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Original Research

Comparison of afatinib and erlotinib combined with bevacizumab in untreated stage IIIB/IV epidermal growth factor receptor-mutated lung adenocarcinoma patients: a multicenter clinical analysis study

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Abstract

Background: Although bevacizumab in combination with afatinib or erlotinib is an effective and safe first-line therapy for advanced epidermal growth factor receptor (EGFR)-mutated non-small-cell lung cancer (NSCLC), there are very few clinical data comparing afatinib and erlotinib combined with bevacizumab. We performed a retrospective multicenter analysis for the comparison of two combination therapies.

Methods: Between May 2015 and October 2020, data of 135 stage IIIB/IV EGFR-mutated NSCLC patients receiving first-line afatinib or erlotinib combined with bevacizumab combination therapy in Linkou, Keelung, Chiayi, and Kaohsiung Chang Gung Memorial Hospitals were retrieved and retrospectively analyzed.

Results: In all, 67 patients received afatinib plus bevacizumab, and 68 patients received erlotinib plus bevacizumab. Afatinib combined with bevacizumab had an objective response rate (ORR) of 82.1% and a disease control rate (DCR) of 97.0%, and the ORR and DCR were 83.8 and 95.6%, respectively, in the erlotinib combined with bevacizumab group ($p = 0.798$ and $p = 1.000$). The median progression-free survival was 20.7 and 20.3 months for the afatinib plus bevacizumab group and the erlotinib plus bevacizumab group, respectively [hazard ratio (HR) = 1.02; 95% confidence interval (CI), 0.891–1.953; $p = 0.167$). The overall survival was 41.9 and 51.0 months for the afatinib plus bevacizumab group and erlotinib plus bevacizumab group, respectively (HR = 1.42; 95% CI, 0.829–2.436; $p = 0.201$). The secondary EGFR-T790M mutation rates after disease progression were 44% in the afatinib plus bevacizumab group and 58.8% in the erlotinib plus bevacizumab group ($p = 0.165$). Skin toxicity was the most frequent treatment-related adverse event (AE) in both treatment groups. Diarrhea, an AE, occurred significantly more frequently in the afatinib plus bevacizumab group than in the erlotinib plus bevacizumab group ($p < 0.05$).

Conclusion: Afatinib combined with bevacizumab was equally as effective as erlotinib combined with bevacizumab for untreated advanced EGFR-mutated NSCLC. Prospective clinical studies that explore bevacizumab combined with afatinib or erlotinib for advanced EGFR-mutated NSCLC are warranted.

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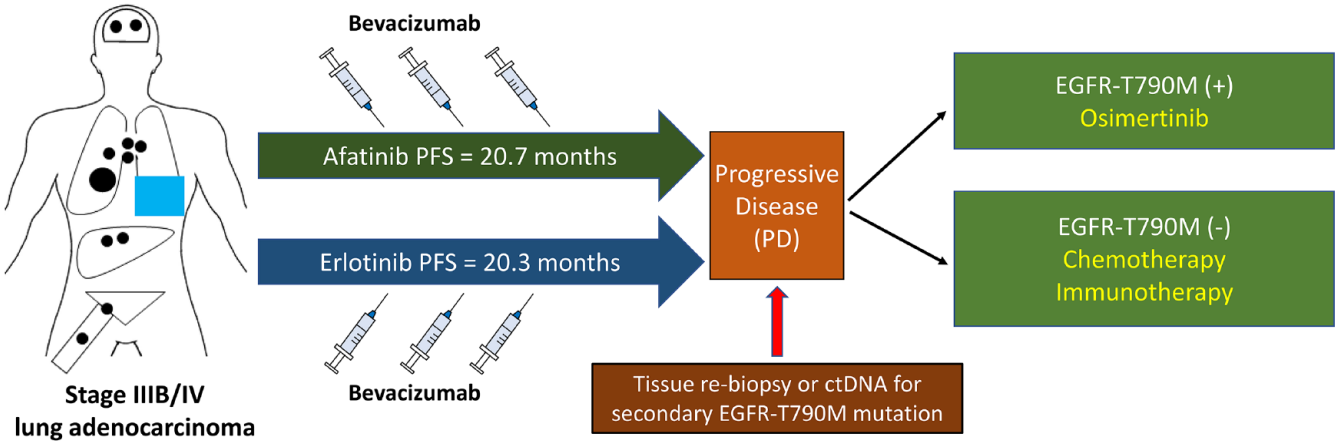

Graphic abstract

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Introduction

Epidermal growth factor receptor (EGFR) mutation is an oncogenic driver mutation that most frequently appears in East Asian non-small-cell lung cancer (NSCLC) patients (ranging from 45% to 55%).[1,2] EGFR tyrosine kinase inhibitor (TKI)-targeted therapies have been developed and used worldwide to treat advanced NSCLC harboring EGFR mutations.[1–4] In-frame deletions in exon 19 (exon 19 deletion) and leucine-to-arginine substitution point mutations at codon 858 in exon 21 (L858R) account for approximately 90% of activating EGFR mutations in NSCLC.[3,4] Other EGFR point mutations, including exon 18 Gly719Xaa (G719X), exon 20 Ser768Ile (S768I), and exon 21 Leu861Gln (L861Q), are uncommon (1−3% of EGFR mutations) but respond to EGFR-TKI therapy.[5,6]

The first-generation EGFR-TKI erlotinib has been approved as a standard first-line therapy for advanced EGFR-mutated NSCLC patients based on the results shown in previous clinical trials (EURTAC and OPTIMAL).[7,8] The two prospective clinical trials show that erlotinib is a promising treatment for advanced EGFR-mutated NSCLC because it has 60–80% objective response rates (ORRs) and a median progression-free survival (PFS) of 10–13 months.[7,8] Afatinib is classified as a second-generation EGFR-TKI because of its characteristic of irreversible covalent binding to pan-ErbB receptors.[2,6] Previous prospective clinical studies (LUX-Lung 3, 6, and 7 trials) have shown that afatinib is promising for untreated advanced NSCLC with EGFR mutations (55–70% ORRs and 11 months of PFS).[9,10] Therefore, afatinib has been approved as a first-line therapy for patients with EGFR-mutated advanced NSCLC.

Bevacizumab is a humanized monoclonal antibody that inhibits vascular endothelial growth factor (VEGF).[11,12] A previous preclinical study demonstrated that plasma VEGF and tumor VEGF mRNA levels increased after erlotinib treatment in an NSCLC xenograft murine model. Increasing VEGF levels in tumors and plasma are associated with tumor progression and erlotinib resistance in an NSCLC xenograft murine model.[12] In the same study, Naumov *et al.* showed that, by combining erlotinib and bevacizumab, dual inhibition of the EGFR and VEGF pathways increased the antitumor effect compared with bevacizumab or erlotinib alone. The experimental results presented by Naumov *et al.* indicated that erlotinib in combination with bevacizumab overcomes primary or acquired resistance to EGFR-TKIs and suggested that this combination should be explored in

clinical NSCLC patients.[12] The efficacy and safety of bevacizumab in combination with first- or second-generation EGFR-TKIs for the treatment of advanced EGFR-mutated NSCLC patients have been explored in several previous studies.[13–19] Three previous prospective clinical trials (JO25567, NEJ026, and BEVERLY trials) showed that erlotinib combined with bevacizumab therapy had significantly longer PFS than erlotinib alone in untreated EGFR-mutated advanced NSCLC patients.[13–16] A phase I clinical trial, the Okayama Lung Cancer Study Group Trial 1404, reported that the combination of afatinib and bevacizumab therapy was feasible and safe for the treatment of chemo-naïve advanced EGFR-mutated NSCLC patients.[17] In the same trial, afatinib combined with bevacizumab showed 24.2 months of PFS in chemo-naïve advanced EGFR-mutated NSCLC patients.[17] Another previous retrospective study showed that the combination of afatinib and bevacizumab had an 87.7% ORR and 23.9 months PFS as first-line therapy in advanced EGFR-mutated lung adenocarcinoma patients.[18]

Although previous clinical studies demonstrated that bevacizumab combined with afatinib or erlotinib was an effective treatment for advanced EGFR-mutated NSCLC, few studies have investigated the comparison between afatinib and erlotinib combined with bevacizumab in untreated advanced EGFR-mutated lung adenocarcinoma patients. In this study, we sought to perform a retrospective multicenter clinical analysis that compares the efficacy, safety, and acquired resistance of first-line afatinib or erlotinib combined with bevacizumab in EGFR-mutated lung adenocarcinoma patients.

Methods

Patients and treatment

The study patients were screened and retrospectively retrieved from the cancer center database in Linkou, Kaohsiung, Chiayi, and Keelung Chang Gung Memorial Hospitals. Between May 2015 and October 2020, 1264 histologically diagnosed stage IIIB/IV lung adenocarcinomas with EGFR mutations were screened, and 135 patients were ultimately retrieved for analysis. The inclusion criteria of this study were as follows: (a) patients with L858R, exon 19 deletion, or other uncommon sensitive EGFR mutations; (b) systemic treatment-naïve patients (no chemotherapy, targeted therapy, or immunotherapy prior to the combination therapy); (c) patients who received afatinib or erlotinib combined with bevacizumab as front-line therapy; and (d) bevacizumab should be administered for at least three cycles during afatinib or erlotinib therapy. The patients were excluded for the following reasons: (a) patients who previously received any systemic treatment (chemotherapy, targeted therapy, or immunotherapy), (b) the appearance of the *de novo* EGFR T790 M mutation, or (c) bevacizumab was not administered in the afatinib or erlotinib therapy course or fewer than three cycles of bevacizumab therapy were administered. The summary and screening of the study patients who underwent analysis are summarized in Figure 1.

All study patients underwent contrast medium enhancement computed tomography (CT), brain magnetic resonance imaging (MRI), and fluorodeoxyglucose positron emission tomography (PET) scans at the initial diagnosis to determine the baseline stages. All study patients received at least whole-body CT scans as the follow-up image every 3–4 months during treatment to evaluate the treatment responses. The clinical physicians were allowed to order additional images, such as chest plain film, sonogram, MRI, and PET scan, during treatment follow-up to assist in the judgment of disease status as needed.

Treatment responses, including complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD), were assessed based on Response Evaluation Criteria in Solid Tumors version 1.1. PFS was defined as the duration of time from the first EGFR-TKI dosing date to the date of first PD images or last follow-up. The duration of overall survival (OS) was defined as the length of time from the diagnosis to the recorded death date. If patients survived through the last follow-up time point (30 November 2021), the OS was censored at the date of the last clinical visit. Treatment-related adverse events (AEs) were graded based on the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

EGFR mutations, including primary mutations or secondary mutations (T790 M) with acquired resistance to treatment, were detected by direct sequencing, amplified refractory mutation system–Scorpion (ARMS/S) assays, or next-generation sequencing (NGS). The details of the NGS panel used in this study are shown in Supplemental Table S1.

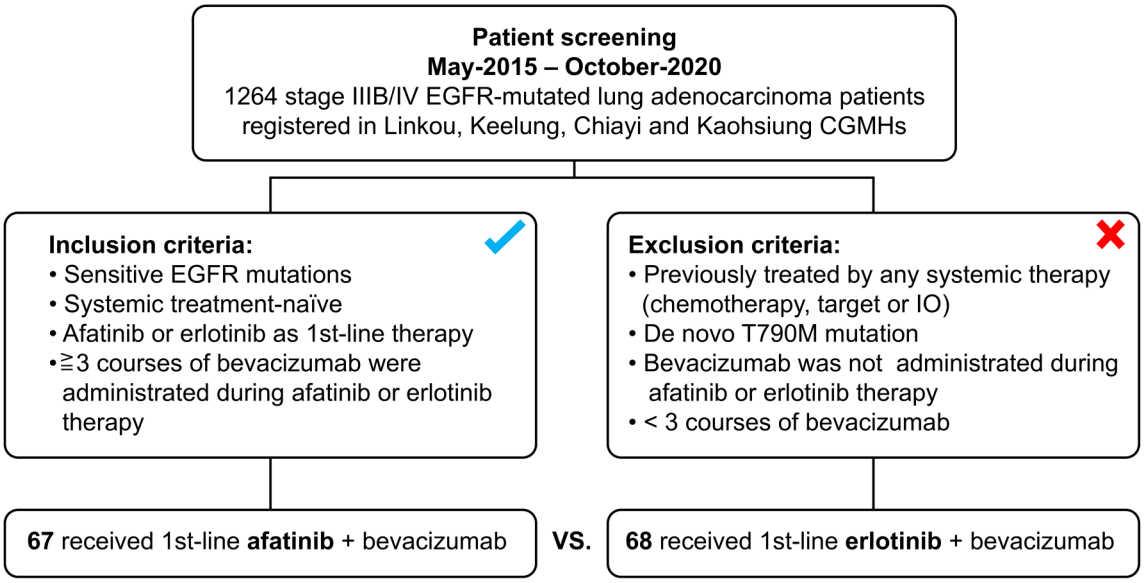

Figure 1. Summary of the inclusion and exclusion criteria for patient retrieval in this study.

Statistical analysis

The statistical significance of continuous variables between two treatment groups was assessed by the Mann–Whitney test. The comparison of categorical variables between two treatment groups was determined using chi-square and Fisher's exact tests. Kaplan–Meier survival curves were generated to estimate and compare PFS and OS between the two treatment groups. The *p* values were all two-sided and defined as statistically significant when they were smaller than 0.05. GraphPad Prism (version 5.0; GraphPad Software, San Diego, CA, USA) was used to perform statistical analyses and plot survival curves in this study.

Results

Baseline patient clinical characteristics

Of the 135 untreated advanced EGFR-mutated lung adenocarcinoma patients, 67 received afatinib combined with bevacizumab, and the other 68 received erlotinib combined with bevacizumab as first-line therapy. The comparison of baseline clinical characteristics between the two treatment groups is summarized in Table 1. For the methods used for primary EGFR detection, ARMS/S was the most frequently used in both treatment groups [65 (97%) in erlotinib plus bevacizumab group; 63 (92.6%) in afatinib plus bevacizumab], and some patients received direct sequencing [two (3%) in afatinib plus bevacizumab group; five (7.4%) in erlotinib plus bevacizumab]. Five patients [three (4.5%) in the afatinib plus bevacizumab group; two (2.9%) in the erlotinib plus bevacizumab] in this study received additional NGS tests, and all five patients also received ARMS. There was no statistically significant difference in EGFR detection methods used between the two treatment groups.

In the afatinib combined with bevacizumab group, two (3%) patients had uncommon EGFR mutations: one (1.5%) was G719X and another (1.5%) was S768I. The baseline diagnosis of distant metastatic sites, including the brain, bone, and liver, in the two treatment groups was analyzed, and there was no significant difference recorded in distant metastatic sites between the two groups of patients.

Only one (1.5%) patient received bevacizumab at a dose of 15 mg/kg in combination with afatinib. Regarding the dose de-escalation of EGFR-TKIs, more patients in the afatinib group had dose de-escalation than those in the erlotinib group [30 (44.8%) and 11 (16.2%) patients, $p < 0.001$].

The ORR, PFS, and OS in bevacizumab combined with afatinib or erlotinib

Among the 67 patients who received first-line afatinib plus bevacizumab, 1 (1.5%) had a CR,

Table 1. Comparison of patient baseline clinical characteristics between the two treatment groups in this study.

	Afa + Bev ***N*** **= 67**	**Erl + Bev** ***N*** **= 68**	***p*** **Value**
Sex			0.188
Male/female	28/39	21/47	
Age (mean ± SD)	57.3 ± 11.0	59.9 ± 11.0	0.358
ECOG PS			0.157
0–1	67 (100%)	66 (97%)	
2	0	2 (3%)	
Smoking			0.884
Former + current	17 (25.4%)	18 (26.5%)	
Non-smoker	50 (74.6%)	50 (73.5%)	
Histology			
Adenocarcinoma	67 (100%)	68 (100%)	
Stage			0.393
IIIB/IV	4/63	2/66	
EGFR mutations			0.355
Exon 19 deletion	31 (46.3%)	33 (48.5%)	
L858R	34 (50.7)	35 (51.5%)	
Others*	2 (3%)	0	
EGFR mutations detection methods			0.468
Direct sequencing	2 (3%)	5 (7.4%)	
ARMS Scorpion	65 (97%)	63 (92.6%)	
NGS	3 (4.5%)	2 (2.9%)	
Brain metastasis	19 (28.4%)	24 (35.3%)	0.387
Bone metastasis	30 (44.8%)	26 (38.2%)	0.441
Liver metastasis	10 (14.9%)	15 (22.1%)	0.286
Starting dose of EGFR-TKIs			
Afa			
40 mg/day	57 (85.1%)	0	
30 mg/day	10 (14.9%)	0	
Erl			
150 mg/day	0	68 (100%)	

(Continued)

Table 1. (Continued)

	Afa + Bev *N* = 67	Erl + Bev *N* = 68	*p* Value
100 mg/day	0	0	
Bev dose			
7.5 mg/kg	66 (98.5%)	68 (100%)	0.496
15 mg/kg	1 (1.5%)	0	
Dose de-escalation			
Afa (40 mg → 30 mg)	30 (44.8%)	0	0.001
Erl (150 mg → 100 mg)	0	11 (16.2%)	
Local radiation therapy			
Brain	11 (16.4%)	12 (17.6%)	0.759
Bone	17 (25.4%)	14 (20.6%)	1.000

Afa, afatinib; ARMS, amplified refractory mutation system; Bev, bevacizumab; ECOG PS, Eastern Cooperative Oncology Group Performance Status; EGFR-TKIs, epidermal growth factor receptor-tyrosine kinase inhibitors; Erl, erlotinib; NGS, next-generation sequencing; SD, standard deviation.
*G719X and S768I.

54 (80.6%) had PRs, 10 (14.9%) had SD, and 2 (3.0%) had PD to the combination therapy. In the 68 patients in the erlotinib plus bevacizumab group, 1 (1.5%) had a CR, 56 (82.3%) had PRs, 8 (11.8%) had SD, and 3 (4.4%) had PD to the combination therapy. The comparison of objective response and disease control rates between the two treatment groups is shown in Figure 2, and no statistical significance was observed ($p = 0.798$).

The median PFS was 20.7 months for the afatinib plus bevacizumab group and 20.3 months for the erlotinib plus bevacizumab group [hazard ratio (HR) = 1.02; 95% confidence interval (CI), 0.891–1.953; $p = 0.167$; Figure 3(a)]. The median OS was 41.9 months for the afatinib plus bevacizumab group and 51.0 months for the erlotinib plus bevacizumab group (HR = 1.42; 95% CI, 0.829–2.436; $p = 0.201$; Figure 3(b)). No statistical significance was noted in the comparisons of PFS and OS between the two treatment groups. The patients with and without brain metastasis at baseline diagnosis were analyzed. In all patients receiving bevacizumab combined with afatinib or erlotinib, the median PFS was 16.1 months for the brain metastasis group and 22.0 months for the without brain metastasis group (HR = 1.38; 95% CI, 0.885–2.159; $p = 0.155$; Figure 3(c)). Among the patients with brain metastasis, the median PFS was 16.1 months for the afatinib plus bevacizumab group and 16.1 months for the erlotinib plus bevacizumab group (HR = 1.78; 95% CI, 0.838–3.757; $p = 0.476$; Figure 3(d)). There was no statistical significance noted in the comparisons of PFS between with and without brain metastasis groups and the two treatment groups with brain metastasis.

Secondary EGFR-T790M mutations and subsequent treatments after progression of first-line bevacizumab combined with afatinib or erlotinib

In all, 53 patients had disease progression in the afatinib plus bevacizumab and erlotinib plus bevacizumab groups, and information on secondary EGFR-T790M mutations and subsequent treatments is summarized in Table 2. Most patients in both groups underwent tissue re-biopsy or circulating tumor-DNA for secondary EGFR-T790M mutation tests [50 (94.3%) in afatinib plus bevacizumab and 51 (96.2%) in erlotinib plus bevacizumab]. Among the 95 (89.6%) patients receiving tissue re-biopsy, none were recorded to have histological transformation of small-cell lung cancer (SCLC). In the patients receiving subsequent second-line osimertinib, SCLC transformation

developed in two (1.9%) patients after disease progression and was confirmed by tissue re-biopsy [one (0.9%) in the afatinib plus bevacizumab group and the other one (0.9%) in the erlotinib plus bevacizumab group].

In the 95 (89.6%) patients receiving tissue re-biopsy, ARMS/S was the most frequent method used for the detection of EGFR-T790M mutation [91 patients (95.8%)]. Eight (8.4%) patients received NGS tests from tissue re-biopsy, and 4 (4.2%) had both ARMS/S and NGS tests [three (3.2%) in the afatinib plus bevacizumab group and the other (1.0%) in the erlotinib plus bevacizumab group]. There was no statistically significant difference in the EGFR-T790M mutation detection methods used between the two treatment groups. The secondary EGFR-T790M mutation rates were 44% (22 patients) for the afatinib plus bevacizumab group and 58.8% (30 patients) for the erlotinib plus bevacizumab group. In all, 23 patients (43.4%) in the afatinib plus bevacizumab group took the third-generation EGFR-TKI osimertinib as second-line therapy, and 31 (58.5%) in the erlotinib plus bevacizumab group took second-line osimertinib.

In all, 19 patients (17.9%) received antiangiogenic agents, including bevacizumab and ramucirumab, in combination with subsequent osimertinib, chemotherapy, or immune checkpoint inhibitors [ICIs; 13 (24.5%) in the afatinib plus bevacizumab group and 6 (11.3%) in the erlotinib plus bevacizumab group]. Among 54 (50.9%) patients who took second-line osimertinib, seven (6.6%) received bevacizumab combined with osimertinib, and 4 (3.8%) received ramucirumab combined with osimertinib. Three patients (2.8%) received second-line therapy of atezolizumab in combination with carboplatin, paclitaxel, and bevacizumab [two (1.9%) in the afatinib plus bevacizumab group and one (0.9%) in the erlotinib plus bevacizumab group]. The remaining five (4.7%) patients received either bevacizumab or ramucirumab in combination with second-line chemotherapy.

A total of 12 (11.3%) patients with PD treated with first-line bevacizumab combined with afatinib or erlotinib did not receive subsequent systemic therapy and received supportive care only [eight (7.5%) in the afatinib plus bevacizumab group and 4 (3.8%) in the erlotinib plus bevacizumab group].

(a)

Clinical response to first-line afatinib or erlotinib + bevacizumab

	Afa+ Bev n (%)	Erl+ Bev n (%)	P-value
CR	1 (1.5)	1 (1.5)	
PR	54 (80.6)	56 (82.3)	
SD	10 (14.9)	8 (11.8)	
PD	2 (3.0)	3 (4.4)	
RR (%)	82.1	83.8	0.798
DCR (%)	97.0	95.6	

(b)

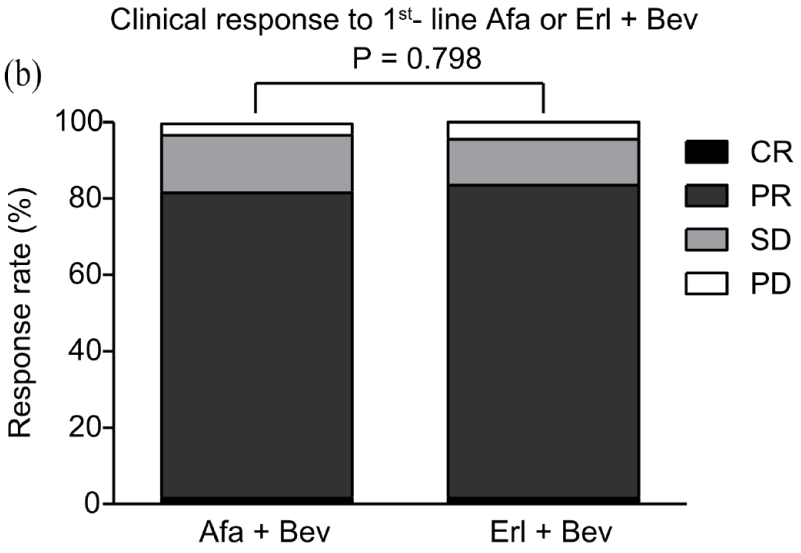

Figure 2. (a) Analysis of the clinical treatment response to bevacizumab combined with afatinib or erlotinib and (b) treatment response comparison between afatinib plus bevacizumab and erlotinib plus bevacizumab for untreated advanced epidermal growth factor receptor (EGFR)-mutated lung adenocarcinoma patients ($p = 0.798$).

The afatinib plus bevacizumab group had a trend of more patients receiving antiangiogenic agents than the erlotinib plus bevacizumab group in second-line therapy, but statistical significance was not achieved ($p = 0.076$). Overall, there was no statistically significant difference in secondary EGFR-T790M mutation rates and second-line systemic therapies between the two treatment groups.

First-line bevacizumab combined with afatinib or erlotinib therapy-related AEs

The comparisons of the combination of bevacizumab with afatinib or erlotinib therapy-related

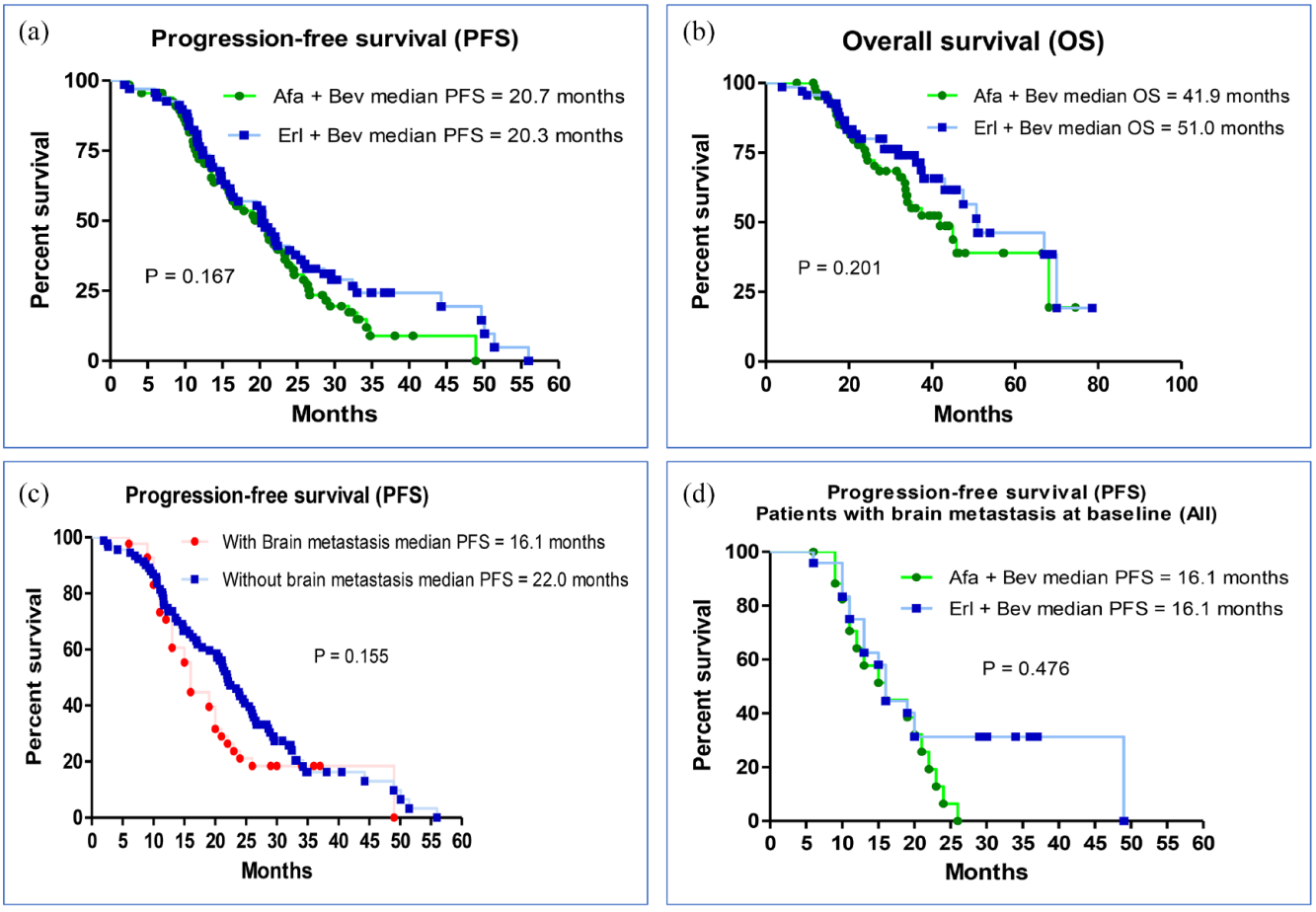

Figure 3. Analysis of progression-free survival (PFS) and overall survival (OS) by Kaplan–Meier survival curve. (a) Comparison of PFS between afatinib plus bevacizumab and erlotinib plus bevacizumab [hazard ratio (HR) = 1.02; 95% CI, 0.891–1.953; $p = 0.167$). (b) Comparison of OS between afatinib plus bevacizumab and erlotinib plus bevacizumab (HR = 1.42; 95% CI, 0.829–2.436; $p = 0.201$). (c) Comparison of PFS between patients with and without brain metastasis at baseline diagnosis (HR = 1.38; 95% CI, 0.885–2.159; $p = 0.155$). (d) Comparison of PFS between afatinib plus bevacizumab and erlotinib plus bevacizumab in patients with brain metastasis at baseline diagnosis (HR = 1.78; 95% CI, 0.838–3.757; $p = 0.476$).

AEs are summarized in Table 3. Among the 67 patients in the afatinib plus bevacizumab group, the most frequent AE was skin toxicity (65, 97%), followed by diarrhea (63, 94.0%), paronychia (49, 73.1%), and stomatitis (38, 56.7%). Among the 68 patients in the erlotinib plus bevacizumab group, skin toxicity was also the leading treatment-related AE in 53 (77.9%), followed by paronychia in 42 (61.8%) and stomatitis in 30 (44.1%). Treatment-related diarrhea was significantly higher in the afatinib plus bevacizumab group than in the erlotinib plus bevacizumab group in both grade 1 and grade 2 or higher than grade 3 toxicity ($p < 0.05$).

Regarding the side effects of hypertension induced by bevacizumab therapy, the incidences were similar in both treatment groups [16 (23.9%) for the afatinib plus bevacizumab group and 18 (26.5%) for the erlotinib plus bevacizumab group]. AE hypertension was limited to grades 1 and 2 and manageable. Some patients experienced grade 3 hypertension, which could be controlled by antihypertensive drugs.

Among the four (3.0%) patients with AEs of hemorrhage in this study, one (0.7%) patient in the afatinib combined with bevacizumab group had epistaxis that subsided after skipping one bevacizumab treatment course. In the three (2.2%) patients in the erlotinib combined with bevacizumab group, two (1.5%) had hemorrhoid bleeding, and the other patient had nasal bleeding. All three (2.2%) patients with AEs of bleeding were treated with topical medication and skipping one cycle of bevacizumab treatment. In

Table 2. Information on secondary EGFR-T790 M mutations and subsequent treatment after disease progression and first-line treatment with bevacizumab + EGFR-TKIs.

	Afa + Bev *n* = 53	Erl + Bev *n* = 53	*p* Value
EGFR-T790 M mutation tests	50 (94.3%)	51 (96.2%)	1.000
Tissue re-biopsy	47 (88.7%)	48 (90.6%)	1.000
ct-DNA	3 (5.7%)	3 (5.7%)	
EGFR-T790 M mutation detection methods			
ARMS Scorpion	45 (90%)	46 (90.2%)	0.778
NGS	5 (10%)	3 (5.9%)	
ct-DNA PCR	3 (6%)	3 (5.9%)	
EGFR-T790 M mutation			
Positive	22 (44%)	30 (58.8)	0.165
Negative	28 (56%)	21 (41.2%)	
Unknown (no re-biopsy and ctDNA)	3 (5.7%)	2 (3.8%)	
Systemic therapy following first-line Bev + EGFR-TKIs			
Osimertinib	23 (43.4%)	31 (58.5%)	0.174
Chemotherapy			
Platinum-based doublet	18 (34.0%)	13 (24.5%)	0.548
Single agent chemotherapy	4 (7.5%)	5 (9.4%)	
Anti-PD-1/PD-L1 immune checkpoint inhibitors	6 (11.3%)	2 (3.8%)	0.269
Antiangiogenic agents	13 (24.5%)	6 (11.3%)	0.076
Bev	8 (15.1%)	4 (7.5%)	
Ramucirumab	5 (9.4%)	2 (3.8%)	
Supportive care (no systemic treatment)	8 (15.1%)	4 (7.5%)	0.359

Afa, afatinib; ARMS, amplified refractory mutation system; Bev, bevacizumab; ct, circulating tumor; EGFR-TKIs, epidermal growth factor receptor-tyrosine kinase inhibitors; Erl, erlotinib; NGS, next-generation sequencing; PD-1, programmed cell death protein-1; PD-L1, programmed cell death ligand-1.

this study, no serious bleeding events, such as massive hemoptysis, bowel perforation, or intracranial hemorrhage, occurred during bevacizumab in combination with afatinib or erlotinib therapies.

Most AEs were manageable by medical treatment, and the doses and treatment course were adjusted in both treatment groups in this study. Patients in the afatinib plus bevacizumab group experienced significantly increased AEs of diarrhea compared with those in the erlotinib plus bevacizumab group.

Discussion

The results of this study provide a comparison between afatinib (second-generation EGFR-TKI) and erlotinib (first-generation EGFR-TKI) combined with bevacizumab therapy in untreated advanced EGFR-mutated lung adenocarcinoma patients. First-line combination therapy of

Table 3. AEs induced by first-line Bev + EGFR-TKIs.

	Afa + Bev *N* = 67	**Erl + Bev *N* = 68**	***p* Value**	**Afa + Bev *N* = 67**	**Erl + Bev *N* = 68**	***p* Value**
AE	Grade 1–2 *n* (%)			Grade 3–4 *n* (%)		
Skin rash/acne	57 (85.1%)	50 (73.5%)	0.137	8 (11.9%)	3 (4.4%)	0.128
Paronychia	44 (65.7%)	39 (57.4%)	0.378	5 (7.5%)	3 (4.4%)	0.493
Diarrhea	50 (74.6%)	11 (16.2%)	0.001	13 (19.4%)	4 (5.9%)	0.02
Stomatitis	35 (52.2%)	30 (44.1%)	0.391	3 (4.5%)	0	0.119
Nausea or vomiting	11 (16.4%)	7 (10.3%)	0.323	0	0	
Increased liver transaminases	3 (4.5%)	5 (7.4%)	0.718	0	0	
Hypertension	14 (20.9%)	14 (20.6%)	1.000	2 (3.0%)	4 (5.9%)	0.680
Hemorrhage	1 (1.5%)	3 (4.4%)	0.619	0	0	

AEs, adverse events; Afa, afatinib; Bev, bevacizumab; EGFR-TKIs, epidermal growth factor receptor-tyrosine kinase inhibitors; Erl, erlotinib.

afatinib or erlotinib with bevacizumab was equally effective in advanced EGFR-mutated lung adenocarcinoma patients regarding the ORR, PFS, and OS. In the treatment-related toxicity analysis, more patients in the afatinib combined with bevacizumab group experienced diarrhea as a side effect than those in the erlotinib combined with bevacizumab group. Most of the AEs in both treatment groups were within grade 1 or 2, and all the AEs were controllable and reversible.

Previous studies showed that the combination of first- and second-generation EGFR-TKIs and antiangiogenic agents had 60–80% ORR and 13–24 months of PFS as first-line therapy in advanced EGFR-mutated NSCLC.[13–18,20–23] Although the addition of bevacizumab to erlotinib has been shown to improve PFS, no impact on OS was seen in previous clinical trials (JO25567, NEJ026, and BEVERLY).[13–15] The treatment options for advanced NSCLC have increased in the last decade, and new drugs, such as chemotherapy, third-generation EGFR-TKIs (osimertinib), and ICIs, have been developed.[24,25] A previous clinical analysis showed that postprogression survival in NSCLC trials progressively increased over time, which suggested that salvage therapy contributes to an increase in postprogression survival.[24] Subsequent therapies after first-line protocol treatment in the JO25567 and NEJ026 studies were reported and analyzed. In the JO25567 trial, 39 of 75 (52%) patients in the erlotinib plus bevacizumab group and 42 of 77% patients in the erlotinib alone group received three or more regimens after the trial treatment protocol. In the NEJ026 trial, 30 of 112 (27%) patients in the erlotinib plus bevacizumab group and 46 of 112 (41%) patients in the erlotinib alone group received three or more regimens after the trial treatment protocol. In addition, the additional bevacizumab increased treatment-related AEs, and some patients in the JO25567 and NEJ026 trials experienced discontinuation of antiangiogenic agents because of treatment-related AEs (41% in JO25567 and 29% in NEJ026).[13,14,26] Together, the increasing subsequent therapies and treatment-related toxicities may explain why the addition of bevacizumab to erlotinib in first-line therapy did not have a positive impact on OS.[24,26] The OS data of RELAY are not currently available because it was immature at the data cutoff time.[20,27]

Osimertinib is classified as a third-generation EGFR-TKI with irreversible covalent binding to the tyrosine kinase domain of mutated EGFR and activates the T790M mutation. In a large pivotal clinical trial FLAURA, osimertinib had significantly longer PFS and OS than gefitinib or erlotinib as a first-line therapy for advanced EGFR-mutated NSCLC. Therefore, osimertinib was proven to be used as the first-line treatment for advanced EGFR-mutated NSCLC.[28] The addition of bevacizumab to osimertinib for

Table 4. Clinical studies investigating the combination of EGFR-TKIs and antiangiogenic agents in untreated EGFR-mutated NSCLC.

Study name	Study regimens	ORR (%)	Median PFS (months)	Median OS (months)
JO25567 Seto *et al.*[13]	Erlotinib + bevacizumab *versus* erlotinib alone	69 *versus* 63	16.4 *versus* 9.8 HR = 0.52, $p < 0.001$	47.0 *versus* 47.4 HR = 0.81, $p = 0.33$
NEJ026 Saito *et al.*[14]	Erlotinib + bevacizumab *versus* erlotinib alone	72.3 *versus* 66.1	16.9 *versus* 13.3 HR = 0.60, $p = 0.02$	50.7 *versus* 46.2 HR = 1.0
BEVERLY Piccirillo *et al.*[15]	Erlotinib + bevacizumab *versus* erlotinib alone	Not reported	15.4 *versus* 9.7 HR = 0.60, $p = 0.0039$	28.4 *versus* 23.0 HR = 0.70, $p = 0.12$
BELIEF Rosell *et al.*[21]	Erlotinib + bevacizumab	78	13.2	28.2
NCT01532089 Stinchcombe *et al.*[22]	Erlotinib + bevacizumab *versus* erlotinib alone	83 *versus* 81	17.9 *versus* 13.5 HR = 0.81, $p = 0.39$	32.4 *versus* 50.6 HR = 1.41, $p = 0.33$
RELAY Nakagawa *et al.*,[20] Kawashima *et al.*[26]	Erlotinib + ramucirumab *versus* erlotinib + placebo	76 *versus* 75	19.4 *versus* 12.4 HR = 0.59, $p < 0.0001$	Not reached
Okayama Lung Cancer Study Group Trial 1404 Ninomiya *et al.*[17]	Afatinib + bevacizumab	81.3	24.2	Not reached
Hsu *et al.*[18]	Afatinib + bevacizumab	87.7	23.9	45.9
Huang *et al.*[23]	Afatinib + bevacizumab *versus* erlotinib + bevacizumab	77.8 (all study patients)	21.6 *versus* 17.1 $p = 0.617$	59.6 (all study patients)
WJOG9717L Kenmotsu *et al.*[29]	Osimertinib + bevacizumab *versus* osimertinib alone	82 *versus* 86	22.1 *versus* 20.2 HR = 0.86, $p = 0.213$	Not available

EGFR-TKIs, epidermal growth factor receptor-tyrosine kinase inhibitors; HR, hazard ratio; NSCLC, non-small-cell lung cancer; ORR, objective response rate.

untreated advanced EGFR-mutated NSCLC was also explored in a phase II clinical trial WJOG9717 L, but the WJOG9717 L trial failed to show an improvement in PFS by the combination therapy.[29] TheWJOG9717L and ETOP 10–16 BOOSTER trials also failed to show that osimertinib combined with bevacizumab improved PFS in NSCLC patients with acquired T790M mutation.[30,31] According to the results of BELIEF, the combination of bevacizumab and erlotinib is effective for the treatment of EGFR T790M-mutated NSCLC.[21] The secondary EGFR-T790M point mutation accounts for most acquired resistance in EGFR-mutated NSCLC patients receiving first- and second-generation EGFR-TKI therapy (ranging from 30% to 60%).[32–34] The mechanism of acquired resistance to osimertinib is more complex than that of acquired resistance to first- and second-generation EGFR-TKIs. Previous studies showed that loss of T790M mutations occurred in most patients with acquired resistance to osimertinib, and SCLC transformation and other resistance genetic alterations appeared.[35,36] Taken together, the results may explain why the combination of bevacizumab and osimertinib did not increase efficacy compared to osimertinib alone. The main studies of combination of first- to third-generation EGFR-TKIs and antiangiogenic agents in untreated advanced EGFR-mutated NSCLC are summarized in Table 4.

Patients with brain metastasis at initial diagnosis were included in our study (28.4% in the afatinib plus bevacizumab group and 35.4% in the erlotinib plus bevacizumab group). A previous study conducted by Feng *et al.* reported that bevacizumab combined with EGFR-TKIs had better

efficacy in brain metastasis control and prevention from brain metastasis progression than EGFR-TKIs alone in metastatic EGFR-mutated NSCLC patients.[37] Our results are compatible with the data shown by Feng *et al.* and the median PFS of patients with brain metastasis at the baseline diagnosis in this study was not inferior to those without brain metastasis at the baseline diagnosis. Bevacizumab in combination with afatinib or erlotinib was equally effective in patients with brain metastasis at the baseline diagnosis in this study.

Regarding the bevacizumab dose administered in this study, only one patient received bevacizumab at a dose of 15 mg/kg, and all the other patients received bevacizumab at a dose of 7.5 mg/kg. In all previous clinical trials, bevacizumab was administered at a dose of 15 mg/kg,[13–15,17,19] which was different from most real-world clinical studies where bevacizumab was given at a dose of 7.5 mg/kg.[16,18,27] In a previous pivotal clinical trial (AVAiL trial), the efficacy of bevacizumab at doses of 15 or 7.5 mg/kg in combination with cisplatin plus gemcitabine chemotherapy was investigated in Asian patients with advanced non-squamous NSCLC.[38,39] Subgroup analysis of the AVAiL trial demonstrated that bevacizumab at a dose of 7.5 mg/kg was also as effective as a dose of 15 mg/kg in addition to chemotherapy for non-squamous NSCLC Asian patients.[38,39] Another concerning point is that bevacizumab is not reimbursed by governmental health insurance for lung cancer treatment in most Eastern Asian countries, so patients receiving additional bevacizumab must meet their economic condition.[40,41] Taken together, these results explained why physicians in our study and other real-world clinical studies administer bevacizumab at a dose of 7.5 mg/kg rather than 15 mg/kg.[16,18,27]

To our knowledge, this is the first study that showed a direct comparison between afatinib plus bevacizumab and erlotinib plus bevacizumab in untreated advanced EGFR-mutated lung adenocarcinoma patients. The comparisons between the two combination groups in our study included the clinical response rate, OS, secondary T790M mutation rate, and treatment-related AEs. A previous study conducted by Huang *et al.* showed that there was no significant difference in PFS between afatinib plus bevacizumab and erlotinib plus bevacizumab combination therapies.[23] The study of Huang *et al.* mainly focused on the clinical analysis of bevacizumab combined with different EGFR-TKIs, not on the comparisons of OS, acquired resistance and treatment-related AEs. In addition, the number of study subjects in Huang *et al.* was smaller than that in our study (36 *versus* 135 in total).[23]

In the analysis of previous large cohort studies, the acquired EGFR-T790M mutation rate was higher in NSCLC patients taking first-generation EGFR-TKIs (gefitinib and erlotinib) than in those taking the second-generation EGFR-TKI afatinib.[42,43] The secondary EGFR-T790M mutation rates in our study were similar to those in previous cohort studies, and our results indicated that the addition of bevacizumab to first- and second-generation EGFR-TKIs did not affect the frequency or the trend of acquired EGFR-T790M mutation after disease progression.

Combination therapy-induced side effects should also be noted. The occurrence rates of skin toxicity, nail changes, and oral mucositis were similar in both treatment groups in this study. Diarrhea was a side effect that occurred more frequently in the afatinib plus bevacizumab group than in the erlotinib plus bevacizumab group. A previous clinical trial showed that diarrhea was one of the major treatment-related toxicities in EGFR-mutated NSCLC patients receiving afatinib therapy.[44] Regarding the increased toxicity of diarrhea in the afatinib plus bevacizumab group, the diarrhea AE led to significantly more patients in this treatment group needing dose de-escalation than those in the erlotinib plus bevacizumab group. Hypertension and hemorrhage were AEs that occurred when using bevacizumab in addition to chemotherapy or TKIs.[13–18,38–40] Based on AE reports in previous clinical trials, most clinical physicians are aware of severe and fatal complications due to hypertension and bleeding induced by bevacizumab therapy in clinical practice. Therefore, patients with uncontrolled underlying cardiovascular disease, history of hemoptysis, gastrointestinal hemorrhage history, and cancer with great vessel encasement may be excluded from the use of bevacizumab.[13–18] Therefore, the patients who experienced treatment-related hypertension in this study could be managed by adjusting the treatment course or medication without severe complications. Patients who had hemorrhage AE in this study only needed an interrupted course of bevacizumab therapy and local therapy, and none in this study received major surgery for bleeding AEs.

The study subjects included in this study were all East Asians, and whether there are different

efficacies and side effects between the two combination therapies in ethnic groups other than East Asians may need to be investigated in future studies. In addition, whether bevacizumab at a dose of 15 mg/kg in combination with afatinib or erlotinib has different results also needs further study.

Conclusion

The combination of bevacizumab (7.5 mg/kg) with afatinib or erlotinib as front-line therapy for advanced EGFR-mutated lung adenocarcinoma was equally effective regarding the ORR and PFS. Patients receiving a combination of afatinib and bevacizumab therapy had increased diarrhea compared with those receiving erlotinib combined with bevacizumab. More prospective studies in the future to compare different generations of EGFR-TKIs combined with bevacizumab in EGFR-mutated NSCLC are warranted.

Declarations

Ethics approval and consent to participate

This multicenter retrospective study was approved by the institutional review board (IRB) (no. 202000137B0 and no. 202100379B0) of the Chang Gung Medical Foundation. All study subjects were retrieved retrospectively from the database of Linkou, Keelung, Chiayi, and Kaohsiung Chang-Gung Memorial Hospital (CGMH) cancer centers. All study patients received standard cancer therapy and care based on the protocol of the Linkou, Keelung, Chiayi, and Kaohsiung CGMH cancer centers. Due to the retrospective nature of this study, the need to obtain consent was waived by the IRB of the Chang-Gung Medical Foundation. All study procedures were performed in accordance with the Helsinki Declaration. No identifiable subjective information, such as personal ID and birthday, was presented in this paper.

Consent for publication

All authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship in this manuscript, take responsibility for the integrity of the work, and have given their approval for this version to be published.

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Competing Interests

The authors declare that there is no conflict of interest.

Availability of data and materials

The datasets generated and analyzed in this study are not publicly available because of local regulations related to medical confidentiality. The data

can be available from the corresponding author on reasonable request.

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Supplemental material

Supplemental material for this article is available online.

References

1. Ha SY, Choi SJ, Cho JH, *et al.* Lung cancer in never-smoker Asian females is driven by oncogenic mutations, most often involving EGFR. *Oncotarget* 2015; 6: 5465–5474.
2. Hsu PC, Wang CW, Kuo SC, *et al.* The co-expression of programmed death-ligand 1 (PD-L1) in untreated EGFR-mutated metastatic lung adenocarcinoma. *Biomedicines* 2020; 8: 36.
3. Sharma SV, Bell DW, Settleman J, *et al.* Epidermal growth factor receptor mutations in lung cancer. *Nat Rev Cancer* 2007; 7: 169–181.
4. Paz-Ares L, Soulières D, Moecks J, *et al.* Pooled analysis of clinical outcome for EGFR TKI-treated patients with EGFR mutation-positive NSCLC. *J Cell Mol Med* 2014; 18: 1519–1539.
5. Li K, Yang M, Liang N, *et al.* Determining EGFR-TKI sensitivity of G719X and other uncommon EGFR mutations in non-small cell lung cancer: perplexity and solution (Review). *Oncol Rep* 2017; 37: 1347–1358.
6. Li T, Wang S, Ying J, *et al.* Afatinib treatment response in advanced lung adenocarcinomas harboring uncommon mutations. *Thorac Cancer* 2021; 12: 2924–2932.
7. Rosell R, Carcereny E and Gervais R. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* 2012; 13: 239–246.
8. Zhou C, Wu YL, Chen G, *et al.* Final overall survival results from a randomised, phase III study of erlotinib versus chemotherapy as first-line treatment of EGFR mutation-positive advanced non-small-cell lung cancer (OPTIMAL, CTONG-0802). *Ann Oncol* 2015; 26: 1877–1883.
9. Yang JC, Wu YL, Schuler M, *et al.* Afatinib versus cisplatin-based chemotherapy for EGFR mutation-positive lung adenocarcinoma (LUX-Lung 3 and LUX-Lung 6): analysis of overall survival data from two randomised, phase 3 trials. *Lancet Oncol* 2015; 16: 141–151.
10. Park K, Tan EH, O'Byrne K, *et al.* Afatinib versus gefitinib as first-line treatment of patients with EGFR mutation-positive non-small-cell lung cancer (LUX-Lung 7): a phase 2B, open-label, randomised controlled trial. *Lancet Oncol* 2016; 17: 577–589.
11. Bi J, Dixit G, Zhang Y, *et al.* Advantages of tyrosine kinase anti-angiogenic cediranib over bevacizumab: cell cycle abrogation and synergy with chemotherapy. *Pharmaceuticals* 2021; 14: 682.
12. Naumov GN, Nilsson MB, Cascone T, *et al.* Combined vascular endothelial growth factor receptor and epidermal growth factor receptor (EGFR) blockade inhibits tumor growth in xenograft models of EGFR inhibitor resistance. *Clin Cancer Res* 2009; 15: 3484–3494.
13. Seto T, Kato T, Nishio M, *et al.* Erlotinib alone or with bevacizumab as first-line therapy in patients with advanced non-squamous non-small-cell lung cancer harbouring EGFR mutations (JO25567): an open-label, randomised, multicentre, phase 2 study. *Lancet Oncol* 2014; 15: 1236–1244.
14. Saito H, Fukuhara T, Furuya N, *et al.* Erlotinib plus bevacizumab versus erlotinib alone in patients with EGFR-positive advanced non-squamous non-small-cell lung cancer (NEJ026): interim analysis of an open-label, randomised, multicentre, phase 3 trial. *Lancet Oncol* 2019; 20: 625–635.
15. Piccirillo MC, Bonanno L, Garassino MCC, *et al.* Bevacizumab + erlotinib vs erlotinib alone as first-line treatment of pts with EGFR mutated advanced non squamous NSCLC: final analysis of the multicenter, randomized, phase III BEVERLY trial. In: *European Society for Medical Oncology (ESMO) congress 2021*, Paris Expo Porte de Versailles, France, 16–21 September 2021, abstract 1207O.
16. Wang CC, Chiu LC, Tung PH, *et al.* A real-world analysis of patients with untreated metastatic epidermal growth factor receptor

(EGFR)-mutated lung adenocarcinoma receiving first-line erlotinib and bevacizumab combination therapy. *Oncol Ther* 2021; 9: 489–503.

17. Ninomiya T, Nogami N, Kozuki T, *et al*. Survival of chemo-naïve patients with EGFR mutation-positive advanced non-small cell lung cancer after treatment with afatinib and bevacizumab: updates from the Okayama Lung Cancer Study Group Trial 1404. *Jpn J Clin Oncol* 2021; 51: 1269–1276.
18. Hsu PC, Huang CY, Wang CC, *et al*. The combination of afatinib and bevacizumab in untreated EGFR-mutated advanced lung adenocarcinoma: a multicenter observational study. *Pharmaceuticals* 2020; 13: 331.
19. Ninomiya T, Ishikawa N, Inoue K, *et al*. Phase 2 study of afatinib alone or combined with bevacizumab in chemonaive patients with advanced non-small-cell lung cancer harboring EGFR mutations: AfaBev-CS study protocol. *Clin Lung Cancer* 2019; 20: 134–138.
20. Nakagawa K, Garon EB, Seto T, *et al*. Ramucirumab plus erlotinib in patients with untreated, EGFR-mutated, advanced non-small-cell lung cancer (RELAY): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 2019; 20: 1655–1669.
21. Rosell R, Dafni U, Felip E, *et al*. Erlotinib and bevacizumab in patients with advanced non-small-cell lung cancer and activating EGFR mutations (BELIEF): an international, multicentre, single-arm, phase 2 trial. *Lancet Respir Med* 2017; 5: 435–444.
22. Stinchcombe TE, Jänne PA, Wang X, *et al*. Effect of erlotinib plus bevacizumab vs erlotinib alone on progression-free survival in patients with advanced EGFR-mutant non-small cell lung cancer: a phase 2 randomized clinical trial. *JAMA Oncol* 2019; 5: 1448–1455.
23. Huang YH, Hsu KH, Chin CS, *et al*. The clinical outcomes of different first-line EGFR-TKIs plus bevacizumab in advanced EGFR-mutant lung adenocarcinoma. *Cancer Res Treat* 2022; 54: 434–444.
24. Rutkowski J, Saad ED, Burzykowski T, *et al*. Chronological trends in progression-free, overall, and post-progression survival in first-line therapy for advanced NSCLC. *J Thorac Oncol* 2019; 14: 1619–1627.
25. Wang M, Herbst RS and Boshoff C. Toward personalized treatment approaches for non-small-cell lung cancer. *Nat Med* 2021; 27: 1345–1356.
26. Kawashima Y, Fukuhara T, Saito H, *et al*. Bevacizumab plus erlotinib versus erlotinib alone in Japanese patients with advanced, metastatic, EGFR-mutant non-small-cell lung cancer (NEJ026): overall survival analysis of an open-label, randomised, multicentre, phase 3 trial. *Lancet Respir Med* 2022; 10: 72–82.
27. Ponce Aix S, Novello S, Garon EB, *et al*. RELAY, ramucirumab plus erlotinib versus placebo plus erlotinib in patients with untreated, EGFR-mutated, metastatic non-small cell lung cancer: Europe/United States subset analysis. *Cancer Treat Res Commun* 2021; 27: 100378.
28. Ramalingam SS, Vansteenkiste J, Planchard D, *et al*. Overall survival with osimertinib in untreated, EGFR-mutated advanced NSCLC. *New Engl J Med* 2020; 382: 41–50.
29. Kenmotsu H, Wakuda K, Mori K, *et al*. Primary results of a randomized phase II study of osimertinib plus bevacizumab versus osimertinib monotherapy for untreated patients with non-squamous non-small cell lung cancer harboring EGFR mutations: WJOG9717L study. In: *European Society for Medical Oncology (ESMO) congress 2021* Paris Expo Porte de Versailles, France, 16–21 September 2021, abstract LBA44.
30. Akamatsu H, Toi Y, Hayashi H, *et al*. Efficacy of osimertinib plus bevacizumab vs Osimertinib in patients with EGFR t790m-mutated non-small cell lung cancer previously treated with epidermal growth factor receptor-tyrosine kinase inhibitor: West Japan Oncology Group 8715L Phase 2 randomized clinical trial. *JAMA Oncol* 2021; 7: 386–394.
31. Soo RA, Han JY, Dafni U, *et al*. A randomised phase II study of osimertinib and bevacizumab versus osimertinib alone as second-line targeted treatment in advanced NSCLC with confirmed EGFR and acquired T790M mutations: the European Thoracic Oncology Platform (ETOP 10-16) BOOSTER trial. *Ann Oncol* 2022; 33: 181–192.
32. Seto T, Nogami N, Yamamoto N, *et al*. Real-world EGFR T790M testing in advanced Non-Small-Cell lung cancer: a prospective observational study in Japan. *Oncol Ther* 2018; 6: 203–215.
33. Wu S, Shi X, Si X, *et al*. EGFR T790M detection in formalin-fixed paraffin-embedded tissues of patients with lung cancer using RNA-based in situ hybridization: a preliminary feasibility study. *Thorac Cancer* 2019; 10: 1936–1944.
34. Kobayashi N, Katakura S, Kamimaki C, *et al*. Resistance mechanisms of epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer patients: a meta-analysis. *Thorac Cancer* 2021; 12: 1096–1105.

35. Oxnard GR, Hu Y, Mileham KF, *et al.* Assessment of resistance mechanisms and clinical implications in patients with EGFR t790m-positive lung cancer and acquired resistance to osimertinib. *JAMA Oncol* 2018; 4: 1527–1534.

36. Roper N, Brown AL, Wei JS, *et al.* Clonal evolution and heterogeneity of osimertinib acquired resistance mechanisms in EGFR mutant lung cancer. *Cell Rep Med* 2020; 1: 100007.

37. Feng PH, Chen KY, Huang YC, *et al.* Bevacizumab reduces s100a9-positive MDSCs linked to intracranial control in patients with EGFR-mutant lung adenocarcinoma. *J Thorac Oncol* 2018; 13: 958–967.

38. Reck M, von Pawel J, Zatloukal P, *et al.* Overall survival with cisplatin-gemcitabine and bevacizumab or placebo as first-line therapy for nonsquamous non-small-cell lung cancer: results from a randomised phase III trial (AVAiL). *Ann Oncol* 2010; 21: 1804–1809.

39. Mok TS, Hsia TC and Tsai CM. Efficacy of bevacizumab with cisplatin and gemcitabine in Asian patients with advanced or recurrent non-squamous non-small cell lung cancer who have not received prior chemotherapy: a substudy of the Avastin in Lung trial. *Asia Pac J Clin Oncol* 2011; 2: 4–12.

40. Ahn MJ, Tsai CM, Hsia TC, *et al.* Cost-effectiveness of bevacizumab-based therapy versus cisplatin plus pemetrexed for the first-line treatment of advanced non-squamous NSCLC in Korea and Taiwan. *Asia Pac J Clin Oncol* 2011; 7 Suppl 2: 22–33.

41. Chien CR and Shih YC. Economic evaluation of bevacizumab in the treatment of non-small cell lung cancer (NSCLC). *Clinicoecon Outcomes Res* 2012; 4: 201–208.

42. Wagener-Ryczek S, Heydt C, Süptitz J, *et al.* Mutational spectrum of acquired resistance to reversible versus irreversible EGFR tyrosine kinase inhibitors. *BMC Cancer* 2020; 20: 408.

43. Huang AC, Huang CH, Ju JS, *et al.* First- or second-generation epidermal growth factor receptor tyrosine kinase inhibitors in a large, real-world cohort of patients with non-small cell lung cancer. *Ther Adv Med Oncol* 2021; 13: 17588359211035710.

44. Yang JC, Hirsh V, Schuler M, *et al.* Symptom control and quality of life in LUX-Lung 3: a phase III study of afatinib or cisplatin/pemetrexed in patients with advanced lung adenocarcinoma with EGFR mutations. *J Clin Oncol* 2013; 31: 3342–3350.