ORIGINAL ARTICLE

Longitudinal Analyses of Circulating Tumor DNA for the Detection of *EGFR* Mutation-Positive Advanced NSCLC Progression During Treatment: Data From FLAURA and AURA3

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ABSTRACT

Introduction: EGFR tyrosine kinase inhibitor (EGFR-TKI)-sensitizing and -resistance mutations may be detected in plasma through circulating tumor DNA (ctDNA). Circulating tumor DNA level changes reflect alterations in tumor burden and could be a dynamic indicator of treatment effect. This analysis aimed to determine whether longitudinal *EGFR*-mutation ctDNA testing could detect progressive disease (PD) before radiologic detection.

Methods: This was a retrospective, exploratory ctDNA analysis in two phase 3 trials (FLAURA, NCT02296125; AURA3, NCT02151981). Patients had treatment-naïve

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(FLAURA) or EGFR-TKI pre-treated (AURA3) advanced NSCLC with *EGFR* mutations and on-study PD (RECIST [Response Evaluation Criteria in Solid Tumors]), with a baseline ctDNA result and *EGFR*-mutation ctDNA monitoring beyond Cycle 3 Day 1. Patients received osimertinib versus comparator EGFR-TKIs (FLAURA) or chemotherapy (AURA3). Outcomes included time from ctDNA PD to RECIST PD and the first subsequent treatment (FLAURA only).

Results: Circulating tumor DNA PD preceded or co-occurred with RECIST-defined PD in 93 out of 146 patients (64%) in FLAURA and 82 out of 146 patients (56%) in AURA3. Median time from ctDNA PD to RECIST-defined PD (mo) was 3.4 and 2.6 in the osimertinib and comparator EGFR-TKI arms (FLAURA) and 2.8 and 1.5 in the osimertinib and chemotherapy arms (AURA3). In FLAURA, the median time from ctDNA PD to the first subsequent treatment (mo) was 6.0 and 4.7 in the osimertinib (n = 51) and comparator EGFR-TKI arms (n = 70).

Conclusions: Among patients with *EGFR* mutation-positive advanced NSCLC receiving EGFR-TKI or chemotherapy with ctDNA data and RECIST-defined PD, ctDNA PD preceded/co-occurred with RECIST-defined PD in approximately 60% of cases. Longitudinal ctDNA monitoring may detect PD before radiologic PD.

Keywords: Circulating tumor DNA; *EGFR* mutations; Non-small cell lung cancer; Osimertinib; Resistance

Introduction

Osimertinib is a third-generation, irreversible, oral EGFR tyrosine kinase inhibitor (EGFR-TKI) that potently and selectively inhibits both EGFR-TKI-sensitizing mutations (including *EGFR* exon 19 deletion [Ex19del] and L858R; *EGFR* mutation-positive [*EGFR*m]) and *EGFR* T790M-resistance mutations.[1] Osimertinib has demonstrated efficacy in *EGFR*m advanced NSCLC including central nervous system (CNS) metastases.[2–5] Osimertinib was first approved for the treatment of patients with *EGFR* T790M-positive advanced NSCLC whose disease had progressed during or after EGFR-TKI therapy.[6] The randomized, phase 3 AURA3 clinical trial (NCT02151981) compared osimertinib and platinum-based chemotherapy in this patient population.[4] Patients treated with osimertinib had significantly improved progression-free survival (PFS) compared with those treated with platinum-based chemotherapy (hazard ratio [HR] = 0.30; 95% confidence interval [CI]: 0.23–0.41], $p < 0.001$; median 10.1 versus 4.4 mo),[4] although no statistically significant benefit in overall survival was observed (HR = 0.87 [95% CI: 0.67–1.12], $p = 0.277$; median 26.8 versus 22.5 mo).[7] Osimertinib was subsequently assessed as a first-line treatment for patients with *EGFR*m-positive advanced NSCLC in the randomized, phase 3 FLAURA trial (NCT02296125).[2] In FLAURA, patients who received osimertinib had significantly improved PFS compared with comparator EGFR-TKI (erlotinib/gefitinib; HR = 0.46 [95% CI: 0.37–0.57], $p < 0.001$; median 18.9 versus 10.2 mo).[2] This translated into a statistically significant and clinically meaningful overall survival benefit (HR = 0.80 [95.05% CI: 0.64–1.00], $p = 0.046$; median 38.6 versus 31.8 mo) with longer follow-up despite the cross-over design of the study.[8] As a result, osimertinib is the preferred first-line treatment in patients with *EGFR*m-positive advanced NSCLC.[9]

Optimal use of targeted therapies requires practical and timely methods to detect and monitor targetable genotypes. Despite being standard in NSCLC molecular characterization, challenges in routine tissue biopsy-based tumor genotyping have led to growing interest in less invasive alternative liquid biopsy approaches, including the analysis of circulating tumor DNA (ctDNA).[9,10] Assays for ctDNA are currently used in clinical practice for the detection of EGFR-TKI-sensitizing and resistance mutations in patients with insufficient tumor tissue specimens or in patients from whom tissue specimens are not obtainable at diagnosis or progressive disease/relapse (PD).[11] Changes in ctDNA levels during EGFR-TKI treatment may reflect variations in tumor burden and could represent a dynamic indicator of treatment effect.[12] Post-hoc analyses of FLAURA and AURA3 indicated that ctDNA clearance of *EGFR*m after 3 or 6 weeks of EGFR-TKI treatment was associated with a favorable prognosis.[13]

We hypothesized that serial longitudinal *EGFR*m testing of ctDNA could be used to detect PD before radiologic or clinical PD. In this exploratory analysis of the phase 3 FLAURA and AURA3 studies, we assessed serial *EGFR*m ctDNA testing of common (Ex19del or L858R) and resistance (T790M [FLAURA only] and C797S) *EGFR* mutations for advanced molecular detection of PD and resistance mechanisms using a cost-effective, clinical-grade droplet digital polymerase chain reaction (ddPCR) assay. Specifically, we assessed the lead times between PD defined by EGFRm and resistance ctDNA biomarkers (ctDNA PD) and radiologic RECIST version 1.1-defined PD (RECIST PD). For FLAURA, we also assessed the lead time between ctDNA PD and the start of the first subsequent treatment (FST).

Materials and Methods

Trial Designs

Full details of the FLAURA and AURA3 trials have been published previously.[2,4] Briefly, FLAURA was a

phase 3, double-blind, randomized study that assessed the efficacy and safety of osimertinib versus comparator EGFR-TKIs (erlotinib or gefitinib) in treatment-naïve patients with *EGFR*m-positive advanced NSCLC. Patients with CNS metastases whose condition was neurologically stable were eligible. Patients were stratified based on *EGFR* mutation status (Ex19del or L858R) and race (Asian or non-Asian), and randomly assigned (1:1) to receive oral osimertinib 80 mg once daily (QD) or comparator EGFR-TKI (gefitinib 250 mg or erlotinib 150 mg QD). AURA3 was a randomized, open-label, phase 3 trial that assessed the efficacy and safety of osimertinib versus platinum-based doublet chemotherapy in patients with *EGFR* T790M-positive advanced NSCLC whose disease progressed after first-line EGFR-TKI therapy. Patients with CNS metastases whose condition was neurologically stable were eligible. Patients were stratified based on race (Asian or non-Asian) and randomly assigned (2:1) to receive oral osimertinib (80 mg QD) or platinum-based doublet chemotherapy (pemetrexed 500 mg/m^2 plus either carboplatin target area under the curve five, or cisplatin 75 mg/m^2) every three weeks for up to six cycles. Patients who did not have PD after four cycles of platinum therapy plus pemetrexed could continue maintenance pemetrexed according to the approved label. In both studies, treatment was continued until PD, development of unacceptable adverse events, or withdrawal of consent. Treatment beyond PD (assessed by the investigator according to RECIST version 1.1) was allowed if there was continued clinical benefit, as judged by the investigator.

FLAURA and AURA3 were conducted in accordance with the principles outlined in the Declaration of Helsinki, Good Clinical Practice, and local regulatory requirements and were approved by the institutional review boards or independent ethics committees of the participating study centers. The data underlying the findings described in this manuscript may be obtained in accordance with AstraZeneca's data-sharing policy.

Objectives

The objective of this retrospective longitudinal ctDNA analysis was to investigate ctDNA samples for the detection of both common (Ex19del, L858R) and acquired resistance (T790M [FLAURA only] and C797S) *EGFR* mutations for advanced molecular detection of PD and acquired resistance mechanisms in patients treated with osimertinib versus comparator EGFR-TKI (FLAURA) or platinum-based doublet chemotherapy (AURA3).

Participants

The provision of informed consent was mandatory for all patients who underwent plasma sampling. Samples from patients who withdrew their consent were excluded from the analysis. Patients included from FLAURA had either RECIST PD by June 2017 or had a PD/treatment discontinuation ctDNA sample available by March 2018, or both. Patients included from AURA3 had either RECIST PD by April 2016 or had a PD/treatment discontinuation ctDNA sample available by March 2019, or both. Due to export limitations, samples from patients enrolled in People's Republic of China were excluded from our analyses.

To be evaluated, patients were required to have a valid baseline ctDNA result and valid longitudinal *EGFR*m ctDNA monitoring beyond Cycle 3 Day 1. Gaps in ctDNA sampling were part of the exclusion criteria in this exploratory study and defined as when no samples were available for more than 56 days (two consecutive sample time points plus 2-week sampling window). Patients with sampling gaps were excluded unless the gap was after detection of ctDNA PD or at least one ctDNA sample with a valid (absence of technical failure) result was collected after the gap but before ctDNA PD was detected. The rationale for using sampling gaps as part of the exclusion criteria was based on the possibility that a patient may have ctDNA PD during the gap. If such ctDNA PD is detected later, the time of ctDNA PD detection and associated lead times would be biased.

Sampling and Testing

Plasma samples were collected on Cycle 1, Days 1, 8, and 15; Cycles 2–7, Day 1; Cycles 8+, every 6 weeks from Day 1 until treatment discontinuation. One cycle was defined as 21 days of treatment. Blood was collected into tubes containing ethylenediaminetetraacetic acid, mixed thoroughly, and centrifuged for 10 minutes at 2000 g at 4°C, within 4 hours of collection. The plasma supernatant was removed and placed into a new tube and then cleared by centrifugation under the same conditions. The resulting cleared plasma samples were stored in cryovials at −70°C until use.

Detection of *EGFR* mutations (Ex19del, L858R, T790M, and C797S) in ctDNA was performed using ddPCR (Biodesix GeneStrat®) analysis. Only samples collected after Cycle 3 Day 1 were assessed for C797S because acquired C797S mutations were not expected in the first 6 weeks of treatment.

Assessments

Clinical outcomes assessed in this exploratory analysis included the following lead times: time from ctDNA

PD (presence of Ex19del or L858R, in FLAURA and AURA3) to RECIST PD or when the patient received their FST (FLAURA only); and time from ctDNA resistance marker identification (T790M [FLAURA only] or C797S) to RECIST PD and FST (FLAURA only). Circulating tumor DNA PD was analyzed in relation to the presence of Ex19del or L858R *EGFR* mutations and defined with respect to the nadir ctDNA result and its proximity to the ddPCR detection limit and lower limit of quantification (Table 1). Further details on the definition of ctDNA PD are provided in the Supplementary Methods.

Tumor assessments according to RECIST version 1.1 were performed at baseline, every 6 weeks (±1 wk) for 18 months, and then every 12 weeks (±1 wk) until PD in FLAURA and at baseline and then every 6 weeks (±1 wk) until PD in AURA3.

Progression-free survival and time to FST (TFST; FLAURA only) were included as overall measures. Progression-free survival was determined by investigator assessment according to RECIST version 1.1 and was defined as the time from randomization to objective PD, or death, irrespective of withdrawal from the trial or treatment with another anticancer therapy before progression. TFST was defined as the time from randomization to the start date of FST after the discontinuation of randomized treatment or death.

Statistics

Data analysis variables included lead times for common *EGFR*m; for FLAURA resistant C797S and T790M ctDNA mutation detection was compared to RECIST PD and FST, for AURA3 resistant C797S ctDNA mutation detection was compared to RECIST PD only. The sample size was determined by the availability of plasma samples collected for ctDNA analysis. Descriptive statistics (medians and interquartile ranges [IQRs]) were used in this analysis.

Results

Patient Disposition (FLAURA and AURA3)

Of the 556 patients randomized in FLAURA, 176 had valid longitudinal ctDNA monitoring (without gaps) beyond Cycle 3 Day 1 using a clinical-grade *EGFR*m focused ddPCR assay (Biodesix GeneStrat®). Of these, 173 were eligible for ctDNA PD to FST lead-time analysis (TFST-eligible population), 146 were eligible for ctDNA PD to RECIST PD lead-time analysis (PFS-eligible population) and 57 patients with a resistance mutation detected were eligible for ctDNA resistance mutation detection to FST lead-time analysis (Fig. 1*A*). Of the 419 patients randomized in AURA3, 146 had valid longitudinal ctDNA monitoring (without gaps) beyond Cycle 3 Day 1. All of these 146 patients were eligible for ctDNA PD to RECIST PD lead-time analysis and 12 patients with a resistance C797S mutation detected were eligible for ctDNA resistance mutation detection to RECIST PD lead-time analysis (Fig. 1*B*).

Patient Demographics and Disease Characteristics (FLAURA and AURA3)

Patient demographics and disease characteristics of the PFS- and TFST-eligible populations in FLAURA and the PFS-eligible population in AURA3 are shown in Supplementary Tables 1 and 2, respectively. Patient demographics and disease characteristics per treatment arm in FLAURA (PFS- and TFST-eligible) and AURA3 (PFS-eligible) are presented in Supplementary Tables 3 and 4, respectively.

ctDNA Disease Progression Analysis

Lead Time From ctDNA PD to FST (FLAURA Only). Among 173 eligible patients in FLAURA with a TFST recorded event, 121 patients (70%) across treatment arms met our criteria for ctDNA PD (Supplementary Table 5) and 52 patients (30%) did not have ctDNA PD detected. Circulating tumor DNA PD was detected at the same time, or earlier than the start of FST in all 121 patients with ctDNA PD detected. Median lead time from ctDNA PD to FST was 4.9 months (IQR: 3.2–8.0) in all patients; 6.0 months (IQR: 3.7–8.4) in the osimertinib arm and 4.7 months (IQR: 3.0–7.7) in the comparator EGFR-TKI arm (Fig. 2*A*). Lead times for each patient are shown in Figure 2*B* and Supplementary Figure 1*A*.

Lead Time From ctDNA PD to RECIST PD (FLAURA and AURA3). Among 146 eligible patients in FLAURA with a RECIST PD event, 106 patients (73%) across treatment arms met our criteria for ctDNA PD whereas 40 patients (27%) with RECIST PD did not have ctDNA

Table 1. Nadir ctDNA Results and Respective Disease Progression Thresholds

Nadir ctDNA Result	ctDNA PD Threshold
Positive (above LLOQ)	A 100% increase in mutant allele fraction at one time point
Positive (below LLOQ) or non-confirmed negative	Any positive result above the LLOQ
Confirmed negative (at two consecutive time points and could include the baseline sample) (i.e., clearance)	Two consecutive positive results (below LLOQ) OR any one positive result (above LLOQ)

LLOQ is defined as ≥30 mutant droplets.
ctDNA, circulating tumor DNA; LLOQ, lower limit of quantification; PD, progressive disease.

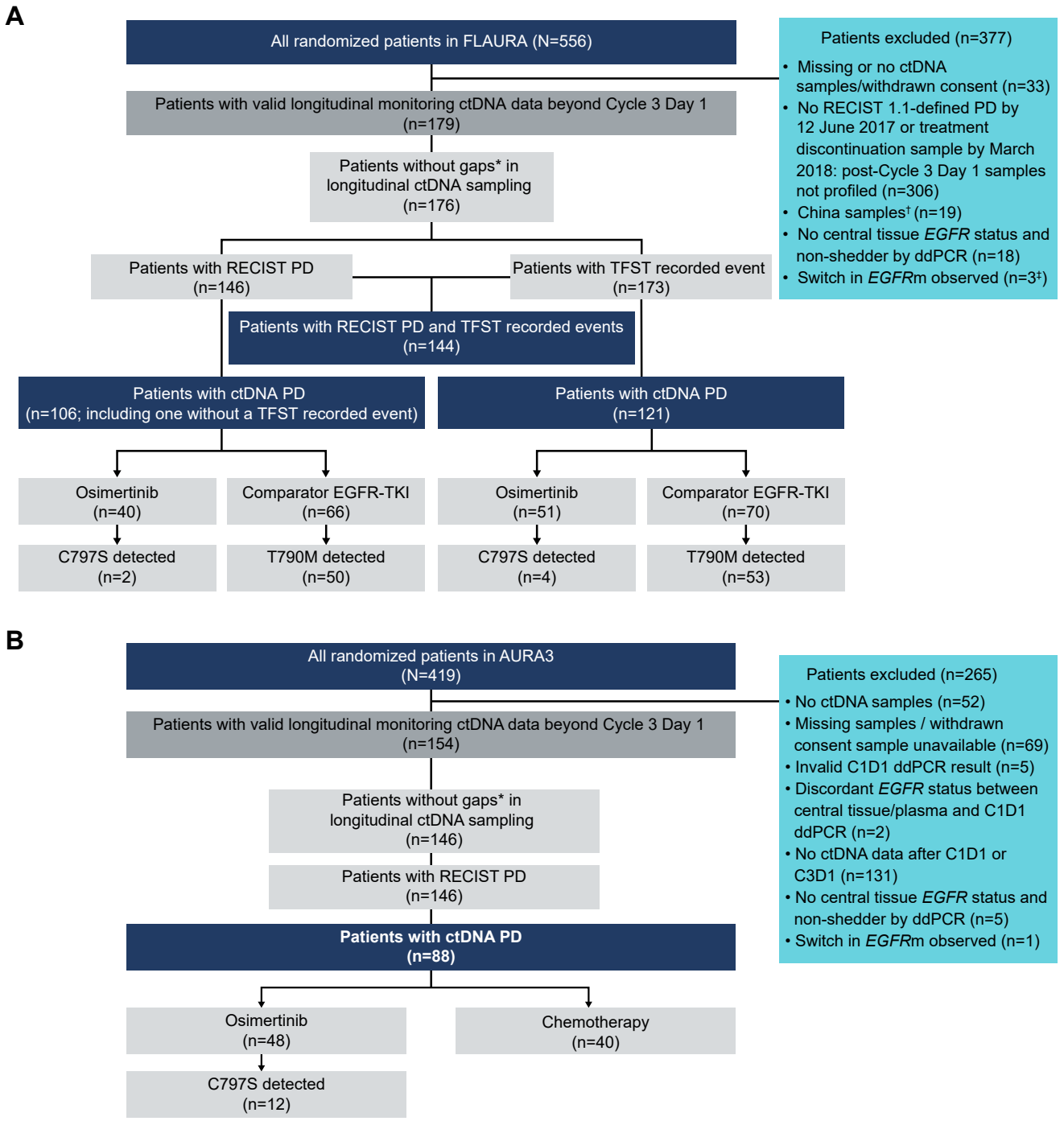

Figure 1. Patient disposition for exploratory analyses in FLAURA (*A*) and AURA3 (*B*). *Sampling gaps were defined as no samples within >56 days (due to the possibility that patients may have ctDNA PD within the sampling gap). A gap in ctDNA sampling was only allowed in the following scenarios: 1) the gap was after the detection of ctDNA PD or 2) at least one ctDNA sample with a valid result was available after the gap, but before ctDNA PD was detected; †Not tested due to sample export limitations; ‡Includes two patients who were also excluded by multiple criteria. ctDNA, circulating tumor DNA; ddPCR, droplet digital polymerase chain reaction; *EGFR*m, EGFR mutation-positive; PD, progressive disease; RECIST, Response Evaluation Criteria in Solid Tumors; TFST, time to first subsequent treatment; TKI, tyrosine kinase inhibitor.

PD detected by treatment discontinuation. ctDNA PD was detected at the same time as, or earlier than, RECIST PD in 93 out of 146 patients (64%), with a median lead time between ctDNA PD to RECIST PD of 2.7 months (IQR: 1.2–5.1). Of these 93 patients, ctDNA PD was detected at the same time as RECIST PD (defined as within 1 week of RECIST PD) in 10 patients (11%) and was detected earlier than RECIST PD in 83 patients

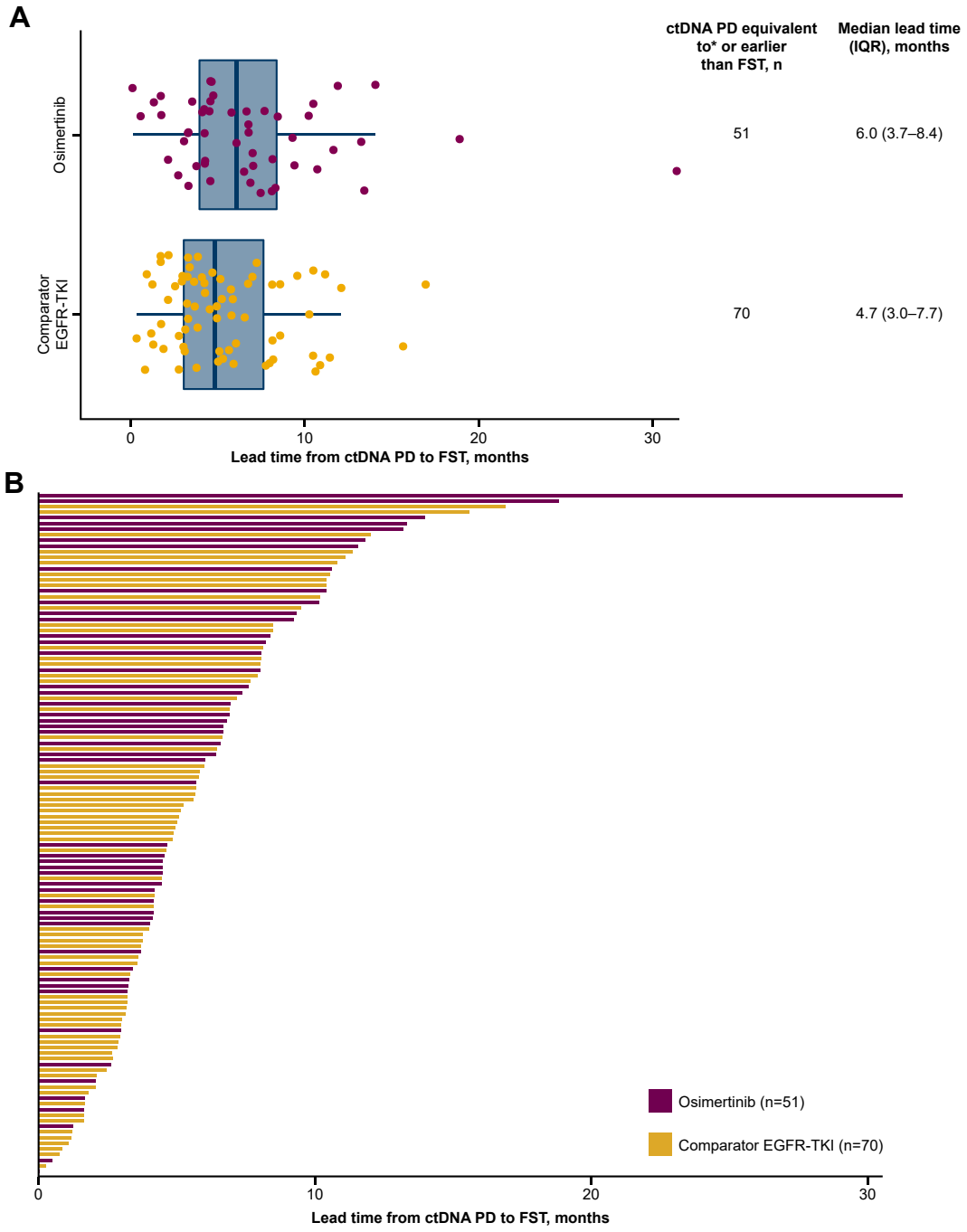

Figure 2. Lead time from ctDNA PD to start of FST in FLAURA. Lead times per treatment arm are presented as a box plot (*A*) and individual patient lead times as a waterfall plot (*B*). In Panel A, the outer box lines represent the 25th and 75th quartiles, the center box line represents the median, and the whiskers represent the minimum (calculated as Q1 minus 1.5 multiplied by IQR) and maximum (calculated as Q3 plus 1.5 multiplied by IQR). *Equivalent indicates ctDNA PD detection within 1 week of RECIST PD to account for the ctDNA sampling window. ctDNA, circulating tumor DNA; FST, first subsequent treatment; IQR, interquartile range; PD, progressive disease; RECIST, Response Evaluation Criteria in Solid Tumors; TKI, tyrosine kinase inhibitor.

(89%). ctDNA PD at the same time as, or earlier than, RECIST PD was observed in 35 out of 54 (65%) and 58 out of 92 (63%) patients in the osimertinib and comparator EGFR-TKI arms, respectively. Median lead time from ctDNA PD to RECIST PD was 3.4 months (IQR: 1.4–5.3) in the osimertinib treatment arm and 2.6 months (IQR: 1.1–4.9) in the comparator EGFR-TKI arm (Fig. 3*A*). ctDNA PD was observed ahead of RECIST PD except in 13 out of 146 patients (9%); lead times varied between patients (Fig. 3*B* and Supplementary Fig. 1*B*).

In AURA3, 88 of 146 eligible patients (60%) across treatment arms met our criteria for ctDNA PD (Supplementary Table 6) whereas 58 patients (40%) did not have ctDNA PD detected by treatment discontinuation. ctDNA PD was detected at the same time as, or earlier than, RECIST PD in 82 out of 146 patients (56%), with a median lead time of 1.9 months (IQR: 0.7–3.1). Of these 82 patients, ctDNA PD was detected at the same time as RECIST PD in 14 patients (17%) and was detected earlier than RECIST PD in 68 patients (83%). ctDNA PD at the same as, or earlier than, RECIST PD was observed in 43 out of 86 patients (50%) and 39 out of 60 patients (65%) in the osimertinib and chemotherapy arms, respectively. The median lead time from ctDNA PD to RECIST PD was 2.8 months (IQR: 0.7–3.7) with osimertinib, and 1.5 months (IQR: 0.8–2.7) with chemotherapy (Fig. 3*C*). ctDNA PD was observed ahead of RECIST PD except in

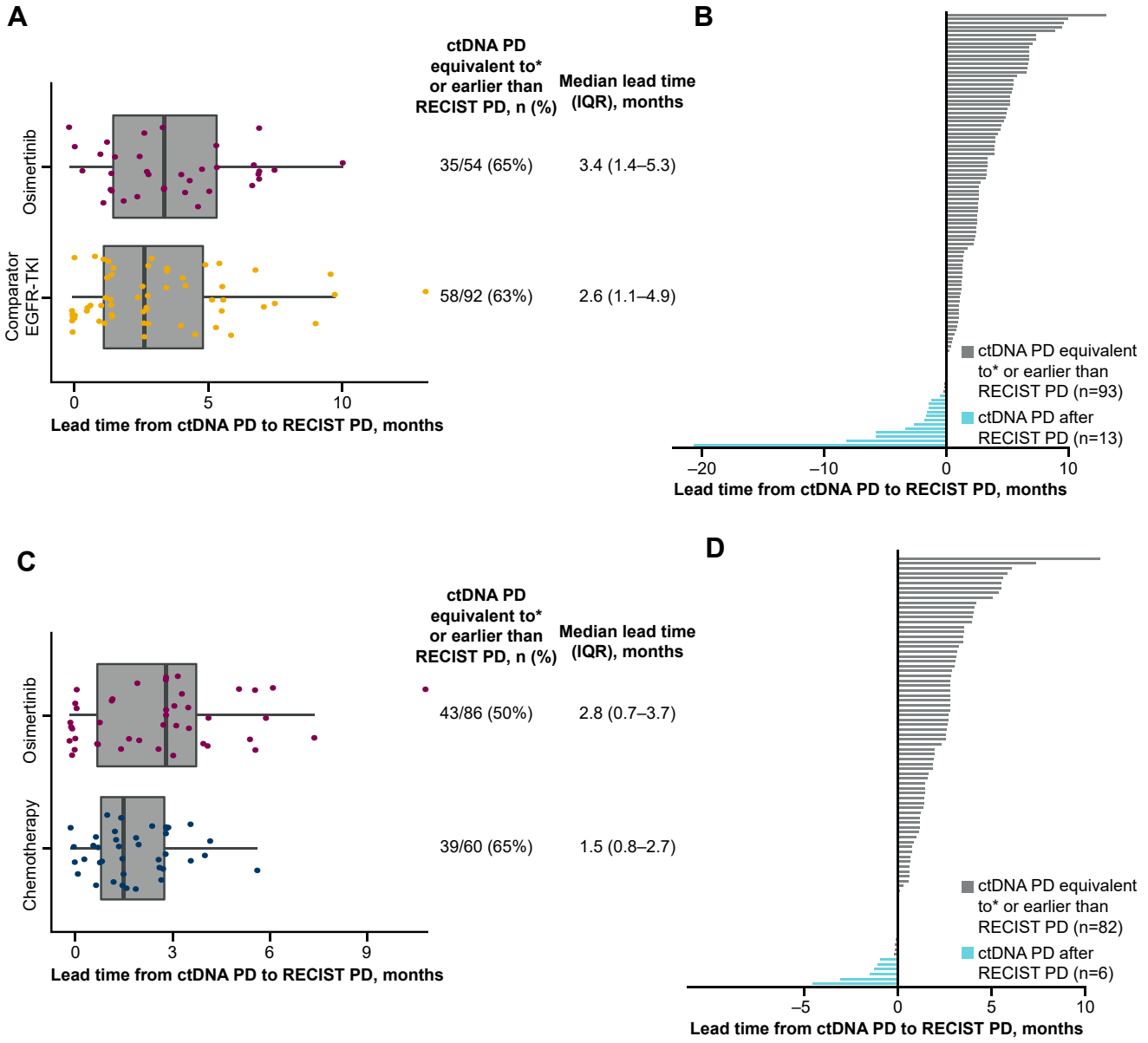

Figure 3. Lead-time from ctDNA PD to identification of RECIST PD in FLAURA and AURA3. Data from FLAURA and AURA3 studies, respectively, presented as box plots showing lead time per treatment arm among patients with ctDNA PD equivalent to or earlier than RECIST PD (*A* and *C*) and as waterfall plots showing lead time per patient among those with ctDNA PD and RECIST PD (*B* and *D*). In panels *A* and *C*, the outer box lines represent the 25th and 75th quartiles, the center box line represents the median, and the whiskers represent the minimum (calculated as Q1 minus 1.5 multiplied by IQR) and maximum (calculated as Q3 plus 1.5 multiplied by IQR). *Equivalent indicates ctDNA PD detected within 1 week of RECIST PD to account for the ctDNA sampling window. ctDNA, circulating tumor DNA; IQR, interquartile range; PD, progressive disease; RECIST, Response Evaluation Criteria in Solid Tumors; TKI, tyrosine kinase inhibitor.

6 out of 146 (4%) patients; lead times varied between patients (Fig. 3*D*).

ctDNA Resistance Analysis

Lead Time From ctDNA Resistance Detection to FST (FLAURA Only). Acquired C797S (in the osimertinib arm) or T790M (in the EGFR-TKI comparator arm) resistance mutations were detected in 4 out of 51 patients (8%) and 53 out of 70 patients (76%) with ctDNA PD in the osimertinib and comparator EGFR-TKI arms, respectively. The median time from treatment start to C797S detection in the osimertinib arm was 16.7 months (IQR: 11.1–20.1), whereas the median time from treatment start to T790M detection in the comparator EGFR-TKI arm was 8.3 months (IQR: 5.6–12.4). Median lead time from detection of acquired C797S or T790M in patients with ctDNA PD to when patients received their FST was 3.8 months (IQR: 2.3–6.0; Fig. 4*A*). Lead times from ctDNA resistance marker detection to start of FST per patient are shown in Figure 4*B* and Supplementary Figure 1*C*.

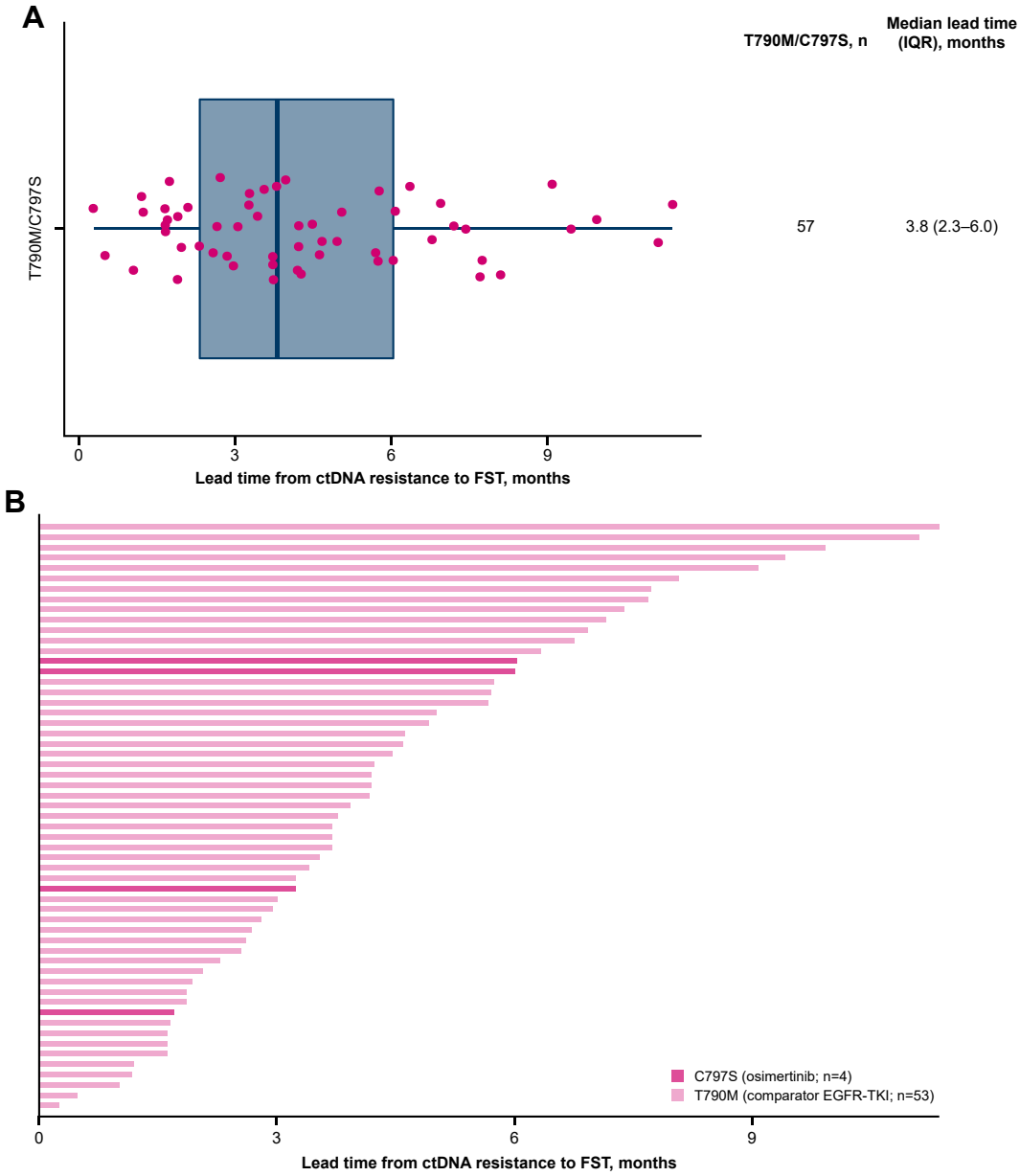

Figure 4. Lead time from ctDNA resistance marker identification to start of FST in FLAURA. Lead times in patients with a detected resistance mutation presented as a box plot (*A*) and a waterfall plot (*B*). In panel *A* the outer box lines represent the 25th and 75th quartiles, the center box line represents the median, and the whiskers represent the minimum (calculated as Q1 minus 1.5 multiplied by IQR) and maximum (calculated as Q3 plus 1.5 multiplied by IQR). ctDNA, circulating tumor DNA; FST, first subsequent treatment; IQR, interquartile range; TKI, tyrosine kinase inhibitor.

Lead Time From ctDNA Resistance Detection to RECIST PD (FLAURA and AURA3). Of 106 patients with ctDNA PD and RECIST PD in FLAURA, resistance mutations were detected in 52 patients (two patients receiving osimertinib had C797S detected and 50 patients receiving comparator EGFR-TKI had T790M detected). Eleven of these patients were not included in this lead time analysis because they did not have ctDNA resistance detected at the same time as, or earlier than, RECIST PD. Among the 41 patients remaining (two with C797S detected and 39 with T790M), the median lead time from detection of acquired C797S or T790M to identification of RECIST PD was 1.4 months (IQR: 0.5–3.4) (Fig. 5*A*). A summary of the results for individual patients is provided in Figure 5*B* and Supplementary Figure 1*D*.

Analysis of resistance mutations in AURA3 was restricted to C797S in the osimertinib arm (as all patients had tumors with *EGFR* T790M). Of 48 osimertinib-treated patients with ctDNA PD and RECIST PD, C797S was detected in 12 patients (25%). Two of these patients had a sampling gap and were excluded from the lead time analyses. The median time from treatment start to detection of C797S was 8.6 months (IQR: 8.3–10.4). Most of the acquired C797S mutations were detected in ctDNA after RECIST PD (6/10, 60%; Supplementary Fig. 2).

Discussion

We report serial longitudinal ctDNA monitoring in patients with *EGFR*m-positive advanced NSCLC who were treated with first-line osimertinib versus comparator EGFR-TKIs, or second-line osimertinib versus platinum-based doublet chemotherapy in the phase 3 FLAURA and AURA3 studies, respectively. Levels of ctDNA are principally a function of two factors: tumor bulk and tumor cell turnover.[14–16] Initial reduction in *EGFR*m ctDNA levels may indicate that tumors are responding to treatment, whereas subsequent increase in *EGFR*m ctDNA levels may indicate PD and potential resistance. Of note, serial ctDNA monitoring in this study was performed using a simple, plasma-only, Clinical Laboratory Improvements Amendments / College of American Pathologists assay in a relatively cost-effective manner.

In *EGFR*m-positive NSCLC, longitudinal monitoring of *EGFR*m ctDNA offers insight into treatment effects and a potential opportunity for advanced detection of molecular PD and the development of resistance mechanisms.[15,17] Our findings in the evaluable FLAURA population demonstrated that *EGFR*m ctDNA PD was observed in 106 out of 146 eligible patients (73%) with RECIST PD. In AURA3, 88 out of 146 eligible patients (60%) with RECIST PD had ctDNA PD. ctDNA PD preceded, or co-occurred with, the identification of RECIST

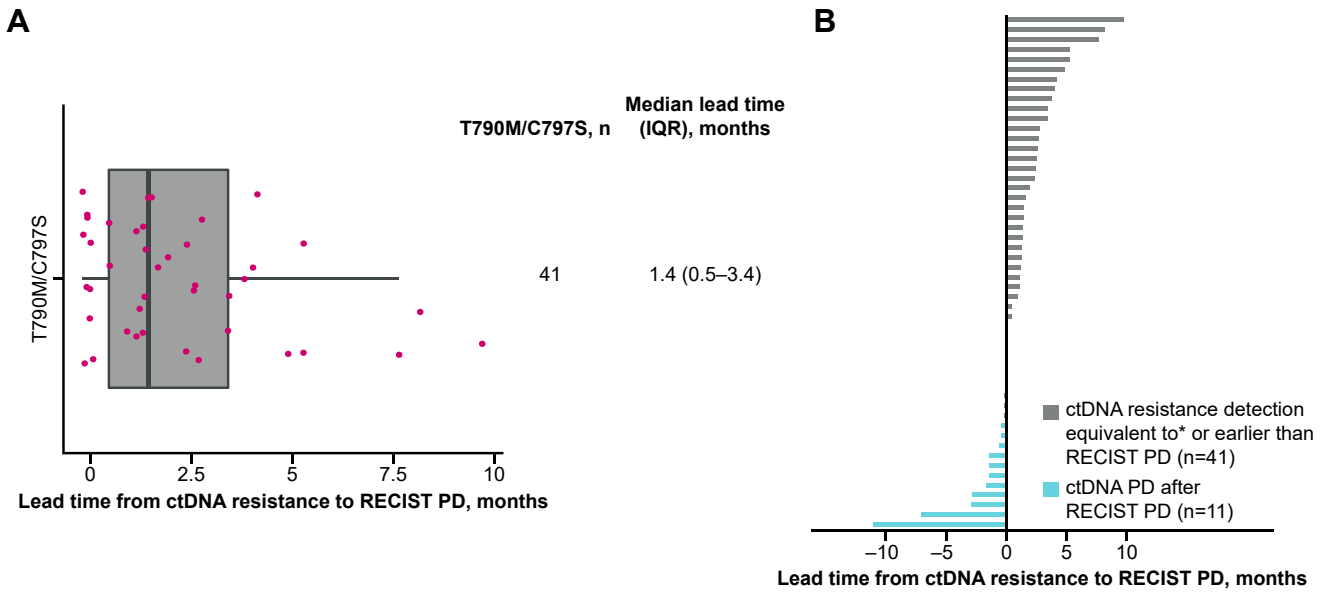

Figure 5. Lead time from ctDNA resistance marker identification to RECIST PD in patients with resistance mutations in FLAURA. Data from FLAURA presented as resistance lead time in patients with a detected resistance mutation as a box plot (*A*) and waterfall plot (*B*). In panel *A* the outer box lines represent the 25th and 75th quartiles, the center box line represents the median, and the whiskers represent the minimum (calculated as Q1 minus 1.5 multiplied by IQR) and maximum (calculated as Q3 plus 1.5 multiplied by IQR). *Equivalent indicates ctDNA PD detected within 1 week of RECIST PD to account for the ctDNA sampling window. ctDNA, circulating tumor DNA; IQR, interquartile range; PD, progressive disease; RECIST, Response Evaluation Criteria in Solid Tumors.

PD in approximately 60% of cases. Among these patients, the median time from ctDNA PD detection to RECIST PD in patients receiving osimertinib was 3.4 months in FLAURA and 2.8 months in AURA3. We also evaluated the time from ctDNA PD to FST in the FLAURA trial. ctDNA PD preceded FST in all cases, with a median lead time from ctDNA PD to FST in the osimertinib arm of 6.0 months. A similar analysis of ctDNA PD relative to FST in AURA3 was not conducted owing to the second-line setting of this study.

Overall, our findings suggest that the detection of dynamic changes in ctDNA levels may serve as a marker preceding PD in patients with *EGFR*m-positive advanced NSCLC who are undergoing treatment. This finding supports previous analyses from the AURA3 and FLAURA trials, demonstrating that clearance of ctDNA after 3 and 6 weeks of EGFR-TKI therapy was associated with improvements in PFS.[13] The longer lead time between ctDNA and FST compared with RECIST-defined PD was expected, owing to the continuation of osimertinib beyond RECIST-defined PD due to ongoing clinical benefit or limited second-line treatment options. This means that the lead time between ctDNA PD and RECIST PD is representative with respect to the availability of second-line treatment options.

The lead time advantage between ctDNA molecular PD and RECIST-defined PD or FST may provide patients and physicians with a much-needed opportunity to discuss subsequent therapy options while the current therapy is still providing clinical and radiologic benefits. This extra time can be significant, as patients often value discussions concerning the next lines of treatment or participation in clinical trials, enabling them to make well-informed decisions.[18,19] Furthermore, it may provide clinicians more time to perform comprehensive biomarker profiling, for example, using next-generation sequencing (NGS) for resistance markers, to better inform subsequent treatment selection,[20,21] including osimertinib in combination with chemotherapy or targeted therapies, or consider enrollment into clinical trials. In addition, molecular detection of disease progression may allow clinicians to organize more frequent positron emission tomography investigative scans to identify the development of oligometastases before further systemic spread, which can be treated with stereotactic ablative radiotherapy.[22] However, the cost-effectiveness of using ctDNA to detect PD before radiologic PD (including its impact on subsequent imaging, treatment, and clinical outcomes) needs to be determined. Since this was an exploratory, retrospective analysis based on patients who had either RECIST PD or had discontinued study treatment, or both, further prospective studies are needed to confirm these lead-time findings. Identifying progression earlier might alter the natural history of the disease and improve survival due to more timely clinical intervention.

In addition to using ctDNA to define PD, we explored the utility of ctDNA analysis for advanced detection of resistance markers. In the current analysis, the T790M mutation was identified in 76% of patients in the comparator EGFR-TKI arm of FLAURA. This is higher than the prevalence reported in a separate ctDNA analysis using NGS in FLAURA (T790M prevalence in comparator arm, 44%)[23] and in other studies and reviews (T790M prevalence, ~50%–60%).[24,25] This difference may reflect an enrichment of patients with early PD in our analysis compared with the overall FLAURA population, as patients must have had a disease progression/discontinuation sample collected relatively early in the study. In addition, different techniques may affect rates of T790M detection.[26] In this analysis, C797S resistance mutations were detected in 8% of osimertinib-treated patients in the FLAURA study and 25% of those in AURA3. This is consistent with other reports that identification of C797S is more common in the second-line setting (~20% of patients) than in the first-line setting.[27–30] In terms of lead times, acquired resistance mutations in the FLAURA trial (C797S in the osimertinib arm and T790M in the EGFR-TKI comparator arm) could be detected a median of 1.4 months ahead of RECIST PD and 3.8 months ahead of FST; lead times varied between individual patients. It should be noted that the majority of resistance mutations detected in FLAURA were T790M from the comparator EGFR-TKI arm, hence the lead times between the detection of acquired resistance mutations and RECIST PD or FST, are predominantly based on T790M. Interestingly, in AURA3, detection of acquired C797S tended to occur after RECIST PD, likely related to the compressed progression timeline in the second-line setting. The clinical implications of these ctDNA lead times are currently unclear.

Although our study presents informative results, there are some limitations that should be considered. Firstly, our analysis focused on *EGFR* ctDNA profiling; with ctDNA-based detection of PD based solely on *EGFR*m, other potentially relevant genes (e.g., MET) were not explored. In addition, traditional tumor markers (e.g., CEA) were not explored in this analysis; however, previous studies have demonstrated the greater sensitivity and specificity of ctDNA analysis compared with tumor markers in solid tumors.[31,32] We used a single mutation-genotyping technology (ddPCR), whereas recent technologies such as NGS may be of more value as they can rapidly sequence multiple mutation types across many genes simultaneously.[33–35] Moreover, depending on the panel details, NGS can detect rare and previously uncharacterized alterations in sequenced genes.[20] This will increase the understanding of tumor

evolution during treatment, and the predictive capacity of liquid biopsy-based ctDNA analysis and may have the potential to better inform clinical decision-making along the patient journey.[36] In addition, the focus on changes in ctDNA levels in patients with PD limited the conduction of specificity analyses, as false-positive ctDNA PD events could not be recorded. However, given the conservative definition of ctDNA PD (Supplementary Methods), coupled with the enduring ctDNA signals within this analysis, false positives are believed to be unlikely. Furthermore, this focus meant that there was an inherent imbalance in data between treatment arms, with most cases of PD occurring in the comparator/standard of care arms of the original studies. In addition, the lead time from ctDNA PD to RECIST PD analysis is biased as the sampling schedule for plasma collection included early time points at Cycle 1, Day 1, 8, and 15, Cycle 2 Day 1, and Cycle 3 Day 1, whereas RECIST tumor assessments were every 6 weeks; as such, if RECIST assessments occurred earlier, or more frequently, the lead time may be reduced. However, no patients had ctDNA PD detected before Cycle 3 Day 1 (time point of the first RECIST scan), and thereafter sampling was aligned with RECIST assessment timings. Moreover, the lead times from ctDNA PD detection to RECIST PD of 3.4 and 2.8 months with osimertinib in FLAURA and AURA3, respectively, are longer than the gap between scan intervals, suggesting this had a limited impact. This also demonstrates the advantage of more frequent plasma collection compared with the current frequency of radiographic clinical monitoring as more regular sampling can detect molecular disease progression sooner. Importantly, the lead times reported here are specific for the schedules of both FLAURA and AURA3, and it is possible that the lead time may differ with different sampling schedules.

As brain scans were only mandated in patients with known or suspected CNS metastases at baseline in both FLAURA and AURA3, the baseline CNS status in these studies is incomplete. In addition, RECIST PD was not recorded in sufficient detail to differentiate cases of CNS-only PD from PD that included a CNS component, among other sites. Therefore, no comment on the association of ctDNA detection with CNS metastases can be made; however, we acknowledge that CNS-only PD may potentially explain cases of apparent PD without a corresponding rise in ctDNA. This analysis only included data from plasma samples; further studies and analyses with paired tissue samples will elucidate further insights into intrinsic resistance and acquired resistance mechanisms. Another limitation was that although the TFST data were mature, many FLAURA-enrolled patients were excluded (n = 306) because they did not have RECIST PD or a treatment discontinuation sample at data cutoff, leading to an imbalance in the number of patients in each treatment arm. This may indicate that our data are likely to be more representative of early progressors than the overall FLAURA patient population.[2] Further studies should be undertaken to cover broader patient populations.

In conclusion, our results indicate that longitudinal ctDNA monitoring has the potential to detect molecular signs of PD and resistance, often preceding RECIST PD. This presents the opportunity for a lead-time, ctDNA-driven management approach, enabling more timely clinical interventions or proactive planning for appropriate clinical studies.

Data Availability Statement

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.

Data for studies directly listed on Vivli can be requested through Vivli at www.vivli.org.

Data for studies not listed on Vivli could be requested through Vivli at https://vivli.org/members/enquiries-about-studies-not-listed-on-the-vivli-platform/.

AstraZeneca Vivli member page is also available outlining further details: https://vivli.org/ourmember/astrazeneca/.

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Disclosure

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at https://doi.org/10.1016/j.jtho.2024.07.008.

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