Assessment of Anti-tumor Efficacy of Osimertinib in Non–Small Cell Lung Cancer Patients by Liquid Biopsy Using Bronchoalveolar Lavage Fluid, Plasma, or Pleural Effusion

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Purpose This study was to evaluate anti-tumor efficacy of osimertinib in patients positive for acquired epidermal growth factor receptor (EGFR) T790M mutation in liquid biopsy using plasma, bronchoalveolar lavage fluid (BALF) or bronchial washing fluid (BWF), and pleural effusion.

Materials and Methods Among patients benefited from previous EGFR–tyrosine kinase inhibitor (TKI) treatment followed by treatment failure, patients in whom T790M mutations are detected in at least one of the samples including tumor tissues, BALF/BWF, plasma, and pleural effusion were enrolled. T790M mutation was detected by extracting cell free DNA from liquid biopsy samples, using PANA Mutyper. Objective response rate (ORR) and progression-free survival (PFS) with osimertinib treatment were evaluated.

Results Between January 2018 and December 2019, 63 patients were enrolled and received osimertinib. Mean age was 63 years, and 38 (60.3%) were female. Twenty-six patients had T790M mutation in both liquid and tissue samples (group A), 19 patients had only in tissue biopsy samples (group B), and 18 patients had T790M mutation only in liquid biopsy samples (group C). ORR in overall population was 63.5%, and was 61.5% in group A, 68.4% in group B, and 61.1% in group C, respectively. Median PFS in overall patients was 15.6 months (95% confidence interval, 10.7 to 24.2). There was no significant difference in ORR or PFS between groups.

Conclusion Osimertinib showed favorable efficacy in lung cancer patients with acquired resistance to prior EGFR-TKI therapies, who screened positive for harboring T790M mutation detected from cell free DNA extracted from plasma, BALF/BWF, and pleural effusion.

Key words Non-small cell lung carcinoma, Osimertinib, T790M, Liquid biopsies

Introduction

First-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) have conferred significant clinical benefits in patients with advanced EGFR mutant non–small cell lung cancer (NSCLC), thus being the standard first-line treatment options. However, majority of patients ultimately develop disease progression after 12-24 months of treatment, most commonly due to acquisition of Thr790Met (T790M) EGFR-TKI resistance mutation [1,2].

Osimertinib is a novel drug that potently inhibits signaling pathways and cellular growth in both EGFR mutation–positive and EGFR /T790M mutation–positive cell lines. Based on the results of the prior AURA phase III study demonstrating an efficacy of the drug with objective response rate (ORR) of 71% and the median progression-free survival (PFS) of 10.1 months [3] and phase III FLAURA trial confirming ORR of 80% and PFS of 18.9 months [4], osimertinib is approved for first-line treatment of patients with metastatic NSCLC harboring EGFR-sensitizing and T790M resistant mutations [5].

In South Korea, positivity of T790M mutation is pre-requisite for reimbursement of the drug for the second-line treatment in EGFR-positive progressive or metastatic NSCLC patients [6]. Accordingly, to diagnose T790M mutation positivity, repeated tumor biopsies should be performed in patients with acquired resistance when those patients develop disease progression following prior therapy with EGFR-TKI. However, such tissue biopsies are invasive methods accompanying discomfort and risk of procedure-associated complications and may not always supply enough tumor tissues for genetic profiling.

To overcome these limitations regarding tissue biopsies, new technologies called ‘liquid biopsy’ using circulating tumor DNA (ctDNA) in plasma have emerged [7-9]. The Korea National Health Insurance Service (NHIS) has covered ctDNA tests for EGFR mutations in advanced NSCLC since 2018, but only plasma or pleural fluid sample is indicated for reimbursement of EGFR-TKIs due to the limited diagnos-
tic efficacy of other types of body fluid [6]. Therefore, efforts to improve diagnostic efficacy and clinical utility of liquid biopsy should be done by diversifying body fluid specimens including especially bronchoalveolar lavage fluid (BALF) or bronchial washing fluid (BWF). Recent studies reported BALF/BWF based EGFR genotyping have superior diagnostic performance to plasma [10,11], but specific detection for T790M mutation was not conducted in these studies and feasibility of liquid biopsy along with clinical response to osimertinib was not confirmed.

Therefore, this study was to evaluate diagnostic performance of liquid biopsy along with anti-tumor efficacy of osimertinib in patients who test positive for T790M mutations in liquid biopsy using at least one of the samples such as plasma, BALF/BWF and pleural effusion (especially focusing on liquid biopsy using BALF/BWF vs. other types of biopsy).

**Materials and Methods**

1. Study population and patient selection

This was a phase II, open-label, single-arm, single-center study to evaluate anti-tumor efficacy of osimertinib in NSCLC in whom T790M mutations are detected by liquid biopsy using at least one of the samples such as plasma, BALF/BWF, and pleural effusion. Among patients diagnosed and treated for NSCLC at Asan Medical Center between January 2018 and December 2019, we prospectively enrolled 63 patients who met following inclusion criteria: (1) patients who are aged ≥ 20 years and histologically or cytologically diagnosed as inoperable stage IIIb or IV NSCLC according to the 7th edition of the TNM staging system by the international association for the study of lung cancer, and patients who understand information about the trial and voluntarily agree to participate in the trial; (2) patients with EGFR sensitizing mutation (E19Del, L858R, L861Q, G719X) positive, who had shown clinical benefits (complete responders [CR] or partial response [PR] and stable disease ≥ 6 months) from EGFR-TKIs and had developed progressive disease; (3) patients in whom T790 mutations are detected in at least one of the samples including tumor tissues, BALF/BWF, and plasma (cell-free DNA), and pleural effusion (cell-free DNA). Patients who received drugs targeting T790M mutations prior to enrolment, who have coexisting malignancies, severe or unstable medical conditions, who previously received other treatments including chemotherapy, radiotherapy, or surgery with less than 2 weeks of time interval at the time of starting study treatment were excluded.

2. Liquid biopsy and tissue sample preparation

All patients willing to be enrolled for the study underwent bronchoscopy with or without endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) for obtaining tumor tissues and BALF/BWF. At least 20 mL of BALF/BWF was taken by instilling 100 mL of sterile 0.9% saline by wedging the bronchoscope at the lung cancer site. If the obtained BALF/BWF specimen was less than 5 mL, an additional specimen was obtained by bronchial washing. Fifteen to twenty milliliters of blood sample was also obtained in heparin bottle from subjects at the time of screening for eligibility. Twenty milliliters of pleural fluid was also obtained in the patients with pleural effusion.

For liquid biopsy samples, centrifugation of samples was performed immediately after collection of the liquid specimens and 1 mL of supernatant was used for ctDNA extraction. ctDNA was purified using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) [12]. The purity and concentration of DNA was measured using a NanoDrop machine (Thermo Fisher Scientific, Waltham, MA). *EGFR* mutation analysis were conducted using PANA Mutyper (Panagene, Daejeon, Korea) with the peptide nucleic acid–mediated PCR clamping method [13] according to the instructions from manufacturers.

DNA of tumor tissue was extracted from paraffin sections, by deparaffinizing sections with xylene and alcohol.

3. Therapeutic methods

Osimertinib was administered as 80 mg once daily, and dose reduction to 40 mg once daily was permitted under physician’s judgement based on individual safety and tolerability. A cycle of study treatment was defined as 28 days, day 1 of next cycle being 29 day of previous cycle, and the time window for each visit being ±7 days. Each cycle was scheduled as D29±7 (cycle 2), D57±7 (cycle 3), D85±7 (cycle 4), D113±7 (cycle 5) from cycle 1 day 1, and then every 8 weeks. Response evaluation was performed every 8 weeks (±7 days) from day 1 of first cycle. Each subject was recommended to continue the study drug until disease progression or manifestation of unacceptable toxicity.

4. Study variables and endpoints

Baseline demographic and clinical characteristics such as age, sex, smoking history, histologic subtype, *EGFR* mutation status, and the presence or absence of previous surgery or irradiation were extracted from each patient’s medical record.

ORR was defined as the proportion of patients achieving a best clinical response to osimertinib of either CR or PR, as recorded in the patient’s medical record, based on Response Evaluation Criteria in Solid Tumors ver. 1.1. PFS was defined

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Cancer Res Treat. 2022;54(4):985-995
as the time (in months) from the first date of Osimertinib treatment until the date of objective disease progression or death, whichever comes first.

Adverse events (AEs) related to osimertinib treatment were reported according to the Common Terminology Criteria for Adverse Events (CTCAE), ver. 4.03. If a patient experienced a CTCAE of grade 3 or higher and/or unacceptable toxicity (any grade) that was associated with osimertinib, drug interruption was permitted for up to 3 weeks. If the toxicity resolved or reverted 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 re-escalated at future cycles.

5. Statistical Analysis
Subject number was calculated using z-test based on the non-inferiority test. We assumed the null hypothesis as ORR 35% and alternative hypothesis as ORR 60%, adopted from the AURA phase I study [14]. We intended to prove the alternative hypothesis that the difference between ORRs would be lower than 0.25 versus the null hypothesis that the difference between ORRs would be higher than 0.25 using the level of significance of 2.5%. When ORR difference is lower than 0.25, 56 subjects were estimated to be needed to have the power of the test of 80% for rejecting the null hypothesis. However, considering a halfway dropout-rate of 10%, a total of 63 subjects were thought to be needed. Among them, given that the likelihood of detecting T790M mutation in TKI-acquired-resistant patients is around 60%, about 105 patients are expected to be tested for T790M mutation status and 63 patients would be administered osimertinib. The diagnostic performance of each method for detecting mutations in plasma or BALF/BWF samples was expressed in terms of the sensitivity, specificity, and accuracy, with the mutation status determined in tissue sample as the reference standard. Analysis variables were summarized and were stratified by the type of biopsy samples which were detected to harbor T790M mutation (tissue or liquid biopsy). We grouped the subjects as they harbor T790M mutation detected in both tissue and liquid biopsy samples (group A), only in tissue sample (group B), or only in liquid biopsy samples (group C). Significant differences in descriptive variables between these groups were assessed with the chi-squared or Fisher exact tests for qualitative variables and Student’s t test for quantitative variables. p < 0.05 was considered statistically significant for all tests. All analyses were conducted using the IBM SPSS ver. 25.0 (IBM Corp., Armonk, NY) or the R statistical package ver. 3.5.3 (Institute for Statistics and Mathematics, Vienna, Austria; http://www.R-project.org).

Fig. 1. Patient flowchart. One hundred twenty-four patients who previously benefited from EGFR-TKI treatment and eventually experienced disease progression were enrolled. From 78 T790M detected in either tissue or liquid biopsy specimens, 63 patients were enrolled and received osimertinib. BALF, bronchoalveolar lavage fluid; ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; NSCLC, non–small cell lung cancer; T790M, (c.2369C>T; p.Thr790Met); TKI, tyrosine kinase inhibitor.
Table 1. Baseline characteristics

|                     | Total  | Group A | Group B | Group C |
|---------------------|--------|---------|---------|---------|
| No.                 | 63     | 26      | 19      | 18      |
| Age (yr)            | 63 (45-84) | 60.3 (47-74) | 63.7 (45-84) | 66.9 (54-81) |
| Female sex          | 38 (60.3) | 15 (57.7) | 11 (57.9) | 15 (66.7) |
| ECOG                |         |         |         |         |
| 0-1                 | 59 (93.7) | 25 (96.2) | 17 (89.5) | 17 (94.4) |
| 2-3                 | 4 (6.3) | 1 (3.8) | 2 (10.5) | 1 (5.6) |
| Previous surgery    | 12 (19.0) | 3 (11.5) | 2 (10.5) | 7 (38.9) |
| Previous RTx        | 21 (33.3) | 10 (38.5) | 7 (36.8) | 4 (22.2) |
| Extrathoracic metastasis | 37 (58.7) | 15 (57.7) | 12 (63.2) | 10 (55.6) |
| Brain               | 18 (28.6) | 9 (34.6) | 4 (21.1) | 5 (27.8) |
| Extrathoracic visceral metastases | 28 (44.4) | 11 (42.3) | 10 (52.6) | 7 (38.9) |
| Coexisting EGFR mutation |     |         |         |         |
| E19del              | 45 (71.4) | 23 (88.5) | 13 (68.4) | 9 (50.0) |
| L858R               | 16 (25.4) | 3 (11.5) | 5 (26.3) | 8 (44.4) |
| G719X               | 2 (3.2) | 1 (3.8) | 1 (5.3) | 0         |
| Other (L861Q, S768I) | 1 (1.6) | 0       | 0       | 1 (5.6) |
| T790M positivity    |         |         |         |         |
| Plasma              | 18      | 13      | 0       | 5        |
| BALF/BWF            | 32      | 19      | 0       | 13       |
| Pleural effusion    | 8       | 4       | 0       | 4        |
| Tissue              | 45      | 26      | 19      | 0        |
| Reason for absence of EGFR mutation test in tissue sample |         |         |         |         |
| Unable to conduct tissue biopsy | - | - | - | 7 (38.9) |
| Inadequate amount of sample | - | - | - | 3 (16.7) |
| No malignant cells  | -       | -       | -       | 4 (22.2) |

Values are presented as number (%) or median (range). EGFR mutation: T790M, (c.2369C>T; p.Thr790Met); E19del, (c.2235del15; p.E746_A750del); L858R, (c.2573T>G; p.Leu858Arg); G719X, (c.2155G>A; p.Gly719Ser); L861Q, (c.2582T>A; p.Leu861Gln); S768I, (c.2303G>T; p.Ser768Ile). Group A, patients who have T790M mutation detected in both tissue and liquid biopsy samples; Group B, patients who have T790M mutation detected only in tissue; Group C, patients who have T790M mutation detected only in liquid biopsy samples. BALF, bronchoalveolar lavage fluid; BWF, bronchial washing fluid; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; RTx, radiotherapy.

Results

1. Clinical characteristics of study population

One hundred twenty-four patients with acquired resistance after treatment with EGFR-TKIs were screened for the T790M resistance mutation in any of samples including tissue, BALF/BWF, plasma, or pleural effusion from January 2018 to December 2019. After screening procedure, 63 patients were finally enrolled and received osimertinib treatment (Fig. 1). Median age was 63 years old (range, 45 to 84 years) and 38 (60.3%) were female. From the enrolled subjects, 56 tissue samples were obtained via bronchoscopy or EBUS-TBNA at the time of screening procedure. Among them, three samples had inadequate amount to perform EGFR mutation test and four specimens showed no malignant cells, being unable to undergo mutation test. Therefore, 45 cases out of 49 showed T790M mutation detected from tissue sample. In terms of liquid biopsy samples, 32 BALF/BWF, 18 plasma, and eight pleural fluid samples had T790M positivity (Table 1).

Among the enrollees, 26 patients had T790M mutation detected in both tissue and liquid biopsy samples (group A), 19 only in tissue sample (group B), and 18 only in liquid biopsy samples (group C) (Fig. 2). Subjects in group C seemed to be older, and had more frequent history of previous surgery and L858R coexistence of EGFR mutation along with T790M mutation compared with group A and B, but there was no statistically significant difference (p-value for age difference=0.356, previous surgery=0.063, and L858R coexistence=0.064) (Table 1).

2. Diagnostic performance of liquid biopsy specimen for detection of EGFR mutation

We compared the diagnostic yields of BALF/BWF and
Fig. 2. Grouping of the subjects according to T790M mutation status in tissue or liquid biopsy samples. T790M positivity by the type of biopsy sample in overall population are shown as a diagram. Group A, patients who have T790M mutation detected in both tissue and liquid biopsy samples; Group B, patients who have T790M mutation detected only in tissue; Group C, patients who have T790M mutation detected only in liquid biopsy samples. BALF, bronchoalveolar lavage fluid; PE, pleural effusion; T790M, (c.2369C>T; p.Thr790Met).

Table 2. Diagnostic performance of liquid biopsy according to EGFR mutation

|                  | T790M            | E19del          | L858R           | All EGFR mutations |
|------------------|------------------|-----------------|-----------------|-------------------|
|                  | Plasma BALF/BWF  | p-value         | Plasma BALF/BWF | p-value           | Plasma BALF/BWF | p-value |
| Sensitivity      | 28.9 (16.4-44.3) | 0.083           | 52.8 (35.5-69.6) | 0.026             | 40.0 (12.2-73.8) | 0.381   |
| Specificity      | 75.0 (19.4-99.4) |                 | 92.3 (64.0-99.8) |                 | 97.4 (86.5-99.9) |         |
| Accuracy, n (%)  | 16/49 (32.7)     |                 | 63.3 (31.4/49)  |                 | 42/49 (85.7)  |         |
| TP               | 13               |                 | 19              |                 | 4               |         |
| TN               | 3                |                 | 12              |                 | 38              |         |
| FP               | 1                |                 | 1               |                 | 1               |         |
| FN               | 32               |                 | 17              |                 | 6               |         |

EGFR mutation: T790M, (c.2369C>T; p.Thr790Met); E19del, (c.2235del15; p.E746_A750del); L858R, (c.2573T>G; p.Leu858Arg). BALF, bronchoalveolar lavage fluid; BWF, bronchial washing fluid; CI, confidence interval; EGFR, epidermal growth factor receptor; FN, false negative; FP, false positive; TN, true negative; TP, true positive.
plasma samples for detecting *EGFR* mutations in 49 cases with adequate tissue samples. Sensitivity for predicting tissue T790M mutation using BALF/BWF was 42.2%, higher compared to that of plasma (28.9%), but was not significantly better (p=0.077). Similar results were shown in detecting tissue L858R (80.0% vs. 40.0%, p=0.381). Sensitivity for E19del and overall *EGFR* mutations was significantly superior using BALF/BWF compared to plasma. Specificity, however, was lower in BALF/BWF (25.0%) for detecting T790M mutation than in plasma (75%). There was no difference in specificity for the diagnosis of E19del or L858R, evaluated by plasma and BALF/BWF (Table 2). When combining the results of BALF/BWF and plasma ctDNA tests, sensitivity for detection of T790M was 51.1%, specificity was 25.0%, and accuracy was 49.0%. The sensitivity for predicting T790M mutation using both BALF/BWF and plasma was significantly higher than using plasma (p < 0.001), but was not significant compared to using BALF/BWF (p=0.125). Similar results were observed in detection of E19del and overall *EGFR* mutations (S1 Table). Ten out of 63 patients underwent additional bronchial washing, and exclusion of these cases did not result any significant difference in diagnosis rate for T790M in BALF/BWF (sensitivity 43.8% [95% confidence interval (CI), 26.4 to 62.3], specificity 25% [95% CI, 0.63 to 80.6], accuracy 41.7%, p=0.830).

3. Clinical efficacy of osimertinib according to T790M positivity status in tissue or liquid biopsy

The response to osimertinib was evaluable in all 63 enrolled patients at the time of data analysis. In the overall population, CR was not observed, PR was observed in 40 patients (ORR, 63.5%) (Table 3, Fig. 3). Subjects with group A (n=26) had ORR of 61.5%, while group B (n=19) and C (n=18) showed ORR of 68.4% and 61.1%, respectively. Although patients who harbor T790M mutation only in tissue have shown the highest ORR among the three groups, the intergroup difference was not significant (p=0.970 comparing A and C, p=0.642 comparing B and C) (Figs. 3 and 4).

Response to osimertinib in patients of group C was not significantly different according to the type of liquid biopsy samples. Patients with T790M detected in both BALF/BWF and plasma, ORR was 100%; for BALF/BWF only, 44.4%; plasma only, 100%; pleural effusion only, 50% (p > 0.05) (S2 Table, S3 and S4 Figs.). ORRs by coexisting *EGFR* mutation status (E19del and L858R) along with T790M mutation (E19del and L858R) was 9.8% (95% CI, 0.06 to 0.46); specificity 90% (95% CI, 0.77 to 0.97), accuracy 90.3%, p=0.830).

Table 3. Clinical efficacy of osimertinib treatment by T790M positivity status in tissue or liquid biopsy samples

|                | Total     | Group A  | Group B  | Group C  |
|----------------|-----------|----------|----------|----------|
| No.            | 63        | 26       | 19       | 18       |
| Type of response |           |          |          |          |
| CR             | 0         | 0        | 0        | 0        |
| PR             | 40        | 16       | 13       | 11       |
| SD             | 21        | 10       | 6        | 5        |
| PD             | 2         | 0        | 0        | 2        |
| Response rate (CR+PR) (95% CI, %) | 63.5 (51.3-75.7) | 61.5 (42.4-80.6) | 68.4 (46.9-89.9) | 61.1 (37.9-84.3) |
| PFS (95% CI, mo) | 15.6 (10.7-24.2) | 10.7 (7.2-16.7) | NR       | 20.3 (11.1-24.4) |

Group A, patients who have T790M mutation detected in both tissue and liquid biopsy samples; Group B, patients who have T790M mutation detected only in tissue; Group C, patients who have T790M mutation detected only in liquid biopsy samples. CI, confidence interval; CR, complete response; NR, not reached to median; PD, progression of disease; PFS, progression-free survival; PR, partial response; SD, stable disease; T790M, (c.2369C>T; p.Thr790Met).
Fig. 4. Tumor response to osimertinib by T790M positivity status in tissue or liquid biopsy samples. Waterfall plot according to Response Evaluation Criteria in Solid Tumors in the response evaluable population are shown by T790M positivity in tissue or liquid biopsy samples. (A) Overall patients (n=63). (B) Group A (n=26): patients who have T790M mutation detected in both tissue and liquid biopsy samples. (C) Group B (n=19): patients who have T790M mutation detected only in tissue. (D) Group C (n=18): patients who have T790M mutation detected only in liquid biopsy samples. PD (blue bar), progression of disease; PR (red bar), partial response; SD (green bar), stable disease; T790M, (c.2369C>T; p.Thr790Met).
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The final analysis of PFS was performed on the data cut-off date of December 3, 2020 and the median follow-up duration was 20.6 months (95% CI, 17.2 to 24.0). The median PFS in overall population was 15.6 months (95% CI, 10.7 to 24.2) (Fig. 5A). PFS according to T790M mutation status of biopsy samples was as follows: group A, 10.7 months (95% CI, 7.2 to 16.7); group B, not reached to median; group C, 20.3 months (95% CI, 11.1 to 24.4). Although patients in group B and C seemed to have numerically better PFS than group A, there were no statistical difference in PFS between groups (p=0.137) (Fig. 5B).

4. Safety assessment of osimertinib

AEs of grade 3 to 5 related to osimertinib treatment developed in six patients (9.5%). Two patients experienced grade 3 neutropenia and one patient experienced grade 3 hyponatremia, which resulted in drug interruption for 2 weeks, and AEs were resolved. One patient had prolongation of QTc interval related to osimertinib, which resolved with permanent drug withdrawal. Pneumonia developed in two patients treated with osimertinib, and worsened despite of drug withdrawal, resulting in death (Table 4).

Discussion

This was the novel prospective trial evaluating the clinical efficacy of osimertinib as 3rd generation EGFR–TKI in patients with NSCLC who harbor EGFR T790M mutation detected from either tissue or liquid biopsy samples, especially in BALF/BWF. In the present study, there is indication that osimertinib may have favorable efficacy in patients who had T790M mutation detected only in liquid biopsy samples, which is not inferior compared to other group of the patients.

After acquiring resistance to EGFR-TKIs, demonstration of T790M mutation is mandatory to use osimertinib, which requires re-biopsy usually based on tumor genotyping. However, obtaining adequate tissue through re-biopsy is clearly limited in clinical practice, due to inaccessible tumor...
site, poor performance status of patients, and potential complications related to procedure [15-17]. Liquid biopsies using ctDNA in blood, which are less invasive and more convenient than conventional tissue biopsy therefore had been approved for the alternative tests [18,19]. In this prospective study, although the number of patients was only five, ORR in patients with T790M-positive plasma and T790M-negative tumor sample was 100%, supporting the promising role of plasma T790M detection as a feasible biomarker to osimertinib treatment outcome. However, some limitations remain regarding feasibility of liquid biopsy. Proportion of ctDNA in blood samples is generally low, and half-life of ctDNA is short, casting challenges with respect to low sensitivity and high false-negative rates. For example, detecting mutations with plasma ctDNA has widely ranged sensitivity, with 39%-86% sensitivity for EGFR mutations and 27%-75% sensitivity for T790M mutation [20-23]. A post hoc analysis of AURA phase III trial demonstrated detection rate of T790M as 51 to 66% (51% by cobas plasma, 58% by droplet digital polymerase chain reaction (ddPCR), and 66% by next-generation sequencing) [24]. In this prospective study, sensitivity of T790M mutation by cobas plasma test (51%) was lower than the values for E19del (82%) and L858R (68%). In the current study, we used PANAMutyper probe only and sensitivity of plasma ctDNA for detecting T790M mutations and overall EGFR mutation was 28.9% and 54.2%, respectively. Detection rate of plasma T790M mutation in our study is noticeably low when compared to the study of Park et al. [25], which reported same detection rate of plasma T790M mutation by either PANAMutyper or cobas test as 45.9% (17/37 patients). We assume that relatively lower disease burden in our study population could explain the low sensitivity of plasma ctDNA test. As shown in Table 1, our patients seem to have lower proportion of extrathoracic metastasis (28.6% of brain metastasis and 44.4% of extrathoracic visceral metastasis) than that of AURA3 (33% of brain metastasis and 52% of extrathoracic visceral metastasis) [3] or study of Park et al. (52.4% of brain metastasis) [25]. In addition to tumor burden, DNA instability while processing plasma or difference in analytic methods could be related to the low sensitivity of plasma T790M detection in our study. Nevertheless, varied sensitivity in detecting T790M mutation from plasma requires further utilization of other types of liquid biopsy along with blood sample.

BALF/BWF plays a supporting role in the diagnosis of lung cancer and the detection of EGFR mutations. Diagnostic yield of BALF/BWF identifying malignant cells in adenocarcinoma was 77% in previous study [26]. Park et al. [27] suggested that BALF/BWF might be effective for determining the EGFR genotype, with high concordance rate (91.7%) between BALF/BWF and tissue using PANAMutyper in 20 patients. Hur et al. [28] reported BALF/BWF extracellular vesicle (EV)-based EGFR genotyping had average sensitivity and specificity of 76% and 87%, respectively. Lee et al. [11] compared diagnostic yields of plasma and BWF for detecting EGFR-TKI sensitizing mutations (E19del and L858R) by ddPCR, reporting superior diagnostic performance of BWF (sensitivity and specificity being 68.42% and 98.15% for E19del, 89.47 and 96.30 for L858R) compared to plasma (sensitivity and specificity being 31.58% and 94.44% for E19del, 47.37% and 98.15% for L858R). In our study, similar superior sensitivity of BALF/BWF compared to plasma was observed in detecting E19del and overall EGFR mutations, but not significant in T790M. Also, sensitivity in detection of T790M mutation of BALF/BWF (42.2%) was considerably low compared to previous studies which used BALF/BWF or plasma ctDNA [11,24,28], which suggest role of ctDNA in BALF/BWF may not fully substitute for tissue biopsy. This relatively low diagnostic performance might be associated to DNA instability in the BALF/BWF, difference in the detection method, location of targeted tumor lesion, or spatial heterogeneity of tumor. Still, sensitivity for T790M detection has been significantly improved from 28.9% to 51.1% when we combined the results of plasma ctDNA and BALF/BWF tests, which reveals the additive effect of BALF on plasma ctDNA test. Indeed, the cost and risks for complication of bronchoscopic procedures must be considered. However, we suggest active measurement of ctDNA from BALF/BWF would enable more patients to be detected as harboring T790M mutation, thus, to be benefited for osimertinib.

Kiura et al. [29] assessed ORR as 75% in Japanese cohort of AURA Phase I study, which contained 12 subjects who had positive T790M result from BALF/BWF samples. In the current study, patients who showed T790M positivity only in BALF/BWF demonstrated ORR of 44.4%, and when combined with patients who harbor T790M mutation in both plasma and BALF/BWF, patients demonstrated ORR of 61.5%. Furthermore, ORR in five patients with T790M-positive plasma and T790M-negative tumor sample was 100%. The number of each patient for T790M positivity in various liquid biopsy samples was too small to draw any firm conclusion, we carefully assume that this relatively low ORR despite of high sensitivity of BALF/BWF was not related to coexisting EGFR activating mutation status according to the data on S2 Table. Rather it could be related to tumor burden, which was not fully evaluated in this study.

Malignant effusion was also under consideration of our study, but number of patients who harbor T790M mutation in pleural fluid was small, limiting exact assessment of diagnostic performance and ORR. EGFR genotyping using both EV DNAs (DNA inside the EV shed by tumor cells, protected...
by dual lipid membranous coating) and ctDNAs from supernatant of pleural effusion resulted in 100% agreement with tissue EGFR genotyping in both EGFR-TKI naive patients and patients who had acquired resistance to EGFR-TKI in a recent study [30], suggesting pleural effusion is also a useful liquid biopsy sample.

Safety profile of osimertinib in the current study was consistent with previous reports of AURA trials [3,31]. Osimertinib was well tolerated, and no dose reductions were needed related to AEs in current study. However, interruptions and discontinuation of the drug did occur, and two mortality cases (3.2% of overall population) developed. The incidence of pneumonia in patients with disease progression in the central nervous system was 3%-5% in previous trial, which is consistent with our data.

This study has several limitations. We only used ctDNA, which is known to have relatively low sensitivity compared to EV-derived liquid biopsy tests. But ctDNA is simple and cost-effective, and our study showed permissive sensitivity and specificity in detecting T790M mutation using diverse liquid biopsy samples. In addition, due to the study maturation was not fully achieved, the median overall survival of this study was not evaluated. However, this is the first study prospectively evaluating efficacy of osimertinib in patients who harbor T790M mutations in liquid biopsy samples, reflecting real world practice setting.

In conclusion, osimertinib had favorable efficacy in patients with NSCLC harboring T790M mutation detected in liquid biopsy samples, which is non-inferior to those detected in tissue, supporting feasibility of liquid biopsy as another tool for re-biopsy for identifying T790M mutation. BALF/BWF have non-inferior diagnostic performance in detecting T790M mutation compared to plasma.

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

Ethical Statement
The study protocol was approved by the Institutional Review Board of Asan Medical Center (approval number: 2017-0295) and written informed consent was obtained from all patients.

Author Contributions
Conceived and designed the analysis: Ji WJ, Lee JC, Choi CM. Collected the data: Ji WJ, Lee JC, Choi CM. Contributed data or analysis tools: Ji WJ, Lee JC, Chun SM, Choi CM. Performed the analysis: Kim YJ, Chun SM, Choi CM. Wrote the paper: Kim YJ, Choi CM.

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Conflicts of Interest
This study was funded by Astrazeneca, Inc. (study sponsor). The authors have no conflicts of interest to declare otherwise.

Acknowledgments
This work was supported by the Korea Medical Device Development Fund grant funded by the Korea Government (the Ministry of Science and ICT, the Ministry of Trade, Industry and Energy, The Ministry of Health & Welfare, Republic of Korea, the Ministry of Food and Drug Safety) (Project Number: 202011B13).

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