



Original Article

A Phase II Trial of Osimertinib as the First-Line Treatment of Non–Small Cell Lung Cancer Harboring Activating *EGFR* Mutations in Circulating Tumor DNA: LiquidLung-O-Cohort 1

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Purpose Osimertinib is a potent, irreversible third-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor for both *EGFR*-activating and T790M resistant mutation. The treatment efficacy of osimertinib was assessed in previously untreated patients with metastatic non–small cell lung carcinoma (NSCLC) harboring activating *EGFR* mutations in circulating tumor DNA (ctDNA) as well as tumor DNA.

Materials and Methods Patients with activating *EGFR* mutations in their tumor DNA underwent screening with ctDNA analysis using Mutyper and Cobas v2 assays. Enrolled subjects received osimertinib 80 mg, once daily. Primary endpoint was objective response rate (ORR) and secondary endpoints were ctDNA test sensitivity, progression-free survival (PFS), duration of response (DoR), and safety.

Results Among 39 screened patients, 29 were ctDNA positive for activating *EGFR* mutations and 19 were enrolled (ex19del, n=11; L858R/L861Q, n=7; G719A, n=1). Median age was 70 and most patients had brain metastases (15/19, 79%). ctDNA test sensitivity for activating *EGFR* mutations was 74% using both methods and 62% (Mutyper) or 64% (Cobas v2) for individual methods. ORR was 68% (13/19), median PFS was 11.1 months (95% confidence interval [CI], 0.0 to 26.7), and median DoR was 17.6 months (95% CI, 3.5 to 31.7). ORR and median PFS were significantly superior with ex19del (91%; 21.9 months; 95% CI, 5.5 to 38.3) than with L858R/L861Q (43%; 5.1 months; 95% CI, 2.3 to 7.9). One patient discontinued the drug because of drug-related interstitial pneumonitis.

Conclusion Osimertinib had favorable efficacy in the first-line treatment of metastatic NSCLC harboring activating *EGFR* mutations in ctDNA as well as tumor DNA.

Key words Osimertinib, *EGFR* mutation, Circulating tumor DNA, Non–small cell lung carcinoma

Introduction

Osimertinib is a potent, irreversible third-generation epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) for both *EGFR*-activating and T790M resistant mutations [1-7]. In the FLAURA and AURA trials, osimertinib showed promising efficacy in treatment-naïve non–small cell lung cancer (NSCLC) patients with activating *EGFR* mutations and had a favorable safety profile compared with the first-generation EGFR-TKIs [5-8]. In tissue-based assays, the objective response rate (ORR) ranged from 67% to 80%, the median duration of response (DoR) ranged from 17.2 to 19.3 months, and the median progression-free survival (PFS) ranged from 18.9 to 22.1 months in first-line treatment with osimertinib (80 mg). Furthermore, previous studies showed improved activity and efficacy of osimertinib against brain

metastasis, compared with platinum-based chemotherapy or standard EGFR-TKIs [4,5,8-10]. Thus, osimertinib is currently becoming the standard of care for the first-line treatment of *EGFR*-mutated NSCLC.

Circulating tumor DNA (ctDNA) can be used as a suitable substitute for tumor DNA in the analysis of *EGFR*-activating and T790M mutation status. Moreover, plasma genotyping using ctDNA has been shown to be a highly sensitive and specific technique for the detection of *EGFR* mutations, with an excellent positive predictive value [11-15]. In our previous study, in which osimertinib was administered as a second-line treatment, the sensitivity of plasma ctDNA tests for *EGFR*-activating and T790M mutations was 65% (52/80) and 57% (21/37), respectively, and osimertinib showed favorable ORR (67%) [14], with almost equivalent efficacy to that of tissue analysis. In a recent report on tissue and plasma *EGFR* muta-

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tion analysis in the FLAURA trial, the sensitivity of plasma tests for the detection of exon 19 deletion (ex19del) and the L858R mutation was 79% and 68%, respectively [16].

Although osimertinib showed favorable efficacy in NSCLC patients with *EGFR*-activating and T790M resistant mutations detected in ctDNA, there is a concern that patients with tumor DNA shedding tend to have a poorer outcome compared to those without tumor DNA shedding. In subgroup analysis of FLAURA and AURA3 trials, the median PFS in the osimertinib arm was shorter in ctDNA-positive vs. -negative patients (FLAURA, 15.2 months vs. 23.5 months; AURA3 trials, 8.2 months vs. 10.1 months) [4,16]. In an exploratory analysis in the BELIEF trial of erlotinib and bevacizumab, detection of *EGFR* mutations in ctDNA was associated with poor survival at any time point during treatment (baseline, response evaluation, and progression) [17].

This trial aimed to assess the treatment efficacy of osimertinib in previously untreated patients with these metastatic NSCLCs which shed the tumor *EGFR*-activating mutations into circulation.

Materials and Methods

1. Trial design and subjects

This was a phase II, open-label, single-center study of osimertinib in *EGFR*-TKI-naïve patients with metastatic NSCLC harboring activating *EGFR* mutations detected in both ctDNA and tumor DNA. Between February 2017 and March 2018, we recruited patients who were histologically or cytologically diagnosed with NSCLC harboring activating *EGFR* mutations (ex19del, L858R, L861Q, and G719X), but without prior exposure to *EGFR*-TKIs.

All patients were required to provide a blood sample at screening, to test for activating *EGFR* mutations in plasma. Sample collection and subsequent ctDNA analysis were performed as described for the ctDNA T790M mutation cohort (Liquid-Lung-O Cohort 2) [14]. Fifteen milliliters of peripheral blood was withdrawn and centrifuged immediately before storage. ctDNA extraction and *EGFR* mutation analysis were performed using the PANA Mutyper R *EGFR* assay (Mutyper, Panagene Inc., Daejeon, Korea), based on peptide nucleic acid (PNA)-mediated polymerase chain reaction (PCR) clamping and melting technology and the Cobas *EGFR* Mutation Test v2 (Cobas v2, Roche Molecular Systems, Pleasanton, CA), based on a real-time PCR technique. Patients who harbored an activating *EGFR* mutation, detected by either of the two methods, were enrolled in this trial. For genotyping tumor tissue and cytology samples, the PNA Clamp *EGFR* mutation detection kit (Panagene Inc.) was used (Fig. 1).

Eligibility criteria are provided in detail in Supplementa-

ry Material, and key criteria are as follows: age > 18 years, locally advanced or metastatic NSCLC, no prior exposure to *EGFR*-TKIs, activating *EGFR* mutations detected from tumor tissue or cytology specimen and ctDNA by Mutyper or Cobas test, World Health Organization performance status of 0-2, and a life expectancy \geq 12 weeks. Patients with central nervous system (CNS) metastases were enrolled if their disease was asymptomatic or stable after local therapy, including surgery or radiotherapy, before the first dose of osimertinib.

The trial was registered at clinicaltrials.gov (NCT02769286).

2. Trial procedure, assessment, and treatment

Eligible patients received 80 mg of osimertinib once a day, regardless of food ingestion, and treatment continued until disease progression, as defined by the Response Evaluation Criteria in Solid Tumors (RECIST) ver. 1.1 [18]; unacceptable toxicity; or another valid reason for ceasing treatment occurred. Response evaluation was performed every 8 weeks for the first three assessments (24 weeks), followed by every 12 weeks for subsequent assessments. Regular brain imaging was performed in patients with known brain metastases. For the rest of the patients, brain imaging was only performed when there were symptoms or signs of suspected CNS metastasis. Patients could continue osimertinib after RECIST 1.1-defined progression if they maintained clinical benefits, as assessed by an investigator.

The primary objective was determining the ORR (assessed by RECIST 1.1) of osimertinib in *EGFR*-TKI-naïve NSCLC patients with activating *EGFR* mutations detected in ctDNA. The secondary objective was to compare the sensitivity of the Mutyper and Cobas v2 methods for detecting *EGFR* mutations in ctDNA. In addition, PFS, DoR, and the safety profile with tolerability parameters of osimertinib were measured. PFS was defined as the time (in months) from the first dose of osimertinib until objective disease progression or death, regardless of whether the patient was withdrawn from therapy or received another anticancer therapy prior to progression. DoR was defined as the time (in months) from the documentation of the tumor response to disease progression or death in a patient who had the best overall complete or partial remission response.

Adverse events (AEs) were measured from the beginning of drug administration, throughout the treatment period, until 28 days after the last dose of osimertinib. AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), ver. 4.0. If a patient experienced an AE of CTCAE grade 3 or higher and/or unacceptable toxicity (any grade) that was considered to be associated with osimertinib, dosing could be interrupted for up to 3 weeks. If the toxicity resolved or reverted

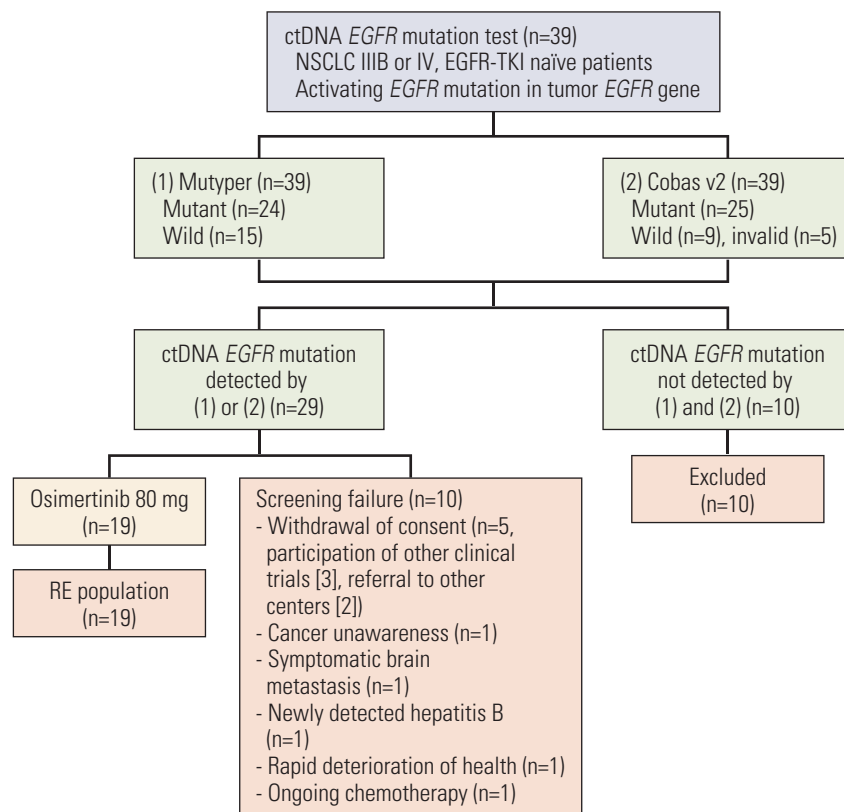


Fig. 1. Study subjects. ctDNA, circulating tumor DNA; EGFR-TKIs, epidermal growth factor receptor tyrosine kinase inhibitors; NSCLC, non-small cell lung cancer; RE, response-evaluable.

to CTCAE grade ≤ 2 within 3 weeks of onset, osimertinib could be restarted at the same dose (80 mg, daily) or a lower dose (40 mg, daily), excluding cases with any grade of pulmonary toxicity, symptomatic corrected QT interval prolongation, or corneal ulceration. Once a dose had been reduced, it was not increased in future cycles.

3. Statistical analysis

To prove a 60% ORR of osimertinib, compared to the 30% ORR in the null hypothesis, and considering a 10% drop-out rate, 19 patients were required to be enrolled in this study (H_0 proportion, 0.3; H_1 proportion, 0.6; sample size, 17; power, 0.8; significance, 0.05; one-sided). The value of the ORR in the null hypothesis (30%) was based on the ORR of platinum doublet chemotherapy as first-line treatment in cases where the *EGFR* mutation was not detected by tumor or ctDNA genotyping. The sensitivity of the *EGFR* mutation test using ctDNA was expected to be 60% among the screening population [19-21]. Thus, it was calculated that 32 patients ($19/0.6=32$) needed to be screened.

Baseline characteristics and safety data were analyzed in an intent-to-treat (ITT) population, which was defined as the

subjects who had received at least one dose of treatment and for whom AEs were monitored ($n=19$) (Fig. 1). According to the study protocol, the ORR was assessed in the response-evaluable (RE) population, defined as subjects who had received at least one dose of treatment and for whom response evaluations were available. PFS and DoR were analyzed in the ITT population. In this study, the ITT and RE populations were identical.

Intergroup comparisons were performed using a Mann-Whitney U test for continuous variables and Pearson's chi-square or Fisher exact tests for categorical variables. Survival times were estimated for each group using the Kaplan-Meier method. Statistical analyses were performed using SPSS ver. 25 (IBM Corp., Armonk, NY) and p-values < 0.05 were used to indicate statistical significance.

Results

1. Patient characteristics

Thirty-nine patients with *EGFR*-activating mutations detected in tumor DNA were screened from February 2017

Table 1. Baseline characteristics

Characteristic	Value (n=19)
Age (yr)	70 (32-84)
Sex (female/male)	13 (68)/6 (32)
Never smokers	16 (84)
WHO performance status ^{a)}	
0/1/2	1(5)/16 (84)/2 (11)
Histology, adenocarcinoma	19 (100)
Stage IV (by 8th TNM)	19 (100)
Within the thoracic cavity (M1a)	1 (5)
Single extrathoracic metastasis ^{b)} (M1b)	3 (16)
Multiple extrathoracic metastases (M1c)	15 (79)
Brain metastasis	15 (79)
Leptomeningeal metastasis	1 (5)
Brain RT before start of osimertinib	7 (37)
No preceding brain RT (reasons)	8 (42)
Small lesion	6
Old age with poor performance	2
Asymptomatic	2
Line of treatment, 1st	19 (100)
Type of EGFR mutation ^{d)}	
ex19del	11 (58)
L858R/L861Q	7 (37)
G719A	1 (5)

Values are presented as median (range) or number (%). EGFR, epidermal growth factor receptor; RT, radiotherapy. ^{a)}A World Health Organization (WHO) performance status of 0 indicates that the patient is fully active and able to carry out all normal activities without restrictions. A WHO performance status of 1 indicates that the patient is restricted in physically strenuous activity, but is ambulatory and able to carry out work of a light or sedentary nature, such as light housework or office work. A WHO performance status of 2 indicates that the patient is ambulatory and capable of all self-care, but unable to carry out any work activities for more than 50% of waking hours, ^{b)}All cases were brain metastases, ^{c)}Results of the tissue-based test at diagnosis.

to March 2018. The ctDNA of 29 patients was found to be positive for an EGFR-activating mutation and 19 patients were finally enrolled (ex19del, n=11; L858R/L861Q, n=7; G719A, n=1). Ten patients were excluded for the following reasons: withdrawal of consent (n=5), cancer unawareness (n=1), symptomatic brain metastasis (n=1), newly detected hepatitis B (n=1), rapid deterioration of health (n=1), and ongoing chemotherapy (n=1) (Fig. 1). Among them, six patients received other EGFR-TKI therapy (gefitinib in five patients and afatinib in a patient).

Baseline characteristics of the enrolled patients are shown in Table 1. The median age was 70 years (range, 32 to 84 years) and female and patients without a history of smoking were dominant. The majority of patients had brain metastases (15/19, 79%) and approximately half of these patients (7/15) received brain radiotherapy before the initiation of osimertinib.

2. Sensitivity of tests for detecting activating EGFR mutations in ctDNA

Activating EGFR mutations were detected using both Mutyper and Cobas v2 methods in 20 cases, only using the Mutyper method in four cases, and only using the Cobas v2 method in five cases (Table 2). There were two cases with discordant mutation types in ctDNA compared with the tissue genotyping results (case 1 with the Mutyper method and case 2 with both methods). The sensitivity of the ctDNA test for activating EGFR mutations was 74% when using both tests, 62% when using only the Mutyper test, and 64% when using only the Cobas v2 test. The concordance rate between two tests in 19 enrolled patients was 68% (13/19) (Table 3). Discordant cases were as follows: wild-type or invalid result in one test (n=4), different mutation type (n=2) which were discordant with the result of tissue genotyping.

3. Response and survival

The response to osimertinib was evaluable in all enrolled

Table 2. Sensitivity of activating EGFR mutation tests using circulating tumor DNA

EGFR mutation	Tissue	Plasma ctDNA				
		Mutyper		Cobas v2		
		Mutant	Wild-Type	Mutant	Wild-Type	Invalid
Mutant	39 (100)	24 (62)	15 (39)	25 (64)	9 (23)	5 (13)
Discordant with tissue						
Case 1	ex19del	ex19del/T790M	-	ex19del	-	-
Case 2	G719X	G719A	-	ex19del	-	-
Sensitivity		24/39 (62)	-	25/39 (64)	-	-
Sensitivity (Mutyper- or Cobas v2-positive)		29/39 (74)	-	-	-	-

Values are presented as number (%). ctDNA, circulating tumor DNA; EGFR, epidermal growth factor receptor.

Table 3. Concordance of activating *EGFR* mutation between two tests using circulating tumor DNA

Concordance in enrolled patients	Cobas v2						Total
	ex19del	L858R/L861Q	ex19del/T790M	G719A	Wild-Type	Invalid	
Mutyper							
ex19del	9	-	-	-	-	-	9
L858R/L861Q	-	4	-	-	1	2	7
ex19del/T790M	1	-	-	-	-	-	1
G719A	1	-	-	-	-	-	1
Wild-type	1	-	-	-	-	-	1
Invalid	-	-	-	-	-	-	0
Total	12	4	0	0	1	2	19

EGFR, epidermal growth factor receptor.

patients. Partial remissions were observed in 13 cases (ORR 68%) and the disease control rate (DCR) was 95% (Table 4, Fig. 2). According to *EGFR* mutation subtype identified by tumor genotyping, patients with the ex19del mutation showed more favorable ORR than those with L858R/L861Q mutations (91% vs. 43%, $p=0.032$). In patients with known brain metastasis, the DCR of the brain metastasis was 100% (15/15).

The final analysis of PFS and DoR was performed at 79% maturity (15/19). The data cut-off date was December 4, 2019 and the median follow-up duration was 25.0 months (95% confidence interval [CI], 23.9 to 26.1). The median PFS and DoR of the enrolled patients were 11.1 months (95% CI, 0.0 to 26.7) and 17.6 months (95% CI, 3.5 to 31.7). Excluding the patients with concomitant *EGFR* mutation and anaplastic lymphoma kinase (*ALK*) rearrangement ($n=2$), ORR was 77% (13/17) and median PFS was 19.2 months (95% CI, 5.9 to 32.5). Most patients showed objective responses within two cycles of osimertinib. The ORR was greater and PFS time was longer in patients without brain metastasis than in patients with brain metastasis (Table 4, S1A Fig.), but the difference was not statistically significant. Based on tumor genotyping, patients with the ex19del mutation (21.9 months; 95% CI, 5.5 to 38.3) showed a more favorable median PFS than patients with the L858R/L861Q mutations (5.1 months; 95% CI, 2.3 to 7.9; $p=0.004$) (S1B Fig.). Data on overall survival were immature at the time of this analysis (21% maturity).

The response to osimertinib was not significantly different according to ctDNA genotyping methods. The ORR and DCR, according to positive results of *EGFR* mutation tests, were as follows: both Mutyper- and Cobas v2-positive, 73% and 93%, respectively; Mutyper only, 67% and 100%, respectively; and Cobas v2 only, 0% and 100%, respectively ($p > 0.05$) (Table 4). With the exclusion of cases that were discordant in ctDNA mutation type (Table 2), ORR showed a sig-

nificant difference according to ctDNA *EGFR* mutation type (ex19del, 100% vs. L858R/L861Q, 25%; $p=0.014$).

4. Pattern of progression and subsequent treatment after osimertinib

Four patients were still receiving osimertinib at the time of data cut-off, and 15 patients discontinued the first-line treatment due to progression (Fig. 3A). Among the patterns of progression, the growth of the primary tumor was the most common, followed by the aggravation of non-target lesions, such as in the bone, pleura, brain, and lymph nodes. All patients with progression to the brain had brain metastases at baseline (Fig. 3B). In total, nine patients (47%) started a second-line therapy after the discontinuation of osimertinib, with cytotoxic chemotherapy and EGFR-TKI other than osimertinib being the most common options (Fig. 3A). Five patients (26%) entered a third-line therapy and all of these patients are still being treated (Fig. 3C).

5. Toxicity and safety

A summary of the toxicity profiles of all enrolled patients who received osimertinib is shown in Table 5. AEs occurred in 18 of 19 patients (95%) and grade 3 or 4 events developed in six patients (32%). Only one patient experienced drug-related interstitial pneumonitis of grade 3, and this resulted in drug discontinuation. The majority of AEs were gastrointestinal and skin manifestations. All AEs associated with osimertinib are described in Table 5.

Discussion

This was a prospective trial for evaluating the treatment efficacy of osimertinib in previously untreated patients with advanced or metastatic NSCLC, harboring activating *EGFR*

Table 4. Clinical efficacy of osimertinib treatment according to brain metastasis

Efficacy	Total (n=19)	Both Mutyper and Cobas v2 (n=15)	Mutyper only (n=3)	Cobas v2 only (n=1)	p-value	Without BM (n=4)	With BM (n=15)	p-value
Type of response, n (%)								
Complete	0	0	0	0	0.501	0	0	0.311
Partial	13 (68)	11 (73)	2 (67)	0		4 (100)	9 (60)	
Stable disease	5 (26)	3 (20)	1 (33)	1 (100)		0	5 (33)	
Progression	1 (5)	1 (7)	0	0		0	1 (7)	
Best % change in target lesion from baseline, median (range)	-40.6 (-82.0 to 51.7)	-44.4 (-82.0 to 51.7)	-39.3 (-44.6 to -26.2)	-	0.578	-47.9 (-82.0 to -41.5)	-40.0 (-68.4 to 51.7)	0.072
Objective response rate, n (%)	13/19 (68)	11/15 (73)	2/3 (67)	0/1 (0)	0.311	4/4 (100)	7/13 (60)	0.255
ex19del	10/11 (91) ^{a)}	9/9 (100) ^{b)}	-	0/1 (0)		3/3 (100)	7/8 (88)	
L858R/L861Q	3/7 (43) ^{a)}	1/4 (25) ^{b)}	2/3 (67)	-		1/1 (100)	2/6 (33)	
G719A	0/1 (0) ^{a)}	- ^{b)}	-	-		-	0/1 (0)	
Disease control rate, n (%)	18/19 (95)	14 (93)	3 (100)	1 (100)	0.869	4/4 (100)	14/15 (93)	> 0.99
CNS response^{c)}, n (%)								
Complete ^{d)}	4 (27)	4 (33)	0	0	0.754	-	4 (27)	-
Non-progression	11 (73)	8 (67)	2 (100)	1 (100)		-	11 (73)	
Progression	0	0	0	0		-	0	
PFS, median (95% CI, mo)	11.1 (0.0 to 26.7)	-	-	-	-	19.2 (2.5 to 35.9)	11.1 (7.4 to 14.8)	0.459
Time to response, median (95% CI, mo)	1.7 (1.6 to 1.8)	-	-	-	-	1.6 (1.6 to 1.8)	1.7 (1.6 to 1.8)	0.666
Duration of response, median (95% CI, mo)	17.6 (3.5 to 31.7)	-	-	-	-	17.6 (0.6 to 34.6)	9.3 (3.7 to 14.9)	0.628

BM, brain metastasis; CI, confidence interval; CNS, central nervous system; ctDNA, circulating tumor DNA; PFS, progression-free survival. ^{a)}Results of the tissue-based test at diagnosis (p=0.032). ^{b)}Results of the ctDNA test at diagnosis (p=0.014). Discordant cases were excluded (n=2). ^{c)}All cases were evaluated as non-target lesions, ^{d)}No cases were treated with radiation to the brain.

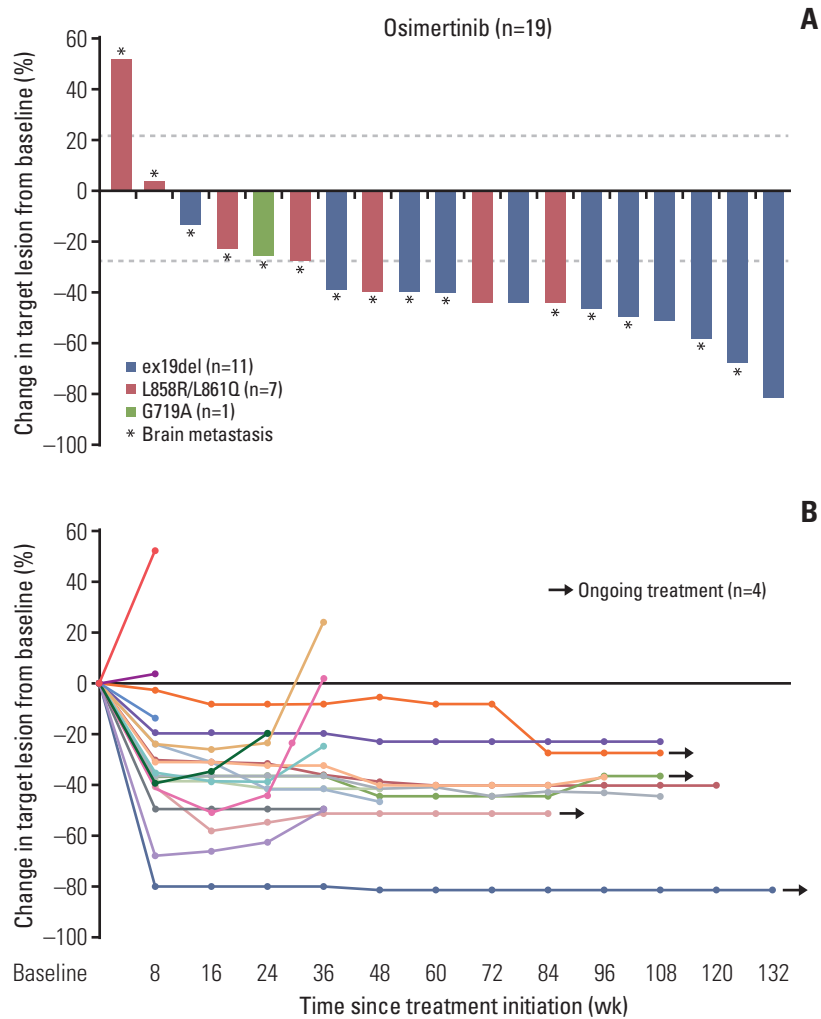


Fig. 2. Response to osimertinib. Waterfall plot (A) and Spider plot (B), according to the Response Evaluation Criteria in Solid Tumors.

mutations in both ctDNA and tumor DNA. In the present study, osimertinib showed favorable efficacy and a safety profile similar to that from previous trials assessing osimertinib as the first-line treatment of *EGFR*-mutated NSCLC [5-8].

An allele-specific PCR assay (Cobas v2) is the only FDA-approved assay for plasma *EGFR* genotyping, and recent advances in highly sensitive technologies have led to the development of novel assays using ctDNA [22]. In the present study, we performed PCR-based analysis of activating *EGFR* mutations. The sensitivity of the ctDNA tests was 74% when using both methods and 62% (Mutyper) or 64% (Cobas v2) when using either method individually. This study showed higher sensitivity for activating *EGFR* mutations compared with our previous trial (65%) [14] and a similar result to that by ctDNA analysis in the FLAURA trial (68%-79%) [16]. Taken together, we demonstrated that plasma *EGFR* genotyping can be a screening tool for the de-

tection of activating *EGFR* mutations at initial diagnosis, as well as T790M resistance mutations at progression after prior *EGFR*-TKI treatment.

There was a case with discordant mutation type in ctDNA between Mutyper (G719A) and Cobas v2 (ex19del), while the result of tissue genotyping was G719X, thus Cobas testing might need a quality control procedure. However, all other cases showed concordant results between the tests and the positive results were translated into clinical efficacy. Considering a previous study of Oxnard et al. [15] highlighting that a well-validated assay has negligible false positives and the clinical outcome would be the best reference standard for a non-invasive assay, the discrepancy may also be explained by a combined *EGFR* mutation attributed to intratumoural heterogeneity.

In the demographics of the present study, the median age (70 vs. 64) and the proportion of patients with known brain

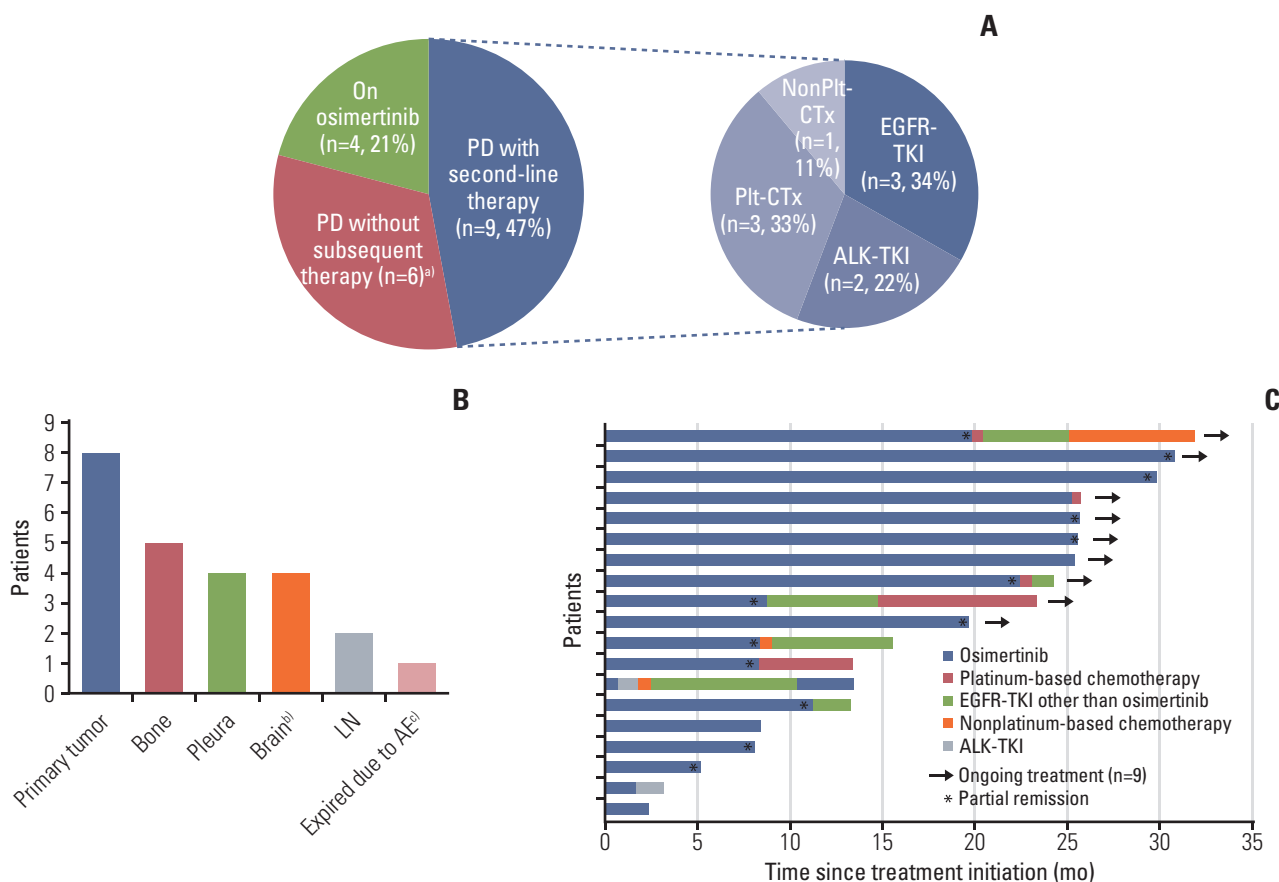


Fig. 3. Subsequent treatment after osimertinib. Overview of the pattern of progression and subsequent treatment after osimertinib. Treatment status of enrolled patients and sequential treatment following first-line osimertinib (A). Patterns of progression after osimertinib (B). The numbers in parentheses refer to the corresponding number of patients. Swimmer plot for sequential treatment after osimertinib (C). AE, adverse event; ALK, anaplastic lymphoma kinase; CTx, chemotherapy; EGFR, epidermal growth factor receptor; LN, lymph node; PD, progressive disease; Plt, platinum; TKI, tyrosine kinase inhibitor. ^{a)}Best supportive care (n=4), death (n=1), referral to another center (n=1), ^{b)}All patients had brain metastasis at baseline, ^{c)}Death due to drug-related interstitial pneumonia.

metastasis (79% vs. 19%) were higher compared with that in the osimertinib arm of the FLAURA trial [5]. In FLAURA and AURA3 trials, osimertinib showed a clear benefit in PFS regardless of CNS metastasis, but there were numerical differences: 19.1 months vs. 15.2 months in FLAURA and 10.8 months vs. 8.5 months in AURA3 [4,5], according to the presence of brain metastases. In the present study, median PFS also showed a numerical difference according to brain metastasis (19.2 months vs. 11.1 months, $p=0.459$). Patients with *EGFR*-mutated NSCLC who have brain metastasis tend to have a poorer prognosis compared to those without brain metastasis [23]. However, as reported in the FLAURA trial, osimertinib showed favorable CNS efficacy, in terms of ORR, PFS, and DoR compared with standard (first-generation) *EGFR*-TKIs, and the benefit was also shown in patients with suspected leptomeningeal metastasis [10]. In the present

study, all patients with progression to the brain after osimertinib treatment had brain metastasis at baseline.

In the subgroup analysis of the FLAURA trial, although there was no difference in hazard ratio (HR) according to the detection of ctDNA *EGFR* mutations [5], the median PFS in the osimertinib arm was numerically shorter in ctDNA *EGFR* mutation-positive vs. -negative patients (15.2 months vs. 23.5 months) [16]. This tendency of worse outcomes in shedding tumors was also seen in the osimertinib arm of the AURA3 trial (8.2 months vs. 10.1 months) [4]. This process of ctDNA shedding is known to be related to tumor biology, such as mitotic rate, necrosis, and the overall burden of the metastatic tumor [22].

The median PFS in the present study was shorter than that in the FLAURA trial. This could be associated with the baseline characteristics of the patients enrolled in the present

Table 5. Safety profile and all adverse events associated with osimertinib treatment

All events	Any grade ^{a)} (n=19)	Grade ≥ 3 ^{a)} (n=19)
Adverse events (AEs)	17 (90)	4 (21)
Drug-related AEs	12 (63)	1 (5) ^{a)}
AEs leading to dose reduction	0	
AEs leading to drug discontinuation ^{b)}	1 (5)	
Serious AEs	7 (37)	
Serious AEs, drug-related ^{b)}	1 (5)	
Rash or acne	3 (16)	0
Pruritus	5 (26)	0
Dry skin	2 (11)	0
Mucositis	5 (26)	0
Paronychia	3 (16)	0
Stomatitis	1 (5)	0
Conjunctivitis	3 (16)	0
Epigastric discomfort	2 (11)	1 (5)
Nausea	2 (11)	0
Vomiting	4 (21)	0
Diarrhea	2 (11)	0
Constipation	2 (11)	0
Headache	3 (16)	0
Dizziness	2 (11)	1 (5)
Unconsciousness	1 (5.3)	1 (5)
Seizure	1 (5.3)	0
Back pain	3 (16)	0
LFT ^{c)} elevation	2 (11)	0
Anemia	1 (5)	1 (5)
Hypocalcemia	2 (11)	1 (5)
Dyspnea	2 (11)	0
Cough	1 (5)	0
Sputum	1 (5)	0
Rhinorrhea	2 (11)	0
Pneumonitis ^{b)}	3 (16)	2 (11)
Pleural effusion	1 (5)	0
Edema	1 (5)	0
Myalgia	1 (5)	0
Urinary tract infection	1 (5)	0
Hair loss	1 (5)	0

Values are presented as number (%). LFT, liver function test.

^{a)}Patients with multiple events in the same category were only counted once in that category. Patients with events in more than one category were counted once in each category, ^{b)}Interstitial pneumonitis, ^{c)}Aspartate aminotransferase or alanine aminotransferase.

study, the majority of whom were older and had developed brain metastases and all of whom were ctDNA-positive. Based on these characteristics, the survival benefit of osimertinib would be underestimated in the present study; how-

ever, this study also provided real-world evidence that osimertinib has favorable efficacy and a tolerable safety profile, even in older patients with brain metastases.

Although the existence of significant differences in the efficacy of EGFR-TKIs between patients with ex19del and L858R mutations may be controversial, most studies of first- and second-generation EGFR-TKIs have demonstrated that patients with ex19del mutations have better ORR and survival rates compared to those with L858R or other uncommon mutations [24,25]. In the FLAURA trial, a subgroup analysis of PFS showed little difference in HR according to *EGFR* mutation subtype (0.43 for ex19del and 0.51 for L858R), while the median PFS in the osimertinib arm was numerically longer in patients with ex19del vs. L858R mutations (21.4 months vs. 14.4 months) [5]. Furthermore, the osimertinib arm showed an OS benefit, compared with standard EGFR-TKIs, in patients with the ex19del mutation (HR, 0.68; 95% CI, 0.51 to 0.90), while there was no difference in OS in patients with the L858R mutation (HR, 1.00; 95% CI, 0.71 to 1.40) [7]. However, in a report on two treatment-naïve cohorts of the AURA phase I trial with different doses of osimertinib (80 mg and 160 mg), there was no difference in median PFS time according to mutation subtype (ex19del, 23.4 months vs. L858R, 22.1 months) [6]. In the present study, patients with the ex19del mutation showed more favorable ORR and PFS than those with the L858R/L861Q mutations, which was consistent with the results of the FLAURA phase III trial. Therefore, osimertinib may be strongly recommend as the first-line treatment for patients with *EGFR*-mutated NSCLC, particularly for those harboring the ex19del mutation in tumor tissue or ctDNA.

The patterns of subsequent treatment following first-line osimertinib treatment in the present study were similar to those of the FLAURA trial [7] and were as follows: subsequent therapy, 47% vs. 48%; no subsequent therapy, 32% vs. 31%; ongoing osimertinib, 21% vs. 22% in the present study and the FLAURA trial, respectively (Fig. 3A). Although two patients received the ALK inhibitor, crizotinib, as the second-line treatment, they had only weakly positive fluorescence results in *in situ* hybridization analysis of ALK (15% and 20%) and the duration of treatment was only 1.5 and 1.1 months.

In the present study, for the majority of patients who received treatment subsequent to osimertinib, the duration of treatment was less than 6 months (Fig. 3C). In the standard EGFR-TKI arm of the FLAURA trial, only 31% of patients received osimertinib by cross-over, as the first subsequent therapy [7]. In a real-world study on a Korean cohort, data collected as part of the ASTRIS trial showed that rebiopsy was performed in 60% (112/188) of screened patients. The detection rate for T790M resistance mutations was 53% (48/90) in rebiopsy tissue from these patients with available *EGFR*

mutation analysis, whereas the detection rate was only 30% (33/112) in plasma ctDNA from patients intending to undergo rebiopsy [26]. In addition, 32% (6/19) of patients in the present study discontinued osimertinib and did not receive the first subsequent therapy, owing to deterioration of health or death in approximately 80% (5/6) of these patients. These results suggested that the most effective therapy for patients with advanced *EGFR*-mutated NSCLC should be the initial treatment. As shown in the swimmer plot (Fig. 3C), most patients who had long-term first-line treatment with osimertinib showed favorable responses initially and remained alive at the end of the follow-up period.

Resistance develops in most *EGFR*-mutated NSCLC patients receiving *EGFR*-TKIs as the first-line treatment. As the use of osimertinib is expected to increase, the mechanism of resistance to osimertinib needs to be identified. In the cohort of the phase I component of the AURA trial, genomic resistance mutations, analyzed by next-generation sequencing of plasma samples, were identified in nine patients who received osimertinib as the first-line treatment and then showed progression [6]. The resistance mutations detected in post-progression plasma samples included *MET*, *EGFR*, and *KRAS* amplifications and *PIK3CA* and *KRAS* mutations, which was similar to previously reported resistance mechanisms of second-line osimertinib treatment. In the present study, we could not perform tissue rebiopsy in any of the patients who progressed after osimertinib treatment, while ctDNA analysis by plasma reverse transcription polymerase chain reaction was performed in four patients who had the ex19del mutation, detected by tissue genotyping at baseline. In the plasma genotyping results, known mutations (ex19del) remained in two patients, but changed to the wild-type sequence in another two patients. A systematic analysis using a larger plasma sample cohort, combined with tissue-based analysis, would be necessary to clarify these results.

There were several limitations to this study. First, the low number of patients were enrolled although the sample size was calculated based on the rational statistics. The comparison with the results from a randomized phase III trial (FLAURA) might overdraw certain issues for the study populations or the efficacy of osimertinib. Second, a serial follow-up of blood samples was not performed during osimertinib treatment, and the monitoring of response or resist-

ance using ctDNA was not attempted by the study protocol. According to an exploratory analysis of FLAURA, clearance of plasma *EGFR* mutation after 3- or 6-weeks of osimertinib and comparator *EGFR*-TKI therapy were associated with improvement in PFS [27].

In conclusion, osimertinib had favorable efficacy as the first-line treatment of metastatic NSCLC in individuals harboring activating *EGFR* mutations, detected in ctDNA as well as tumor DNA. The results of this phase II prospective trial support the up-front use of osimertinib, having favorable efficacy and tolerability, even in elderly patients with brain metastases and tumor DNA shedding into the circulation.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (<https://www.e-crt.org>).

Ethical Statement

This study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by institutional ethics review boards (CNUHH-2016-065) and the Korean Ministry of Food and Drug Safety (30956). All patients were required to provide written informed consent before participating in this study.

Author Contributions

Conceived and designed the analysis: Kim YC.
Collected the data: Park CK, Cho HJ, Choi YD, Oh IJ, Kim YC.
Contributed data or analysis tools: Park CK, Oh IJ, Kim YC.
Performed the analysis: Park CK, Oh IJ, Kim YC.
Wrote the paper: Park CK, Cho HJ, Choi YD, Oh IJ, Kim YC.

Conflicts of Interest

Panagene and Roche molecular diagnostics, Korea supported this study as providing screening kits for ctDNA extraction and *EGFR* mutation analysis. Those companies had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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