Biomarkers of Osimertinib Response in Patients with Refractory, EGFR-T790M–positive Non–Small Cell Lung Cancer and Central Nervous System Metastases: The APOLLO Study

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ABSTRACT

Purpose: Dynamic biomarker monitoring may inform pathways for treating *EGFR*-T790M–positive non–small cell lung cancer (NSCLC) and central nervous system (CNS) metastases with osimertinib. This study aimed to determine the efficacy and safety of osimertinib for real-world patients with *EGFR*-T790M NSCLC and CNS metastases and to explore potential circulating biomarkers of therapeutic response.

Patients and Methods: APOLLO (ClinicalTrials.gov registration: NCT02972333) was a prospective, single-arm, open-label trial which ran from January 2017 to April 2019. Eligible patients had confirmed *EGFR*-T790M–positive NSCLC, prior treatment with an EGFR-tyrosine kinase inhibitor, and CNS metastases. All enrolled patients received oral osimertinib 80 mg once daily until disease progression or intolerable toxicity. Primary outcome was overall progression-free survival (PFSo) and secondary outcomes included objective response rate (ORR) and adverse events (AE). Exploratory biomarker analysis involved collection of plasma and cerebrospinal fluid (CSF) samples for next-generation sequencing and drug penetration analysis.

Results: From January to September 2017, 38 patients were enrolled. After a median follow-up of 8.2 months (range, 0.07–15.6), 23 (60.5%) of 38 patients had disease progression or death. Median PFSo was 8.4 months [95% confidence interval (CI), 5.8–10.9]. Overall ORR was 39.4%. Twelve (31.6%) of 38 patients had ≥1 grade 3–4 AE. Median osimertinib CSF penetration rate was 31.7%. Patients with undetectable plasma *EGFR* mutations at week 6 had improved PFSo compared with those with detectable mutations (not reached vs. 4.5 months; 95% CI, 0.0–1.1; $P < 0.05$).

Conclusions: Osimertinib had potent activity against *EGFR*-T790M–positive NSCLC with CNS metastases. Dynamic monitoring of plasma *EGFR* may suffice for predicting clinical responses, mitigating the need for repeat CSF biopsy.

See related commentary by Marmarelis and Bauml, p. 6077

Introduction

Central nervous system (CNS) metastases, including brain metastases and leptomeningeal metastases, are frequent causes of disease progression and death in patients with non–small cell lung cancer (NSCLC; ref. 1). Patients with NSCLC and mutant epidermal growth factor receptor (*EGFR*) are more likely to develop brain metastasis than patients with wild-type *EGFR* (2), and may be even more likely to develop brain metastasis if treated with first-line EGFR-tyrosine kinase inhibitor (TKI) therapy compared with other first-line treatments (3, 4). Current first- and second-generation EGFR-TKIs have generally displayed low CNS efficacy, which has been hypothesized to be due to their affinity for efflux transport proteins and limited ability to cross the blood–brain barrier (BBB; ref. 5). Of patients treated with first-line gefitinib or erlotinib, 25%–35% showed CNS progression after a median follow-up of 22 months; only 16% of the cohort had prior CNS involvement (6). Therefore, developing treatments for *EGFR*-mutant NSCLC with improved CNS penetration and corresponding response rates is a priority (5).

Osimertinib is a potent, irreversible, third-generation EGFR-TKI that is selective for *EGFR*-sensitizing and T790M resistance mutations (7, 8). In the AURA studies, osimertinib achieved high objective response rates (ORR), promising progression-free survival (PFS), and durable responses in patients with T790M-positive NSCLC who progressed on prior EGFR-TKI therapy (7–9). In AURA3, osimertinib demonstrated an unprecedented median CNS PFS of 11.7 months for advanced T790M-positive patients (10). In a pooled analysis of the AURA extension and AURA2 trials, osimertinib demonstrated CNS

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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 2020;26:6168–75

doi: 10.1158/1078-0432.CCR-20-2081

Translational Relevance

Our study provides evidence guiding the use of cerebrospinal fluid (CSF) and plasma monitoring in patients with non–small cell lung cancer (NSCLC) and central nervous system (CNS) metastases in the real-world setting. APOLLO is a novel study that prospectively examined osimertinib CSF penetration rate with a simultaneous biomarker analysis of cell-free DNA from liquid biopsies (plasma and CSF), correlating *EGFR* mutational load to progression-free survival (PFS) in the NSCLC and CNS metastatic setting. Clinical response outcomes to osimertinib, including PFS, objective response rate, and disease control rate, were similar to those previously reported in patients with NSCLC with CNS disease. We suggest that CSF testing for T790M mutations is warranted in patients with T790M-negative plasma. Dynamic shifts in the presence or absence of detectable *EGFR*-sensitizing mutations in the plasma may predict clinical response to osimertinib.

activity with a high disease control rate (DCR) of 92% and a toxicity profile consistent with the overall patient population (9). Osimertinib is currently approved for first-line treatment of NSCLC with *EGFR*-sensitizing mutations and treatment of EGFR-TKI refractory, *EGFR*-T790M–positive NSCLC by the FDA, and also recommended in the National Comprehensive Cancer Network (NCCN) guidelines for patients with symptomatic brain metastases (11, 12).

Tumor genotyping is performed to confirm the presence of *EGFR* mutations. Difficulty in accessing CNS lesions for biopsy has generated interest in using cell-free DNA (cfDNA) from plasma and cerebrospinal fluid (CSF; refs. 13–15). Plasma cfDNA is a good surrogate for genetic profiling of extracranial tumor tissue, demonstrated in the ASSESS study by the high concordance rate of 89% between tissue and plasma cfDNA in patients with advanced NSCLC (13). Plasma biopsy is currently recommended by the NCCN for T790M testing in NSCLC where tissue biopsy is not feasible (12). However, limited evidence is available to support the use of plasma cfDNA as a surrogate for CNS tumor tissue as, due to the BBB, plasma may not accurately profile lesions confined to the brain and leptomeninges. CSF cfDNA may provide a more sensitive and specific readout of genetic changes in CNS metastases than plasma (14, 16). Interestingly, early research has suggested that temporal changes in the level of *EGFR* mutations in plasma and/or CSF during treatment may correspond to clinical outcomes (17). Hence, it is possible that dynamic monitoring of liquid biopsies could be a useful tool in managing CNS disease treated with EGFR-TKIs (15).

The APOLLO study aimed to prospectively evaluate the efficacy of osimertinib in treating patients with T790M-positive NSCLC and CNS metastases, and to explore the correlation of genetic biomarkers in CSF and/or plasma samples with clinical outcomes.

Patients and Methods

Study design

The APOLLO study was an open-label, single-arm, multicenter, prospective study (NCT02972333) conducted in accordance with legal and regulatory requirements, as well as the general principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human Subjects (Council for International Organizations of Medical Sciences 2002), Guidelines for Good Clinical Practice (International Conference on Harmonization 1996), and the Declaration of Helsinki (World Medical Association 2008). Eligible patients from the real-world ASTRIS clinical access study (NCT02474355) were screened from 10 sites across China. Data cut-off date for the primary analysis was May 3, 2018. Ethics approval for the study was gained at each enrollment site.

Participants

Patients with confirmed *EGFR*-T790M–positive NSCLC and concurrent CNS metastases who failed prior EGFR-TKI therapy were eligible for enrollment. All patients provided written informed consent prior to study procedures. Patients were eligible if aged ≥18 years; had stage IV NSCLC; World Health Organization performance status 0–2 with no deterioration over the previous 2 weeks and a minimum life expectancy of 3 months; and measurable brain metastasis suitable for repeated assessments (≥1 intracranial measurable lesion that, if previously irradiated, had progressed or not responded to radiotherapy). Patients with leptomeningeal metastasis were also eligible, but a confirmed diagnosis by CSF cytology was required, as well as the presence of ≥1 leptomeningeal metastasis lesions that could be assessed repeatedly with MRI. Measurable extracranial disease was not required. Key exclusion criteria were treatment with osimertinib currently or in the previous 6 months; prior whole-brain radiotherapy (WBRT); evidence of severe/uncontrolled systemic diseases; and/or symptomatic CNS metastases that were neurologically unstable or had required increased steroid dosage to manage CNS symptoms <2 weeks prior to osimertinib treatment.

Procedures

Patients received oral osimertinib 80 mg (AstraZeneca) once daily until disease progression (PD), intolerable toxicity, or at patient and/or investigator discretion. Access to osimertinib was provided through the ASTRIS study to all patients until loss of clinical benefit. Concomitant WBRT and stereotactic radiosurgery were permitted, provided patients undergoing radiotherapy had their osimertinib treatment interrupted with a 7- to 10-day washout period before and 1 week after completion of radiotherapy.

Patients were assessed for clinical outcomes at baseline and every 6 weeks until death, loss to follow-up, data cut-off, or withdrawal of consent. Tumor assessments and CT/ultrasound of the chest and abdomen were performed at baseline and every 12 weeks until objective PD, intolerant toxicity, or loss to follow-up in accordance with RECIST, version 1.1. Intracranial and extracranial lesions were assessed separately per RECIST 1.1.

Osimertinib concentration in CSF and plasma samples was determined using protein precipitation (API 4000+ LC/MS-MS System, SCIEX). Blood and CSF samples were collected at baseline, at 6 weeks, and PD for exploratory analyses, which were conducted by Nanjing Geneseeq Technology Inc. Somatic mutation and copy-number alteration (CNA) analyses were performed on cfDNA extracted from blood and CSF samples using next-generation sequencing (NGS; HiSeq 4000 NGS Platforms, Illumina). The average sequencing depth of whole-blood normal control and cfDNA samples was 247× and 2,912×, respectively. Detailed methods are available in the Supplementary Materials and Methods.

Outcomes

The primary endpoint was overall progression-free survival (PFSo), measured until progression of intracranial and/or extracranial disease. Secondary endpoints included intracranial PFS, extracranial PFS, ORR, and DCR for overall, intracranial, and extracranial categories,

overall survival (OS), and adverse event (AE) monitoring. Data cut-off for the primary analysis took place after 60% PFSo occurred. For patients with leptomeningeal metastasis, ORR was defined as at least one response of complete response (CR) or partial response (PR) prior to PD and DCR was defined as confirmed CR, PR, or stable disease (SD). AEs were reported and graded according to the Common Terminology Criteria for AEs, version 4.0.

Exploratory endpoints included rate of T790M-positive mutation, concordance of T790M mutation status between plasma and CSF, change of variant allele frequency (VAF) of somatic mutations before and after treatment, the proportion of each genetic mutation, and osimertinib concentration level in plasma and CSF samples.

Statistical analysis

Statistical analyses were performed at data cutoff. Statistical analysis system, version 9.2, was used. The full analysis set (FAS) included all patients who received ≥1 dose of osimertinib and was used for all analyses, including the safety analysis. Time-to-event data were summarized using the Kaplan–Meier method for PFSo or by the event number and event rate for intracranial and extracranial PFS. Continuous variables were summarized by the number of observations and mean (SD) or median (quartiles) values. Categorical variables were summarized by frequency counts and percentages. The Kaplan–Meier method and log-rank test were used to investigate the association between time-to-event clinical outcomes and biomarkers.

Results

Between January and September 2017, 38 patients with confirmed *EGFR*-T790M–positive NSCLC and concurrent CNS metastases were enrolled (**Fig. 1**). The majority were female (60.5%), nonsmokers (82.9%), and had received at least three prior lines of antitumor therapy (57.9%; **Table 1**). Mean age was 59.1 years (SD, 9.32). All patients had documented T790M mutations combined with an *EGFR*-sensitizing mutation, either 19Del or 21L858R (L858R). All patients presented with CNS metastases either in the brain (92.1%) or leptomeninges (5.3%); 1 patient had both brain metastasis and leptomeningeal metastasis (2.6%), and 31 patients had concurrent extracranial metastases (81.6%).

At data cut-off (May 3, 2018), 12 (31.6%) of 38 patients had died and 26 patients (68.4%) remained in the study, with a median follow-up of 8.2 months (range, 0.1–15.6). Median PFSo was 8.4 months [95% confidence interval (CI), 5.8–10.9; **Fig. 2**; Supplementary Table S1]. Progression events occurred in 16 (42.1%) of 38 patients, including 7 patients who subsequently died (**Fig. 2**; Supplementary Table S1). Of the 16 patients with PD, 7 (43.8%) of 16 had only intracranial PD, 5 (31.3%) had only extracranial PD, and 4 (25.0%) had both intracranial and extracranial PD. Kaplan–Meier analyses were not performed for intracranial and extracranial subgroups because of limited event numbers.

Overall and extracranial tumor evaluation data were available for 33 patients and intracranial data for 32 patients (Supplementary Table S1). Overall ORR was 39.4% (13/33; 95% CI, 22.9–57.9); all 13 patients demonstrated PR (**Fig. 2**; Supplementary Table S1). Of the 13 patients with objective response, 6 (46.2%) had subsequently progressed or died at the time of analysis, with the remaining 7 (46.2%) continuing to receive osimertinib (**Fig. 2**). A numerically higher intracranial ORR of 68.8% (22/32; 95% CI, 50.0–83.9) was achieved, including 3 patients who achieved CR (2 patients had brain metastasis and 1 had leptomeningeal metastasis) and 19 patients who achieved PR (18 patients had brain metastasis and 1 had both leptomeningeal metastasis and brain metastasis). Overall DCR was 90.9% (30/33; 95% CI, 75.7–98.1; Supplementary Table S1), including 17 patients with a best response of SD (**Fig. 2**). Of the 30 patients with controlled disease, 8 patients (26.7%) with SD and 6 patients (20.0%) with PR had subsequently progressed or died at the time of analysis.

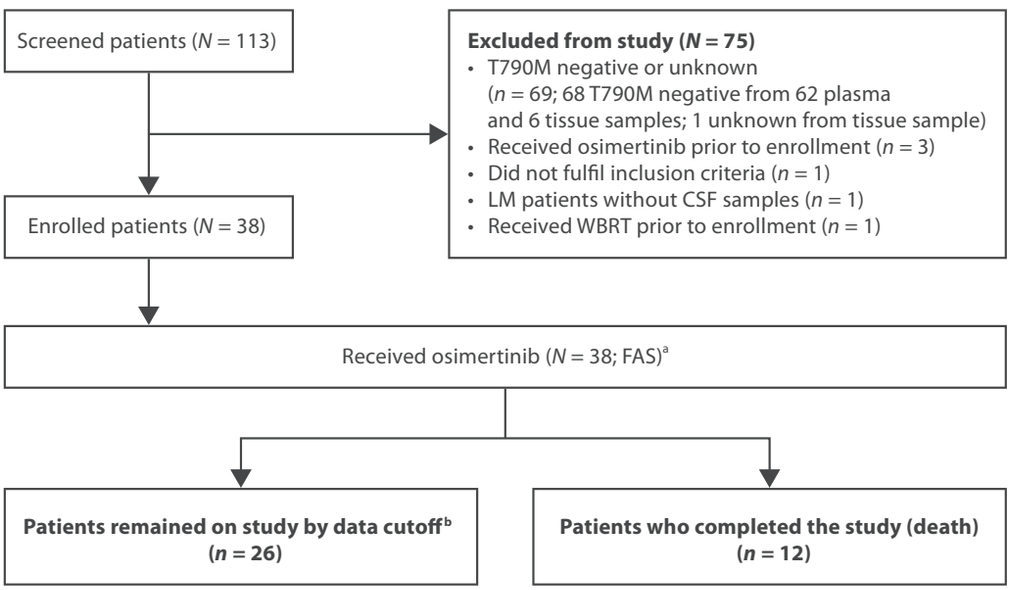

Figure 1.
Study patients. [a]Two patients had important protocol deviations, including having received osimertinib prior to enrollment ($n = 1$), and withdrawal from the study after receiving the first dose but prior to tumor evaluation ($n = 1$). As both patients received ≥1 dose of osimertinib, these patients were included in the FAS. There were no differences between outcomes for the per-protocol population and FAS, hence only FAS results are reported. [b]Data cutoff occurred at 60% PFSo (May 3, 2018). LM, leptomeningeal metastases; WBRT, whole-brain radiotherapy.

Table 1. Baseline characteristics and demographics.

	FAS ($N = 38$)
Demographics	
Age in years, median (range)	62.0 (37–74)
Female sex, *n* (%)	23 (60.5)
Smoking status	
Nonsmoker, *n* (%)	29 (82.9)
Smoker, *n* (%)	6 (17.1)
Missing, *n*	3
Disease characteristics	
Duration of NSCLC in months, median (range)	23 (0.3–94.0)
Histologic type, *n* (%)	
Adenocarcinoma	38 (100)
Location of metastases, *n* (%)	
Intracranial only	7 (18.4)
Extracranial and intracranial	31 (81.6)
Type/s of CNS metastases, *n* (%)	
BM only	35 (92.1)
LM only	2 (5.3)
BM and LM	1 (2.6)
EGFR mutation status, *n* (%)	
EGFR-T790M mutation positive	38 (100)
Combined *T790M* + *19Del* mutation	22 (57.9)
Combined *T790M* + *L858R* mutation	16 (42.1)
Sample type for *EGFR*-T790M mutation assessment, *n* (%)	
Blood	25 (65.8)
Tissue	13 (34.2)
WHO performance status, *n* (%)	
0	7 (18.4)
1	29 (76.3)
2	2 (5.3)
NSCLC treatment history	
Prior lines of antitumor therapy, *n* (%)	
1	13 (34.2)
2	3 (7.9)
≥3	22 (57.9)
Prior EGFR-TKI, *n* (%)	
Erlotinib	6 (15.8)
Gefitinib	24 (63.2)
Icotinib	7 (18.4)
Afatinib	1 (2.6)
Dacomitinib	1 (2.6)
History of brain radiotherapy, *n* (%)	4 (10.5)

Abbreviations: BM, brain metastasis; LM, leptomeningeal metastasis; WHO, World Health Organization.

For the intracranial disease subgroup, DCR was 96.9% (32/33; 95% CI, 83.8–99.9). At data cutoff, 12 patients (31.6%) had died. Data for the OS analysis were not mature at the time of analysis.

The safety profile of osimertinib was consistent with previous studies (Supplementary Table S2). AEs occurred in 34 of 38 patients (89.5%), including 10 patients (26.3%) with serious AEs and 22 patients (57.9%) with adverse drug reactions. Twelve patients (31.6%) experienced AEs of grade ≥3, of which 3 were drug related. Further details are included in Supplementary Table S2.

Clinical characteristics of the 12 patients with paired plasma and CSF samples included in the exploratory analyses are presented in Supplementary Table S3. Median CSF penetration rate of osimertinib at 6 weeks was 31.7% (range, 19.8%–57.8%), with a median CSF concentration of 10.8 nmol/L (range, 5.2–30.4 nmol/L; Supplementary Table S4). Overall ORR was 41.7% (5/12; 95% CI, 15.2–72.3) and the overall DCR was 83.3% (10/12; 95% CI, 51.6–97.9; Supplementary Table S5). Median PFSo was 8.3 months (95% CI, 2.7–NA; Supplementary Table S5). There was a strong correlation between the concentration of osimertinib in CSF and plasma at week 6 ($r = 0.83$; $P < 0.0001$; **Fig. 3**). The degree of correlation between the concentration of osimertinib in CSF to free plasma and PFS was low (Supplementary Table S6). There was also a higher median CSF penetration rate of osimertinib in patients who achieved intracranial CR and PR (36.5%) than in patients who achieved SD and PD (25.8%; Supplementary Table S7).

All 12 patients had T790M mutation–positive tumor tissue, 10 (83.3%) with T790M mutation–positive plasma and 2 (16.7%) with T790M mutation–positive CSF (**Fig. 4A**). There was low concordance between CSF and plasma T790M status at baseline (1/12, 8.3%). However, concordance was high between CSF and plasma

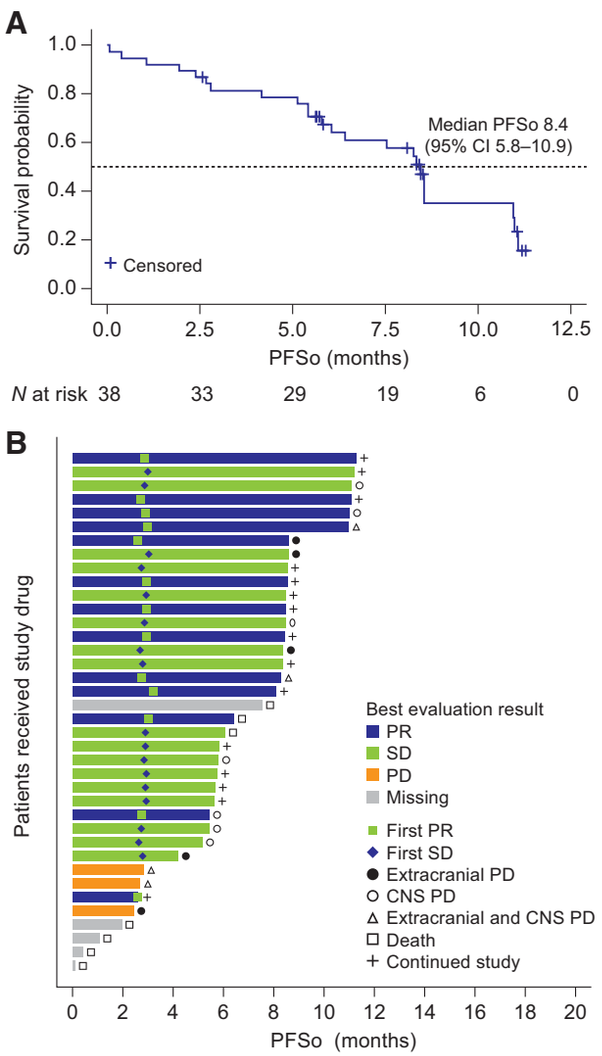

Figure 2.

PFS outcomes. **A,** Kaplan–Meier estimation of median PFSo, defined as the time elapsed between first dose of osimertinib and first progression of CNS and/or extracranial lesions. **B,** Documentation of tumor responses, progression of disease, and death in patients from the FAS. Patients arranged from best to worst tumor response to osimertinib.

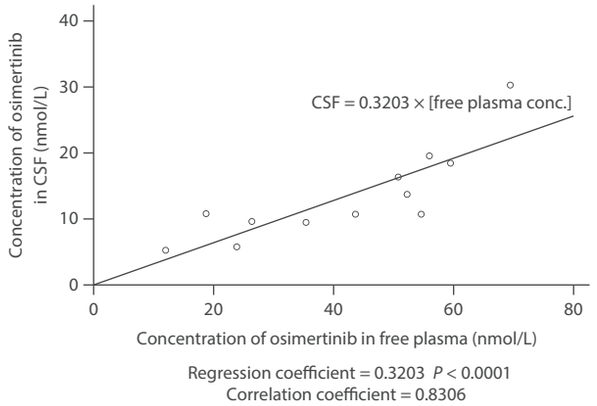

Figure 3.
Concentration of osimertinib in free plasma and CSF. Scatter plot of osimertinib concentration in free plasma and CSF following 6 weeks of osimertinib treatment.

samples for L858R (5/7, 71.4%) and 19Del (4/5, 80.0%) *EGFR*-sensitizing mutations (**Fig. 4A**). *EGFR*-sensitizing mutations were detected in 100.0% (12/12) and 75.0% (9/12) of plasma and CSF samples, respectively (Supplementary Table S8). Patients with 19Del had a higher intracranial response rate than L858R (ORR, 75.0% vs. 57.1%; DCR, 100.0% vs. 85.7%; Supplementary Table S9); PFS was slightly worse in patients with 19Del compared with L858R (Supplementary Fig. S1).

We further compared all somatic alterations and identified 69 and 41 mutations from plasma and CSF, respectively, with 34 mutations overlapping (Supplementary Fig. S2; Supplementary Table S10). In contrast, cfDNA from CSF revealed more CNAs that were not detected from plasma (16 vs. 12 CNAs, with five shared; Supplementary Fig. S2; Supplementary Table S10).

VAF of T790M was dramatically decreased in both plasma and CSF samples after 6 weeks of treatment with osimertinib. Furthermore, 75.0% (9/12) and 88.9% (8/9) of patients with *EGFR*-sensitizing mutations showed decreased VAF in plasma and CSF samples, respectively (**Fig. 4B**; Supplementary Table S8). Two PD patients (2/2, 100.0%) had detectable plasma *EGFR*-sensitizing mutations at week 6. In contrast, only 40.0% (2/5) of PR and 20.0% (1/5) of SD patients had detectable *EGFR*-sensitizing mutations (Supplementary Tables S8 and S11). Patients without detectable *EGFR*-sensitizing mutations in plasma after 6 weeks had significantly improved PFSo (HR, 0.2; 95% CI, 0.0–1.1; $P < 0.05$; **Fig. 4C**). No significant change in PFSo was observed on the basis of the absence of detectable *EGFR*-sensitizing mutations in CSF at week 6 ($P = 0.68$). However, patients with T790M detected in CSF samples at baseline ($n = 2$, one with T790M-negative plasma) displayed partial CNS response and a trend toward improved PFSo (**Fig. 5**). Monitoring the response and progression to osimertinib treatment in a representative patient with T790M detected in both baseline CSF and plasma samples are shown in Supplementary Fig. S3.

Discussion

Osimertinib provided robust clinical benefits in the APOLLO study, with a median PFSo of 8.4 months, and no new safety or tolerability concerns for patients with advanced, T790M-positive, EGFR-TKI refractory NSCLC and CNS metastases. Median CSF penetration rate was 31.7%, correlating with a 68.8% ORR for CNS lesions. In the NGS biomarker analysis, marked heterogeneity was found between T790M status in CSF versus plasma liquid biopsies, while dynamic monitoring of plasma *EGFR* mutational load corresponded with PFSo outcome. This study monitored osimertinib CNS activity, compared T790M cfDNA profiles in plasma and CSF samples, and included a dynamic biomarker analysis correlating with clinical response.

Despite the poor prognosis for patients with EGFR-TKI refractory NSCLC and CNS metastases, osimertinib has provided consistently good response rates in this setting (10, 18). The median PFSo of 8.4 months achieved in our study aligns with two retrospective real-world studies of osimertinib in patients with CNS metastases, which found PFSo of 8.5 months (19) and 9.7 months (20), respectively. However, APOLLO emphasizes the importance of T790M testing in CSF and plasma in guiding osimertinib treatment. In AURA3, a phase III, randomized controlled trial evaluating osimertinib and platinum-pemetrexed chemotherapy in patients who had progressed on first-line EGFR-TKI, patients with CNS metastases achieved a similar PFS of 8.5 months (7). More than half the patients in APOLLO had already failed three or more prior EGFR-TKI therapies, suggesting that osimertinib has comparable efficacy regardless of prior therapy failures.

The intracranial response rate (68.8%) in this study was also similar to the findings of AURA3 (10). Complete CNS tumor response was found in 3 patients (9.4%) in our study, compared with 7%–20% reported in the literature (9, 10, 19). Interestingly, the intracranial response rate and PFS may also be affected by the presence of *EGFR*-sensitizing mutations, 19Del and L858R. However, possibly due to short follow-up and small sample size, no significance was reported (Supplementary Fig. S1; Supplementary Table S8). Particularly strong responses for CNS metastases may be explained by the high penetrating capacity of osimertinib. A recent study of radio-labeled osimertinib in healthy human subjects found that osimertinib rapidly penetrated the BBB and was distributed throughout the brain (21). In APOLLO, the standard 80 mg osimertinib dosage achieved an excellent CSF penetration rate of 31.7%. Patients with intracranial responses had a higher median CSF penetration rate (36.5%) than patients with stable or progressive disease (25.8%). To our knowledge, this is the highest reported CSF penetrance for any EGFR-TKI. A previous single-arm study of 80 mg osimertinib reported a penetration rate of only 2.5%, believed to be due to different computation methods (22). The ongoing BLOOM study, evaluating refractory NSCLC with CNS metastases, reported 16% penetrance for the higher, 160 mg dosage of osimertinib (APOLLO used the same penetration rate calculation method as the BLOOM study; ref. 23). The higher CSF penetration rate in our study may be attributable to the presence of patients with leptomeningeal metastasis and brain metastasis, whereas the BLOOM study only enrolled patients with leptomeningeal metastasis (23). As we only had a small sample of patients, caution is advised when interpreting these results. In comparative studies, preclinical data in mice and nonhuman primates showed that osimertinib achieved greater CSF penetration and extended exposure in the brain than other EGFR-TKIs, including gefitinib (24). Osimertinib is not only more penetrative than first-generation EGFR-TKIs, but also third-generation EGFR-TKIs such as avitinib, which recently demonstrated a penetration rate <0.15% in a study of 16 patients with T790M-positive NSCLC, 7 of whom had brain metastases (25).

Currently, *EGFR* mutation analysis from tissue biopsy is the gold standard for predicting tumor response to osimertinib treatment in EGFR-TKI refractory patients. cfDNA from plasma or CSF can be an

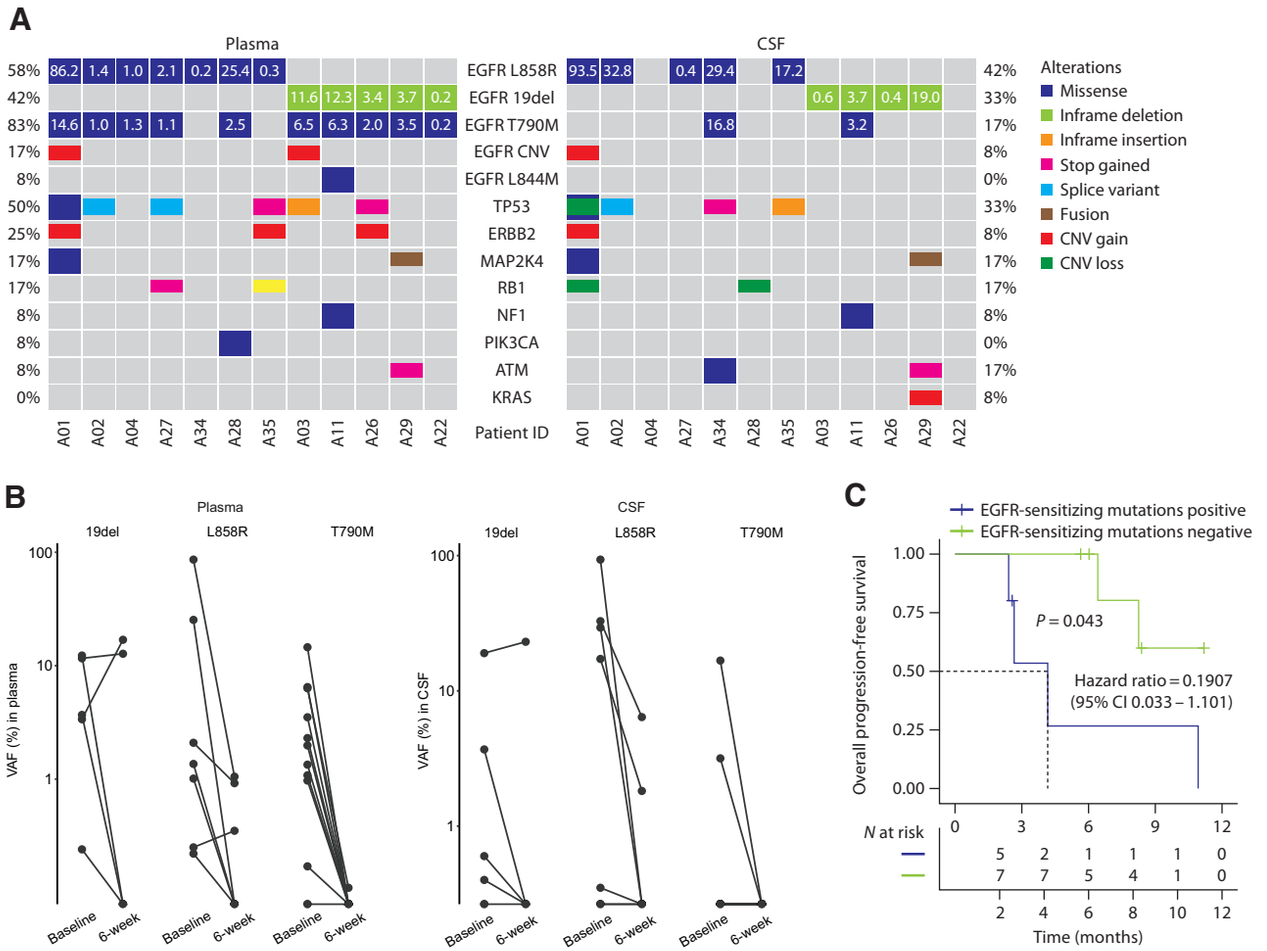

Figure 4.

Exploratory NGS analysis from CSF and plasma samples. NGS analysis of cfDNA from CSF and plasma. **A,** OncoPrint of somatic mutations and copy-number variations (CNV) in baseline samples of 12 patients. Genes altered in ≥2 samples and NSCLC driver genes (including *EGFR, TP53, ERBB2, BRAF, MET, PIK3CA, KRAS*, and *NF1*) altered in ≥1 sample are shown. Frequency of the alterations in 12 plasma (left) and CSF (right) samples is shown. VAFs of *EGFR* mutations are presented for each sample and percentages rounded to the nearest whole number. **B,** VAF changes for *EGFR*-sensitizing and *EGFR* resistance mutations from baseline to 6 weeks after osimertinib treatment. **C,** PFSo based on the detection of *EGFR*-sensitizing mutations in plasma 6 weeks after osimertinib treatment.

alternative for mutational profiling of CNS tumors inaccessible to tissue biopsy (12). However, we found a high level of heterogeneity between *EGFR*-T790M mutation status in baseline plasma and CSF, with concordance of 8.3%. Previous studies also suggest low concordance of T790M status between plasma and CSF samples (14, 26). In addition to T790M, heterogeneity of other somatic alterations was common between plasma and CSF samples (Supplementary Fig. S2; Supplementary Table S10). Two patients from our study had T790M-positive CSF at baseline, and both demonstrated prolonged PFSo and robust intracranial response to osimertinib (**Fig. 5**; Supplementary Fig. S3), despite 1 patient having T790M-negative plasma. In separate case studies, 2 patients with leptomeningeal metastasis, T790M-positive CSF, and T790M-negative plasma, results demonstrated neurologic improvement and leptomeningeal metastasis response with osimertinib treatment (27, 28). Our exploratory data and these case studies suggest that T790M-positive CSF cfDNA at baseline may predict a response to osimertinib regardless of whether plasma cfDNA is T790M positive or negative. Thus, when CNS tumor biopsy is not feasible and the *EGFR*-T790M mutation status is negative in plasma, CSF testing should be considered as an alternative to avoid missing T790M-positive CNS metastases with a likelihood of responding to osimertinib therapy. Furthermore, the overall mutational profiles between CSF and plasma can be quite different as previously demonstrated, with CSF potentially revealing unique and more representative genetic profiles of the tumor than plasma (29).

Dynamic monitoring of *EGFR* mutational load via plasma cfDNA may help predict clinical outcomes (17). In the FLAURA study, which compared osimertinib with erlotinib and gefitinib as a first-line therapy for patients with locally advanced or metastatic NSCLC, early clearance of plasma *EGFR* mutations correlated with better prognosis (30). In the AURA17 trial, early clearance of *EGFR*-sensitizing mutations at 3 or 6 weeks after osimertinib treatment correlated with favorable PFS and ORR in Chinese patients with

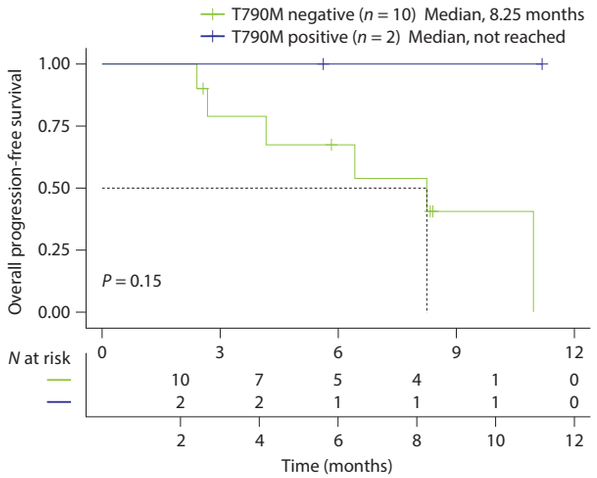

Figure 5.

PFSo based on *EGFR*-T790M detection in CSF at baseline. Kaplan–Meier estimation of median PFSo, defined as the time elapsed between the first dose of osimertinib and first progression of CNS and/or extracranial lesions, separated by detection of *EGFR*-T790M in the CSF at baseline.

T790M-positive NSCLC who had progressed on previous EGFR-TKI therapy (31). Similarly, patients in our study without detectable *EGFR*-sensitizing mutations in plasma after 6 weeks had significantly improved PFSo. Our results add further to early evidence supporting a role for dynamic monitoring of *EGFR* mutational load through cfDNA.

In APOLLO, the VAF of *EGFR*-T790M was dramatically reduced in both plasma and CSF cfDNA after 6 weeks. Seventy-five percent and 89% of *EGFR*-sensitizing mutations showed decreased VAF at 6 weeks in plasma and CSF samples, respectively. The high CNS penetration rate of osimertinib in the APOLLO study may explain the strong concordance between *EGFR* mutation clearance from the CSF and plasma. Thus, while our data suggest CSF sampling should be considered for determining baseline *EGFR*-T790M status of CNS metastases, recommending repeat CSF biopsies for monitoring osimertinib response would not be practical. Our data suggest that plasma sampling may suffice for monitoring osimertinib response over time, mitigating the requirement for CSF rebiopsy.

This study has several limitations. As a single-arm trial, APOLLO has limited generalizability, cannot be used to compare treatment strategies, and is subject to increased internal variability and biases as compared with controlled trials. Baseline features of the cohort were variable, including prior EGFR-TKI treatment and performance status. Small sample size is another key limitation of the study, increasing the margin of error and meaning certain statistical analyses could not be performed. Furthermore, only a subset of patients had paired plasma and CSF samples available for the exploratory biomarker analysis, which should be scaled-up for future studies, in particular to examine more patients with divergent CSF and plasma T790M status. APOLLO provides a snapshot of osimertinib use in patients with advanced NSCLC from China and highlights potential biomarkers for future investigation. Up-scaled, randomized controlled trials are warranted, guided by the dynamic mutational load of *EGFR*-sensitizing and resistance mutations, in patients with NSCLC with CNS metastases.

To conclude, osimertinib demonstrated robust PFS and tumor responses in patients with *EGFR*-T790M–positive NSCLC with CNS metastases refractory to prior EGFR-TKI treatment. Because of molecular heterogeneity between plasma and CSF samples, assessing *EGFR*-T790M status in CSF may be an important avenue for determining suitability of osimertinib treatment in patients with CNS metastases where tissue biopsy is not feasible. Dynamic monitoring of the *EGFR* mutational landscape using plasma cfDNA may be an effective tool for predicting clinical outcomes and guiding treatment decisions for patients with NSCLC with CNS metastases.

Disclosure of Potential Conflicts of Interest

H. Bao reports personal fees from Geneseeq Technology Inc. (employment) during the conduct of the study. Y. Shao reports employment with Nanjing Geneseeq Technology Inc. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

AstraZeneca had no role in design and conduct of the study; collection, management, analysis, and interpretation of the data; or the decision to submit the article for publication.

Authors' Contributions

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Acknowledgments

This study was partially funded by AstraZeneca Pharmaceuticals. The study was also supported by funds from The National Key Research and Development Projects of China (2018YFC1312201), the Key Research and Development Program of Shandong Province (2016CYJS01A03), the Innovation Project of Shandong Academy of Medical Sciences (2019-04), and the Academic Promotion Program of Shandong First Medical University (2019ZL002). Medical writing support was provided by Kristen Perry (Nucleus Global) and funded by AstraZeneca China. We thank the patients and their families, staff members at all study sites, and investigators, pharmacists, and clinical research associates who helped with this trial. We also thank AstraZeneca, China for scientific support (Yao Wu, Yan Hu, Sheng-Mei Feng, Fei Liu, Hong-Yan Shang, Zhi-Gang Ma, Li Xu, and Hai-Ying Yang).

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Received May 28, 2020; revised July 16, 2020; accepted August 13, 2020; published first August 17, 2020.

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