Plasma ctDNA monitoring during epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor treatment in patients with EGFR-mutant non-small cell lung cancer (JP-CLEAR trial)

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Abstract

Background: Osimertinib, a third generation epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI), is active against EGFR-mutant non-small cell lung cancer (NSCLC) resistant to first-/second-generation EGFR-TKIs with the T790M mutation. T790M monitoring in plasma circulating tumor DNA (ctDNA) in patients receiving EGFR-TKIs is less invasive than re-biopsy and could provide valuable clinical information.

Methods: Patients with advanced or postoperative recurrent NSCLC with sensitizing EGFR mutations who were planned to receive or were receiving first-/second-generation EGFR-TKI treatment without disease progression were eligible for enrollment. Plasma samples at baseline and every 1–2 months thereafter were analyzed for EGFR mutation status using the cobas® EGFR Mutation Test v2.

Results: Between September 2016 and March 2017, 122 patients at 15 Japanese institutions were enrolled. In August 2018, 1291 plasma samples from 121 patients were analyzed for EGFR mutation status. At baseline, a sensitizing EGFR mutation was detected in 29 (23.9%) of 121 patients and T790M mutation was detected in three (2.5%). At follow-up, 66 (54.5%) patients experienced disease progression and 64 (52.9%) discontinued first-line EGFR-TKI treatment. Twenty-two (18.2%) patients showed T790M in plasma ctDNA, of which 15 (68.2%) received osimertinib. Although 31 patients received re-biopsy to examine EGFR status at disease progression, T790M was detected in only nine (22.0%) patients, of which 7 (77.8%) received osimertinib.

Conclusions: ctDNA monitoring during EGFR-TKI treatment is useful for detecting T790M mutation. The efficacy of osimertinib treatment based on T790M status in plasma ctDNA remains to be established, warranting further research.

Key words: liquid biopsy, EGFR-TKI, ctDNA, T790M, osimertinib
Introduction

Treatment with first- or second-generation epidermal growth factor (EGFR)-tyrosine kinase inhibitors (TKIs) is effective for non-small cell lung cancer (NSCLC) patients harboring a sensitizing EGFR mutation. However, acquired resistance is inevitable after 9–14 months (1–6).

The most common mechanism of resistance to first- and second-generation EGFR-TKIs in the first-line setting is the EGFR T790M mutation, which accounts for approximately 60% of cases (7,8). Osimertinib, a third-generation EGFR-TKI targeting EGFR T790M mutation, is reported to be highly active against T790M-positive NSCLC (9). To detect the T790M mutation in patients progressing during EGFR-TKI treatment, tumor re-biopsy is necessary. However, re-biopsies with invasive procedures (bronchoscopy or needle biopsy) are often infeasible in standard care of NSCLC patients (10,11).

Circulating tumor DNA (ctDNA) detected in plasma is recognized as a noninvasive biomarker for the molecular analysis of NSCLC (12). The cobas® EGFR Mutation Test (Roche Diagnostics K.K., Switzerland.) is a companion diagnostic test for the detection of EGFR mutations in plasma specimens and has been approved to identify such patients with NSCLC (13–15). T790M monitoring in plasma ctDNA of patients receiving EGFR-TKIs could yield valuable clinical information. We conducted an observational study to estimate the usefulness of plasma ctDNA monitoring in NSCLC patients with EGFR mutations receiving EGFR-TKIs.

Patients and methods

Study population

Patients with histologically and/or cytologically confirmed advanced or postoperative recurrent NSCLC harboring sensitizing EGFR mutations were eligible if they were at least 20 years old and were receiving or planned to receive first-line EGFR-TKIs (gefitinib, erlotinib, or afatinib). Sensitizing EGFR mutations were defined as follows: (1) Exon 19 deletion; (2) Exon 21 L858R; and (3) other minor mutation (i.e., Exon 18 G719X). The co-existence of T790M was not excluded. Patients were required to have an Eastern Cooperative Oncology Group performance status of 0 to 2. Patients were excluded if they had undergone prior EGFR-TKI treatment with disease progression or had hepatitis B virus (HBsAg), hepatitis C virus (HCV-RNA), or HIV.

Plasma ctDNA analysis

Plasma to assess the EGFR genotype of circulating ctDNA was collected at baseline and every 1–2 months. The following events were particularly noted: (1) radiological disease progression; (2) clinical disease progression; (3) re-biopsy at disease progression; and (4) treatment change. At each institution, 10-mL samples of blood were centrifuged within 4 hours of plasma collection. Plasma ctDNA was analyzed at SRL Laboratory (Tokyo, Japan) using the cobas® EGFR Mutation Test version 2 (v2) to detect sensitizing EGFR mutations and the T790M resistance mutation.

Re-biopsy and EGFR mutation analysis

When disease had progressed, re-biopsy was recommended. The EGFR genotypes of re-biopsied materials were analyzed at each hospital using the peptide nucleic acid-locked nucleic acid clamp method (16) or the cobas® EGFR Mutation Test v2.

Clinical data collection

Case report forms (CRFs) were collected at 6 and 12 months after registration. The CRF included clinical information about radiological disease progression (PD; date, site of disease progression), clinical PD (date, pattern of PD), survival (date last verified), death (date), cause of death, and adverse events. Radiological PD was assessed according to the Response Evaluation Criteria in Solid Tumors v1.1 at each institution. Clinical PD was defined as follows: clinical symptoms with disease progression; worsening of performance status due to disease progression; main organ dysfunction (lymphangitis carcinomatosa, bone marrow metastasis, meningeit carcinomatosa, or liver metastasis with hepatic dysfunction); and other clinically meaningful multiple metastasis. Adverse events were assessed according to the Common Terminology Criteria for Adverse Events v4.0.

Statistical analysis

This study is an observational study to estimate the usefulness of ctDNA monitoring in NSCLC patients with EGFR mutations who received first-line EGFR-TKIs. The primary analysis was designed to estimate the plasma ctDNA T790M-positivity rates using the cobas® EGFR Mutation Test in patients with T790M-positive tumors and at each clinical point. In the prior CSPOR-LOC02 study (observational study of treatment of EGFR mutation-positive advanced or recurrent NSCLC: UMIN 000010538), radiological PD was documented in approximately 80% of the patients (17). Among the patients (80%) who acquired resistance to EGFR-TKIs, approximately 60% were presumed to have T790M. This study used descriptive statistics and was set to 120 cases in consideration of feasibility of research. Median time to progression was estimated based on the Kaplan–Meier method.

Ethical considerations

This study protocol was approved by the institutional review board at each participating institution. Declaration of Helsinki ethical standards and local and national regulations were followed. All patients provided written informed consent before participation.

Results

Patients

A total of 122 Japanese patients were enrolled between September 2016 and March 2017 at 15 sites in Japan. One patient was ineligible because of first-line EGFR-TKI treatment failure. A total of 121 patients were registered. Patient characteristics are shown in Table 1. At the data cut-off date of this study (30 August 2018), CRFs were collected from 121 and 108 patients at 6 and 12 months after registration, respectively. Median(range) follow-up time was 369 (9–438) days. During the follow-up period, 66 (54.5%) patients experienced disease progression and 64 (52.9%) discontinued first-line EGFR-TKI treatment (Fig. 1). Median (95% CI) time to progression, which was defined as radiological or clinical PD since first-line EGFR-TKI treatment, was 663 (512–916) days.

Frequency of T790M detection in re-biopsied samples

Forty-one samples obtained from 33 patients (at disease progression during first-line EGFR-TKI treatment, n = 31, and at disease progression during post-discontinuation treatment, n = 2) were collected to analyze the EGFR genotype. DNA was extracted from various materials including lung tissue (n = 19), lymph nodes (n = 4), pleural effusions (n = 8), and other tissues (n = 10). A sensitizing EGFR mutation and T790M were detected in 24 (72.7%) and nine (27.3%) patients, respectively. EGFR mutations were not detected
Table 1. Patient characteristics

| Characteristic | Value |
|---------------|-------|
| Age           | Median (range) 72 (40–92) |
| Sex           | Male 42 (34.7) |
|              | Female 79 (65.0) |
| PS            | 0 64 (52.9) |
|              | 1 54 (44.6) |
|              | 2 3 (2.5) |
| Smoking status| Never 80 (66.1) |
|              | Current/former 39 (32.3) |
|              | Unknown 2 (1.7) |
| Histology     | Adenocarcinoma 118 (97.5) |
|              | Others 3 (2.5) |
| EGFR genotype | Ex 19 del 61 (50.4) |
|              | Ex 21 L858R 55 (43.5) |
|              | Others 4 (3.36) |
|              | Ex 21 L858R + other 1 (0.8) |
| Clinical stage of NSCLC | IIA 1 (0.8) |
|              | IIIB 3 (2.5) |
|              | IV 78 (64.5) |
|              | recurrence 39 (32.2) |
| EGFR-TKI      | Gefitinib 50 (41.3) |
|              | Erlotinib 40 (33.1) |
|              | Afatinib 31 (25.6) |

Data are n (%), unless otherwise stated.

EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; PS, performance status; TKI, tyrosine kinase inhibitor.

Figure 1. CONSORT flow diagram of this study.

Timing of T790M detection in plasma ctDNA

Of 22 patients with T790M detected in plasma ctDNA, 19 patients experienced disease progression and discontinued first-line EGFR-TKI treatment. Three patients with T790M detected in plasma did not experience clinical/radiological disease progression and continued first-line EGFR-TKI at 12 months. The timing of T790M detection in plasma ctDNA in 19 patients with disease progression is shown in Figure 2. T790M detection in plasma ctDNA preceded and/or appeared at disease progression in 15 (78.9%) of 19 patients; however, in four (21.1%) patients, T790M was detected for the first time after the disease had progressed.

Treatment after first-line EGFR-TKI failure and T790M detection

Among the 64 patients who discontinued first-line EGFR-TKI treatment, 50 received post-discontinuation treatment. Of these patients, 20 (40%) received osimertinib as the second-line treatment following first-line EGFR-TKI failure. Of nine patients with T790M detected in re-biopsied materials, seven (77.8%) received osimertinib. Of 22 patients with T790M detected in plasma ctDNA, 15 (68.1%) received osimertinib, three (13.6%) continued first-line EGFR-TKI therapy, and two (9.0%) switched to platinum-based chemotherapy.

Discussion

At a median follow-up of 1 year, T790M was detected in 29 patients. T790M was detected in the plasma ctDNA only, re-biopsied materials only, and both the plasma ctDNA and the re-biopsied materials of 20, 7, and 2 patients, respectively. The concordance rate of T790M detection in re-biopsied materials and plasma was 54.8%. T790M detection in plasma ctDNA preceded and/or appeared at disease progression in 15 (78.9%) of 19 patients with disease progression.

Repeated monitoring of circulating ctDNA in this study increased the frequency of T790M detection and the proportion of osimertinib treatment in the second-line setting of patients with advanced EGFR mutation-positive NSCLC. Seto et al. reported in the REMEDY study that T790M were detected in only 19.7% of plasma samples and the frequency of T790M detection and the proportion of the patients treated with osimertinib were approximately 25.8% and 23.7%, respectively, in the real-world setting (18). In this study, T790M was detected in 22 (33.3%) of 66 patients at disease progression during first-line EGFR-TKI treatment and 20 (30.3%) patients received osimertinib treatment in the second-line setting. Although we analyzed plasma ctDNA using the PCR-based cobas® EGFR Mutation Test v2, next-generation sequencing (NGS) can reach higher values of sensitivity compared with PCR-based methods (19,20). The NGS concordance rate with tumor tissue for EGFR alterations is very high. According to the International
but also a spectrum of alterations (able of detecting not only the common resistance mutation T790M is preferred and recommended over PCR-based methods as it is cap-
Association for the Study of Lung Cancer, an NGS multiplex panel

ease progression. Timing of T790M detection (○) and no T790M detection (●) in patients with disease progression. Timing of T790M detection (●) and no T790M detection (○): duration of first-line EGFR-TKI treatment (orange bar), osimertinib (yellow bar), afatinib (green), cytotoxic chemotherapy (gray bar), and immune checkpoint inhibitor (blue bar). Letters A–S represent each patient.

Figure 2. Timing of T790M detection in plasma ctDNA in patients with disease progression. Timing of T790M detection in plasma ctDNA in patients with disease progression and after disease progression during discontinuation of first-line EGFR-TKI treatment in four (21.1%) patients in this study. Zheng et al. reported that 45% of patients harboring a T790M mutation could have this alteration detected before progressive disease through ddPCR assays (22). Further investigation is necessary to elucidate the clinical benefits of switching to osimertinib from first-/second-generation EGFR-TKIs when T790M is detected in patients’ plasma ctDNA without disease progression.

As first-line use of osimertinib becomes the standard of care in the first-line setting of advanced EGFR mutation-positive NSCLC with the results of the FLAURA trial (23), the frequency of T790M will decline, but understanding the mechanisms of resistance to osimertinib will likely be of clinical utility to patients in the near future. Oxnard et al. reported that the persistent existence of T790M at osimertinib treatment failure is a good prognostic factor of patients with T790M mutation treated with osimertinib (24). Del Re et al. reported that the T790M/activating EGFR mutant allele frequency ratio is a prognostic factor of osimertinib treatment (25). These data suggest that ctDNA monitoring of sensitizing EGFR mutations and T790M may be useful in monitoring the efficacy of osimertinib treatment.

This study has several limitations. First, this study was an observational study rather than one designed to define treatment strategies when T790M is detected in plasma. Second, tissue re-biopsy upon disease progression was not mandatory but recommended in this study, and hence the frequency of re-biopsy to estimate EGFR status (31/66, 47.0%) and T790M detection in re-biopsied materials (9/31, 29.0%) was low. Additionally, the sample size in this study is small for assessing the concordance rate of T790M detection in tissue and plasma. Finally, we could not assess the levels of sensitizing EGFR mutations and T790M as well as other resistance mechanisms of first- and second-generation EGFR-TKIs including MET and erb-b2 receptor tyrosine kinase 2 amplification.

In conclusion, plasma ctDNA monitoring using the cobas® EGFR Mutation Test v2 increased the frequency of T790M detection in patients with a sensitizing EGFR mutation during first- and/or second-generation EGFR-TKI treatment. Further investigation is necessary to evaluate the clinical usefulness of starting treatment with the third-generation EGFR-TKI osimertinib based on T790M detection in plasma ctDNA.

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Conflict of interest statement

Dr. Naka has received personal fees from AstraZeneca. Dr. Ohashi has received personal fees from Taiho. Dr. Kunitoh has received personal fees from AstraZeneca, Boehringer Ingelheim, Chugai, Taiho, Daichi-sankoyo, Johnsonand Johnson. All remaining authors have declared no conflicts of interest.

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