Classification and regression tree for estimating predictive markers to detect T790M mutations after acquired resistance to first line EGFR-TKI: HOPE-002

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Abstract

Background and objective: Osimertinib as first-line treatment for patients with non-small cell lung cancer (NSCLC) harboring epidermal growth factor (EGFR) mutations remains controversial. Sequential EGFR-tyrosine kinase inhibitor (TKI) might be superior to the first line osimertinib in patients at risk of developing acquired T790M mutations.

Methods: We enrolled consecutive patients with *EGFR*-mutated (deletion 19 or L858R) advanced NSCLC treated with first-line drugs and evaluated predictive markers using classification and regression tree (CART) for the detection of T790M mutations based on patient backgrounds prior to initial treatment.

Results: Patients without acquired T790M mutations had worse outcomes than those with T790M mutations (median OS: 798 days vs. not reached; HR: 2.70; $P < 0.001$). CART identified three distinct groups based on variables associated with acquired T790M mutations (age, CYF, WBC, liver metastasis, and LDH; AUROC: 0.77). Based on certain variables, CART identified three distinct groups in deletion 19 (albumin, LDH, bone metastasis, pleural effusion, and WBC; AUROC: 0.81) and two distinct groups in L858R (age, CEA, and ALP; AUROC: 0.80). The T790M detection frequencies after TKI resistance of afatinib and first-generation EGFR-TKIs were similar (35.3% vs. 37.4%, $P = 0.933$). Afatinib demonstrated longer PFS (398 vs. 279 days; HR: 0.67; $P = 0.004$) and OS (1053 vs. 956 days; HR: 0.68; $P = 0.051$) than first-generation EGFR-TKIs.

Conclusion: Identification of patients at risk of acquiring T790M mutations after EGFR-TKI failure may aid in choice of first-line EGFR-TKI. Furthermore, afatinib may be the more effective 1st-line EGFR-TKI treatment for patients at risk of developing T790M as initial EGFR-TKI resistance.

Introduction:

Epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (TKIs) are key drugs for patients with non-small cell lung cancer (NSCLC) harboring *EGFR* mutations. First-, second, and third generation (1st-, 2nd-, and 3rd-G) EGFR-TKIs have been developed and evaluated for toxicity and efficacy in three randomized trials (LUX-Lung 7, ARCHER 1050, and FLAURA). All three trials showed more clinical benefits in the 2nd- and 3rd-G than the 1st-G EGFR-TKIs. While all studies showed that the 2nd- and 3rd-G EGFR-TKIs improved progression-free survival (PFS), only osimertinib in FLAURA exhibited a statistically significant improvement in overall survival (OS). While OS improvement was also observed with dacomitinib in ARCHER1050, statistical analysis was not performed in this study due to adoption of the gatekeeping method [1–3]. Therefore, osimertinib is the most strongly recommended drug for patients with common *EGFR* mutations. Japanese guidelines have judged the quality of this evidence as B and the strength of this recommendation as 1 [4].

Osimertinib was developed as an anti-cancer drug due to its activity against Thr790Met (T790M) mutations through covalent binding [5]. T790M is the most common resistance mechanism that impairs the activity of TKIs detected in approximately 50% of patients with 1st and 2nd-G EGFR-TKI-refractory

tumors [6, 7]. Thus, osimertinib is effective against T790M-mediated acquired resistance (AR) [8], making it a reasonable option for several *EGFR*-mutant patients initially treated with 1st- or 2nd-G *EGFR*-TKIs.

At present, there are two main first-line strategies for advanced *EGFR*-mutant NSCLC: first-line osimertinib and salvage osimertinib for T790M-positive AR initially treated with first-line 1st- or 2nd-G *EGFR*-TKIs. However, prevention of the development of AR must also be considered as a key therapeutic strategy. Importantly, first-line and salvage therapy have different mechanisms of the development of osimertinib AR [9–12] that are more complicated than those of 1st- or 2nd-G *EGFR*-TKIs.

Treatment strategies for AR to first-line osimertinib, are currently still limited. On the other hand, salvage osimertinib therapy was found to be a simple and effective treatment for patients with T790M detected after the development of AR to 1st- or 2nd-G *EGFR*-TKIs. However, the occurrence of T790M after developing AR to first-line 1st- or 2nd-G *EGFR*-TKIs was found to have a low incidence (approximately 30%) in real-world FLAURA trial data [3, 13]. To more effectively utilize salvage osimertinib therapy for these cases, the detection rate of T790M must be increased.

In the present study, we aimed to increase rates of T790M detection after first-line 1st- or 2nd-G *EGFR*-TKI therapy by evaluating predictive markers for T790M mutation detection in patients prior to first-line *EGFR*-TKI treatment.

Method:

Study design

We conducted a multicenter, retrospective cohort study across nine medical institutes belonging to the Hanshin Oncology Clinical Problem Evaluation group (HOPE) in Japan. The clinical data of the patients were retrospectively extracted from their medical charts and added to a database. Because this was a retrospective observational study, sample size calculation based on hypothesis testing was not performed.

This study was approved by the ethical review board or institutional review board of each participating institute. Informed consent was not required owing to the retrospective nature of the study, and an opt-out method was utilized so that patients and their families could refuse to participate in the study.

Patient selection

Patients >20 years of age were consecutively enrolled if they had pathologically confirmed stage IV non-squamous NSCLC (excluding recurrent cases, such as those who had undergone post-operation or post-chemoradiation therapy) with sensitizing *EGFR* mutations (deletion 19 or L858R) and had received gefitinib, erlotinib, or afatinib as first-line therapies between January 1, 2015 and March 31, 2017.

The patients were classified as either never-smokers (those reported to have never smoked), current smokers (those who had smoked within 1 year of diagnosis), or former smokers (the remaining). The

clinical stages of all the patients were determined according to the eighth edition of the tumor, node, and metastasis classification of malignant tumors. Anti-tumor responses were assessed using the RECIST version 1.1. The intervals between dates of commencing EGFR-TKI therapy and disease progression or death (PFS) and overall survival (OS) of the patients were calculated. The cutoff date for data collection was set at August 31, 2018.

Statistical analysis

Data were analyzed by independent statisticians. We evaluated predictive markers for T790M mutation detection based on the patients' backgrounds before first-line EGFR-TKI treatment using a classification and regression tree (CART). The Gini coefficient was used to determine the best split. The data collected included sex, age, type of *EGFR* mutation, smoking history, Eastern Cooperative Oncology Group (ECOG) performance status (PS), type of EGFR-TKI, site of metastasis (brain, bone, liver, adrenal, lung, and pleural effusion), and laboratory data.

Fisher's exact test was used for categorical comparisons of data. Kaplan-Meier curves were used to evaluate PFS and OS. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using the Cox proportional hazards model. All statistical analyses were conducted using R software (version 4.0.5; <http://R-project.org>; The R Foundation for Statistical Computing, Vienna, Austria). The following R libraries were used: *rpart* (version 4.1.15), *rpart.plot* (version 3.0.9), *pROC* (version 1.17.0.1), *survival* (version 3.2.10), and *survminer* (version 0.4.9). Differences were considered statistically significant at $P < 0.05$.

Results:

Patient demographics

A total of 289 consecutive stage 4 NSCLC patients (287 eligible) were enrolled at nine medical institutes belonging to the HOPE in Japan from January 1, 2015 to March 31, 2017. Among them, two patients with *de novo* T790M mutations were excluded. A total of 225 patients experienced disease progression (PD) after first-line EGFR-TKI treatment, of which 166 underwent re-biopsy by tissue or plasma. Among these patients, 147 patients remained for CART method analysis after excluding patients with missing background data, such as programmed cell death-ligand 1 (PD-L1), thyroid transcription factor-1 (TTF-1), sialylated carbohydrate antigen KL-6 (KL-6), neuron specific enolase (NSE), pro-gastrin releasing peptide (Pro-GRP) patients missing $\geq 10\%$ data (Figure 1).

The characteristics of the 166 patients are shown in Table 1. The median age of the patients was 69 years. Of the 166 patients, 71.7% were male, 31.9% had histories of smoking, 48.8% had L858R mutations and exon 19 deletions, and 2.4% had common and other uncommon mutations. Approximately 30.7% were treated with afatinib, 25.9% with erlotinib, and 43.4% with gefitinib as first-line treatments.

Development of T790M mutation after PD in first line EGFR-TKI therapy

A total of 36.7% (61/166) of the patients acquired T790M mutations after first-line EGFR-TKI therapy. The frequencies of T790M after each first-line EGFR-TKI failure were as follows: gefitinib, 34.7% (25/72); erlotinib, 41.9% (18/43); and afatinib, 35.3% (18/51). The frequencies of T790M in the 1st-G EGFR-TKIs, including gefitinib and erlotinib (37.4%; 43/115) were similar to that of afatinib ($P=0.862$). On the other hand, the frequency of T790M in patients with deletion 19 (44.4%; 36/81) tended to be higher than that in patients with L858R (44.4%, 36/81 vs 30.9%, 25/81; $P=0.104$).

Evaluation of predictive markers for detecting T790M through CART

First, we evaluated predictive markers for the detection of T790M in the patients included by CART analysis ($N=147$). CART identified three distinct groups of patients based on variables strongly associated with acquired T790M mutations (age, cytokeratin 19 fragmen (CYF), white blood cell count (WBC), liver metastasis, and lactate dehydrogenase (LDH)), with an area under the receiver operating characteristic (AUROC) of 0.77 (95% CI: 0.69–0.84) (Figure 2a). Although we classified the three groups according to the frequency of T790M, the AUROC was found to be low. Next, we analyzed predictive markers for detecting T790M mutations with each type of *EGFR* mutation since a previous analysis found that its frequency varies according to *EGFR* type [14]. The CART identified three distinct groups in deletion 19 based on certain variables (albumin (Alb), LDH, bone metastasis, pleural effusion, and WBC), with an AUROC of 0.81 (95% CI: 0.71–0.91) (Figure 2b). Furthermore, CART identified two distinct groups in L858R based on certain variables (age, carcinoembryonic antigen (CEA), and ALP), with an AUROC of 0.80 (95% CI: 0.69–0.90) (Figure 2c). The detection rates of the highest groups in deletions 19 and L858R were $\geq 80\%$ and $\geq 60\%$, respectively.

Effect of T790M on OS

The median OS across all patients ($N=289$) was 1041 days (Figure 3a). The T790M mutation was detected in 64 patients. Furthermore, more patients without acquired T790M mutations died compared with those with acquired T790M mutations throughout the observation period (116/225 vs. 18/64; $P<0.01$). Additionally, the patients without acquired T790M mutations had worse outcomes than patients with the T790M mutation (median OS: 798 days vs. not reached; HR: 2.70 [95% CI: 1.64–4.55]; $P<0.001$) (Figure 3b).

PFS and OS of first-line EGFR-TKIs

As mentioned above, the T790M mutation affected OS, and the frequency of the development of T790M after developing EGFR-TKI resistance was similar between afatinib and 1st-G EGFR-TKIs. However, afatinib was previously reported to have better outcomes than gefitinib [1]. We evaluated which first-line

EGFR-TKI had the highest PFS and OS based on real-world data. Compared with 1st-G EGFR-TKIs, afatinib had a longer PFS (median PFS: 398 vs. 279 days; HR: 0.67 [95% CI: 0.50–0.88]; P=0.004) (Figure 4a) and tended to have a longer OS (median OS: 1053 vs. 956 days; HR: 0.68 [95% CI: 0.46–1.01]; P=0.051) (Figure 4b).

Discussion:

This is the first analysis to identify predictive markers using CART for the detection of T790M mutations based on patient backgrounds prior to first-line EGFR-TKI treatment. This study also identified markers that distinguish between *EGFR* mutation types, leading to more accurate predictions of T790M detection. CART classified the groups according to the detection rate of T790M based on certain variables (age, CYF, WBC, liver metastasis and LDH). Furthermore, deletion 19 mutations were classified into three distinct groups based on certain variables (Alb, LDH, bone metastasis, pleural effusion, and WBC), and L858R mutations were classified into two distinct groups based on certain variables (age, CEA, and ALP).

As first-line treatment, osimertinib has been found to be associated with longer PFS and OS than 1st-G EGFR-TKIs against advanced NSCLC harboring *EGFR* mutations (exon-19 deletion and L858R) [3]. However, in the Asian subset (especially in the Japanese subset) analysis of OS in the FLAURA study, osimertinib was not observed to be superior to 1st-G EGFR-TKIs [15]. Furthermore, no additional molecular targets for therapy are known due to the heterogeneity of resistance mechanisms that are not well understood [11, 16]. As a result, in the clinical care of most patients following cancer progression after osimertinib treatment, chemotherapy is the only remaining option for second-line treatment. In contrast, the most common mechanism of the development of resistance to 1st- and 2nd-G EGFR-TKI treatment is the T790M mutation. Fortunately, osimertinib overcomes the T790M mutation and provides significantly longer PFS compared to standard platinum-based chemotherapy in advanced T790M positive NSCLC patients with AR to first line 1st or 2nd-G EGFR-TKIs (median PFS: 10.1 vs. 4.4 months; HR: 0.30 [95% CI: 0.23–0.41]) [8]. In particular, a non-interventional GioTag study demonstrated clinical benefit with sequential afatinib and osimertinib in patients with EGFR mutation-positive NSCLC with T790M-acquired resistance; this trend was more pronounced among the Asian population [17].

In this study, we showed that patients with acquired T790M mutations had better outcomes than patients without T790M mutations after AR to first-line 1st- or 2nd-G EGFR-TKIs. Therefore, the use of osimertinib as first-line treatment for NSCLC patients harboring EGFR mutations remains controversial in practice. Furthermore, sequential EGFR-TKI treatment may be superior to first-line osimertinib in patients who will likely develop acquired T790M mutations. When considering sequential therapy, the benefit is likely to be diminished if T790M is not detected since osimertinib remains an important and beneficial drug that is essential for patients with advanced NSCLC harboring EGFR mutations. However, the incidence of T790M after AR to first-line first line 1st- or 2nd-G EGFR-TKIs was found to be low based on real-world and FLAURA trial data [3, 13].

Previous reports have shown the value of performing a re-biopsy since patients who initially present as T790M-negative exhibited T790M-positive conversion after repeated re-biopsy. In these studies, performing re-biopsies increased their T790M detection rates from 36–80% and 45–67% [14, 18]. While this method increases the frequency of T790M detection, it also increases the number of invasive procedures done on patients. Liquid biopsies, a type of re-biopsy, are less invasive for patients but have lower T790M detection rates [19]. On the other hand, the droplet digital polymerase chain reaction (ddPCR) is another method that increases the sensitivity and rate of T790M [20, 21]. These studies suggest that patients with low T790M allele frequency had longer PFS with osimertinib than those with high T790M allele frequency. Thus, and detection and measurement of T790M using ddPCR is an effective method for increasing T790M detection rates that guarantees the efficacy of osimertinib. However, the use of ddPCR is non-reimbursable and impractical for daily clinical practice. Therefore, the development of other methods to increase the rate of T790M detection after first-line 1st- or 2nd-G EGFR-TKI therapy remains important.

We evaluated predictive markers for the detection of T790M mutations based on patient backgrounds prior to first-line EGFR-TKI treatment through CART. CART analysis is a prediction model constructed by recursively partitioning a dataset and fitting a simple mode with machine learning methods for constructing prediction models from data [22]. CART classified three distinct groups of patients based on variables that were strongly associated with detecting acquired T790M mutations (age, CYF, LDH, and liver metastasis); however, the AUROC was not satisfactory. In our study, the T790M detection rates between cases with deletion 19 and L858R mutations were different, in which is consistent with a previous report [14, 23, 24]. Accordingly, we decided to analyze predictive markers for the detection of T790M mutations for each type of EGFR mutation. We demonstrated that CART highly stratified the T790M detection markers according to EGFR type, with a more satisfactory AUROC than that of the total population. This may be due to the biological and clinical differences between deletion 19 and L858R mutations. The affinity between ATP and EGFR in cases with deletion 19 mutations is higher than that of L858R [25]. The phosphorylation of Akt and Erk in cases of deletion 19 mutations, along with downstream signals of *EGFR*, are inhibited in a concentration-dependent manner compared with in cases of L858R [26]. Furthermore, a difference in the mechanisms of EGFR activation has been reported [27], and differences in the efficacy of EGFR-TKI treatment have been reported in clinical practice [3, 28].

The present study had some limitations. First, this was a retrospective study, in which only Japanese patients were eligible for the analysis. Thus, our findings may not be generalizable to other ethnic populations. Second, the patients were treated according to the physician's choices, and the treatment and examinations may not have been standardized. Therefore, this study was with a little missing data within eligible group that would limit the applicability of results. Third, the patients underwent various assays for primary and secondary EGFR mutations in this study. The sensitivity of each assay may have differed. However, this cohort is representative of true real-world practice.

In conclusion, identification of patients at risk of acquiring the T790M mutation after failure of EGFR-TKI may help in the selection of first-line EGFR-TKI treatment options. Furthermore, prediction of T790M

mutations after initial EGFR-TKI resistance aids in recommending afatinib as the more effective first-line EGFR-TKI treatment compared with 1st-G EGFR-TKIs.

Declarations

Author Declarations

O Ethics approval and consent to participate: This study was approved by the ethical review board or institutional review board of each participating institute, and main institutional review board (IRB) approval for this study was obtained from the Medical Research Ethics Committee of Osaka International Cancer Institute.

O Consent for publication: All authors approved final manuscript.

O Availability of data and materials: The data that support the findings of this study are openly available in University hospital Medical Information Network at <https://www.umin.ac.jp>, reference number UMIN000041474.

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O Authors' contributions: (I) Conception and design: M.T., K.F., A.T., and S.T., (II) Administrative support: M.T., K.F., A.T., and S.T., (III) Provision of study materials or patients: All authors,, (IV) Collection and assembly of data: All authors, (V) Data analysis and interpretation: M.T., K.F., H.S., A.T., and S.T., (VI) Manuscript writing: All authors,

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• Compliance with Ethical Standards

O Disclosure of potential conflicts of interest: This study was approved by the ethical review board or institutional review board of each participating institute. Informed consent was not required owing to the retrospective nature of the study, and an opt-out method was utilized so that patients and their families could refuse to participate in the study.

O Research involving Human Participants and/or Animals: Research involving Human Participants

O Informed consent: Informed consent was not required owing to the retrospective nature of the study, and an opt-out method was utilized so that patients and their families could refuse to participate in the study.

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Tables

Table 1. Patient demographics.

Data are N (%) or median (range).

		T790M (+) N=61	T790M (-) N=105	Total N=166
Gender	Male	43 (36)	76 (64)	119
	Female	18 (38)	29 (62)	47
Age		68 (37–85)	70 (35–91)	69 (35–91)
EGFR Type	Del19	36 (44)	45 (56)	81
	L858R	25 (31)	56 (69)	81
	Other	0 (0)	4 (100)	4
Smoking	Non-smoker	43 (38)	70 (62)	113
	Ex-smoker	11 (28)	29 (72)	40
	Current	7 (54)	6 (46)	13
Histology	Adeno	61 (38)	100 (62)	161
	Non-adeno	0 (0)	5 (100)	5
Performance status	0	15 (44)	19 (56)	34
	1	37 (34)	73 (66)	110
	2	6 (55)	5 (45)	11
	3	2 (22)	7 (78)	9
	4	1 (50)	1 (50)	2
First-line EGFR TKI	Afatinib	18 (35)	33 (65)	51
	Erlotinib	18 (42)	25 (58)	43
	Gefitinib	25 (35)	47 (65)	72
Brain meta	Positive	19 (34)	37 (66)	56
	Negative	42 (38)	68 (62)	110
Bone meta	Positive	30 (42)	41 (58)	71
	Negative	31 (33)	64 (67)	95
Liver meta	Positive	11 (61)	7 (39)	18
	Negative	50 (34)	98 (66)	148
Adrenal meta	Positive	4 (33)	8 (67)	12
	Negative	57 (37)	97 (63)	154

Lung meta	Positive	25 (41)	36 (59)	61
	Negative	36 (34)	69 (66)	105
Pleural effusion	Positive	25 (40)	38 (60)	63
	Negative	36 (35)	67 (65)	103
LDH (U/L)		202 (83–1115)	204 (124–525)	203 (83–1115)
ALP (U/L)		n=59	n=102	n=161
		264 (114–1311)	282.5 (104–6519)	276 (104–6519)
CRP (mg/dL)		n=61	n=104	n=165
		0.2 (0–7.4)	0.2 (0–18.7)	0.2 (0–18.7)
ALB (g/dL)		n=59	n=103	n=162
		3.9 (2.7–4.6)	3.9 (2.1–5.1)	3.9 (2.1–5.1)
WBC (×10³/μL)		6.5 (2.5-18.3)	6.7 (3.7-22.8)	6.5 (2.5-22.8)
Neut (×10³/μL)		n=61	n=104	n=165
		4.7 (1.6-15.2)	4.3 (1.8-19.6)	4.4 (1.6-19.6)
Lym (/μL)		n=61	n=103	n=164
		1400 (200–3000)	1300 (100–3700)	1400 (100–3700)
CEA (ng/mL)		33.8 (1–2230)	14.5 (0.7–4747.3)	18.4 (0.7–4747.3)
CYF (ng/mL)		n=56	n=102	n=158
		4 (0.6-144)	3.5 (0.5-78.9)	3.7 (0.5-144)
PD-L1	Negative	9 (39)	14 (61)	23
	1–49%	5 (45)	6 (55)	11
	≥50%	1 (14)	6 (86)	7

Table 2. Frequency of T790M mutations after first line EGFR-TKI failure (N=166).

Type of EGFR-TKI	Gefitinib	Erlotinib	Afatinib	Total
Frequency of T790M mutations	25/72	18/43	18/51	61/166
(%)	34.7% + 41.9% = 37.4%		35.3 %	36.7 %

Figures

Figure 1.

※ Due to Exclude variables in patient background with 10% or more missing data

Figure 1

CONSORT diagram. EGFR m+: epidermal growth factor receptor mutation positive, EGFR-TKI: epidermal growth factor receptor-tyrosine kinase inhibitor, PD: disease progression, PD-L1: programmed cell death-ligand 1, TTF-1: thyroid transcription factor-1, KL-6: sialylated carbohydrate antigen KL-6, NSE: neuron specific enolase, Pro-GRP: pro-gastrin releasing peptide.

Figure 2

The predictive markers for the detection of T790M by CART analysis. (a) in the patients included by CART analysis, (b) in deletion 19, (c) in L858R. CYF: cytokeratin 19 fragmen, WBC: white blood cell count, Liver: liver metastasis , LDH: lactate dehydrogenase, AUROC: area under the receiver operating characteristic, Alb: albumin, Bone: bone metastasis, Pleural: pleural effusion, CEA: carcinoembryonic antigen, ALP: alkaline phosphatase, +: existence, -: nonexistence.

Figure 3

Kaplan-Meier plots for (a) overall survival (OS) in eligible patients. The median OS was 1041 days. (b) OS between T790M negative and T790M positive in eligible patients. The median OS were T790M (-) :798 days, T790M (+) :Not reached [HR = 2.70 (95%CI: 1.64 – 4.55), P < 0.001].

Figure 4a.

Figure 4b.

Figure 4

Kaplan-Meier plots for (a) progression-free survival (PFS) and (b) OS between afatinib and 1st-generation EGFR-TKIs (gefitinib or erlotinib). The median PFS: 398 vs. 279 days; HR: 0.67 [95% CI: 0.50–0.88]; P=0.004. The median OS: 1053 vs. 956 days; HR: 0.68 [95% CI: 0.46–1.01]; P=0.051.