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Original Article

T790M detection rate after first-line combination therapy with bevacizumab and EGFR-TKIs in advanced NSCLC (TERRA Study)

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Abstract: Real-world data regarding the T790M mutation rate after acquiring resistance to first-line combination therapy with epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) and bevacizumab in patients with advanced non-small-cell lung cancer (NSCLC) are limited. The present study was aimed at analyzing predictors of acquired T790M mutations in this patient group. A total of 107 patients who received first-line combination therapy with EGFR-TKIs and bevacizumab at 11 tertiary referral centers in Taiwan were enrolled in this multicenter retrospective study. Survival data and genomic test results after acquiring resistance were analyzed. We discovered that patients who received a combination of afatinib, a second generation EGFR-TKI, and bevacizumab showed better progression-free survival (PFS). After disease progression, 59 patients (55.1%) were confirmed to test positive for EGFR T790M. A longer duration of first-line therapy could be a predictor of subsequent T790M mutations. To our knowledge, this is one of the few and early studies to demonstrate the T790M mutation rate after first-line combination therapy with an EGFR-TKI and bevacizumab. Whether the longer PFS afforded by the addition of bevacizumab could lead to subsequent T790M mutations needs further investigation.

Keywords: NSCLC, T790M, EGFR-TKI, bevacizumab, PFS

Introduction

Non-small-cell lung cancer (NSCLC), which accounts for more than 80% of lung cancer cases, is one of the most common, fatal cancers [1]. Somatic activating mutations in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) are present in approximately 50% of Asian patients with advanced adenocarcinoma, which is the most common histology subtype of NSCLC [2, 3]. For patients with EGFR-mutant NSCLC, tyrosine kinase inhibitors (TKIs), which can efficiently target the ATP-binding pocket of the EGFR-tyrosine kinase domain to block downstream signaling pathways, have become the major treatment modality [4]. Both first- and second-generation EGFR-TKIs have been demonstrated good treatment efficacy against EGFR mutation positive NSCLC [5-8]. On acquiring treatment resistance, more than 50% of patients have a new Thr790Met point mutation (T790M) in the EGFR kinase domain, which causes steric hindrance that affects the binding of EGFR-TKIs [9]. Second-line use of the third-generation EGFR-TKI osimertinib was proven to exert better treatment efficacy than that of chemotherapy in the phase 3 AURA3 trial [10, 11].

More recently, the phase 3 FLAURA study revealed that first-line treatment with osimertinib had superior clinical benefits in terms of progression-free survival (PFS) and overall survival (OS) compared with erlotinib and gefitinib, which led to its approval as a first-line treatment option [12, 13]. However, no randomized-controlled trial has compared the treatment efficacy of osimertinib and a second-generation EGFR-TKI. A multicenter cohort study demonstrated that afatinib provided better PFS and OS in patients with EGFR exon 21 L858R substitution and without brain metastasis [14]. Moreover, the GioTag study reported that sequential therapy with afatinib followed by osimertinib could provide Asian patients with a median OS of 44.8 months [15], which also implied that sequential treatment with a first- or second-generation EGFR-TKI followed by osimertinib might be a better choice. According to the ARISE study, a longer duration of first-line therapy with an EGFR-TKI was associated with higher chance of subsequent T790M emergence. All the above data highlight the importance of prolonging the treatment efficacy of first-line therapy [15].

The tumor exosomes-mediated intercellular transfer of EGFR signaling would upregulate VEGF expression in the endothelial cells through the activation of MAPK and Akt pathways, which support the combination of EGFR-TKIs and antiangiogenic therapy would improve the survival of EGFR-mutant NSCLC patients [16-18]. One such approach was to combine EGFR-TKIs with bevacizumab, an anti-angiogenic agent targeting vascular endothelial growth factor (VEGF). The first randomized phase II trial, JO25567, that compared bevacizumab and erlotinib combination therapy with erlotinib monotherapy in treatment-naive patients with advanced EGFR-mutant NSCLC showed a PFS advantage [19]. This result was further confirmed by another phase 3 randomized trial, NEJ026 [20], which showed an improvement in PFS from 13.3 months (erlotinib alone) to 16.9 months (bevacizumab combined with erlotinib). It is important to note the proportion of patients acquired T790M mutation after combined therapy with erlotinib and bevacizumab. Although a prospective study [21] aimed to answer this question, the issue is yet to be elucidated. Therefore, in this study, we retrospectively reviewed patients who acquired resistance to first-line combination treatment with a first- or second-generation EGFR-TKI and bevacizumab, and then underwent tissue or liquid biopsy for repeated EGFR mutation tests. The primary objective was to determine the T790M mutation rate and its predicting factor after combination therapy in a real-world setting. As a pan-HER inhibitor, afatinib exhibited clinical efficacy against HER2 alterations, identified as a form of bypass tract activation upon disease progression [22]. The use of afatinib as a first-line treatment may lead to a higher acquisition rate of T790M [23, 24]. However, studies comparing the rates of acquired T790M mutations between patients who received first- and second-generation EGFR-TKIs have yielded controversial results. Thus, the choice of different EGFR-TKIs was also analyzed as a predictive factor.

Methods

Study design

This retrospective, multicenter study was conducted in Taiwan. Patients with disease progression after first-line combination therapy with bevacizumab and a first- or second-gener-

ation EGFR-TKI for advanced EGFR-mutant NSCLC at 11 tertiary referral centers, between January 1, 2014 and June 30, 2021, were identified. Among these, patients harboring EGFR mutation subtypes other than an exon 19 deletion and exon 21 L858R substitution, and those with de novo T790M mutations, were excluded. Demographic data at the initial diagnosis, including age, sex, smoking status, performance status, disease stage, medical history, site of metastasis, histologic findings, and EGFR mutation subtype, were recorded.

Statistical and survival analysis

Frequencies and descriptive statistics of the demographic and clinical variables were calculated. Non-categorical data are expressed as mean ± standard deviation (SD) values, while categorical data are expressed as percentages. The objective response rate was defined as the proportion of patients with a complete response or partial response to treatment according to Response Evaluation Criteria in Solid Tumors (RECIST) v1.1. The PFS was estimated using the Kaplan-Meier method and compared using the log-rank test. PFS was calculated from the initiation of first-line treatment until the date of radiological progression, according to the RECIST v1.1. To identify the prognostic factors for PFS, Cox proportional hazard regression analysis was performed. Age, sex, disease stage, performance status, history of smoking, metastatic sites, EGFR mutation subtypes, and first-line therapy were chosen as prognostic factors.

Emergence of an acquired T790M mutation

Upon disease progression, detailed information on re-biopsy and the subsequent T790M mutation testing and status was recorded. The detection methods of acquired T790M including polymerase chain reaction (PCR)-based methods, MassARRAY genotyping (Agena Bioscience, California, USA), next generation sequencing, and laboratory-developed test. The PCR based methods include COBAS EGFR mutation test v2 (COBAS; Roche Molecular Systems Inc., New Jersey, USA), Therascreen EGFR RGQ PCR Kit (Therascreen; Scorpions & amplification refractory mutation system [ARMS], Qiagen Manchester Ltd., Manchester, UK), Beads, Emulsion, Amplification and Magnetics (BEAMing) digital PCR (dPCR) assay (BEAMing; OncoBEAM EGFR assay; Sysmex Inostics, Inc., Maryland, USA), competitive allele-specific TaqMan polymerase chain reaction (TaqMan; Life Technologies; Thermo Fisher Scientific, Inc., Massachusetts, USA). The next generation sequencing includes the Oncomine Comprehensive Assay Plus (Thermo Fisher Scientific, Waltham, USA), ACTOnco panel (ACT Genomics, Taipei, Taiwan) and FoundationOne® CDx (Foundation Medicine Inc., MA, USA). The laboratory-developed test for the detection of EGFR mutation included peptide nucleic acid locked nucleic acid sequencing (PNA-sequencing) and direct sequencing [25, 26]. Multivariate logistic regression analysis was performed to determine predictive factors for the emergence of acquired T790M mutations. Age, sex, disease stage, performance status, history of smoking, metastatic sites, EGFR mutation subtypes, and the choice and duration of first-line therapy were chosen as predictive factors. All statistical analyses were performed using SAS version 9.4 (SAS Institute). The reported *P*-values are two-sided.

Ethics statement

The study complied with the guidelines of the International Conference on Harmonization Tripartite Guideline for Good Clinical Practice and relevant local regulations. It was reviewed and approved by the review board and ethics committee of each tertiary referral center, and all data were anonymized according to approved guidelines and the tenets of the Declaration of Helsinki.

Results

Patients and clinical characteristics

The median age of the 107 enrolled patients was 60.0 (range: 36-87) years. Most of the enrolled subjects were female (n = 70, 65.4%), never-smokers (n = 83, 77.6%), and had a good performance status (n = 52, 48.6%). Most patients had advanced or metastatic disease at the initial diagnosis (n = 95, 88.8%), and all patients had the pathologic subtype of adenocarcinoma. Sixty-three (58.9%) patients harbored an exon 19 deletion mutation, while the remaining 44 (41.1%) had an exon 21 L858R substitution. Regarding metastatic burden, 58 (54.2%) and seven (6.5%) patients had brain and liver metastases, respectively, at the initial diagnosis. In addition, 47 (43.9%) patients had pleural effusion and two (1.9%) patients had pericardial effusion. The bevacizumab dose

Table 1. Baseline demographic data

Characteristics	Total population N = 107	First-generation EGFR-TKIs N = 77	Second-generation EGFR-TKIs N = 30	*P*-value
Age				0.711
Mean ± SD	60.0 ± 10.9	60.4 ± 10.5	59.1 ± 10.4	
Median (range)	60.0 (36-87)	61.0 (54-67)	59.5 (42-78)	
Sex				0.462
Female	70 (65.4%)	52 (67.5%)	18 (60.0%)	
Male	37 (34.6%)	25 (32.5%)	12 (40.0%)	
Smoking status				0.707
Current	4 (3.7%)	3 (3.9%)	1 (3.3%)	
Former	20 (18.7%)	15 (19.5%)	5 (16.7%)	
Never	83 (77.6%)	59 (76.6%)	24 (80.0%)	
ECOG PS				0.541
0	52 (48.6%)	36 (46.8%)	16 (53.3%)	
1	45 (42.1%)	32 (41.6%)	13 (43.3%)	
2	10 (9.3%)	9 (11.6%)	1 (3.3%)	
Stage				0.712
IIIB	3 (2.8%)	2 (2.6%)	1 (3.3%)	
IV	95 (88.8%)	69 (89.6%)	26 (86.7%)	
Recurrence	9 (8.4%)	6 (7.8%)	3 (10.0%)	
EGFR mutation type				0.307
Exon 19 deletion	63 (58.9%)	43 (55.8%)	20 (66.7%)	
Exon 21 L858R substitution	44 (41.1%)	34 (44.2%)	10 (33.3%)	
Pleural effusion				0.037
Absence	60 (56.1%)	48 (62.3%)	12 (40.0%)	
Presence	47 (43.9%)	29 (37.7%)	18 (60.0%)	
Pericardial effusion				0.485
Absence	105 (98.1%)	76 (98.7%)	29 (96.7%)	
Presence	2 (1.9%)	1 (1.3%)	1 (3.3%)	
Brain metastasis				< 0.001
Absence	49 (45.8%)	27 (35.1%)	22 (73.3%)	
Presence	58 (54.2%)	50 (64.9%)	8 (26.7%)	
Liver metastasis				0.367
Absence	100 (93.5%)	73 (94.8%)	27 (90.0%)	
Presence	7 (6.5%)	4 (5.2%)	3 (10.0%)	
Bevacizumab dose				0.682
Standard dose (15 mg/kg)	5 (4.7%)	4 (5.2%)	1 (3.3%)	
Reduced dose (7.5 mg/kg)	102 (95.3%)	73 (94.8%)	29 (96.7%)	

EGFR, epidermal growth factor receptor; TKIs, tyrosine kinase inhibitors; ECOG PS, Eastern Cooperative Oncology Group performance status.

was based on the physician's discretion, and 102 patients (95.3%) received a reduced dose (7.5 mg/kg). In most patients (77, 72.0%), first-generation EGFR-TKIs were used as first-line therapy. As dacomitinib was not reimbursed until the end of 2020, all patients who received a second-generation EGFR-TKI were treated with afatinib. Baseline characteristics were mostly similar between patients who received first- and second-generation EGFR-TKIs. However, those who received first-generation EGFR-TKIs had higher incidences of brain metastasis and lower incidence of malignant pleural effusion ($P < 0.001$ and $P = 0.037$, respectively). Detailed demographic data are presented in **Table 1**.

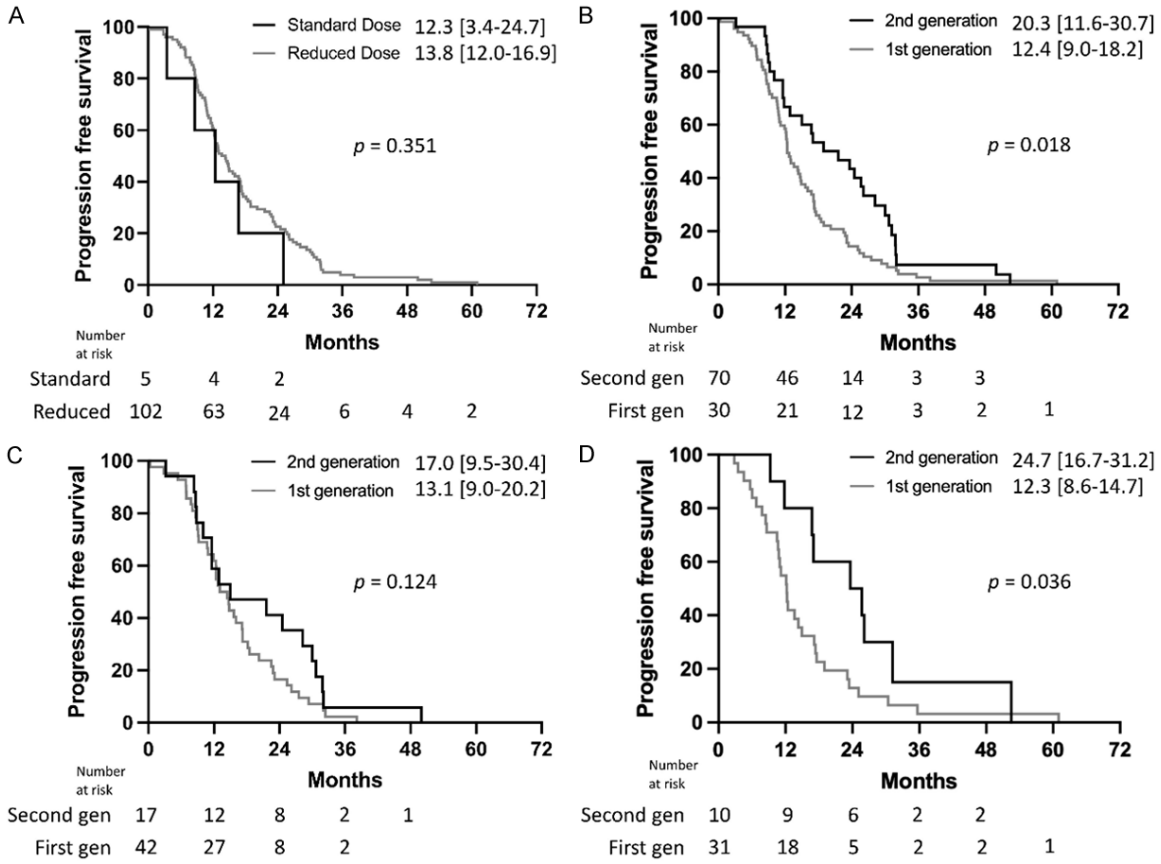

Figure 1. Progression-free survival of (A) patients who received different doses of bevacizumab, (B) patients who received different EGFR-TKIs, (C) subgroup patients with exon 19 deletions who received different EGFR-TKIs, and (D) subgroup patients with exon 21 L858R substitutions who received different EGFR-TKIs. EGFR-TKIs: epidermal growth factor receptor-tyrosine kinase inhibitors.

Statistical and survival analysis

The ORR was 67.3% for the total population and was similar between patients who received the standard dose and those who received a reduced dose of bevacizumab. The ORR was also similar among patients using first- or second-generation EGFR-TKIs and those with different metastatic burdens. Detailed information on tumor response is shown in Supplementary Table 1. The median PFS of the total population was 13.4 (range: 12.0-16.8) months (Supplementary Figure 1), which was similar to that among patients who received different doses of bevacizumab (**Figure 1A**). In contrast, the PFS of patients who received the second-generation EGFR-TKI afatinib was 20.3 months, which was significantly longer than that of patients who received the first-generation EGFR-TKIs gefitinib or erlotinib (P = 0.018, **Figure 1B**). In addition, when the patients were classified by EGFR mutation subtypes, improvement in PFS was more significant in patients with an exon 21 L858R substitution than in those with an exon 19 deletion (**Figure 1C** and **1D**).

Regarding the metastatic site, the presence of pleural effusion, liver metastasis, or brain metastasis did not affect the ORR and PFS (**Figure 2**; Supplementary Table 1). Although patients with pericardial effusion seemed to have longer PFS, the number of patients was limited (**Figure 2**; Supplementary Table 1). Cox proportional hazard regression analysis performed to eliminate potential confounding factors showed that the use of second-generation EGFR-TKIs was an independent and good prognostic factor for PFS (hazard ratio [HR] 0.46, 95% CI: 0.28-0.77, P = 0.003) (**Table 2**). In contrast, older age and male gender were independent and poor prognostic factors for PFS (HR:

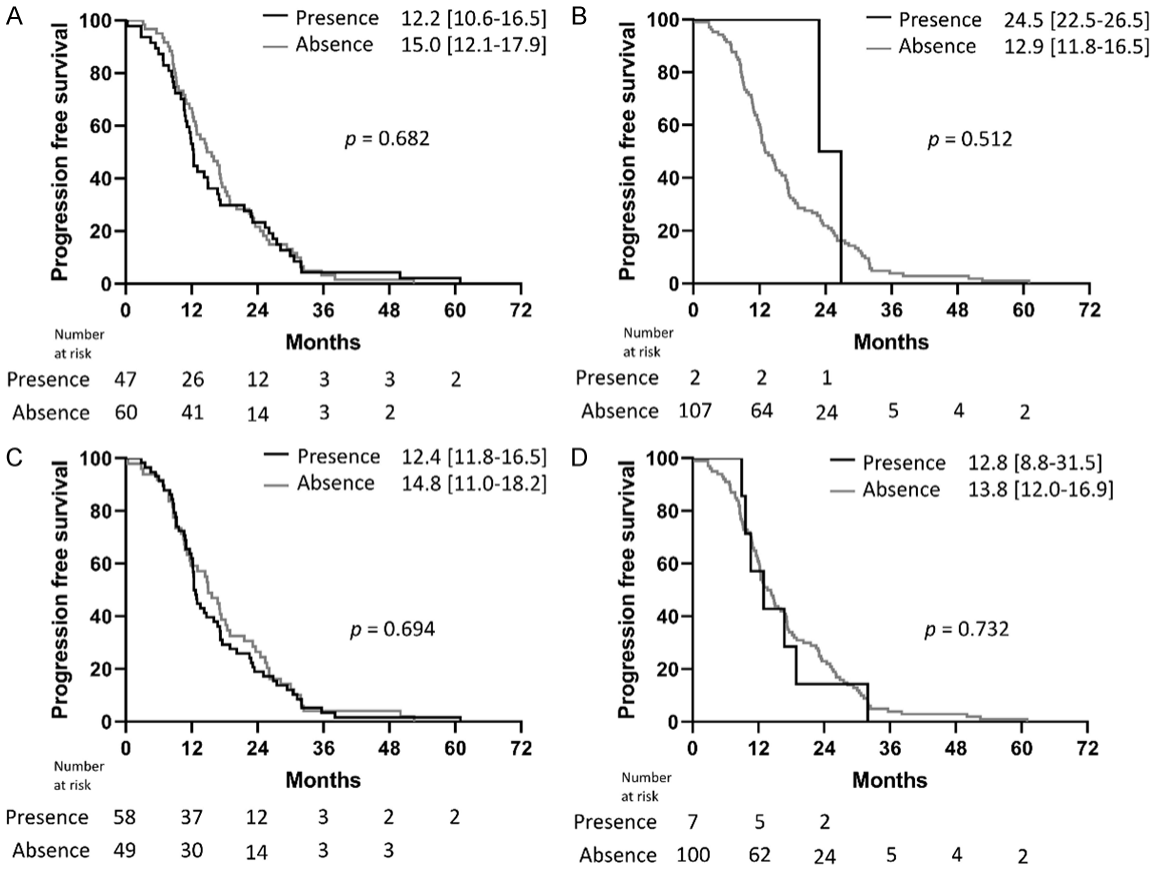

Figure 2. Progression-free survival of patients with the presence and absence of (A) pleural effusion, (B) pericardial effusion, (C) brain metastasis, and (D) liver metastasis.

1.67 and 1.60, P = 0.020 and P = 0.049, respectively; **Table 2**).

Emergence of acquired T790M mutations

After acquiring resistance to first-line combination therapy, 71 of the 107 patients (66.3%) underwent tissue biopsy for subsequent T790M testing, and the remaining 36 patients (33.7%) underwent liquid biopsy. Fifty-nine patients (55.1%) were confirmed to test positive for EGFR T790M. The positivity rate for T790M was similar between tissue and liquid biopsies (40/71 [56.3%] and 19/36 [52.8%], respectively). Similarly, the T790M positivity rates were 54.5% (42/77) and 56.7% (17/30) among patients who received first- and second-generation EGFR-TKIs, respectively. And there was no significant difference between these two groups. However, patients harboring exon 19 deletions had a relatively higher T790M positivity rate (39/63, 61.9%) than that in patients with exon 21 L858R substitutions (20/44, 45.5%; P = 0.092). The T790M positivity rate was significantly higher among patients who achieved PFS of more than 12 months with first-line combination therapy (43/65, 66.2%) than those who achieved PFS of less than 12 months (16/42, 38.1%; P = 0.004). Although there are various testing platforms available, our results indicate that there is no significant difference in the positivity rate of T790M among different institutions (Supplementary Table 3) or testing platforms (Supplementary Table 4). The detailed data of the re-biopsy and subsequently acquired T790M mutations are summarized in **Table 3**. Multivariate logistic regression analysis showed that female gender (odds ratio [OR]: 2.92, 95% CI: 1.10-7.73, P = 0.031) and PFS of more than 12 months (OR: 3.24, 95% CI: 1.35-7.75, P = 0.008) were independent predictors of T790M positivity (**Table 4**).

Adverse events

The adverse event profile of the patients is summarized in Supplementary Table 2. All

Table 2. Cox proportional hazard regression analysis of progression-free survival

	Hazard ratio (95% CI)	P
Age		
≥ 60 years versus < 60 years	1.67 (1.09-2.56)	0.020
Sex		
Male versus female	1.60 (1.00-2.54)	0.049
Stage		
Recurrence versus newly diagnosed	0.59 (0.28-1.23)	0.156
Performance status		
ECOG PS ≥ 1 versus < 1	1.24 (0.82-1.87)	0.313
Smoking status		
Current/Former versus never-smoker	0.82 (0.49-1.36)	0.432
Brain metastasis		
Presence versus absence	0.83 (0.52-1.32)	0.428
Pleural effusion		
Presence versus absence	1.40 (0.91-2.14)	0.129
EGFR mutation		
L858R versus Del19	0.95 (0.63-1.43)	0.795
First-line therapy		
Second- versus first-generation EGFR-TKIs	0.46 (0.28-0.77)	0.003

EGFR, epidermal growth factor receptor; TKIs, tyrosine kinase inhibitors; ECOG PS, Eastern Cooperative Oncology Group performance status.

Table 3. T790M mutation status of the total population and each subgroup

	No. of patients (%)
Repeat biopsy specimen information	
Blood sample	36 (33.6%)
Tissue sample	71 (66.3%)
T790M status after acquired resistance	
Negative	48 (44.9%)
Positive	59 (55.1%)
Repeat biopsy specimen	
Blood sample (n = 36)	19 (52.8%)
Tissue sample (n = 71)	40 (56.3%)
First-line therapy	
Second-generation EGFR-TKIs (n = 30)	17 (56.7%)
First-generation EGFR-TKIs (n = 77)	42 (54.5%)
EGFR mutation type	
Exon 21 L858R substitution (n = 44)	20 (45.5%)
Exon 19 deletion (n = 63)	39 (61.9%)
PFS of first-line therapy	
< 12 months (n = 42)	16 (38.1%)
≥ 12 months (n = 65)	43 (66.2%)

EGFR, epidermal growth factor receptor; PFS, progression-free survival; TKIs, tyrosine kinase inhibitors.

adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0. The most frequent adverse event was skin rash (78.5%), followed by paronychia (45.8%) and diarrhea (43.9%). Most adverse events were of grade 1 or 2. The most common grade 3 adverse events were rash (6.5%), paronychia (3.7%), and proteinuria (3.7%). The other grade 3 adverse events included hypertension (1.9%), diarrhea (0.9%), mucositis (0.9%), palmar plantar pain (0.9%), nephrotic syndrome (0.9%), and alanine aminotransferase (ALT) elevation (0.9%).

Discussion

In this retrospective study, patients were enrolled from 11 tertiary referral centers in Taiwan. Median PFS was significantly longer among patients who received combination treatment with second-generation EGFR-TKI and bevacizumab than among those who received combination treatment with bevacizumab and first generation EGFR-TKI. Upon disease progression, 55.1% (59/107) of the patients acquired T790M alterations. Although the biopsy method and EGFR mutation subtypes did not affect the emergence of acquired T790M, longer PFS afforded by first-line therapy was an independent predictor of T790M positivity. This result implies that it is important to improve the efficacy of first-line treatments and that the addition of bevacizumab could be a good approach.

The efficacy of different EGFR TKIs in the first-line setting has been well studied. The phase 2 LUX-Lung 7 study found that patients who received afatinib had better PFS than those who received gefitinib; the difference in the median PFS between these two groups was only 0.1 months [27]. In contrast, there is growing real-world evidence demonstrating longer PFS in patients taking afatinib [28-31]. To the best of our knowledge, no previous study ever compared the treatment efficacy of the combi-

Table 4. Multivariate analysis of predictive factors for the emergence of acquired T790M mutation

Factors	Number of patients	Odds ratio (95% CI)	P
Age			
< 60 years	54	1	
≥ 60 years	53	1.25 (0.52-2.94)	0.608
Sex			
Male	37	1	
Female	70	2.92 (1.10-7.73)	0.031
Stage			
Newly diagnosed	98	1	
Recurrence	9	1.18 (0.25-5.55)	0.830
Performance status			
ECOG PS < 1	52	1	
ECOG PS ≥ 1	55	0.87 (0.37-2.06)	0.750
Smoking status			
Never-smoker	83	1	
Current/Former smoker	24	1.59 (0.52-4.84)	0.414
Brain metastasis			
Absence	49	1	
Presence	58	0.94 (0.36-2.45)	0.902
Pleural effusion			
Absence	60	1	
Presence	47	1.39 (0.57-3.40)	0.473
EGFR mutation			
Exon 19 deletion	63	1	
Exon 21 L858R substitution	44	0.87 (0.37-2.06)	0.083
First-line treatment			
First-generation EGFR-TKIs	77	1	
Second-generation EGFR-TKIs	30	0.93 (0.34-2.52)	0.881
First-line treatment duration			
< 12 months	42	1	
≥ 12 months	65	3.24 (1.35-7.75)	0.008

EGFR, epidermal growth factor receptor; TKIs, tyrosine kinase inhibitors; ECOG PS, Eastern Cooperative Oncology Group performance status.

nation of different EGFR-TKIs with bevacizumab.

In the present study, we found that, therapy with the combination of a second-generation EGFR-TKI and bevacizumab could provide significantly better PFS (20.3 months) than that provided by combined therapy with a first-generation EGFR-TKI and bevacizumab (12.4 months). The improvement in PFS was more significant in patients with exon 21 L858R substitutions. Median PFS in the second-generation EGFR-TKI group in our study was consistent with that in previous observational studies [32]. However, PFS in the first-generation EGFR-TKI group was shorter than the PFS results in previous clinical trials [20, 33], which may be due to the higher incidence of brain metastasis in the present study. Using Cox proportional hazard regression analysis to adjust for the potential confounding effects of brain metastasis, we were able to identify that the use of second-generation EGFR-TKIs is still an independent and good prognostic factor for PFS. Moreover, the better treatment efficacy of afatinib in patients with exon 21 L858R substitution was consistent with that in a previous clinical trial [27] and cohort study [34]. To the best of our knowledge, this is the first real-world study to compare the efficacy of combination treatments involving different EGFR-TKIs and bevacizumab.

Regarding the dose of bevacizumab, a previous study found that the dose of 7.5 mg/kg was as effective as 15 mg/kg in combination with chemotherapy among Asian patients with NSCLC [35, 36]. However, data regarding the combination of different doses of bevacizumab and EGFR-TKIs remains limited. Although the number of patients who received a standard dose of bevacizumab was limited in the present study, we found that the dose of bevacizumab did not affect treatment efficacy.

Our results showed that the proportion of patients with subsequent T790M mutations was 55.1%. A previous meta-analysis focusing on EGFR-TKI monotherapy demonstrated a similar T790M positivity rate in the total population, and the rate was higher in a subgroup analysis of patients with exon 19 deletions [37]. In another single-center cohort study, long duration of first-line therapy was shown to be a predictor of the subsequent emergence of T790M [38]. This result was further confirmed

in a multicenter retrospective cohort study [39]. Similarly, in the present study, multivariate logistic regression also showed that a longer duration of first-line therapy was associated with a higher incidence of subsequent T790M mutations. Given the prolonged PFS afforded by bevacizumab use in a previous clinical trial [20], and the relatively few adverse events in the present study, future study is warranted to validate whether the addition of bevacizumab could potentially increase the possibility of receiving second-line therapy with osimertinib.

This retrospective study has some limitations. First, about one third of the patients who received combined therapy did not undergo repeat biopsy after completion of first-line treatment. Furthermore, although bevacizumab has been approved as combined therapy with EGFR TKIs in Taiwan, it has not been covered by the national health insurance for lung cancer patients. Thus, the number of patients receiving combined therapy was limited. In addition, the number of patients in the present study was also too small to perform subgroup analysis with balanced baseline characteristics. Further prospective studies are warranted to validate these results. Second, the baseline characteristics of the patients receiving first- and second-generation EGFR-TKIs were imbalanced. A higher proportion of patients who received first-generation EGFR-TKIs had brain metastasis, which might affect the outcome analysis. However, we performed Cox proportional hazard regression analysis to eliminate potential confounding factors, and found the use of second-generation EGFR-TKIs was an independent and good prognostic factor. Third, although we found that the longer PFS achieved with first-line therapy was associated with a higher rate of subsequent T790M mutations, we did not compare the T790M mutation rate between patients receiving combined therapy and those who received EGFR-TKI monotherapy. Whether prolonged first-line treatment with bevacizumab will lead to a higher incidence of subsequent T790M mutations still warrants future investigation. Nevertheless, the current study provides meaningful information regarding T790M alterations after first-line combination therapy. To the best of our knowledge, this is the first real-world study to investigate T790M alterations after first-line combination therapy with different EGFR-TKIs and bevacizumab. Forth, the patients enrolled in present study only had single gene test at initial diagnosis. Whether the co-existing driver mutations, would interfere the treatment outcome could not be assessed by present study. However, the co-existing driver oncogene was not common [40], which might not cause significant influence on the survival outcome. Fifth, low socioeconomic status continues to be a negative prognostic factor for lung cancer patients [41, 42]. Given that we only enrolled patients who could afford self-paid medications, generalizing the data from this study to the broader population might present a challenge. However, since all participants in our study had similar baseline characteristics, we can still emphasize the significance of choosing the most effective first-line therapy to achieve longer progression-free survival. This approach is potentially predictive of a higher rate of acquired T790M mutation. Sixth, we did not include patients who received Osimertinib as their first-line therapy. It should be noted that the combination of Osimertinib and Bevacizumab has not been demonstrated any survival benefit compared to Osimertinib monotherapy in either first-line [43] or second-line settings [44]. Thus, we did not enroll patients who received the Osimertinib and Bevacizumab combination. Further research is necessary to compare the treatment efficacy between Osimertinib monotherapy and the combination of first- or second-generation EGFR-TKIs with Bevacizumab.

Conclusions

In the present study, we demonstrated that second-generation EGFR-TKIs could provide better PFS when combined with bevacizumab. When disease progression of the patients receiving 1st line combination therapy occurred, approximately half of these patients showed subsequent T790M mutations. Although the use of second-generation EGFR-TKIs was not a predictor of T790M, longer duration of first-line combination therapy could be a predictor of the emergence of acquired T790M mutations. These results highlight the importance of improving the efficacy of first-line therapy. Further prospective studies are warranted to investigate the association between bevacizumab use and subsequent T790M mutations.

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Disclosure of conflict of interest

CS-K received speaker honoraria from AstraZeneca, Boehringer Ingelheim, Roche, Pfizer, Eli Lilliy, Novartis, OnO Pharma, Chugai, Merck and Guardant Health. CS-K provided consultation for AstraZeneca, Boehringer Ingelheim, Eli Lilliy, Merck, Chugai, Takeda and Novartis. C-L C had received honoraria from AstraZeneca, Boehringer Ingelheim, Pfizer, and Roche. C-C H received honoraria from Boehringer Ingelheim, AstraZeneca, Eli Lilly, Roche/Genentech/Chugai, MSD, Merck, Pfizer, Novartis, Takeda, BMS and Ono pharmaceutical, Merck, Pfizer, Novartis, Takeda. J-Y H received honoraria from Boehringer Ingelheim, AstraZeneca, Eli Lilly, Roche/Genentech/Chugai, MSD, Merck, Pfizer, Novartis, Takeda, BMS and Ono pharmaceutical, Merck, Pfizer, Novartis, Takeda.

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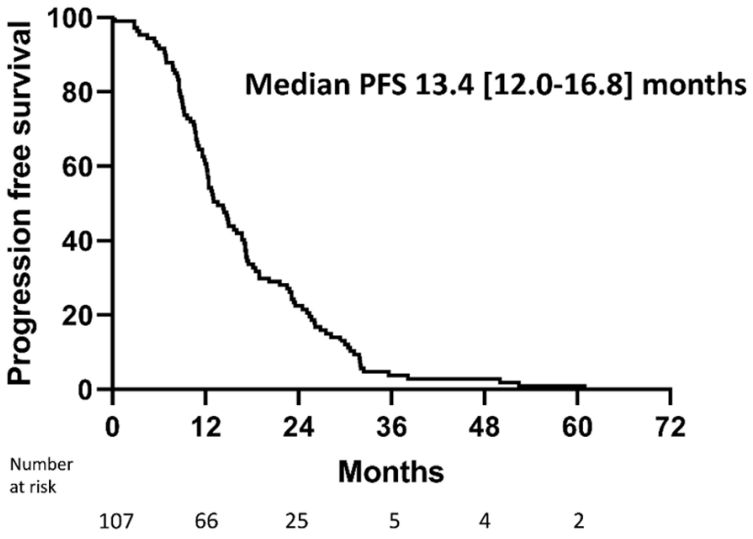
Supplementary Table 1. The summary of treatment efficacy

	Overall Response Rate, % (95% CI)	TKI DoT (months), median (95% CI)	TKI PFS (months), median (95% CI)
Total population	67.3 (57.9-75.5)	13.0 (11.8-16.3)	13.4 (12.0-16.8)
Bevacizumab Dosage			
Standard dose (15 mg)	60.0 (23.1-88.2)	13.1 (3.4-26.4)	12.3 (3.4-24.7)
Half dose (7.5 to 10 mg)	67.6 (58.1-75.9)	12.8 (11.8-16.3)	13.8 (12.0-16.9)
First-line TKI			
Second-generation	70.0 (52.1-83.3)	18.6 (12.1-26.0)	20.3 (11.6-30.7)
First-generation	67.7 (55.4-78.0)	12.4 (10.8-16.3)	12.4 (9.0-18.2)
History of pleural effusion			
No (n = 60)	66.7 (54.1-77.3)	15.4 (12.3-17.7)	15.0 (12.1-17.9)
Yes (n = 47)	68.1 (53.8-79.6)	11.9 (10.4-14.1)	12.2 (10.6-16.5)
History of cardiac effusion			
No (n = 105)	66.7 (57.2-75.0)	12.7 (11.7-16.0)	12.9 (11.8-16.5)
Yes (n = 2)	100.0 (34.2-100.0)	24.5 (22.5-26.5)	24.5 (22.5-26.5)
History of brain metastasis			
No (n = 49)	65.3 (51.3-77.1)	14.8 (11.9-18.9)	14.8 (11.0-18.2)
Yes (n = 58)	69.0 (56.2-79.4)	12.3 (10.8-15.2)	12.4 (11.8-16.5)
History of liver metastasis			
No (n = 100)	68.0 (58.3-76.3)	12.8 (11.8-16.0)	13.8 (12.0-16.9)
Yes (n = 7)	57.1 (25.0-84.2)	16.3 (10.3-33.4)	12.8 (8.8-31.5)

DoT, duration of therapy; PFS, progression-free survival; TKI, tyrosine kinase inhibitor.



Supplementary Figure 1. Progression-free survival of the total population.

Supplementary Table 2. Summary of adverse events

Adverse event	Patient number, n (%)			
	Any grade	Grades 1-2	≥ Grade 3	Unknown
Rash	84 (78.5)	77 (72.0)	7 (6.5)	
Paronychia	49 (45.8)	45 (42.1)	4 (3.7)	
Diarrhea	47 (43.9)	46 (43.0)	1 (0.9)	
Mucositis	32 (29.9)	31 (29.0)	1 (0.9)	
Hypertension	23 (21.5)	21 (19.6)	2 (1.9)	
Liver function: ALT elevation	21 (19.6)	20 (18.7)	1 (0.9)	
Liver function: AST elevation	18 (16.8)	18 (16.8)		
Proteinuria	17 (15.9)	13 (12.1)	4 (3.7)	
Pulmonary infiltrates	16 (15.0)	16 (15.0)		
Hemorrhage	9 (8.4)	9 (8.4)		
Renal function: CCr	7 (6.5)	7 (6.54)		
Fatigue	4 (3.7)	4 (3.7)		
Injection reaction	3 (2.8)	3 (2.8)		
Nausea	3 (2.8)	3 (2.8)		
Pruritus	3 (2.8)	3 (2.8)		
Neuropathy	2 (1.9)	2 (1.9)		
Palmar plantar erythrodysesthesia	2 (1.9)	1 (0.9)	1 (0.9)	
Pneumonitis	2 (1.9)	2 (1.9)		
Vomiting	2 (1.9)	2 (1.9)		
Alopecia	1 (0.9)	1 (0.9)		
Increased ALP	1 (0.9)	1 (0.9)		
Anemia	1 (0.9)	1 (0.9)		
Constipation	1 (0.9)	1 (0.9)		
Creatinine increased	1 (0.9)	1 (0.9)		
Duodenal ulcer	1 (0.9)	1 (0.9)		
Dysgeusia	1 (0.9)	1 (0.9)		
Ileus	1 (0.9)	1 (0.9)		
Insomnia	1 (0.9)			1 (0.9)
Nephrotic syndrome	1 (0.9)		1 (0.9)	
Neutropenia	1 (0.9)	1 (0.9)		
Platelet count decreased	1 (0.9)	1 (0.9)		
Pulmonary fibrosis	1 (0.9)	1 (0.9)		
Soreness	1 (0.9)			1 (0.9)

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate transaminase; CCr, creatinine clearance.

Supplementary Table 3. The results of T790M testing upon disease progression varied across different institutions

Institution	T790M test		*P* value
	Positive	Negative	
CGMH-KS	10	3	0.200
CGMH-LK	13	7	
EDH	2	2	
KMUH	0	1	
MMH	3	1	
NCKUH	8	10	
NTUH	12	11	
NTUH-HC	2	8	
NTUH-YL	2	0	
VGH-TC	4	4	
VGH-TPE	3	1	

Supplementary Table 4. The results of T790M testing upon disease progression varied across different testing platform

T790M testing platform	T790M test		*P* value
	Positive	Negative	
COBAS EGFR mutation test v2	10	10	0.965
Therascreen EGFR RGQ PCR Kit	8	3	
Beads, Emulsion, Amplification and Magnetics (BEAMing) digital PCR (dPCR) assay	5	4	
competitive allele-specific TaqMan polymerase chain reaction	5	3	
Oncomine Comprehensive Assay Plus	5	4	
FoundationOne® CDx	4	4	
ActGenomics	6	5	
MassARRAY genotyping	6	4	
Laboratory-developed test	10	11	