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Osimertinib plus anlotinib for advanced NSCLC with acquired EGFR T790M mutation: results from a multicenter phase II study with ctDNA analysis

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Abstract

Background Osimertinib is a standard treatment for first- or second-line therapy in patients with non-small cell lung cancer (NSCLC) harboring mutations in the epidermal growth factor receptor (EGFR). However, options are limited for patients with acquired EGFR T790M mutations resistant to first- or second-generation EGFR-tyrosine kinase inhibitors (TKIs). This study assessed the efficacy and safety of combining osimertinib with anlotinib in this patient population and explored circulating tumor DNA (ctDNA) as a biomarker of treatment outcomes.

Methods In this prospective, single-arm, phase II trial, 31 patients with advanced NSCLC resistant to prior first- or second-generation EGFR-TKIs therapy received osimertinib (80 mg daily) and anlotinib (12 mg daily on days 1–14 of each 21-day cycle). Efficacy endpoints included progression-free survival (PFS) and overall survival (OS). ctDNA was analyzed using next-generation sequencing (NGS) to monitor mutation status and treatment response.

Results The median PFS was 16.2 months (95% confidence interval [CI] 9.8–23.6, 90% CI 14.2–20.9), and the median OS was 31.4 months (95% CI 27.3–not reached). The objective response rate (ORR) was 45.2% (95% CI 30.6–66.6%), with a disease control rate (DCR) of 96.8% (95% CI 86.3–100.0%). ctDNA analysis showed that activating EGFR mutation clearance after two treatment cycles correlated with significantly longer PFS and OS. The regimen was well-tolerated, with no grade 4 or higher adverse events observed.

Conclusions Osimertinib combined with anlotinib demonstrates promising long-term efficacy and manageable safety in EGFR T790M-positive NSCLC. Clearance of ctDNA, particularly of EGFR mutations, could serve as a valuable predictive biomarker, supporting the implementation of personalized treatment strategies.

Trial registration ClinicalTrials.gov, NCT04029350.

Keywords EGFR T790M mutation, Osimertinib, Anlotinib, CtDNA, Non-small cell lung cancer

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Background

Mutations in the epidermal growth factor receptor (EGFR) are key drivers of non-small cell lung cancer (NSCLC) [1]. These mutations occur in approximately 40% of Asian patients with NSCLC [2, 3]. Osimertinib is a third-generation irreversible oral EGFR-tyrosine kinase inhibitor (EGFR-TKI) that effectively and selectively inhibits activating EGFR mutations [exon 19 deletion (ex19 del) and L858R; aEGFR mutations] as well as the EGFR T790M mutation in NSCLC [4].

The FLAURA study established osimertinib as a first-line treatment [5]. A subset of patients, particularly in China, still receives first- or second-generation EGFR-TKIs, despite the growing adoption of third-generation EGFR-TKIs, and those who develop resistance often present with the T790M mutation. The AURA3 study showed that patients with the EGFR T790M mutation treated with osimertinib had a longer median progression-free survival (PFS) compared to those treated with platinum-based doublet chemotherapy [10.1 months vs. 4.4 months, hazard ratio (HR) 0.3, $P < 0.001$] [6]. To explore more effective treatment options, studies have found that combined inhibition of the EGFR and VEGF pathways may delay or overcome resistance to EGFR-TKIs [7, 8]. Although first-generation EGFR-TKI therapy combined with anti-angiogenic agents has shown promising efficacy in treating EGFR-mutant lung adenocarcinoma patients, as demonstrated by the JO25567 and NEJ026 clinical trials showing that the combination of bevacizumab with erlotinib prolongs median PFS compared with erlotinib alone [9, 10], some studies suggest that osimertinib combined with anti-angiogenic agents may not synergize effectively [11–13].

Currently, the efficacy of second-line osimertinib combined with anti-angiogenic treatment remains controversial. Notably, there is limited research on predictive biomarkers for the efficacy of osimertinib and anti-angiogenic treatment combination therapy and the selection of advantageous patient populations. Therefore, this study aims to evaluate the efficacy and safety of second-line osimertinib in combination with anlotinib, an oral multi-targeted anti-angiogenic TKI that targets VEGFR, PDGFR, FGFR, and c-KIT [14, 15]. Through a comprehensive analysis of clinical data, circulating tumor DNA (ctDNA), and treatment outcomes, we aim to identify the predictive factors for response and potential resistance mechanisms associated with this combination therapy while also seeking to elucidate the applicability of osimertinib and anlotinib across diverse patient populations.

Methods

Study design and participants

This study was an investigator-initiated, multicenter, single-arm, prospective phase II trial to test the efficacy and safety of osimertinib combined with anlotinib in advanced NSCLC with EGFR T790M mutation after failure of prior first- or second-generation EGFR-TKIs therapy. Enrolled patients received osimertinib 80 mg orally daily and anlotinib 12 mg orally daily on days 1–14 of each 21-day cycle. Treatment was continued until the disease progression or treatment intolerance [unacceptable adverse events (AEs) occurred] or the patients withdrew consent. This study followed the Transparent Reporting of Evaluations with Nonrandomized Designs (TREND) reporting guideline [16].

The main inclusion criteria were as follows: (1) histologically proven locally advanced or metastatic non-squamous NSCLC with EGFR activating mutations; (2) unsuitable for radical surgery or failed of radiotherapy, chemotherapy, and first- or second-generation EGFR-TKI; (3) confirmed EGFR T790M mutation detected in tumor tissues or ctDNA after disease progression; (4) aged 18 to 75 years old; (5) Eastern Cooperative Oncology Group (ECOG) performance status score of 0–2, no disease deterioration within 2 weeks before enrollment, estimated survival time of more than 3 months; (6) presence of at least one measurable or evaluable target lesion per Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1); (7) adequate organ function; and (8) written informed consent for inclusion. The main exclusion criteria were as follows: (1) confirmed EGFR C797S mutation; (2) previous receipt of EGFR-TKI targeting the EGFR T790M mutation or failed anti-angiogenic drugs; (3) increased risk of bleeding or embolism; (4) coexistence or history of interstitial lung disease; (5) other severe comorbidities; (6) current pregnancy or lactation; (7) coexistence of other malignant cancers or a history of other malignant cancers within the last 5 years (except for basal cell carcinoma, cervical carcinoma in situ, squamous cell skin cancer, or papillary thyroid cancer).

Ethical considerations

The trial was conducted in accordance with the Declaration of Helsinki (2010), Good Clinical Practice and Chinese regulations on clinical trial research and was approved by the institutional review boards or independent ethics committees of the participating study centers. Each patient fully understood the relevant information for the clinical trial and provided informed consent. This trial was registered at ClinicalTrials.gov (NCT04029350, ALTN-03-II-01).

Evaluation of efficacy and safety

Computed tomography of the chest and upper abdomen was performed every 6 weeks. The primary endpoint was PFS, defined as the time from the date of treatment initiation to documented progression according to RECIST v1.1. Patients without disease progression were censored on the date of their last assessment. The secondary endpoints were overall survival (OS), defined as the time from treatment initiation to death from any cause, and objective response rate (ORR), which was defined as the percentage of patients with partial response (PR) or complete response (CR) as the best overall response during treatment. CR and PR had to be confirmed at least 4 weeks later; the disease control rate (DCR) was defined as the percentage of patients with CR, PR, or stable disease (SD) as the best overall response during treatment; and AEs were recorded from the date of treatment initiation until recovery from any treatment-related AEs and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03 (NCI-CTC AE 4.03).

Biomarker analysis

Longitudinal blood samples were obtained from the patients, and next-generation sequencing (NGS) was used to elucidate the spectrum of gene alterations at each time point. The baseline sample was collected within 7 days before the initiation of therapy (cycle 0, C0), and blood samples were collected on cycle 2, day 21 (C2D21); cycle 8, day 21 (C8D21); cycle 14, day 21 (C14D21); cycle 20, day 21 (C20D21); cycle 26, day 21 (C26D21); and within 7 days of treatment discontinuation. Details of the NGS-based 86 gene panels (Berry Oncology Co., Ltd., Fuzhou, China) are provided in Additional file 1: Table S1. Specific methods and further information are available in Additional file 2: Supplementary Material [17–22].

Statistical analysis

Based on published results in AURA3 on the median PFS (10.1 months) of patients with EGFR T790M who received osimertinib treatment, we set the threshold median PFS at 10.1 months. Combined with the results of ALTER0302 and ALTER0303 studies [15, 23] and the current clinical practice requirements, we hypothesized that the median PFS was 16 months for combination therapy of osimertinib combined with anlotinib. The expected accrual time was 2 years, and a 2-year follow-up period after accrual completion was planned. The sample size calculation was based on 80% power using a one-sample log-rank test with a 5% one-sided alpha level. According to this hypothesis, 48 patients were required for efficacy evaluation, and the study aimed to recruit 53 patients, accounting for a 10% dropout rate.

The median PFS and OS were estimated using the Kaplan-Meier method. Cox regression was used for univariate and multivariate analyses of PFS and OS. Univariate analysis assessed the associations between variables and outcomes, expressed as HRs with 95% confidence intervals (CIs). Significant variables were then included in a multivariate model to control for confounders and identify independent predictors. A two-sided significance level of 0.05 was applied. Statistical analyses and data visualization were performed using R (version 4.3.2, R Foundation for Statistical Computing, Vienna, Austria). A two-sided P value of ≤ 0.05 was considered statistically significant.

Results

Patient population

Due to the slow enrollment caused by the COVID-19 pandemic, a total of 31 patients from Tianjin Medical University Cancer Institute & Hospital, the Fourth Hospital of Hebei Medical University, and Shanxi Provincial Cancer Hospital were enrolled in this study from September 2019 to December 2021 (Fig. 1). Despite the limited enrollment, the study maintained high data integrity, as the enrolled patients had complete follow-up data with minimal data loss, allowing for a reduced sample size without significantly compromising validity of the findings. Additionally, the adoption of third-generation EGFR-TKI therapy as the preferred first-line treatment further reduced the pool of eligible patients, and financial constraints restricted the possibility of extending the enrollment period. Patient characteristics are listed in Table 1. In the full cohort, the median age was 58 years (range, 32–72 years), 22 (71.0%) patients were female, 24 (77.4%) patients were never smokers, and 18 (58.1%) patients had an ECOG status of 0. EGFR exon 19 deletions were detected in 18 patients (58.1%). Thirty patients received treatment with first-generation EGFR-TKIs prior to enrollment, including gefitinib (15 patients), icotinib (11 patients), and erlotinib (4 patients), while one patient was treated with the second-generation EGFR-TKI dacomitinib. At baseline, seven patients (22.6%) had brain metastasis, three patients (9.7%) had liver metastasis, 12 patients (38.7%) had bone metastasis, seven patients (22.6%) had pleural metastasis, and two patients (6.5%) had adrenal gland metastasis.

Follow-up

The cutoff date for the final analysis was December 31, 2023. The median follow-up time was 43.0 months [interquartile range (IQR) 33.0 months–not reached

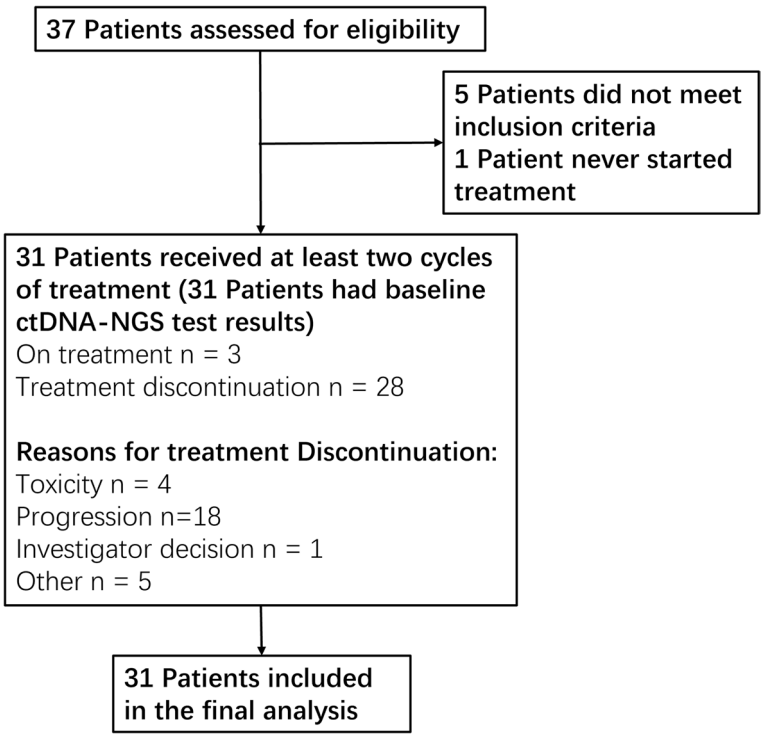

Fig. 1 Flow diagram of patient enrollment and eligibility in the ctDNA analysis. ctDNA, circulating tumor DNA; NGS, next-generation sequencing

(NR)]. Up to the cutoff date, 17 patients (54.8%) had died, and three patients were still on treatment.

Clinical response

The primary endpoint of PFS was met, with a median PFS of 16.2 months (95% CI 9.8–23.6; 90% CI 14.2–20.9) among the 31 enrolled patients (Fig. 2A). The lower limit of the 90% confidence interval (14.2 months) exceeded the pre-specified threshold median PFS of 10.1 months, supporting the clinical efficacy of the combination therapy. The 90% confidence interval was selected to align with the study's primary objective of demonstrating superiority over the threshold PFS. The median OS was 31.4 months (95% CI 27.3–NR, Fig. 2B). Swimmer's plot demonstrated the duration of treatment response in all patients (Fig. 2C). The best overall response during treatment, as assessed by RECIST v1.1, showed that 15 patients (48.4%) achieved PR, which included 14 confirmed PRs (45.2%) and one unconfirmed PR. SD was observed in 16 patients (51.6%). The ORR was 45.2% (95% CI 30.6–66.6%), and the DCR was 96.8% (95% CI 86.3–100.0%). The reduction from baseline in the target lesion size of patients was exhibited in the waterfall plot, and it was obvious that most of the target lesions shrank significantly (Fig. 2D).

Safety

The safety overview is summarized in Additional file 1: Table S2. In this study, 29 patients (93.5%) experienced at least one AE of any grade. No grade 4 or higher AEs occurred. No interstitial pneumonitis was observed as a treatment-related adverse event (TRAE) in our study. Twenty-seven patients (87.1%) experienced at least one TRAE. Grade 3 TRAEs were reported in 18 patients (58.1%). TRAEs leading to treatment discontinuation were reported in 4 patients (12.9%). Serious adverse events (SAEs) of any cause were reported in three patients (9.7%). Treatment-related SAEs were reported in two patients (6.5%). One patient developed grade 3 diarrhea, and another experienced grade 2 oral mucositis, both of which were considered potentially related to the combination of anlotinib and osimertinib. Both patients were hospitalized and showed recovery following appropriate treatment. No TRAE leading to death were observed in this study. The majority of TRAEs were grade 1 or 2. The most common TRAEs included thrombocytopenia (48.4%), hypertension (45.2%), leukopenia (41.9%), and neutropenia (41.9%), among others (Table 2).

ctDNA dynamics and response to therapy

Plasma samples were collected longitudinally pre- and post-treatment for ctDNA-NGS testing, with all 31

Table 1 Baseline characteristics of patients

Characteristics	Patients, no. (%) (*N* = 31)
Age, years	
Median (range)	58 (32–72)
Sex	
Female	22 (71.0)
Male	9 (29.0)
Smoking status	
Former	7 (22.6)
Never	24 (77.4)
ECOG-PS	
0	18 (58.1)
1	13 (41.9)
EGFR mutation type	
Ex19 del	18 (58.1)
L858R	13 (41.9)
Prior EGFR-TKI	
Gefitinib	15 (48.4)
Erlotinib	4 (12.9)
Icotinib	11 (35.5)
Dacomitinib	1 (3.2)
Brain metastasis	
No	24 (77.4)
Yes	7 (22.6)
Liver metastasis	
No	28 (90.3)
Yes	3 (9.7)
Bone metastasis	
No	19 (61.3)
Yes	12 (38.7)
Pleural metastasis	
No	24 (77.4)
Yes	7 (22.6)
Adrenal gland metastasis	
No	29 (93.5)
Yes	2 (6.5)

ECOG-PS, the Eastern Cooperative Oncology Group performance status; *EGFR*, epidermal growth factor receptor gene; *Ex19 del*, exon 19 deletion

patients having test results at baseline (Additional file 3: Fig. S1). Raw sequencing data are publicly available in the Genome Sequence Archive for Human (GSA-Human: HRA005252) [24]. The figure also provides details on the time points when specimens were submitted and the corresponding results at each time point. The types of gene alterations included gene mutations, gene amplifications, and gene fusions. Specifically, patients without detectable aEGFR mutations at baseline had comparable PFS (median PFS 14.4 vs. 16.2 months; log-rank $\chi^2 = 0.4$, $P = 0.6$, Fig. 3A) and OS (median OS 27.1 vs. 32.8 months; log-rank $\chi^2 = 0.1$, $P = 0.8$, Fig. 3B) to those with detectable aEGFR mutations. However, among patients with detectable aEGFR mutations in ctDNA at baseline, those who achieved aEGFR mutations clearance after two treatment cycles (week 6) demonstrated significantly longer PFS (median PFS 18.5 vs. 5.2 months; log-rank $\chi^2 = 11.7$, $P < 0.001$, Fig. 3C) and OS (median OS 43.9 vs. 10.9 months; log-rank $\chi^2 = 8.0$, $P = 0.005$, Fig. 3D) compared to those without mutation clearance. The presence of the EGFR T790M mutation at baseline did not significantly impact PFS (median PFS 19.8 vs. 14.6 months; log-rank $\chi^2 = 0.9$, $P = 0.4$) or OS (median OS 29.6 vs. 38.2 months; log-rank $\chi^2 = 0.1$, $P = 0.8$).

Similarly, baseline TP53 mutation status did not significantly affect PFS (median PFS 16.2 vs. 16.7 months; log-rank $\chi^2 = 0.2$, $P = 0.7$, Fig. 3E) or OS (median OS NR vs. 31.0 months; log-rank $\chi^2 = 1.8$, $P = 0.2$, Fig. 3F). However, patients who achieved TP53 mutation clearance after two treatment cycles exhibited significantly longer PFS (median PFS 18.5 vs. 7.6 months; log-rank $\chi^2 = 7.8$, $P = 0.005$, Fig. 3G) and OS (median OS 38.2 vs. 9.4 months; log-rank $\chi^2 = 5.3$, $P = 0.02$, Fig. 3H) compared to those without clearance.

Univariable and multivariable analysis of PFS and OS

Univariate analysis of the baseline characteristics revealed no factors associated with PFS (Additional file 1: Table S3, Additional file 3: Fig. S2 A, B). However, EGFR mutation type (L858R vs. ex19 del) and brain metastasis were identified as prognostic factors for OS (Table 3, Additional file 3: Fig. S2 C, D). In the corresponding multivariate analysis, the presence of brain metastasis at baseline remained a significant adverse prognostic factor for OS (Table 3).

Analyses of potential resistance mechanisms for combination treatment

In this study, eight plasma samples from patients were sequenced after disease progression on treatment with osimertinib combined with anlotinib. The most frequently detected mutations were in EGFR and TP53. Sensitizing EGFR mutations and TP53 mutations were detected in all eight patients after they experienced progression. The changes in EGFR and TP53 mutation variant allele fractions (VAF) in ctDNA from these eight patients are presented in line graphs (Additional file 3: Fig. S3). Among the eight patients, six (75.0%) exhibited a loss of the T790M mutation post-progression. In the other two patients, the T790M mutation remained detectable from baseline through disease progression. Only one patient had the C797S mutation (c.2390G > C) observed in cis with the T790M mutation. Additionally, other bypass activation mechanisms were observed, such

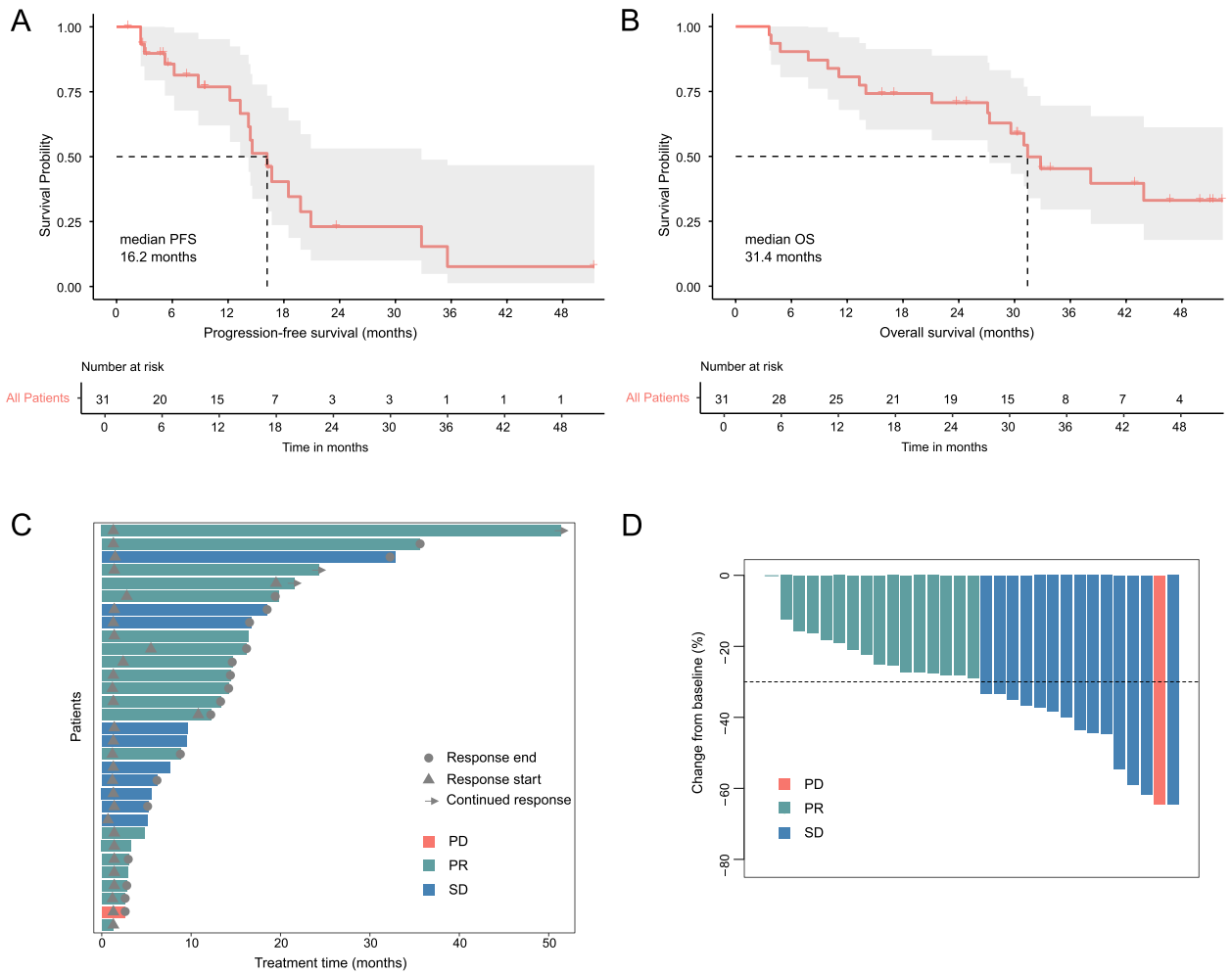

Fig. 2 Clinical outcomes and treatment response evaluation. **A** Kaplan-Meier curve for PFS. The median PFS was 16.2 months (95% CI 9.8–23.6, 90% CI 14.2–20.9). **B** Kaplan-Meier curve for OS. The median OS was 31.4 months (95% CI 27.3–NR). **C** Swimmer's plot showing the duration of treatment response for each patient. Each bar represents one patient, and the length of each bar represents the duration of treatment. **D** Waterfall plot demonstrating the percentage change in target lesion size from baseline. PFS, progression-free survival; OS, overall survival; PR, partial response; SD, stable disease; PD, progressive disease; CI, confidence interval; NR, not reached

as MET amplification in one patient and FGFR3 fusion in another. One patient also had both PIK3CA and VEGFA amplifications. Gene alterations at the time of disease progression are presented in Additional file 1: Table S4.

Discussion

In the field of treating advanced NSCLC with EGFR-sensitive mutations, a wide range of first-line treatment options is available [25], while for patients who develop resistance to prior first- or second-generation EGFR-TKI therapies due to the T790M mutation, the primary second-line treatment option currently mainly involves the third-generation EGFR-TKI osimertinib as monotherapy [26]. Combination therapeutic approaches remain to be explored. This study is the first to report osimertinib combined with anlotinib as a treatment option for advanced NSCLC with acquired EGFR T790M mutations. Specifically, our study met the prespecified primary endpoint of PFS and showed that combination therapy was well tolerated with no unexpected toxicities.

Our study demonstrated a median PFS of 16.2 months and a DCR of 96.8%, consistent with the results from retrospective studies involving osimertinib and anlotinib [27]. However, the ORR of 45.2% observed in our study was lower than the 68% reported in the West Japan Oncology Group (WJOG) 8715L study and the 55% in the European Thoracic Oncology Platform (ETOP 10–16) BOOSTER trial [12, 28]. The WJOG 8715L study was a phase II randomized clinical trial that compared the efficacy of osimertinib monotherapy to osimertinib

Table 2 Most common TRAEs[a]

AE	Grade 1–2 (%)	Grade 3 (%)	Total patients, no. (%)
Thrombocytopenia	14 (45.2)	1 (3.2)	15 (48.4)
Hypertension	5 (16.1)	9 (29.0)	14 (45.2)
Leucopenia	10 (32.3)	3 (9.7)	13 (41.9)
Neutropenia	10 (32.3)	3 (9.7)	13 (41.9)
Diarrhea	8 (25.8)	1 (3.2)	9 (29.0)
Proteinuria	7 (22.6)	2 (6.5)	9 (29.0)
Serum creatinine increased	8 (25.8)	0 (0)	8 (25.8)
Weight loss	7 (22.6)	1 (3.2)	8 (25.8)
Fatigue	6 (19.4)	1 (3.2)	7 (22.6)
Hypertriglyceridemia	7 (22.6)	0 (0)	7 (22.6)
Hand-foot syndrome	6 (19.4)	0 (0)	6 (19.4)
Oral mucositis	6 (19.4)	0 (0)	6 (19.4)
Hyperuricemia	5 (16.1)	0 (0)	5 (16.1)
Hypothyroidism	5 (16.1)	0 (0)	5 (16.1)
Hypercholesterolemia	4 (12.9)	0 (0)	4 (12.9)
Decreased appetite	4 (12.9)	0 (0)	4 (12.9)
Rash	3 (9.7)	1 (3.2)	4 (12.9)

[a] Most common TRAEs are those observed in > 10% of patients treated with osimertinib and anlotinib. TRAEs are defined as events that are possibly, probably, or definitely related to osimertinib, anlotinib, or both. *AEs*, adverse events; *TRAEs*, treatment-related adverse events

combined with bevacizumab in patients with EGFR T790M-mutated NSCLC, finding a higher ORR for the combination therapy (68% vs. 54%) but no significant advantage in median PFS (9.4 months vs. 13.5 months; HR 1.44; $P=$ 0.20) [28]. Similarly, the ETOP 10–16 BOOSTER trial, another phase II randomized study, investigated the same combination and found no significant difference in median PFS between the combination therapy and osimertinib alone (15.4 months vs. 12.3 months; HR 0.96; $P=$ 0.83), with both groups achieving an ORR of 55% [12]. These findings suggest that while our treatment regimen is effective in disease control, it does not achieve the same level of response rate improvement as other combinations. Despite this, the median OS of 31.4 months in our study underscores the long-term benefits of this regimen. The combination of osimertinib and anlotinib in our study differs from bevacizumab-based regimens previously explored in EGFR T790M-mutant NSCLC. Both anlotinib and bevacizumab target angiogenesis, but their targets and mechanisms vary. Bevacizumab, an anti-angiogenic monoclonal antibody targeting the VEGF signaling pathway, has shown added efficacy in combination with first-line platinum-based chemotherapy in non-squamous NSCLC [29, 30], while anlotinib, a multi-targeted TKI that blocks VEGFR, FGFR, PDGFR, and c-KIT, simultaneously affects angiogenesis and tumor cell proliferation [14, 15]. This broader target profile of anlotinib may contribute to its potential advantages when combined with EGFR-TKIs. This may also partially explain the long-term benefits observed with this regimen in our study. Further large-scale randomized controlled trials are needed to evaluate the relative efficacy of this combination therapy and identify the patients who can truly benefit from it. This led to our ctDNA analysis to more accurately identify potential responders.

In this study, AEs and TRAEs occurred in 29 (93.5%) and 27 (87.1%) patients, respectively. Grade 3 TRAEs occurred in 18 (58.1%) patients. In the BOOSTER trial, AEs were reported in 100% of patients and grade 3 or higher TRAEs were reported in 47% of the patients [12]. In the WJOG9717L study, grade 3 or higher AEs were reported in 56% of the patients [13]. The incidence of AEs and grade ≥ 3 TRAEs in our study with osimertinib and anlotinib was similar to those observed with osimertinib and bevacizumab. In contrast, Zhou et al. reported that grade 3 or higher TRAEs occurred in 36.4% of the patients treated with anlotinib and osimertinib [27]. The incidences of thrombocytopenia, leukopenia, and neutropenia in our study were lower than those reported in the WJOG8715 study [11]. In addition, our study observed cases of diarrhea, fatigue, proteinuria, liver function abnormalities, and skin toxicity, which were consistent

(See figure on next page.)

Fig. 3 Clinical outcomes associated with aEGFR and TP53 mutation clearance. **A** Kaplan-Meier curves for PFS in patients with and without detectable aEGFR mutations at baseline. Patients without detectable aEGFR mutations had comparable PFS to those with detectable mutations ($P=$ 0.6). **B** OS comparison in patients with and without detectable aEGFR mutations at baseline, showing no significant difference ($P=$ 0.8). **C** PFS for patients with detectable aEGFR mutations who achieved clearance of mutations in ctDNA after two treatment cycles compared to those without clearance, demonstrating significantly longer PFS ($P<$ 0.001). **D** OS comparison between patients with and without aEGFR mutation clearance, showing significantly improved OS in those with mutation clearance ($P=$ 0.005). **E** PFS in patients with and without baseline TP53 mutations, showing no significant difference ($P=$ 0.7). **F** OS in patients with and without TP53 mutations at baseline, also demonstrating no significant difference ($P=$ 0.2). **G** PFS in patients who achieved TP53 mutation clearance after two treatment cycles, showing significantly longer PFS compared to those without clearance ($P=$ 0.005). **H** OS in patients with TP53 mutation clearance, showing significantly improved OS compared to those without clearance ($P=$ 0.02). PFS, progression-free survival; OS, overall survival; ctDNA, circulating tumor DNA; aEGFR mutations, activating EGFR mutations

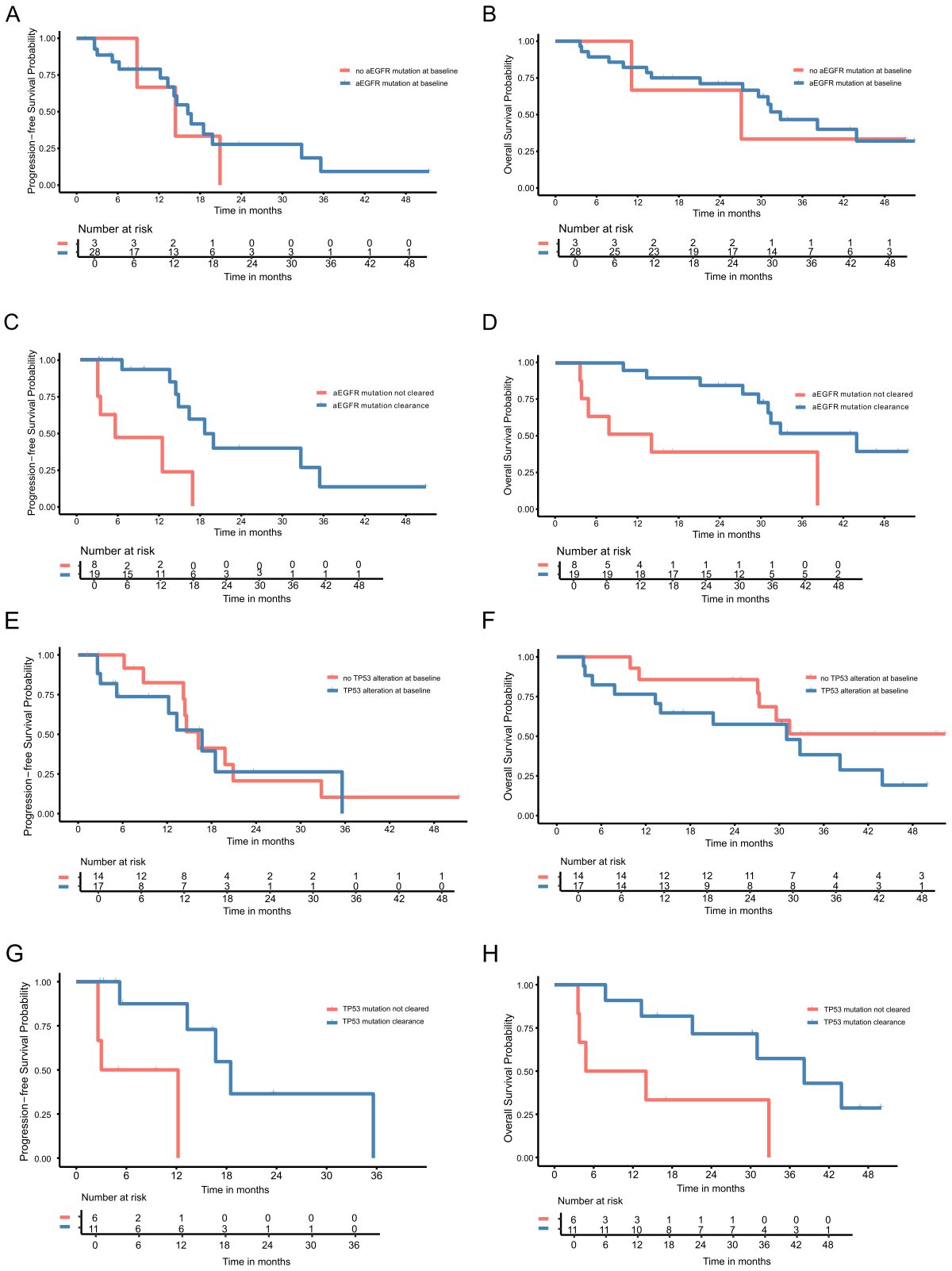

Fig. 3 (See legend on previous page.)

Table 3 Results of univariable and multivariable Cox regression analysis of overall survival

Variable	Univariable regression			Multivariable regression		
	HR	95% CI	*P* value*	HR	95% CI	*P* value
Age, years (≥ 65/< 65)	0.87	0.32–2.36	0.784			
Sex (male/female)	0.76	0.27–2.20	0.617			
Smoking status (former/never)	1.32	0.43–4.08	0.755			
ECOG-PS (1/0)	1.04	0.37–2.89	0.943			
EGFR mutation type (L858R/ex19 del)	1.63	1.01–2.67	**0.049**	1.57	0.92–2.66	0.098
Brain metastasis (yes/no)	2.91	1.08–7.87	**0.035**	4.63	1.39–15.45	**0.013**
Liver metastasis (yes/no)	0.38	0.05–2.89	0.347			
Bone metastasis (yes/no)	2.15	0.82–5.61	0.120			
Pleural metastasis (yes/no)	0.92	0.26–3.25	0.380			
Adrenal gland metastasis (yes/no)	9.49	0.99–91.25	0.051			
aEGFR mutations at baseline (detectable/non-detectable)	0.81	0.19–3.58	0.786			
T790M mutation at baseline (detectable/non-detectable)	0.89	0.31–2.53	0.821			
TP53 alteration at baseline (detectable/non-detectable)	1.94	0.72–5.25	0.193			

* Significant *P* values < 0.05 are in bold. *ECOG-PS*, the Eastern Cooperative Oncology Group performance status; *EGFR*, epidermal growth factor receptor gene; *Ex19 del*, exon 19 deletion; *aEGFR mutations*, activating EGFR mutations

with those reported in previous treatment regimens involving EGFR-TKIs combined with anti-angiogenic therapy [13, 27]. Importantly, no fatal TRAEs occurred during safety evaluation in our study. Overall, the toxic effects of the combination of osimertinib and anlotinib were manageable, indicating that the regimen was safe.

There are limited reports on the efficacy of osimertinib combined with anti-angiogenic or multi-targeted agents and their correlation with dynamic changes in ctDNA during treatment. This study provides valuable evidence in this area. Exploratory analyses from the AURA3 and FLAURA trials indicated that detectable baseline plasma ctDNA EGFR mutations may serve as adverse prognostic factors [31–33]. However, our study did not observe a similar relationship between baseline aEGFR mutations and PFS or OS. The discrepancy may be attributed to the significant difference in sample sizes: the AURA3 and FLAURA studies analyzed 291 and 499 patients with baseline plasma ctDNA EGFR mutations, respectively, whereas our study included only 31 patients, limiting the statistical power of our analysis. On the other hand, exploratory analyses from the AURA3 and FLAURA studies suggested that early clearance of plasma ctDNA EGFR mutations could be associated with improved prognosis [31]. Consistently, in our study, among patients with detectable baseline aEGFR mutations in ctDNA, those who achieved mutation clearance after two treatment cycles experienced significantly longer PFS and OS compared to those without mutation clearance. These findings align with existing evidence suggesting that baseline plasma EGFR mutation status can guide treatment decisions [34, 35]. We hypothesize that for patients without mutation clearance after two treatment cycles, osimertinib combined with anlotinib could be a more effective second-line treatment option. While this hypothesis requires further clinical investigation, our findings support considering this combination therapy in such cases.

The limitations of this study include its single-group, non-randomized design and the potential reduction in statistical power due to the inability to reach the target sample size. This reduction in sample size compromised the statistical power, resulting in wider CIs for the median PFS. In addition to reporting 95% CIs, we also provided 90% CIs, which offer added value by providing a more precise estimate of the treatment effect and helping to refine the interpretation of our findings. However, the reduced precision of these estimates necessitates cautious interpretation of the results. Furthermore, the small sample size may have affected the reliability of the multivariate analysis, which was primarily exploratory in nature and should be regarded as preliminary. Despite these limitations, the study had minimal data loss, included long-term follow-up, and the sample size was sufficient for biomarker analysis. While the findings of this study are promising, they warrant validation in larger, prospective cohorts to confirm their reliability and generalizability.

Conclusions

In summary, the combination of osimertinib and anlotinib appears to be a promising treatment option for patients with acquired EGFR T790M-positive advanced NSCLC. The identification of patients who may benefit

from more aggressive combination therapies could be aided by the clearance of plasma aEGFR mutations. Continued efforts to refine patient selection criteria will be essential for optimizing treatment strategies and improving outcomes in this patient population.

Abbreviations

NSCLC	Non-small cell lung cancer
EGFR	Epidermal growth factor receptor
TKI	Tyrosine kinase inhibitor
T790M	Threonine to methionine at position 790
HR	Hazard ratio
ctDNA	Circulating tumor DNA
AEs	Adverse events
ECOG	Eastern Cooperative Oncology Group
PFS	Progression-free survival
OS	Overall survival
ORR	Objective response rate
DCR	Disease control rate
NGS	Next-generation sequencing
CI	Confidence interval
VAF	Variant allele fractions

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-025-04044-8.

Additional file 1. Tables S1–S4. Table S1 List of genes included in the NGS-based 86 gene panel. Table S2 Safety overview. Table S3 Results of univariable and multivariable Cox regression analysis of progression-free survival. Table S4 Newly acquired gene alterations at the time of disease progression in eight patients.

Additional file 2. Supplementary Material. The specific methods of ctDNA-NGS testing.

Additional file 3. Figures S1–S3. Fig. S1 Heatmap of genomic alterations in 31 patients. Fig. S2 Impact of EGFR type and brain metastasis on PFS and OS. Fig. S3 Dynamic changes in aEGFR mutations and TP53 mutations in ctDNA from eight patients.

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Authors' contributions
X.W.: Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Visualization, Writing- Original draft preparation. Z.L.: Data curation, Formal analysis, Resources, Visualization, Writing-Original draft preparation. L.W.: Data Curation, Investigation, Project Administration. Y.L.: Data Curation, Investigation. C.H.: Data Curation, Investigation. P. C.: Data Curation, Investigation. D.H.: Data Curation, Investigation. X.S.: Data Curation, Investigation. C.D.: Data curation, Investigation, Supervision, Writing-Review& Editing. C.W.: Conceptualization, Funding Acquisition, Supervision, Writing-Review & Editing. R.J.: Conceptualization, Formal Analysis, Funding Acquisition, Investigation, Methodology, Supervision, Writing-Review& Editing. All authors read and approved the final manuscript.

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Data availability
The clinical data generated from this study will not be publicly available due to protection of patient privacy but may be accessible upon reasonable request and subject to approval by the corresponding author. The raw sequencing data reported in this study have been deposited in the Genome Sequence Archive (GSA-Human: HRA005252) at the National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences, and are publicly accessible at https://ngdc.cncb.ac.cn/gsa-human/browse/HRA005252.

Declarations

Ethics approval and consent to participate
This study was first approved by the ethics committee of the Tianjin Medical University Cancer Institute & Hospital, and the approval number was E2019157. The trial was conducted in accordance with the Declaration of Helsinki (2010), Good Clinical Practice and Chinese regulations on clinical trial research and was approved by the institutional review boards or independent ethics committees of the participating study centers. Written informed consent was obtained from all participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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