Genetic diagnostic features after failure of initial treatment with epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors among non-small-cell lung cancer patients harboring EGFR mutations

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Abstract

Background: Osimertinib, a third - generation epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI), can be used as a second-line treatment for lung cancer patients harboring the T790M substitution. Although osimertinib is more effective than the first-generation EGFR-TKIs used for first-line treatment, its efficacy with respect to long-term patient survival remains unclear even upon the administration of a complete sequence of EGFR-TKI therapy, and limited information is available regarding genetic diagnostic approaches after EGFR-TKI naïve treatment. This study investigated the characteristics of EGFR-mutated lung cancer patients harboring the T790M substitution resistance to EGFR-TKIs and the advantages of rebiopsy and liquid biopsy for these patients. Methods: The medical records of patients screened for EGFR mutations were reviewed. Upon failure of naïve treatment with EGFR-TKIs, except for osimertinib, single plexus cobas version 2 was repeatedly used to detect the T790M substitution in EGFR via tissue or liquid biopsy. Results: From April 2016 through May 2019, 113 patients were determined to harbor EGFR mutations. Sixty patients were treated with EGFR-TKIs, among which 46 underwent tissue or liquid biopsy. Twenty-nine of these 46 (63%) patients harbored the T790M substitution. In total, 141 rebiopsies were performed. The T790M substitution was detected in 24 of 43 tissue and 11 of 98 liquid biopsies. If patients displayed an EGFR exon 19 deletion, had new lesions, and were administered gefitinib as first-line therapy, patients harboring an EGFR mutation were suspected of harboring the T790M substitution. Furthermore, the T790M substitution was detected through rebiopsy in patients with co-existing original mutations, brain metastases, tumor enlargement by ≥ 12 mm, or metastases at minor sites. Conclusion: Repeated biopsy can help maximize the detection rate of the T790M substitution. Furthermore, the advantages of repeated tissue or liquid biopsy should be considered among patients with positive T790M factors , and these biopsies can be repeated numerous times .

Introduction

Patients with metastatic non-small-cell lung cancer (NSCLC) harboring epidermal growth factor receptor (EGFR)-sensitizing mutations generally receive EGFR-tyrosine kinase inhibitors (TKIs) as first-line treatment [1]. Five TKIs including first- to third-generation TKIs are available for EGFR-TKI therapy. Although most patients eventually become resistant to EGFR-TKIs, the EGFR p.Thr790Met point mutation (*EGFR* T790M) is detected in 30–50% of patients presenting with disease progression after receiving first- or second-generation TKIs [2, 3]. These patients can be treated with osimertinib, whereas other patients might be treated with cytotoxic chemotherapy. Although osimertinib is generally preferred as first-line therapy because of efficacy and tolerability [4], patients with disease progression upon osimertinib treatment have been administered only cytotoxic chemotherapy [5]. Recent studies have revealed numerous EGFR-TKI-based alternatives for first-line treatment. First-generation EGFR-TKIs have been used in combination with an anti-VEGF antibody or with chemotherapy [6, 7, 8]. These clinical trials reported almost the same high efficacy as that of osimertinib, despite slightly increased toxicities. In these trials, Furthermore, second-generation EGFR TKIs constitute first-line treatment alternatives for EGFR-mutated

advanced NSCLC because no phase III clinical trial has compared the clinical efficacy of second-generation EGFR-TKIs and osimertinib. When NSCLC patients harboring EGFR mutations are administered EGFR-TKIs except osimertinib as first-line treatment, approximately half of the patients qualify for osimertinib therapy. For second- or third-line treatment of patients with osimertinib to maximize the treatment duration for EGFR-TKIs since April 2018 [9], it is important to maximally detect the T790M substitution. Cobas ver. 2 can be used for companion diagnostic examination (CDx) [10]. Limited information is available on maximizing detection of the T790M substitution using this type of CDx. Repeated rebiopsy is considered more effective to reduce “detection overlook” of the T790M mutation when rebiopsy is performed for patients with this mutation and with clinical features of the T790M substitution. The purpose of this study was to investigate the characteristics of EGFR-mutated lung cancer patients harboring the T790M substitution that were resistant to EGFR-TKIs and the advantages of tissue rebiopsy and liquid rebiopsy among these patients

Methods

Patients

From April 2016 to May 2019, consecutive patients screened for *EGFR* mutations were retrospectively reviewed at the National Center for Global Health and Medicine, Japan. The peptide nucleic acid-locked nucleic acid (PNA-LNA) PCR clamp method [11] was used to detect the *EGFR* mutation, using tissue biopsy specimens during the initial diagnosis of non-small non-squamous-cell lung cancer. After EGFR-mutated lung cancer acquired clinical resistance to EGFR-TKIs, the cobas® EGFR Mutation Test (Version 2; Roche Molecular Systems) [10] was repeatedly performed to detect T790M mutation status through tissue or liquid biopsy. Clinical resistance was defined as an increase in monitoring of tumor markers, disease progression through radiological imaging, or clinical disease progression.

Rebiopsy and genetic analysis

All types of clinical rebiopsies were repeated when patients were suspected to be clinically resistant to EGFR-TKIs. If patients were likely to provide tumor tissue through a clinical procedure (e.g. bronchoscopy or CT-guided biopsy) at the time of radiographic disease progression, they underwent tissue biopsy numerous times. Otherwise, liquid biopsy was performed. After each rebiopsy, the cobas® version 2 was used. When a new T790M substitution was detected, patients were administered osimertinib; if not, they were administered treatment other than osimertinib, such as cytotoxic chemotherapy or other molecular-targeted therapy. Among patients not harboring the T790M substitution, tissue or liquid rebiopsies were repeated numerous times until the T790M substitution was detected. Cobas® version 2 is a single plexus real-time PCR procedure to detect EGFR mutations, which potentially uses unstained 5-μm-thick sections obtained from a formalin-fixed paraffin-embed block and mounted on slides or whole blood samples, as previously reported [10]. Mutations were analyzed at the central laboratory of LSI Medience Corporation (Tokyo, Japan).

Data collection

The following data were obtained from each patient's medical records: patient characteristics including age, sex, smoking index, smoking status, comorbidities, and Eastern Cooperative Oncology Group performance status (PS) at diagnosis, as well as oncological data including histologic type, staging in accordance with the 8th edition of the TNM Classification of Malignant Tumors [12], tumor size of biopsy site, number of tumor lesions, metastatic organ, EGFR mutation sites detected via the PNA-LNA PCR clamp method or cobas® version 2, treatment data including surgical treatment, radiotherapy including radical or palliative radiation, pharmacotherapy (gefitinib, erlotinib, afatinib, and osimertinib) at EGFR-TKI naïve line, subsequent systemic therapies including cytotoxic chemotherapy regimens, immunotherapy, or other molecular-targeted therapy, data on the best supportive care, and tumor markers for CEA (ng/mL). Computed tomography (CT), positron-emission CT, and magnetic resolution imaging were performed within 1 month of each biopsy for corresponding biopsy specimens. Patients harboring the T790M substitution were defined under the category of "at least one detection of T790M using single-plexus PCR through any type of clinically available biopsy."

Ethical considerations

The study was conducted in accordance with the tenets of the Declaration of Helsinki. The study protocol was approved by the certified review board of the National Center for Global Health and Medicine (NCGM-G-003361-00). In accordance with the Japanese Ethical Guidelines for Medical and Health Research Involving Human Subjects, we used the opt-out method. We informed the participants about this study and obtained informed consent from subjects by displaying the disclosure document in the hospital as per the approval data by the appropriate review board until 31st January 2020.

Statistical analysis

The primary outcome was the identification of EGFR-mutated lung cancer patients harboring the T790M substitution with acquired clinical resistance to EGFR-TKIs. Secondary outcomes were the identification of factors inducing the T790M substitution through any type of rebiopsy among patients harboring the T790M substitution and factors inducing the T790M substitution through liquid rebiopsy. Fisher's exact test was performed to compare the proportion of subjects with dichotomous outcomes in both groups. For continuous variables, receiver-operator characteristics (ROC) curves were analyzed using SigmaPlot version 14 software (Systat Software, Inc., San Jose CA, USA). At a p-value of < 0.05 ($p < 0.05$), the optimal cutoff values of these continuous variables were set on the basis of a pre-test probability of 0.5 and cost ratio of 1.0.

Logistic regression analysis was performed to assess the aforementioned three factors among patients with EGFR-mutated lung cancer with acquired clinical resistance to EGFR-TKIs, as previously described [13]. To select a model for multivariate analysis, we identified variables with a p-value less than 0.15 based on univariate analysis. Spearman's rank test and clinically clarified dependent variables were used to exclude dependent variables from the aforementioned selected variables. A correlation coefficient (ρ) of more than 0.3 as the absolute value based on Spearman's rank test indicated a significant association. Some models were constructed with only independent variables as candidates. ROC curves were used to

select the best model among candidate models. In the final multivariate analysis using the simultaneous method, statistical significance was determined at a p-value < 0.05 through a two-sided test. All analyses were performed using SPSS Statistics software version 25 (IBM, Armonk NY, USA) or Stata version 15.1 (StataCorp LLC, College Station TX, USA).

Results

Patients

From April 2016 to May 2019, 405 patients consecutively underwent the EGFR mutation test. Among them, EGFR mutations were detected in 113 patients (Figure 1). Five patients decided to undergo only best supportive care and 48 did not experience relapse after local therapy including surgery, radiotherapy, or chemoradiotherapy. Sixty patients harbored activating EGFR mutations and seven were administered osimertinib as TKI-naïve therapy. The remaining patients were treated with TKIs, except for osimertinib. The T790M substitution was detected in 29 of 46 (63%) patients who underwent rebiopsy. Table 1 outlines the demographic characteristics of each group. Thirteen patients presented with post-operative recurrence and four patients presented with post-irradiation recurrence. During the overall study period, 33 patients received chemotherapy. During TKI-naïve treatment, 12 patients received gefitinib, 32 received erlotinib, and eight received afatinib.

Rebiopsy outcomes

To identify patients harboring the T790M substitution, all types of clinically available rebiopsies were performed numerous times (Table 2). Tissue biopsy was repeated four times maximum. During the initial tissue biopsy, the detection rate was 67.8%, with a sensitivity of 80.8%. Thereafter, the detection rate was approximately 30%, with a cumulative sensitivity of 77.4–79.3%. Liquid biopsy was repeated 10 times maximum. During the initial liquid biopsy, the detection rate was 8.1%, with a sensitivity of 13%. During each liquid biopsy, the median detection rate was 8.6%, ranging from 0–25%, and the median cumulative sensitivity was 18.9%, ranging from 16.7–20.6%. In total, we performed 141 rebiopsies, including both tissue and liquid biopsies from 46 patients (Table 3). Among these patients, 29 (63 %) harbored the T790M substitution. The T790M substitution was detected in 35 (cumulative sensitivity = 39.3%) biopsies, including 24 tissue (cumulative sensitivity = 77.4%) and 11 liquid (cumulative sensitivity = 19%) biopsies. Regarding detection of the T790M substitution, significant differences between tissue and liquid biopsy were observed based on Fisher's exact test (P < 0.0001).

Positive factors in patients harboring the T790M substitution

The primary outcome was to identify factors among patients harboring the T790M substitution after patients with EGFR-mutant NSCLC acquired clinical resistance to EGFR-TKIs. We performed logistic

regression analyses for 53 patients treated with EGFR-TKIs other than osimertinib (Figure 1). The results of logistic regression analyses are shown in Table 4. Six variables had p-values > 0.15 based on univariate analyses. Multivariate analysis indicated that significant clinical features associated with patients harboring the T790M substitution were as follows: exon 19 deletion in the original mutation, termination of TKIs owing to detection of new lesions, and gefitinib during TKI-naïve treatment.

Positive factors associated with the T790M substitution upon rebiopsy

One of the secondary outcomes was to identify positive factors associated with the T790M substitution through any type of rebiopsy among patients harboring this alteration. Among these patients, 89 rebiopsies were performed. Table 5 shows the background characteristics of patients harboring the T790M substitution upon rebiopsy. Logistic regression analyses revealed 16 variables with p-values < 0.15 based on univariate analyses. We constructed four sets of multivariate models comprising variables that were not correlated with each other. The best model selected through ROC curve analysis is shown in Table 6. Multivariate analysis indicated that the significant associations detected with the T790M substitution upon rebiopsy among patients harboring T790M were as follows: co-detection of the original mutation, co-occurring brain metastases, tumor enlargement ≥ 12 mm, or involvement of minor site metastases, which means metastases of skin, kidney, ascites, lymphangiosis carcinomatosa, adrenal organ, or others.

Positive factors associated with the T790M substitution upon liquid biopsy

Regarding the detection of the T790M substitution through liquid rebiopsy among patients harboring the T790M substitution as another secondary outcome, 58 liquid biopsies were performed. Background characteristics of patients harboring the T790M substitution through liquid biopsy are shown in Table 7. Eleven variables with p-values < 0.15 were obtained based on univariate logistic regression analyses. Four sets of multivariate model candidates were constructed with each independent variable. The best model selected through ROC curve analysis is shown in Table 8. Based on multivariate analysis, detection of the T790M substitution via liquid biopsy among patients harboring the T790M substitution indicated the following: involvement of bone metastases or new tumor lesions ≥ 4.

Discussion

Our main purpose was to elucidate clinical features at the time of detection of T790M through clinically available mutational analysis. If we could identify these clinical features, we could perform tissue or liquid rebiopsy with more appropriate timing and reduce the frequency of tissue or liquid biopsy while maintaining the maximum detection rate of T790M. For this aim, this study investigated the characteristics of EGFR-mutated lung cancer patients harboring the T790M substitution that were

resistant to EGFR-TKIs and the advantages of tissue rebiopsy and liquid rebiopsy among these patients. In this study, repeated biopsy through single-plexus PCR revealed that 63% of EGFR-mutated NSCLC patients harbored the T790M substitution after acquiring clinical resistance to EGFR-TKIs. Tissue biopsy was superior to liquid biopsy in detecting the T790M substitution ($P < 0.0001$). Because liquid biopsy is a noninvasive biopsy modality for molecular-targeted analysis including the EGFR mutational status, using a plasma sample, it is easily reproducible. The present results indicate that liquid biopsy is associated with more false-negative results in clinical practice at levels of detection of approximately 0.1 to 2% [14, 15]. When liquid biopsy through this test yields negative results for the T790M substitution, it is essential to perform tissue biopsy. To identify patients harboring the T790M substitution, tissue biopsy should receive first priority because of its high sensitivity and high detection rate.

Herein, the primary outcome was to assess the clinical characteristics of patients harboring the T790M substitution. Three clinically relevant factors were detected as follows: exon 19 deletion in the original mutation, TKI termination owing to the detection of new lesions, and gefitinib administration during TKI-naïve treatment (Table 4). When deciding to perform or to repeat biopsy, these three factors should be considered. We estimated the probability of detecting the T790M substitution considering the number of rebiopsies.

The timing and site are important factors to consider for each rebiopsy. Positive factors associated with the T790M substitution through any type of rebiopsy among patients harboring the T790M substitution would provide information regarding the timing and site for the biopsy (Table 6). Based on the timing of the biopsy, patients had brain metastases and minor metastases and their tumor lesions were enlarged by > 12 mm. The enlarged tumor site would be better for tissue biopsy. When patients had bone metastases and harbored ≥ 4 new tumor lesions compared with previous tumor lesions, liquid biopsy was considered to detect the T790M substitution (Table 8).

This study has several limitations. Despite including consecutive patients herein, our study had a single-center, real-world, retrospective design. Although 405 consecutive patients were screened for the *EGFR* mutation for 3 years, these mutations were detected in only 28% of patients (Figure 1). Furthermore, our patient cohort comprised only 53 patients, and was thus a small cohort for obtaining clinical data. Owing to remarkable progress in NSCLC treatment, only minor benefits would be obtained, although the clinical data were obtained over a longer duration. In clinical practice, information from real-world data would be useful for repeated molecular analyses.

Nonetheless, this study also has some strengths. The knowledge of the clinical characteristics of patients harboring the T790M substitution would help select appropriate patients who should undergo and repeat tissue or liquid rebiopsy. Because individuals with drivers receiving a matched targeted agent lived longer [16], we consider that patients with clinical characteristics similar to those harboring the T790M substitution should undergo repeated biopsies until the T790M substitution is detected. Furthermore, these results will help select the type or timing of biopsy based on the positive factors associated with the

T790M substitution. In addition, upon testing for EGFR-mutant tumors by single-plexus PCR, tissue biopsy still received first priority.

Conclusion

This study shows that repeated biopsy helps to maximize the detection rate of the T790M substitution. Upon repeating the tissue or liquid biopsy, it is necessary to consider the positive factors associated with the T790M substitution. *If patients present with these positive factors, rebiopsy should be repeated numerous times.*

List Of Abbreviations

NSCLC: non-small cell lung cancer

EGFR: epidermal growth factor receptor

TKI: tyrosine kinase inhibitor

T790M: EGFR p.Thr790Met point mutation

VEGF: vascular endothelial growth factor

CDx: companion diagnostic examination

PCR: polymerase chain reaction

PNA-LNA PCR clamp method: peptide nucleic acid - locked nucleic acid PCR clamp method

PS: performance status

CT: computer tomography

ROC: receiver-operator characteristics

Declarations

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Contributions

YT performed the analysis and wrote the article. GO, YY, MH, MS, and SI supported the article writing and analysis. YT, GO, and YY performed the analysis and proofread the article. All authors contributed to the interpretation of the data and read and approved the final manuscript.

Ethics declarations

Ethics approval and consent to participate

The study was conducted in accordance with the tenets of the Declaration of Helsinki. The study protocol was approved by the certified review board of the National Center for Global Health and Medicine (NCGM-G-003361-00). In accordance with the Japanese Ethical Guidelines for Medical and Health Research Involving Human Subjects, we used the opt-out method. We informed the participants about this study and obtained informed consent from subjects by displaying the disclosure document in the hospital as per the approval data by the appropriate review board until 31st January 2020.

Consent for publication

Not applicable.

Competing interests

YT has received grants from Boehringer Ingelheim, Chugai Pharmaceutical, outside of this study. The remaining authors declared no competing interests for this work.

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Tables

Please see the supplementary files section to view the tables.

Figures

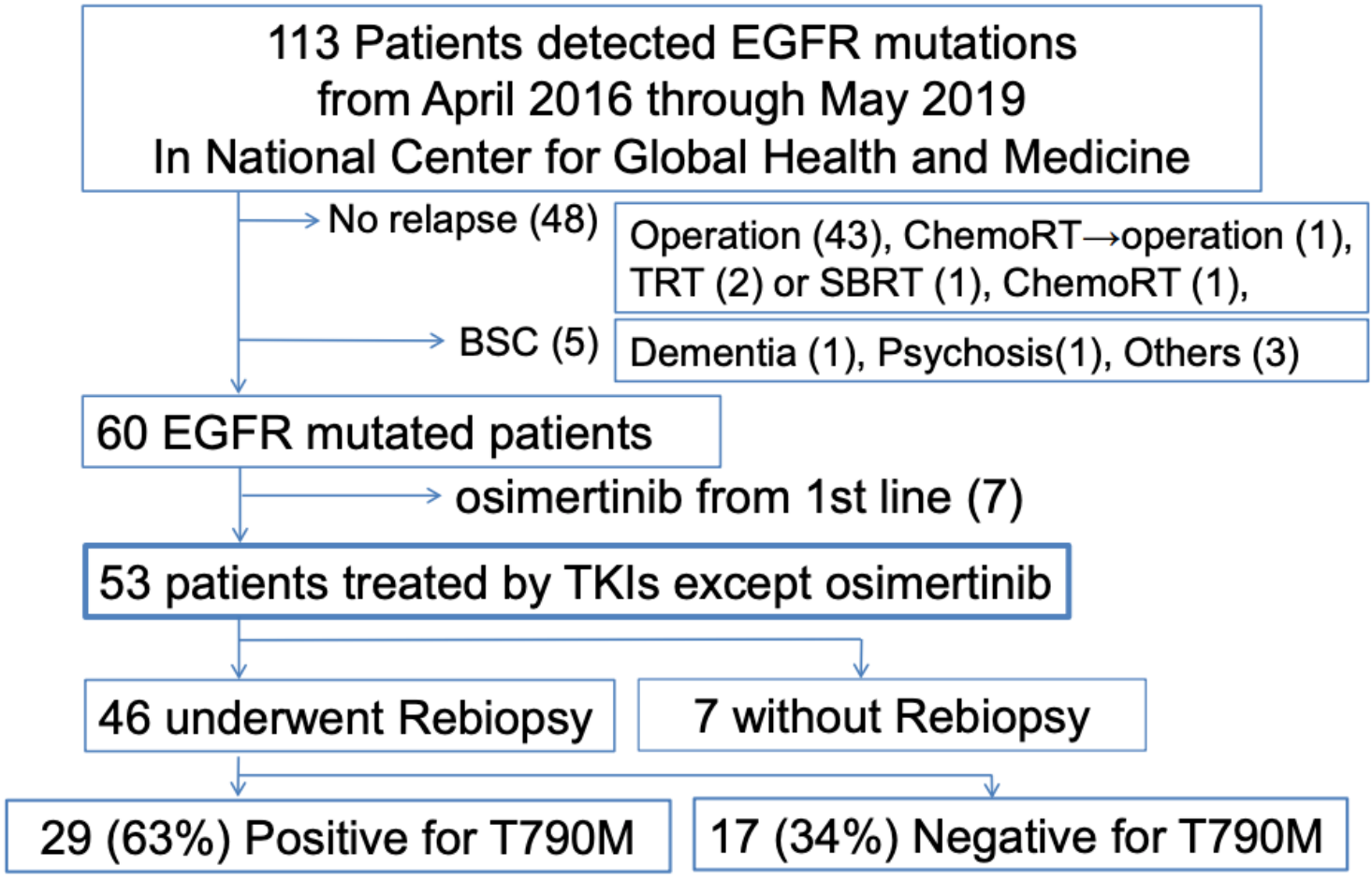

Figure 1

Study cohort. Data are number of patients unless specified otherwise.

Supplementary Files

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