Clinical Cancer Research

Tissue and Plasma EGFR Mutation Analysis in the FLAURA Trial: Osimertinib versus Comparator EGFR Tyrosine Kinase Inhibitor as First-Line Treatment in Patients with EGFR-Mutated Advanced Non–Small Cell Lung Cancer

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Abstract

Purpose: To assess the utility of the **cobas** EGFR Mutation Test, with tissue and plasma, for first-line osimertinib therapy for patients with *EGFR*-mutated (*EGFR*m; Ex19del and/or L858R) advanced or metastatic non–small cell lung cancer (NSCLC) from the FLAURA study (NCT02296125).

Experimental Design: Tumor tissue *EGFR*m status was determined at screening using the central cobas tissue test or a local tissue test. Baseline circulating tumor (ct)DNA *EGFR*m status was retrospectively determined with the central cobas plasma test.

Results: Of 994 patients screened, 556 were randomized (289 and 267 with central and local *EGFR* test results, respectively) and 438 failed screening. Of those randomized from local *EGFR* test results, 217 patients had available central test results; 211/217 (97%) were retrospectively confirmed *EGFR*m positive by central cobas tissue test. Using reference central cobas tissue test results, positive percent agreements with cobas plasma test results for Ex19del and L858R detection were 79% [95% confidence interval (CI), 74–84] and 68% (95% CI, 61–75), respectively. Progression-free survival (PFS) superiority with osimertinib over comparator EGFR-TKI remained consistent irrespective of randomization route (central/local *EGFR*m-positive tissue test). In both treatment arms, PFS was prolonged in plasma ctDNA *EGFR*m-negative (23.5 and 15.0 months) versus -positive patients (15.2 and 9.7 months).

Conclusions: Our results support utility of cobas tissue and plasma testing to aid selection of patients with *EGFR*m advanced NSCLC for first-line osimertinib treatment. Lack of *EGFR*m detection in plasma was associated with prolonged PFS versus patients plasma *EGFR*m positive, potentially due to patients having lower tumor burden.

Introduction

Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) are the recommended first-line treatment for patients with advanced non–small cell lung cancer (NSCLC) harboring an EGFR-TKI–sensitizing mutation (*EGFR*m; refs. 1, 2). Most patients treated with first- or second-generation EGFR-TKIs eventually

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Clin Cancer Res 2019;25:6644–52

doi: 10.1158/1078-0432.CCR-19-1126

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Translational Relevance

This analysis evaluates the prospective clinical utility of the **cobas** EGFR Mutation Test in tissue and plasma samples from the FLAURA trial for selection of first-line osimertinib therapy for patients with EGFR-TKI–sensitizing mutated (*EGFR*m) advanced or metastatic NSCLC. Concordance was generally high between local validated tests and central cobas tissue *EGFR* mutation tests and between cobas tissue and plasma tests for ex19del and L858R mutations individually or in aggregate. PFS superiority of osimertinib over comparator EGFR-TKIs remained consistent irrespective of randomization route (local or central *EGFR*m tissue test) and tissue or plasma ctDNA *EGFR*m status. Lack of *EGFR*m detection in plasma was associated with prolonged PFS versus patients plasma *EGFR*m positive in both treatment arms, potentially due to lower tumor burden and less tumor DNA shedding into the blood. Our results support utilization of cobas tissue and plasma testing to identify patients with *EGFR*m advanced NSCLC for first-line osimertinib therapy.

develop resistance, with the *EGFR* p.Thr790Met point mutation (*EGFR* T790M) resistance mutation detectable in approximately 50% of cases (3–6). Osimertinib is a third-generation, central nervous system (CNS)–active, EGFR-TKI that potently and selectively inhibits both EGFR-TKI–sensitizing and *EGFR* T790M resistance mutations (7–11). Osimertinib is an approved first-line treatment option in several countries, including the United States and European Union, for patients with *EGFR*m advanced NSCLC and patients with T790M-positive NSCLC following disease progression on first-line EGFR-TKIs (12–14).

At initial diagnosis of nonsquamous NSCLC, *EGFR* mutation testing is recommended using tumor tissue biopsies (2, 15). In some clinical settings in which tissue is limited and/or insufficient for molecular testing, physicians may use a plasma circulating tumor (ct)DNA assay to identify *EGFR* mutations. ctDNA is easily obtained through minimally invasive blood sampling and can be a specific and sensitive biomarker for the detection of *EGFR* mutations in patients whose tumors shed DNA (15–21).

The original **cobas** EGFR Mutation Test v1 (Roche Molecular Systems Inc., Pleasanton, CA) and the latest **cobas** EGFR Mutation Test v2 (Roche Molecular Systems, Inc., Pleasanton, CA) are real-time PCR assays. The **cobas** EGFR Mutation Test v1 is for use only with formalin-fixed paraffin-embedded (FFPE) tissue. The **cobas** EGFR Mutation Test v2 can be used with both FFPE tissue and ctDNA from plasma and has been approved by the FDA as a companion diagnostic test for Tagrisso (osimertinib), Tarceva (erlotinib), and Iressa (gefitinib) in the first-line setting to aid in identifying patients with metastatic NSCLC whose tumors or plasma samples have either exon 19 deletion (Ex19del) or L858R mutations. In addition, the **cobas** EGFR Mutation Test v2 is FDA approved as a companion diagnostic test with Tagrisso in the second-line setting and beyond for patients with metastatic NSCLC who test positive for the *EGFR* T790M mutation.

In the FLAURA trial (NCT02296125), a phase III, double-blind, randomized study, treatment with osimertinib resulted in a clinically meaningful and statistically highly significant improvement in progression-free survival (PFS) versus first-generation comparator EGFR-TKI (erlotinib or gefitinib) as first-line treatment for patients with tumor tissue–positive *EGFR*m advanced NSCLC; hazard ratio (HR) of 0.46 [95% confidence interval (CI): 0.37–0.57; $P < 0.0001$; ref. 11]. In this trial, patients with a positive tumor tissue *EGFR*m status confirmed by a validated local or central cobas tissue test were eligible for enrollment. At baseline, patients were required to provide tumor tissue samples for central prospective or retrospective analysis of *EGFR*m status and blood samples for retrospective central cobas plasma ctDNA analysis of *EGFR*m status. The cobas test was used for patient selection in this study as at the time it was being developed as a companion diagnostic for Tagrisso (osimertinib) following its use in previous clinical trials. The cobas test is now approved by the FDA as a companion diagnostic for osimertinib in the first- and second-line settings for patients with an *EGFR*m- or T790M-positive status.

Herein, we report the results of the *EGFR* mutation analysis in tissue (local and central results) and plasma (central results) from the FLAURA trial; furthermore, we describe the clinical efficacy results according to the method of randomization (local vs. central cobas tissue test), uncommon *EGFR*-sensitizing mutations (detected by central cobas tissue test), and by plasma *EGFR*m status.

Materials and Methods

Trial design

Full details of the FLAURA study have been previously published (11). In brief, FLAURA was a randomized (1:1), double-blind, international phase III study assessing the efficacy and safety of osimertinib (80 mg once daily) versus comparator first-generation EGFR-TKI (gefitinib 250 mg once daily or erlotinib 150 mg once daily) in patients with previously untreated, *EGFR*m-positive (Ex19del or L858R) locally advanced or metastatic NSCLC.

Tumor tissue and plasma sampling

*EGFR*m status at screening was confirmed by analyzing freshly sectioned tissue from diagnostic tumor tissue FFPE blocks, either using testing by **cobas** EGFR Mutation Test v1 (cobas tissue test) at a designated central laboratory or using a locally available *EGFR* mutation test performed at Clinical Laboratory Improvement Amendments certified (for U.S. sites) or accredited laboratories (outside of the United States). The **cobas** EGFR Mutation Test v1 can identify 41 mutations, including ex19del and exon 21 (L858R) mutations in the *EGFR* gene. Patients were enrolled on the basis of a tissue Ex19del or L858R *EGFR*m-positive test result confirmed by either a local or central cobas test. Investigators were not required to submit tissue samples for central cobas testing for patients who failed screening based on local *EGFR* mutation test results. Tumor tissue and plasma ctDNA *EGFR* mutation status [positive, negative, unknown (invalid/no sample)], assessed using the central cobas tissue test and cobas plasma test, respectively, were compared for all screened patients with evaluable paired baseline tumor and plasma samples.

Plasma samples were collected at baseline (after randomization but before first dose) for retrospective analysis of *EGFR*m status by plasma ctDNA using the **cobas** EGFR Mutation Test v2 (cobas plasma) assay, performed by a central laboratory (Carolinas HealthCare System Core Laboratory, Charlotte, NC), in accordance with the manufacturer's instructions (Roche Molecular Systems, Inc., Pleasanton, CA; ref. 21). The **cobas** EGFR Mutation Test v2 can identify 42 mutations in exons 18, 19,

20, and 21 of the *EGFR* gene, including G719X, ex19del, S768I, T790M, exon 20 insertions, L858R, and L861Q.

Standard protocol approvals, registration, and patient consents

The FLAURA trial was conducted in accordance with the provisions of the Declaration of Helsinki, Good Clinical Practice guidelines (as defined by the International Conference on Harmonisation), applicable regulatory requirements, and the policy on bioethics and human biologic samples of the trial sponsor, AstraZeneca. The study was approved by the institutional review board or independent ethics committee associated with each study center. Informed consent was obtained from all patients prior to enrollment into the study. The trial was funded by the sponsor and was designed by the principal investigators and the sponsor. Data underlying the findings described in this manuscript may be obtained in accordance with AstraZeneca's data-sharing policy described at https://astrazenecagrouptrials.pharmacm.com/ST/Submission/Disclosure.

Endpoints

The primary endpoint of the FLAURA study was to assess the efficacy of osimertinib compared with comparator EGFR-TKI therapy as measured by PFS determined by investigator assessment, according to Response Evaluation Criteria in Solid Tumors (RECIST; version 1.1). PFS by cobas plasma test status was a secondary endpoint. Exploratory endpoints included concordance between central cobas tissue test and local tissue test results for EGFR-TKI–sensitizing mutations and concordance between the cobas tissue and plasma ctDNA tests for the detection of *EGFR* mutations. The primary objective of the current analysis was to assess the clinical utility of the cobas tissue test and the cobas plasma test as aids in the selection of patients with locally advanced or metastatic NSCLC harboring EGFR-TKI–sensitizing mutations for first-line therapy with osimertinib.

Assessments

Tumor assessments (RECIST; version 1.1) occurred at baseline, every 6 weeks (±1 week) for 18 months, and then every 12 weeks (±1 week) until disease progression. PFS was defined as the time from randomization to objective disease progression or death from any cause in the absence of progression, irrespective of withdrawal from the trial, or treatment with another anticancer therapy before progression.

Statistical analysis

The data cutoff for the FLAURA study was June 12, 2017. The agreement between cobas tissue and cobas plasma test results was calculated using the rates (percentages) with corresponding 95% CIs (Wilson score intervals or, if subgroup was <30, the Clopper–Pearson method) by overall percent agreement (OPA), positive percent agreement (PPA), and negative percent agreement (NPA). PFS analyses were performed on subgroup populations based on screening method (central cobas tissue or local tissue test); additional PFS analyses were performed on all randomized patients with an *EGFR*m-positive cobas tissue test result and in subgroups of cobas plasma–positive patients and cobas plasma–negative patients separately. All statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC).

Results

***EGFR* mutation tissue test results in the FLAURA study**

The disposition of patients in this analysis is summarized in Fig. 1. A positive tissue Ex19del and/or L858R *EGFR* mutation result was required for enrollment. Of the 994 patients screened in FLAURA, 289 were randomized on the basis of central cobas *EGFR* tissue test results, 267 were randomized on the basis of validated local *EGFR* tissue test results, and 438 failed screening. Of the 438 patients who failed screening, 224 patients had a negative *EGFR*

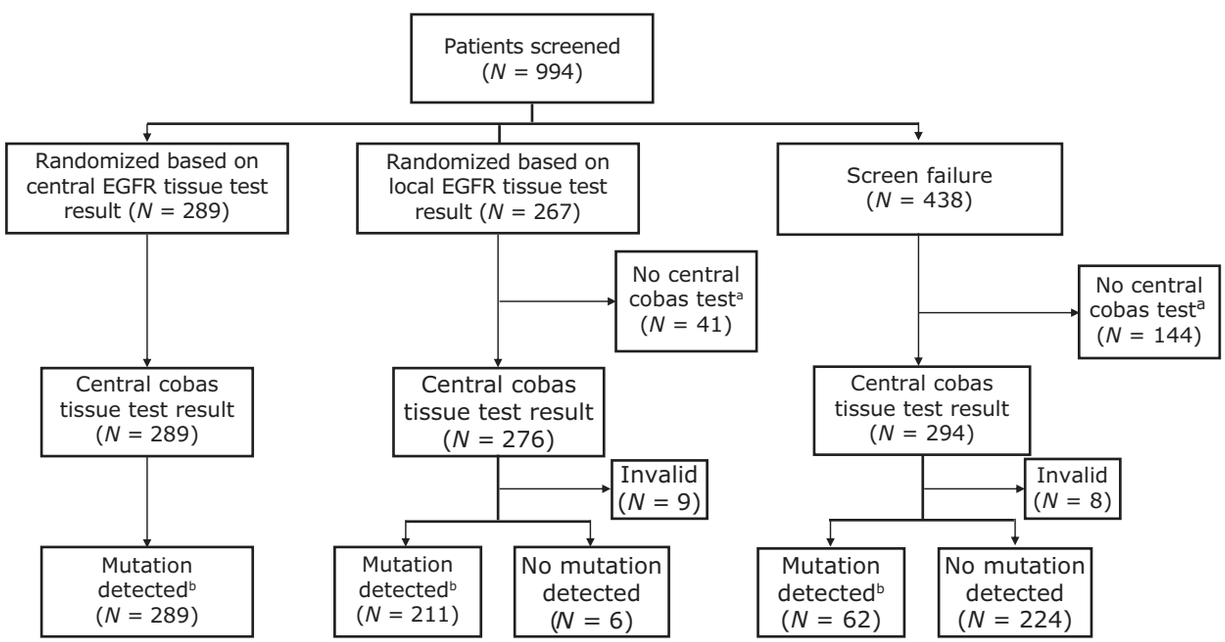

Figure 1.

Patient disposition. [a]Tissue sample not available, insufficient tissue, tissue failed pathology review. [b]Presence of Ex19del or L858R mutation.

Table 1. Comparison of central cobas tissue test and local tissue test results for EGFR-TKI–sensitizing mutations (patients randomized on the basis of a local *EGFR* mutation test result)

		Local *EGFR*m test result[a]		
Central test result		**Ex19del or L858R**	**Ex19del**	**L858R**
Central *EGFR* mutation test result	Mutation detected	211	125	86
	No mutation detected	6	1	5
	Invalid result	9	4	5
	Not tested[b]	41	28	13
	Total	267	158	109
Excluding invalid test results	PPA (95% CI)[c]	97.2% (94.1–99.0)	99.2% (95.7–100.0)	94.5% (87.6–98.2)

NOTE: 95% CIs calculated using Clopper–Pearson exact method for binomial proportions. Lower limit of detection for the cobas central test was <10% mutant allelic fraction.

Abbreviations: CI, confidence interval; EGFR, epidermal growth factor receptor; Ex19del, exon 19 deletion; PPA, positive percent agreement; TKI, tyrosine kinase inhibitor.

[a]Includes Ex19del or L858R.

[b]Includes no tissue available, insufficient tissue, pathology review failure, and insufficient DNA yield.

[c]PPA was calculated as [(local *EGFR*m-positive/central *EGFR*m detected)/(local *EGFR*m-positive/central *EGFR*m detected + local *EGFR*m-positive/central *EGFR*m not detected)] × 100.

tissue test, 142 had no *EGFR* test result available (insufficient or no tissue available, insufficient DNA yield from tissue, or tissue failed pathology review), and 10 had invalid test results. The remaining 62 patients had a positive *EGFR* test result but did not meet other eligibility criteria. Of the 267 patients randomized on the basis of validated local *EGFR* tissue test results, 217 (81%) had a valid retrospective central cobas *EGFR* tissue test result, 41 patients did not have a valid tissue sample for central cobas *EGFR* tissue testing, and the remaining nine patients had invalid central cobas *EGFR* tissue test results. A total of 211 of 217 (97%) patients were retrospectively confirmed to be *EGFR*m positive using the central cobas tissue test (osimertinib $n = 110$, comparator EGFR-TKI $n = 101$). Of those patients who failed screening ($n = 438$) and thus were not randomized to treatment, 294 had a central *EGFR* test, of whom 286 (97%) had a valid central tissue result (Fig. 1).

Among all screened patients with a valid central cobas tissue test result [792/994 (80%)], uncommon *EGFR* mutations (*EGFR* mutations other than Ex19del/L858R, such as T790M, G719X, S768I, and Exon 20 insertion) were detected in 5% (40/792) of patients, including 3% (7/267) of patients randomized on the basis of a local *EGFR* test result, and 2% (5/289) of patients randomized on the basis of a central cobas test result. Among the 40 patients with an uncommon *EGFR* mutation, G719X only ($n = 10$), T790M + L858R ($n = 10$), and Exon 20 insertion only ($n = 7$) occurred most frequently (Supplementary Table S1).

Comparison of central cobas tissue and local tissue test results for Ex19del/L858R mutations

The validated local tissue testing methods used in FLAURA are listed in Supplementary Table S2. High PPA was observed between the central cobas tissue test and local tissue testing methods among patients randomized on the basis of locally available tissue test results for the detection of Ex19del or L858R: 99% (95% CI, 95.7–100.0) and 95% (95% CI, 87.6–98.2), respectively, and 97% (95% CI, 94.1–99.0) in aggregate (excluding invalid results or inadequate samples; Table 1). Overall, six patients had discordant local and central tissue test results (*EGFR*m positive by local testing and *EGFR*m negative by central cobas testing), three patients in each treatment arm. Of these six discordant cases, Cycleave detected L858R in three cases, QIAGEN therascreen detected L858R in two cases, and an unspecified next-generation sequencing (NGS) assay detected Ex19del in one case. Discordant local and central *EGFR*m test results are summarized in Supplementary Table S3.

Clinical efficacy by central or local *EGFR*m tissue test results

In FLAURA, all randomized patients had a confirmed tumor tissue *EGFR*m status by local test or central cobas testing. Osimertinib treatment resulted in a significant improvement in PFS over comparator EGFR-TKI: median PFS 18.9 months versus 10.2 months [HR of 0.46 (95% CI, 0.37–0.57); $P < 0.0001$; ref. 11]. The substantial improvement in PFS was maintained irrespective of *EGFR* testing route (randomized on the basis of local *EGFR* test results; HR of 0.50, 95% CI, 0.35–0.71; $P < 0.0001$; randomized on the basis of central *EGFR* tissue test results, HR of 0.39, 95% CI, 0.29–0.52; $P < 0.001$; Table 2).

In the subgroup of randomized patients with a confirmed central cobas *EGFR*m positive result ($n = 500$), the PFS superiority of osimertinib [HR of 0.43 (95% CI, 0.34–0.54); $P < 0.0001$; ref. 11; Table 2] was comparable to that observed in all randomized patients [FLAURA full analysis set (FAS), $n = 556$; 0.46 (95% CI, 0.37–0.57); $P < 0.001$]. The HR was not calculated in randomized patients with a negative *EGFR*m centrally confirmed cobas test result due to the low number of patients ($n = 6$). In the subgroup of patients randomized by local *EGFR* test result, but in which retrospective central cobas testing yielded an invalid result ($n = 50$), PFS HR was 0.85 (95% CI, 0.38–1.82; $P = 0.6813$), with 27 patients experiencing disease progression ($n = 11$ osimertinib; $n = 16$ comparator EGFR-TKI); median PFS was very similar in both treatment groups, with a large variation, demonstrated by very wide CIs (Table 2). The PFS superiority of osimertinib was consistent irrespective of the type of EGFR-sensitizing mutation at randomization: Ex19del, HR of 0.43 (95% CI, 0.32–0.56; $P < 0.0001$); L858R, HR of 0.51 (95% CI, 0.36–0.71; $P < 0.0001$; ref. 11).

Comparison of central cobas tissue and cobas plasma test results

In total, 486 of the 994 (49%) patients screened had matched valid cobas central tissue and cobas plasma test results; 792 patients had a valid central cobas tissue test result and 554 patients had a valid baseline cobas plasma test result. Using the central cobas tissue test as a reference, the sensitivity (PPA), specificity (NPA), and overall concordance of the cobas plasma test for detection of Ex19del were 79% (95% CI, 74–84), 99%

Table 2. Subgroup analyses of PFS by investigator assessment

Subgroup/central cobas *EGFR* mutation status[a]	Treatment arm	Number of patients	Number (%) of patients with events[b]	Median PFS (months)[c] (95% CI)	Comparison between arms		
					HR[d]	95% CI	Two-sided *P* value
Patients randomized on the basis of a central cobas test result ($N = 289$)							
Mutation detected	Osimertinib	145	70 (48)	17.8 (14.9–NC)	0.39	0.29–0.52	<0.001
	Comparator EGFR-TKI	144	115 (80)	9.7 (8.3–11.0)			
Patients randomized on the basis of a local test result ($N = 267$)							
Mutation detected	Osimertinib	110	54 (49)	20.5 (14.2–23.5)	0.50	0.35–0.71	< 0.001
	Comparator EGFR-TKI	101	73 (72)	11.0 (9.5–13.9)			
No mutation detected	Osimertinib	3	1 (33)	NC (2.7–NC)	NC	NC–NC	NC
	Comparator EGFR-TKI	3	2 (67)	2.8 (1.4–NC)			
Missing[e]	Osimertinib	21	11 (52)	16.5 (11.1–NC)	0.85	0.38–1.82	0.6813
	Comparator EGFR-TKI	29	16 (55)	16.6 (9.7–23.0)			
Randomized patients with a retrospectively confirmed *EGFR*m-positive status by central tissue testing ($N = 500$)							
Mutation detected	Osimertinib	255	124 (49)	18.9 (15.2–21.4)	0.43	0.34–0.54	<0.001
	Comparator EGFR-TKI	245	188 (77)	9.7 (9.5–11.0)			

NOTE: RECIST; version 1.1. Data cutoff: June 12, 2017.

Abbreviations: CI, confidence interval, EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; HR, hazard ratio; NC, not calculated; PFS, progression-free survival.

[a]Ex19del and/or L858R.

[b]Progression events that did not occur within two scheduled visits (plus visit window) of the last evaluable assessment (or randomization) were censored and therefore excluded in the number of events.

[c]Calculated using the Kaplan–Meier technique.

[d]The HR and 95% CI were calculated from the Cox proportional hazards model with no stratification. A HR of < 1 favors osimertinib 80 mg.

[e]With an invalid test result or not tested by the central cobas test.

(95% CI, 96–100), and 87% (95% CI, 84–90), respectively. The sensitivity, specificity, and overall concordance observed for the detection of L858R were 68% (95% CI, 61–75), 99% (95% CI, 97–100), and 88% (95% CI, 85–91), respectively (Supplementary Table S4).

Clinical efficacy in subgroups of patients by cobas plasma test

A PFS benefit was observed with osimertinib compared with comparator EGFR-TKI therapy in patients with an *EGFR*m tissue positive test result, in both plasma ctDNA *EGFR*m-positive and -negative patients. Compared with the comparator EGFR-TKI arm, osimertinib reduced the risk of progression or death by 56% [HR of 0.44 (95% CI, 0.34–0.57; $P < 0.0001$)] in the plasma ctDNA *EGFR*m-positive subgroup (Fig. 2A) and by 52% [HR of 0.48 (95% CI, 0.28–0.80; $P = 0.0047$)] in plasma ctDNA *EGFR*m-negative subgroup (Fig. 2B).

In both the osimertinib and comparator EGFR-TKI arms (*EGFR*m tissue positive), a longer median PFS was observed in the plasma ctDNA *EGFR*m-negative subgroups [osimertinib: 23.5 months (95% CI, 17.8–24.3) and comparator EGFR-TKI: 15.0 months (95% CI, 9.7–18.3), respectively] compared with the plasma ctDNA *EGFR*m-positive subgroups [osimertinib:15.2 months (95% CI, 13.7–20.7) and comparator EGFR-TKI: 9.7 months (95% CI, 8.4–11.1), respectively]. Importantly, at baseline, the median target lesion tumor size was significantly greater in those patients with a cobas plasma *EGFR*m-positive status (55 mm) than those with an *EGFR*m-negative status (35 mm; $P < 0.001$; Table 3).

Clinical efficacy in patients with cooccurring uncommon *EGFR* mutations detected by central cobas tissue test

Of the 556 patients randomized to treatment (FAS), uncommon mutations cooccurring with Ex19del/L858R were detected by the central cobas tissue test in 12 patients (2%; Table 4). *De novo* T790M was present in five patients (osimertinib, $n = 4$; comparator EGFR-TKI, $n = 1$), S768I in four patients (osimertinib, $n = 1$; comparator EGFR-TKI, $n = 3$), and Ex20ins in three patients (osimertinib, $n = 2$; comparator EGFR-TKI, $n = 1$). A progression event occurred in seven of those patients with an uncommon mutation (osimertinib, $n = 3$; comparator EGFR-TKI, $n = 4$); the best objective response was partial response (PR) in nine patients, stable disease in one patient, and progressive disease (PD) in two patients, both of whom received comparator EGFR-TKI. Among the five patients with *de novo* T790M detected, all patients treated with osimertinib achieved a PR. In contrast, the one patient with detectable T790M treated with comparator EGFR-TKI therapy had a best response of PD. Because of the low number of patients with tumors harboring uncommon mutations and/or T790M in this first-line population ($n = 5$ in the FAS based on tissue and/or ctDNA testing), the subgroup analysis based on T790M status was not conducted.

Discussion

In the current analysis of the *EGFR* testing methods used in FLAURA, we found high PPA in tissue test results between local and central (cobas tissue test) *EGFR* mutation testing methods, both for Ex19del and L858R mutations individually and in aggregate. A similar PPA (99%) between the cobas tissue test and local testing methods for the detection of Ex19del (PPA: 99%) and L858R (PPA: 95%) mutations in aggregate was observed in a study that analyzed samples obtained from patients randomized to the expansion cohorts of the AURA phase I trial (22).

In total, only six of 217 patients randomized on the basis of a local *EGFR*m test with a valid retrospective central cobas tissue test had discordant results (local test positive, central test negative). Several factors may have contributed to the six discordant results in our analysis. First, lower limits of detection (LOD) exist among the local testing methods used in the six cases: Cycleave LOD 5% (23), therascreen LOD 1%–7% (24, 25), targeted NGS LOD

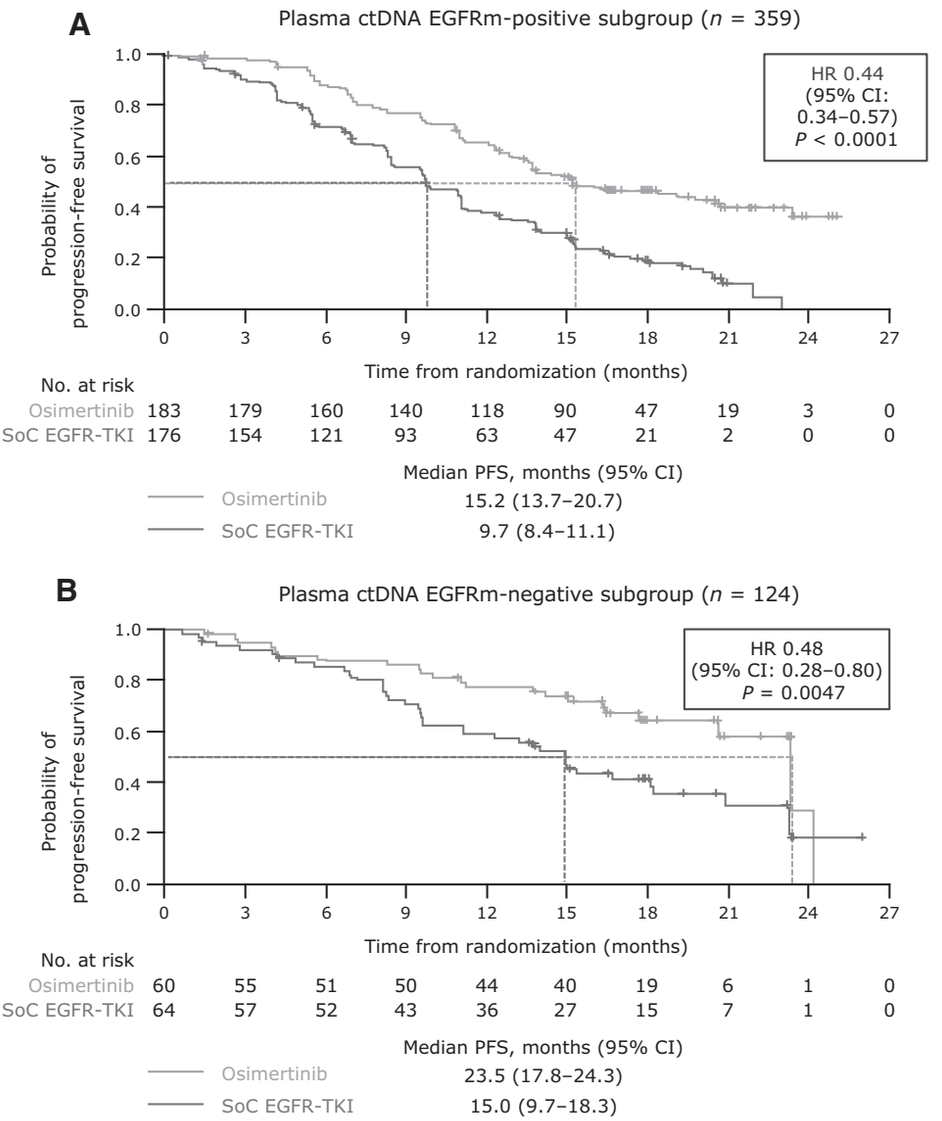

Figure 2.
Investigator-assessed PFS in the plasma ctDNA *EGFR*m-positive subgroup (**A**), the plasma ctDNA *EGFR*m-negative subgroup (**B**). Tick marks indicate censored patients. CI, confidence interval; ctDNA, circulating tumor DNA; EGFRm, epidermal growth factor receptor mutation; HR, hazard ratio; PFS, progression-free survival.

approximately 0.01%–5% (26–28), and cobas LOD 5% (29), which may explain, in part, the discordant results. Second, variation in local methodology and validation protocols, laboratory experience, and analytic standardization, and the involvement of the pathologist can affect assay performance (29) and may lead to false-positive results (30). Finally, intratumoral heterogeneity may have played a role (31).

Table 3. Median baseline target lesion size by *EGFR* mutation status determined by the cobas plasma test (full analysis set)

	Ex19del/L858R status by cobas plasma test		
Target lesion size	**Positive ($n = 359$)**	**Negative ($n = 124$)**	**Unknown ($n = 72$)**
Median baseline target lesion size (mm)	55	35	47
Range (mm)	10–207	10–126	10–176
P[a]	<0.001		

NOTE: Ex19del, exon 19 deletion mutation.
[a]Two-sided *P* value is obtained via Wilcoxon rank-sum test for patients with a positive or negative cobas plasma test result.

In this analysis, the PFS superiority of osimertinib over comparator EGFR-TKI observed in the FLAURA FAS (11) remained consistent irrespective of the route of randomization (local or central *EGFR*m tissue test) or the type of EGFR-TKI–sensitizing mutation detected (Ex19del or L858R) in patients with a valid central cobas tissue test result. These results demonstrate that both certified local tests and the cobas test are acceptable for identification of patients for treatment with first-line osimertinib. The proportion of patients with uncommon mutations detected in their tissue samples (2%) is slightly lower than other reports from larger studies, in which the range is typically 10%–18% (32, 33). Although the sample size is small, those patients with uncommon mutations in the osimertinib arm generally achieved a better response than those in the comparator EGFR-TKI arm (see Table 4); however, it should be noted that osimertinib is currently approved only for the first-line treatment of patients with advanced NSCLC with *EGFR*-sensitizing mutations Ex19del and L858R, and in the second-line setting and beyond for patients with the T790M mutation. Currently, there is no universal

Table 4. PFS and best objective response of patients with uncommon *EGFR* mutations detected by the central cobas *EGFR* mutation tissue test [patients randomized to treatment (full analysis set)]

Patient number	EGFR-TKI–sensitizing mutation detected by central cobas tissue test	Other *EGFR* mutation detected by central cobas tissue test[a]	Treatment arm	Progression event (yes or no)	Best objective response	Days from randomization to progression or censoring (for patients who did not progress)
1	L858R	T790M	Comparator EGFR-TKI	Yes	Progressive disease	40
2	Ex19del	S768I	Osimertinib	No	Partial response	546[b]
3	L858R	T790M	Osimertinib	No	Partial response	421[b]
4	NMD[c]	S768I	Comparator EGFR-TKI	Yes	Progressive disease	42
5	L858R	T790M	Osimertinib	Yes	Partial response	379
6	NMD[d]	S768I	Comparator EGFR-TKI	No	Partial response	211[b]
7	Ex19del	Exon 20 insertion	Osimertinib	Yes	Partial response	603
8	Ex19del	Exon 20 insertion	Comparator EGFR-TKI	Yes	Partial response	376
9	L858R	T790M	Osimertinib	No	Partial response	461[b]
10	L858R	Exon 20 insertion	Osimertinib	Yes	Stable disease	305
11	Ex19del	S768I	Comparator EGFR-TKI	Yes	Partial response	336
12	L858R	T790M	Osimertinib	No	Partial response	458[b]

NOTE: Investigator data presented. RECIST; version 1.1.

Abbreviations: EGFR, epidermal growth factor receptor; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; Ex19del, exon 19 deletion; NMD, no mutation detected; PFS, progression-free survival.

[a]Other *EGFR* mutations include T790M, G719X, S768I, and Exon 20 insertion that are targeted by the cobas EGFR Mutation Test v2.

[b]Censored at the time of last evaluable visit.

[c]Patient was randomized on the basis of a local *EGFR*m (Ex19del) test result.

[d]Patient was randomized on the basis of a local *EGFR*m (L858R) test result.

consensus for the management of patients with uncommon mutations; therefore, there is an unmet need for this patient group. Further explorations with osimertinib in this patient group are warranted.

Fifty patients randomized to FLAURA based on local test results did not have samples available for central cobas testing or had an invalid central cobas tissue result. Within this small subgroup, similar median PFS was observed in both treatment groups (median PFS 16.5 months with osimertinib vs. 16.6 months with comparator EGFR-TKI; HR of 0.85, 95% CI, 0.38–1.82). It is not possible to draw any specific conclusions from this because of low patient numbers, in which any outliers would have a more notable impact on the results.

Although tissue biopsy remains the gold standard for *EGFR* mutation testing, the quality of tissue samples can vary because of difficulty with the acquisition during the biopsy procedure, limited sample tumor size, necrosis, and sample preservation (34). Tumor heterogeneity can also hinder mutation testing and lead to multiple biomarker assessments, which require more residual tissue sample and extend the waiting time for the results. A well-validated plasma test would be beneficial for patients with an inadequate residual tissue sample for molecular testing. In this analysis, plasma samples from patients screened to the FLAURA trial were retrospectively analyzed by the cobas plasma test. The PPA and the NPA between the tissue and plasma testing results for each of the *EGFR* Ex19del- and L858R-sensitizing mutations were consistent with the previously reported agreements with tumor tissue and plasma samples in the phase I AURA and the pooled phase II AURA extension/AURA2 studies (16, 21, 35). These studies support the expectation that a proportion (15%–32%) of patients with NSCLC do not seem to shed detectable ctDNA into the circulation. These patients, sometimes referred to as patients with "nonshedding" tumors, seem to have a better prognosis than patients with detectable ctDNA, as demonstrated in the AURA study and pooled analysis of the AURA extension and AURA2 studies (21, 35). This is also evidenced in the FLAURA study in which patients who were *EGFR*m positive by cobas tissue but ctDNA *EGFR*m negative by cobas plasma had a longer median PFS than the FAS or the plasma ctDNA *EGFR*m–positive subgroup, irrespective of the treatment arm. Previous studies have shown that levels of ctDNA shedding into plasma correlate with tumor burden (34), and lack of detectable ctDNA early in EGFR-TKI therapy is associated with better clinical prognosis (36). Similar trends were observed in the second-line setting of AURA3 in patients with a cobas plasma T790M-negative status, with existing T790M-positive status by cobas tissue test (37). Although we do not have data on ctDNA shedding and tumor burden for this study, we report that patients with a cobas plasma *EGFR*m-positive status had a significantly larger median baseline target lesion size than those patients with the negative plasma test result. Therefore, the improved PFS in patients with an *EGFR*m-negative plasma test result may be, in part, due to lower tumor burden in these patients. In this analysis, osimertinib consistently improved PFS versus comparator EGFR-TKI in both cobas *EGFR*m plasma–positive and –negative patients, with results reflecting those observed in the FLAURA FAS.

Although the cobas test is an FDA-approved companion diagnostic for osimertinib in first-line treatment of patients with NSCLC, NGS is becoming more widely available for optimizing tissue use and has been shown to be feasible in clinical practice for parallel profiling of different genetic alterations (38, 39). Studies have shown that NGS can be used to detect actionable gene mutations with high accuracy in plasma samples (40–42), and new targeted NGS methodologies are being developed that improve the sensitivity and specificity in cases such as samples with low allelic frequencies (43). However, the complexity of NGS workflow and data analysis can be challenging, and a lack of standardization across NGS platforms and assays remains problematic (44). PCR-based tests are more accessible and have a shorter turnaround time and lower cost, and less sample size is required compared with NGS. Other factors that can influence selection of a test include reimbursement and mutation prevalence in the target population. In cases where there is insufficient tissue or DNA in the plasma, single-gene testing could be a useful alternative to screening for multiple mutations.

Our results confirm that the cobas plasma test is robust for the detection of Ex19del and L858R mutations in plasma, with a high PPA (Ex19del 79%; L858R 68%), NPA (Ex19del 99%; L858R 99%), and OPA (99% in aggregate) when comparing with the cobas tissue test as a reference. The cobas plasma test provides a comparable clinical utility for the detection of these mutations to that of tissue in the first-line setting of advanced NSCLC. Nevertheless, several factors such as the lower sensitivity relative to tissue testing can limit the use of plasma ctDNA for *EGFR* mutation detection (45, 46). Thus, in the absence of an initial tissue test result, a negative plasma ctDNA *EGFR*m test result should be followed up with a biopsy and tissue test whenever feasible.

In conclusion, these results support the clinical utility of the **cobas** EGFR Mutation Test (both in tissue and plasma) for selecting patients for first-line osimertinib treatment. In addition, a lack of *EGFR*m detected in plasma ctDNA is associated with improved outcomes, which may be due to these patients having a lower tumor burden.

Disclosure of Potential Conflicts of Interest

J.E. Gray is a consultant/advisory board member for AstraZeneca, Janssen, Genentech, Eli Lilly, Celgene, and Takeda; reports her institution receiving commercial research grants from AstraZeneca, Array, Merck, Epic Sciences, Genentech, Bristol-Myers Squibb, Boehringer Ingelheim, Trovagene, and Novartis; and reports receiving commercial research support from Genentech, AstraZeneca, Merck, Eli Lilly, and Grand Rounds. I. Okamoto reports receiving speakers bureau honoraria from AstraZeneca. J. Vansteenkiste is a consultant/advisory board member for AstraZeneca. Y.-K. Pang reports receiving speakers bureau honoraria from AstraZeneca. K. Kasahara reports receiving speakers bureau honoraria from AstraZeneca, MSD, Chugai Pharmaceuticals, and Eli Lilly Japan K.K., and is a consultant/advisory board member for AstraZeneca. N. Nogami reports receiving speakers bureau honoraria from Pfizer Inc., Chugai Pharmaceutical Co., Eli Lilly, MSD, TAIHO Pharmaceutical, AstraZeneca, Kyowa Hakko Kirin, ONO Pharmaceutical Co., and Bristol-Myers Squibb. G. Zhang is an employee of and has ownership interests (including patents) at Roche Molecular Solution. X. Li-Sucholeiki has ownership interests (including patents) at AstraZeneca. B. Lentrichia is an employee of and has ownership interests (including patents) at AstraZeneca. S. Dearden, S. Jenkins, and M. Saggese have ownership interests (including patents) at AstraZeneca. S.S. Ramalingam is a consultant/advisory board member for AstraZeneca, Bristol-Myers Squibb, Merck, Roche, and Tesaro, and reports receiving commercial research grants from AstraZeneca, Bristol-Myers Squibb, Merck, Amgen, Tesaro, Genmab, and Advaxis. No potential conflicts of interest were disclosed by the other authors.

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Acknowledgments

The authors thank all the patients and their families. The study (NCT02296125) was funded by AstraZeneca, Cambridge, United Kingdom, the manufacturer of osimertinib. The authors would like to acknowledge Helen Brown, Alex Kohlmann, and Milena Kohlmann for supporting diagnostic data collection and interpretation, and Rachel Hodge and Alexander Todd for providing statistical support. The authors acknowledge Roche Molecular Systems Inc. (Pleasanton, CA), the manufacturer of the **cobas** EGFR Mutation Test v1 and v2. The authors also acknowledge Natalie Griffiths, PhD, of iMed Comms, Macclesfield, United Kingdom, an Ashfield Company, part of UDG Healthcare plc for medical writing support that was funded by AstraZeneca, Cambridge, United Kingdom, in accordance with Good Publications Practice (GPP3) guidelines (http://www.ismpp.org/gpp3).

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Received April 5, 2019; revised July 4, 2019; accepted August 13, 2019; published first August 22, 2019.

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