291) in Advanced Non–Small-Cell Lung Cancer**

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**Geoffrey R. Oxnard**
**Honoraria:** Chugai Pharma, AstraZeneca, Boehringer Ingelheim
**Consulting or Advisory Role:** AstraZeneca, Sysmex, ARIAD Pharmaceuticals, Inivata, Boehringer Ingelheim, Genentech
**Patents, Royalties, Other Intellectual Property:** Coauthor of a patent pending entitled "Non-Invasive Blood-Based Monitoring of Genomic Alterations in Cancer." (Inst)

**Kenneth S. Thress**
**Employment:** AstraZeneca
**Stock or Other Ownership:** AstraZeneca

**Ryan S. Alden**
No relationship to disclose

**Rachael Lawrance**
**Employment:** AstraZeneca
**Stock or Other Ownership:** AstraZeneca

**Cloud P. Paweletz**
**Honoraria:** Bio-Rad, Clovis Oncology
**Patents, Royalties, Other Intellectual Property:** Coauthor of a patent pending entitled "Non-Invasive Blood-Based Monitoring of Genomic Alterations in Cancer." (Inst)

**Mireille Cantarini**
**Employment:** AstraZeneca
**Stock or Other Ownership:** AstraZeneca

**James Chih-Hsin Yang**
**Honoraria:** Boehringer Ingelheim, Roche, Chugai Pharma, MSD Oncology, AstraZeneca
**Consulting or Advisory Role:** Boehringer Ingelheim, Novartis, AstraZeneca, Genentech, Clovis Oncology, Eli Lilly, MSD Oncology, Merck Serono, Celgene, Astellas Pharma, Bayer AG, Pfizer, Ono Pharmaceutical, Bristol-Myers Squibb, Boehringer Ingelheim (Inst), AstraZeneca (Inst)

**J. Carl Barrett**
**Employment:** AstraZeneca
**Stock or Other Ownership:** AstraZeneca

**Pasi A. Jänne**
**Stock or Other Ownership:** Gatekeeper Pharmaceuticals
**Consulting or Advisory Role:** Acea Biosciences, AstraZeneca, Boehringer Ingelheim, Chugai Pharma, Merrimack Pharmaceuticals, Pfizer, Roche, ARIAD Pharmaceuticals
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## Appendix

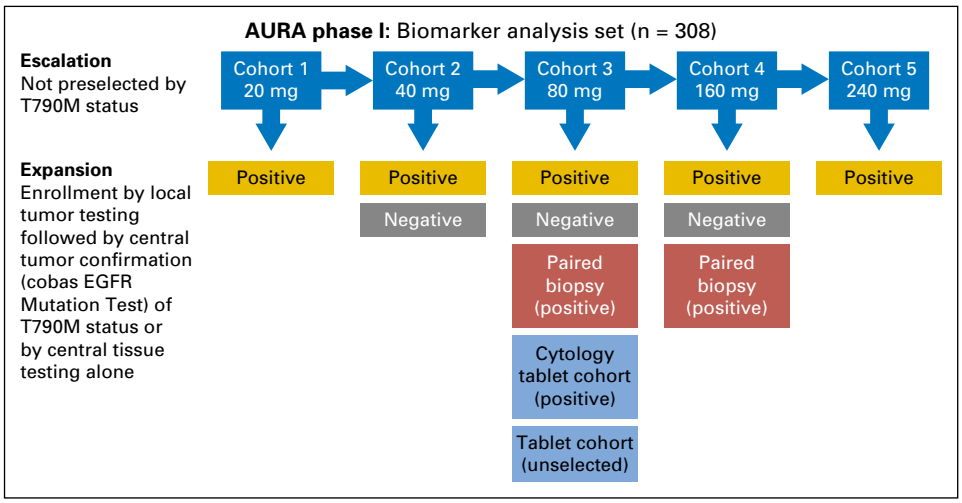


**Fig A1.** AURA phase I cohorts enrolling previously treated patients. Dose escalation cohorts (dark blue) were not preselected by T790M status, and enrolled approximately 6 patients each. Dose expansion cohorts initially enrolled approximately 12 patients each, which was then expanded to approximately 20 patients. Most of these expansion cohorts enrolled patients as locally T790M positive (yellow) or T790M negative (gray), with central T790M testing used for allocation if local testing was unavailable. Additional cohorts included two paired biopsy cohorts enrolling T790M-positive patients (red), and two tablet cohorts (light blue) enrolling patients T790M positive by cytology or T790M unselected. EGFR, epidermal growth factor receptor.

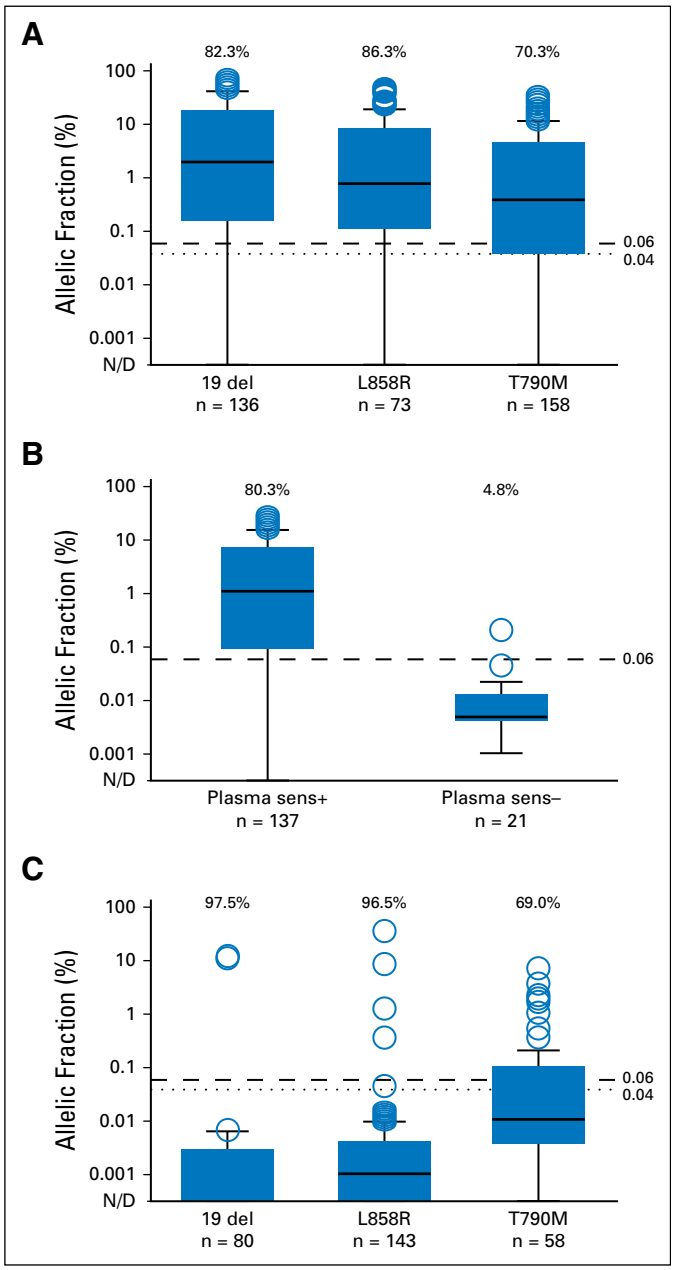


**Fig A2.** Test characteristics and dynamic range of central plasma *EGFR* genotyping using BEAMing. (A) Sensitivity of the assays for exon 19 deletion (19 del), L858R, and T790M, calculated as the proportion of patients positive in plasma for a given mutation out of all patients with positive central tumor genotyping for that mutation. (B) Among patients T790M positive on tumor genotyping, sensitivity of the plasma T790M assay is lower in cases where no *EGFR*-sensitizing mutation is detected in plasma ($P < .001$). (C) Specificity of the assays for 19 del, L858R, and T790M, calculated as the proportion of patients negative in plasma for a given mutation out of all patients with negative central tumor genotyping for that mutation. 0.04% allelic fraction is the positive threshold for *EGFR* exon 19 del and L858R assays, while 0.06% is the positive threshold for the *EGFR* T790M assay. Turkey box plots of allelic fraction are shown; circles indicate outliers, solid lines indicate medians, and boxes represent interquartile ranges. N/D, not determined.

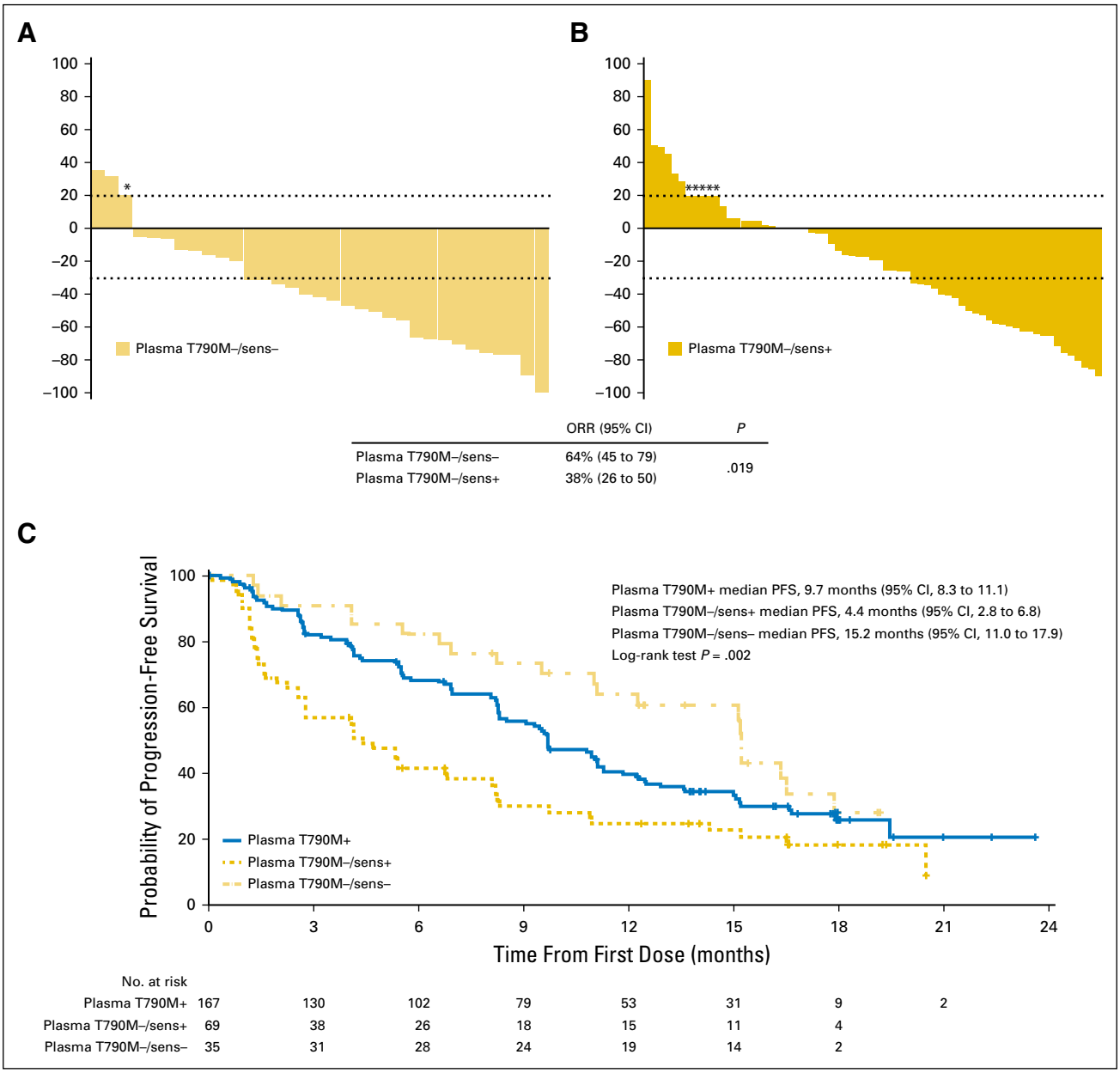


**Fig A3.** Objective response rate (ORR) and progrssion-free survival (PFS) of the plasma T790M negative (T790M−) population using detection of a plasma-sensitizing mutation (sens) as a quality control. (A) Waterfall plots for best percentage change in target lesion size of evaluable patients in the 33 cases whose plasma genotype was T790M−/sens- (yellow dashed) or the 69 patients whose plasma genotype was T790M−/sens+ (yellow dotted) by BEAMing. The dashed lines at 20% represent the boundary for determination of progressive disease, and the dashed line at −30% represents the boundary for determination of partial response. Asterisks represent imputed values: if it was known that a patient had died within 14 weeks (96 days) after the start of treatment and had no assessments of the target lesion that could be evaluated, the best change was imputed as 20%. (B) Kaplan-Meier curves of PFS using detection of a plasma-sensitizing mutation as a quality control for plasma T790M− cases. Three groups can be identified with significant differences in PFS (P = .002). T790M+, T790M-positive.

**Table A1.** Demographics of All Patients in Biomarker Analysis and Subsets Used for Analysis of Tumor Genotype and Outcome, Plasma Genotype and Outcome, and Diagnostic Comparison of Tumor and Plasma Genotyping

| Baseline Characteristic | All Patients (n = 308) | Central Tumor Genotyping Results (n = 237) | Central Plasma Genotyping Results (n = 271) | Both Central Tumor and Plasma Genotyping Results (n = 216) |
|---|---|---|---|---|
| Gender, No. (%) | | | | |
| Male | 116 (37.7) | 91 (38.4) | 104 (38.4) | 84 (38.9) |
| Female | 192 (62.3) | 146 (61.6) | 167 (61.6) | 132 (61.1) |
| Median age, years | 60 | 59 | 60 | 59 |
| Race, No. (%) | | | | |
| White | 97 (31.5) | 77 (32.5) | 86 (31.7) | 66 (30.6) |
| Asian | 191 (62.0) | 148 (62.4) | 165 (60.9) | 138 (63.9) |
| Other | 6 (1.9) | 4 (1.7) | 6 (2.2) | 4 (1.9) |
| No. of prior TKI lines | | | | |
| Missing, No. (%) | 14 (4.5) | 8 (3.4) | 14 (5.2) | 8 (3.7) |
| Min/median/max | 2/3/3 | 2/3/3 | 2/3/3 | 2/3/3 |
| Immediate prior TKI therapy, No. (%) | | | | |
| No | 113 (36.7) | 86 (36.3) | 103 (38.0) | 80 (37.0) |
| Yes | 195 (63.3) | 151 (63.7) | 168 (62.0) | 136 (63.0) |
| Erlotinib | 96 (31.2) | 66 (27.8) | 81 (29.9) | 59 (27.3) |
| Gefitinib | 53 (17.2) | 44 (18.6) | 50 (18.5) | 43 (19.9) |
| Afatinib | 40 (13.0) | 35 (14.8) | 35 (12.9) | 32 (14.8) |
| Rociletinib | 5 (1.6) | 5 (2.1) | 1 (0.4) | 1 (0.5) |
| Dacomitinib | 1 (0.3) | 1 (0.4) | 1 (0.4) | 1 (0.5) |
| Extrathoracic metastatic sites, No. (%) | | | | |
| Liver | 99 (32.1) | 72 (30.4) | 89 (32.8) | 67 (31.0) |
| Brain | 112 (36.4) | 90 (38.0) | 93 (34.3) | 79 (36.6) |
| Bone | 157 (51.0) | 122 (51.5) | 139 (51.3) | 110 (50.9) |
| Osimertinib dose, No. (%) | | | | |
| 20 mg | 21 (6.8) | 13 (5.5) | 21 (7.7) | 13 (6.0) |
| 40 mg | 52 (16.9) | 43 (18.1) | 52 (19.2) | 43 (20.0) |
| 80 mg | 126 (40.9) | 90 (40.0) | 108 (39.9) | 88 (40.7) |
| 160 mg | 92 (30.0) | 78 (32.9) | 73 (26.9) | 59 (27.3) |
| 240 mg | 17 (5.5) | 13 (5.5) | 17 (6.3) | 13 (6.0) |
| *EGFR*-sensitizing mutation,* No. (%) | | | | |
| Exon 19 deletion | 200 (64.9) | 152 (64.1) | 177 (65.3) | 138 (63.9) |
| L858R | 108 (35.1) | 85 (35.9) | 94 (34.6) | 78 (36.1) |
| Central (tissue) T790M mutation, No. (%) | | | | |
| Positive | 179 (58.1) | 179 (75.5) | 158 (58.3) | 158 (73.1) |
| Negative | 58 (18.8) | 58 (24.5) | 58 (21.4) | 58 (26.9) |
| Unknown | 71 (23.1) | | 55 (20.3) | |
| Central (plasma) T790M mutation, No. (%) | | | | |
| Positive | 167 (54.2) | 129 (54.4) | 167 (61.6) | 129 (59.7) |
| Negative | 104 (33.8) | 87 (36.7) | 104 (38.4) | 87 (40.3) |
| Unknown | 37 (12.0) | 21 (8.9) | | |

Abbreviations: EGFR, epidermal growth factor receptor; max, maximum; min, minimum; TKI, tyrosine kinase inhibitor

*Five cases were positive for both exon 19 deletion (del) and L858R on local or plasma genotyping but were positive for exon 19 deletion only on central tumor genotyping; therefore, these were considered patients with exon 19 deletion.

**Table A2.** Sites of Metastatic Disease at Baseline and Sensitivity for Sensitizing Mutation Detection (Exon 19 Del/L858R) by Plasma Test

| | Present | | Absent | | |
|---|---|---|---|---|---|
| Metastatic Site* | Sensitivity, % | No. Detected of Total | Sensitivity, % | No. Detected of Total | *P*† |
| Any thoracic site | 83 | 143 of 172 | 86 | 32 of 37 | .617 |
| Respiratory | 83 | 104 of 126 | 86 | 71 of 83 | .565 |
| Lymph nodes | 86 | 96 of 111 | 81 | 79 of 98 | .251 |
| Pleural effusion | 83 | 64 of 77 | 84 | 111 of 132 | .854 |
| Pericardial effusion | 89 | 8 of 9 | 84 | 167 of 200 | .668 |
| Any extrathoracic site | 86 | 139 of 161 | 75 | 36 of 48 | .062 |
| Bone and locomotor | 88 | 91 of 104 | 80 | 84 of 105 | .142 |
| Brain/CNS | 85 | 63 of 74 | 83 | 112 of 135 | .684 |
| Liver | 94 | 61 of 65 | 79 | 114 of 144 | .008 |
| Adrenal | 91 | 20 of 22 | 83 | 155 of 187 | .335 |
| Skin/soft tissue | 100 | 14 of 14 | 83 | 161 of 195 | .088 |

NOTE. Diagnostic set (n = 209), plasma epidermal growth factor receptor mutation–positive. No adjustment for multiple testing has been performed.
*Metastatic sites as reported on electronic case report form (eCRF) from available list of sites, with the exception of adrenal, which was selected from reported free text on eCRF using "adrenal" search of free-text terms.
†*P* from $2 \times 2$ $\chi^2$ test.

**Table A3.** Genotyping and Treatment Results for 18 Patients Positive for T790M in Plasma but Negative for T790M in Tumor

| Patient No. | Dose | Result of Central Tumor Genotyping for T790M | Result of Central Plasma BEAMing for T790M | T790M AF (BEAMing), % | EGFR Driver | EGFR Driver AF (BEAMing), % | Relative Prevalence Plasma T790M | T790M Detected With Alternative Plasma Assay | Alternative Plasma Assay Used | BOR | Best % Change From Baseline | PFS (month) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12 | 80 mg | Not detected | Detected | 0.19 | L858R | 3.39 | 0.06 | No | ddPCR | SD | −24.1 | 12.25* |
| 11 | 80 mg | Not detected | Detected | 0.34 | 19 del | 5.08 | 0.07 | Yes | Cobas | PR | −56.1 | 11.83 |
| 5 | 80 mg | Not detected | Detected | 1.65 | 19 del | 3.42 | 0.48 | Yes | ddPCR | PR | −56.5 | 9.76* |
| 18 | 80 mg | Not detected | Detected | 0.06 | 19 del | ND | NA | Yes | ddPCR | SD | −34.1 | 9.66 |
| 1 | 80 mg | Not detected | Detected | 7.05 | 19 del | 34.75 | 0.2 | Yes | ddPCR | PR | −50.6 | 6.74 |
| 14 | 80 mg | Not detected | Detected | 0.09 | 19 del | 11.09 | 0.01 | Yes | ddPCR | SD | −22.2 | 5.55 |
| 7 | 160 mg | Not detected | Detected | 0.64 | 19 del | ND | NA | Yes | ddPCR | SD | −21.3 | 5.32 |
| 6 | 80 mg | Not detected | Detected | 1.11 | L858R | 34.14 | 0.03 | Yes | Cobas | SD | −3.8 | 4.34 |
| 17 | 160 mg | Not detected | Detected | 0.07 | L858R | 0.32 | 0.23 | No | Cobas | PR | −43.4 | 4.17 |
| 4 | 80 mg | Not detected | Detected | 2.04 | 19 del | 18.14 | 0.11 | Yes | Cobas | PR | −62 | 4.14 |
| 9 | 20 mg | Not detected | Detected | 0.45 | L858R | 28.61 | 0.02 | Yes | Cobas | SD | 0 | 2.73 |
| 15 | 80 mg | Not detected | Detected | 0.09 | L858R | 4.70 | 0.02 | No | ddPCR | SD | −12.1 | 2.66 |
| 16 | 80 mg | Not detected | Detected | 0.08 | L858R | 0.15 | 0.55 | No | Cobas | SD | −1 | 2.6 |
| 3 | 40 mg | Not detected | Detected | 2.24 | L858R* | 7.73 | 0.29 | Yes | ddPCR | PD | 41.3 | 1.25 |
| 13 | 40 mg | Not detected | Detected | 0.12 | L858R | 0.37 | 0.33 | Yes | Cobas | PD | −2.2 | 1.25 |
| 10 | 40 mg | Not detected | Detected | 0.34 | L858R | 2.05 | 0.17 | Yes | Cobas | PD | −20.4 | 1.05 |
| 2 | 80 mg | Not detected | Detected | 3.45 | 19 del | 20.49 | 0.17 | Yes | ddPCR | PD | 13.6 | 1.02 |
| 8 | 160 mg | Not detected | Detected | 0.59 | 19 del | 3.14 | 0.19 | Yes | ddPCR | PD | 13.3 | 0.36 |

Abbreviations: AF, allelic fraction; BOR, best overall response; ddPCR, droplet digital polymerase chain reaction; EGFR, epidermal growth factor receptor; NA, not applicable, ND, not detected; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

*Subject 3 had discordant tumor and plasma genotyping. Plasma genotyping showed L858R and T790M while tumor genotyping showed G719X and no T790M.