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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, meningitis 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-LC02 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 *EFGR*-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

		N = 121	
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	(45.5)
	Others	4	(3.36)
	Ex 21 L858R + other	1	(0.8)
Clinical stage of NSCLC	IIIA	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.

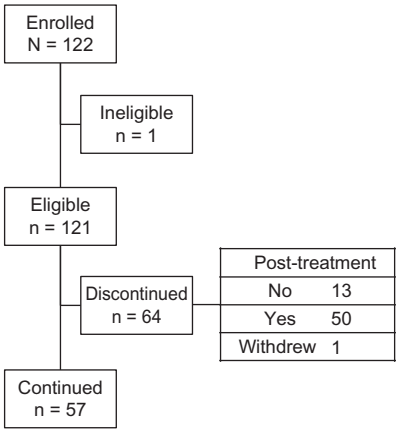

Figure 1. CONSORT flow diagram of this study.

in four (12.1%) patients. There was insufficient DNA to analyze the *EGFR* genotype in the samples from four (12.1%) patients. Only two (22.2%) of nine patients with T790M detected in re-biopsied materials showed T790M in plasma. The sensitivity and specificity of the cobas® *EGFR* Mutation Test v2 to detect T790M mutation in re-biopsied materials by plasma ctDNA in this study were 2/9 (22.2%) and 15/22(68.2%), respectively. The concordance rate of T790M detection in re-biopsied materials and plasma was 54.8%.

Frequency of T790M detection in plasma ctDNA

Because one patient withdrew from this study after registration, a total of 1291 plasma samples were collected from 120 patients. At baseline, a sensitizing *EGFR* mutation (Ex 19 del n = 14, L858R n = 15) was detected in 29 (24.2%) of 120 patients and T790M was detected in three (2.5%) patients. At 12 months, a sensitizing *EGFR* mutation and T790M was detected in the plasma ctDNA of 57 (47.5%) and 22 (18.3%) patients, respectively. The frequency of T790M detection in plasma ctDNA at 12 months was higher in patients with Ex 19 deletions detected in plasma (15/29, 51.7%) than with the L858R mutation (6/28, 21.4%) (chi-square test, P = 0.017). Other EGFR mutations except Ex 19 deletion, L858R, and T790M were not detected in plasma ctDNA.

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

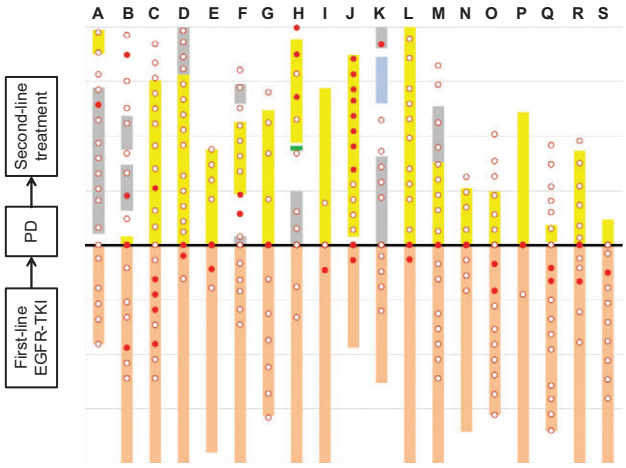

Figure 2. Timing of T790M detection in plasma ctDNA in patients with disease progression. Timing of T790M detection (●) and no T790M detection (○); duration of first-line EGFR-TKI treatment (orange bar), osimertinib (yellow bar), afatinib(green),cytotoxic chemotherapy (gray bar), and immune checkpoint inhibitor (blue bar). Letters A–S represent each patient.

Association for the Study of Lung Cancer, an NGS multiplex panel is preferred and recommended over PCR-based methods as it is capable of detecting not only the common resistance mutation T790M but also a spectrum of alterations (19). The considerable advantage of NGS methods over PCR-based methods could potentially be useful for increasing the frequency of T790M detection and ensuring the appropriate delivery of osimertinib in patients with first- and second-generation EGFR-TKI failure.

T790M detection in plasma could precede radiological progressive disease and could be potentially used to monitor response to first- and second-generation EGFR-TKIs (21); however, T790M detection appeared at unexpected sites and at unexpected moments. T790M detection in plasma ctDNA preceded and/or appeared at disease progression in 15 (78.9%) of 19 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, Daiichi-sankyo, Johnsonand Johnson. All remaining authors have declared no conflicts of interest.

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