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# A phase II trial of anlotinib plus EGFR-TKIs in advanced non-small cell lung cancer with gradual, oligo, or potential progression after EGFR-TKIs treatment (CTONG-1803/ALTER-L001)

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## Abstract

**Background** The study is to evaluate the efficacy and safety of combined anlotinib and EGFR-tyrosine kinase inhibitors (TKIs) in patients with advanced non-small cell lung cancer (NSCLC) who had gradual, oligo, or potential progression after previous EGFR-TKIs treatment.

**Methods** We conducted an open-label, single-arm, multicenter, phase II trial in China. Eligible patients were 18–75 years old with histologically or cytologically confirmed NSCLC who were EGFR mutation positive and showed gradual, oligo, or potential progression after EGFR-TKIs. Anlotinib (12 mg/day) was administered orally for 2 weeks and then off 1 week in a 3-week cycle. EGFR-TKIs were continue used. The primary endpoint was progression-free survival (PFS). The secondary endpoints included 6- and 12-month PFS rate, objective response rate (ORR), disease control rate (DCR), overall survival (OS) and safety.

**Results** From July 2019 to December 2022, 120 patients were enrolled. The median PFS (mPFS) was 9.1 months (95% CI 6.8–11.7). The PFS rates at 6 and 12 months was 68.5% and 38.8% respectively. For 86 patients with first-line 1st /2nd generation EGFR-TKIs, the mPFS was 9.2 months (95% CI 6.7–12.6). For 32 patients with first-line 3rd generation EGFR-TKIs, the mPFS was 10.3 months (95% CI 6.1–13.3). Overall ORR and DCR were 6.7% (95% CI 2.9–12.7) and 87.5% (95% CI 80.2–92.8), respectively. 52.5% of patients had grade 3 or higher treatment-emergent adverse events (TEAEs).

**Conclusion** Anlotinib in combination with continuation of EGFR-TKIs prolonged the clinical benefit of EGFR-TKIs, demonstrating favorable survival outcomes and manageable toxicity in NSCLC treated with EGFR-TKIs and had specific progression modes, such as gradual progression.

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**Trial registration** NCT04007835.

**Keywords** EGFR mutation, Anlotinib, Gradual progression, Oligo progression, Non-small cell lung cancer

## Background

Lung cancer is a leading cause of cancer-related death worldwide, with non-small cell lung cancer (NSCLC) being the most common subtype. Over the years, significant progress has been made in understanding the molecular mechanisms underlying NSCLC, particularly the role of epidermal growth factor receptor (EGFR) mutations. EGFR mutations are found in a subset of NSCLC patients, and they play a critical role in driving cancer growth. Targeted therapies known as EGFR-tyrosine kinase inhibitors (TKIs) have revolutionized the treatment landscape for these patients [1]. Medications like gefitinib, erlotinib, afatinib, dacomitinib and osimertinib have demonstrated remarkable efficacy in controlling the growth of EGFR-mutated tumors, improving patient outcome, as measured by response rate, progression-free survival (PFS), overall survival (OS) and minimizing side effects compared to traditional chemotherapy [2]. However, despite the initial success of EGFR-TKIs, individuals harboring these oncogenic mutations eventually experience disease progression. The optimal approach to the management of such EGFR-TKIs resistance patients remains undefined [3].

One approach is to subtype the progression after EGFR-TKIs treatment which provides a rational approach to both clinical trial design and day-to-day patient management. For instance, Gandara et al. proposed that progressive disease (PD) can be subgrouped into categories such as central nervous system (CNS)-PD, oligo-PD and systemic–PD [4]. Similarly, Yang et al. separated the pattern of PD as dramatic, gradual, and local progression [5]. Their results showed that patients with a gradual progression had better results in the PFS and could represent a subset of patients who benefit from continuing TKIs rather than switching to cytotoxic chemotherapy. In addition, combining EGFR-TKIs with antiangiogenic agents such as bevacizumab showed substantially improve PFS in patients with TKI-naive EGFR-mutant NSCLC [6–8]. Several retrospective studies have explored the combination of bevacizumab and EGFR-TKIs in gradual progression NSCLC patients after first-line EGFR-TKIs [9–11]. Nonetheless, there is still limited understanding regarding whether antiangiogenic agents continue to exhibit synergistic effects with EGFR-TKIs once EGFR-TKIs resistance has developed.

Anlotinib is a multi-targeted small-molecular TKI that inhibits a group of kinases such as VEGFR, c-Kit, PDGFR, and FGFR [12]. In untreated metastatic EGFR-mutated NSCLC patients, the combination of anlotinib and icotinib demonstrated efficacy and good tolerability in the ALTER-L004 study [13], anlotinib plus gefitinib also significantly prolonged PFS when compared with anlotinib plus placebo [14]. The ALTER0303 clinical trial demonstrated favorable outcomes in patients with advanced NSCLC receiving anlotinib as a third line or further therapy [15].Limited retrospective studies have also suggested that the combination of EGFR-TKIs and anlotinib is a feasible treatment option for patients who have developed resistance to EGFR-TKIs [16–19].

However, few prospective trials have focused on evaluating the efficacy and safety of anlotinib in combination with original previous EGFR-TKIs on those NSCLC patients with specific progression modes including gradual progression. In this open-label, single-arm, multicenter phase II trial, we aimed to evaluate the efficacy and safety of combined EGFR-TKIs and anlotinib in patients with gradual, oligo, or potential progression after previous EGFR-TKIs.

## Methods

### Study design and population

CTONG-1803/ALTER-L001 was a prospective single-arm, phase II clinical trial and conducted at 14 hospitals in China. Eligible patients were 18–75 years old with histologically or cytologically confirmed NSCLC who were EGFR mutation positive and had received EGFR-TKIs (1st /2nd /3rd generation in the first-line or 3rd generation in the second-line) and had gradual, oligo, or potential progression; ECOG PS of 0–1; had at least one measurable lesion per Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1. Gradual progression was defined as disease control lasting≥6 months with EGFR-TKIs, no target lesion progression (non-target lesions progression and or emergence of new lesions), and no deterioration in clinical symptoms [5]; Oligo progression was defined as disease control lasting≥3 months with EGFR-TKIs, progression limited to only a few sites (solitary extracranial lesion or limitation in intracranial lesions, covered by a radiation field), and no deterioration in clinical symptoms [5, 20]; Potential progression was defined as blood CEA < 10.0ng/ml, two consecutive tests≥10ng/ml, or blood CEA≥10ng/ml, two consecutive tests gradually increasing (at intervals of not less than 1 month). Main exclusion criteria included those patients with small cell lung cancer; patients with primary resistance to EGFR-TKIs (<3 months of treatment); patients with dramatic progression after EGFR-TKIs (dramatic progression was defined as disease control lasting≥3 months, rapid deterioration in clinical symptoms, and significant targetable alterations); patients with

a tumor lesion ≤ 5 mm from a major blood vessel, or the presence of a centralized tumor that invades a localized major blood vessel tumor; or the presence of obvious cavitary or necrotic tumors in the lungs et al. The present prospective study was approved by the local institutional ethics committee and was conducted according to the principles of the Declaration of Helsinki and Good Clinical Practice requirements. This trial is registered at ClinicalTrial.gov: NCT04007835.

#### Outcomes

The primary endpoint was investigator-assessed PFS, which was defined as the period from first administration to the date of disease progression or death from any cause, whichever occurs first. Key secondary endpoints included: (1) 6-month and 12-month PFS rate, which were defined as survival without progression or death from first administration to month 6 / 12 for all patients; (2) objective response rate (ORR), was defined as the proportion of patients whose tumors shrank to a certain standard and remained so for a certain period of time, and included both CR and PR cases; (3) disease control rate (DCR), was defined as the proportion of patients whose disease is stable and remains so for a certain period of time after treatment, and included cases of CR, PR, and SD; (4) overall survival (OS), which was defined as the period from first administration to the date of death from any cause and (5) safety, which was evaluated according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4.03. In addition, an exploratory endpoint to identify gene mutations at gradual, oligo, or potential progression and after the combination of anlotinib was assessed.

#### Procedures

Anlotinib (12 mg/day) was administered orally for 2 weeks and then off 1 week in a 3-week cycle until disease progression or intolerable toxicities. EGFR-TKIs were continue used followed the original drug until disease progression or intolerable toxicities. Efficacy evaluations were conducted on day 21 of the first cycle and every two cycles (odd-numbered cycles) thereafter. Patients with CR, PR, and SD were reevaluated six weeks after the initial evaluation, and all imaging data were retained for CR, PR, SD, and PD. Patients who discontinued the trial before the onset of PD were followed up every eight weeks with imaging to monitor for tumor progression or until other antitumor therapy was initiated.

#### Sample size calculations

Referring to the mPFS of continuing erlotinib after first-line erlotinib progression is 3.1 months [21], combining with ALTER0303 study [15], ALTER0302 study [22] and the requirements of the current clinical practice, it is assumed that the mPFS of anlotinib combined with EGFR-TKIs after previous EGFR-TKIs treatment is 6 months, and that the mPFS of the historical control is 4 months, and the one-sided $\alpha=0.025$, $\beta=0.2$, enrollment time of 12 months, follow-up time of 12 months, and assuming that the survival curve obeys an exponential distribution, the sample size was calculated to be 107 cases, and taking into account a 15% dropout rate, 126 subjects were proposed to be enrolled in this study.

#### Statistical analysis

Efficacy was analyzed using the full analysis set (FAS), including all enrolled patients who had received at least one dose of study drug according to the intend-to-treat (ITT) principle. Safety was analyzed using the safety analysis set (SS), including all enrolled patients who had received at least one dose of study drug, and have a documented post-dose safety profile. PFS and OS were estimated using the Kaplan-Meier method for median values, and 95% confidence intervals (CI) for median PFS and OS were calculated using the Brookmeyer-Crowley method based on the log-log transformation. 6-month and 12-month PFS rate were obtained based on the PFS curve and 95% CI were calculated using the log-log transformation method. For PFS, patients with no imaging documented after treatment were censored at the date of first administration, patients who did not have disease progression were censored at the date of last imaging, patients who discontinued due to toxicity or other reasons were censored at the date of last imaging prior to discontinuation, and patients who began a subsequent antitumor therapy were censored at the date of last imaging prior to initiation of the subsequent therapy. For OS, patients who did not die were censored at the time of final survival follow-up. The 95% CIs for ORR and DCR were estimated by the Clopper-Pearson method. Adverse events (AEs) were mainly analyzed using descriptive statistics. Statistical analyses were calculated using SAS 9.4.

#### Targeted deep sequencing for circulating tumor DNA and genomic analyses

Within 72 h of collection, peripheral blood samples underwent centrifugation, resulting in the separation of white blood cells (WBCs) and plasma. Circulating tumor DNA (ctDNA) from plasma was then converted into indexed libraries, following the methodology outlined in the previous study [23]. Library construction adhered to the protocols of the KAPA Library Preparation Kit by Kapa Biosystems. Subsequently, capture hybridization was performed according to the manufacturer's instructions. DNA libraries were sequenced using the Geneplus Seq-2000 (GenePlus, Beijing, China) with the panel of 1,021 genes and paired-end read (Geneplus-Beijing,

China). The sequencing data underwent below processes. Initially, they were aligned and mapped to the reference human genome (hg19) by BWA (version 0.5.9) [24]from the Broad Institute. Before alignment, any terminal adaptor sequences and low-quality data were removed. High-quality reads were selected for further analysis. Single nucleotide variants and small insertions and deletions were identified using MuTect (version 1.1.4) [25]. Finally, all final candidate variants underwent manual verification using the integrative genomics viewer browser [26], as part of an in-house workflow based on related read count and location.

## Results

### Patients and treatment

From July 08, 2019 to December 15, 2022, 140 patients were screened and 120 were eligible for inclusion. All patients received at least one dose of study treatment. Baseline demographics and disease characteristics were described in Table 1. There were 49 men (40.8%) and 71 women (59.2%), and the median age was 57.5 (29–75) years. The majority of patients (96.7%) had adenocarcinoma. 86 patients (71.7%) treated with first-line 1st /2nd generation EGFR-TKIs, 32 patients (26.7%) treated with first-line 3rd generation EGFR-TKIs, one patient treated with 1st and 3rd generation EGFR-TKIs, and one patient treated with 2nd and 3rd generation EGFR-TKIs. 14 patients (11.7%) with postoperative recurrence at initial EGFR-TKI. 19 patients (15.8%) received radiotherapy prior to study treatment.109 patients (90.8%) had gradual progression, 6 patients (5.0%) had oligo progression, and 5 patients (4.2%) had potential progression. Before the enrollment, the median time to gradual, oligo, or potential progression was 12.9 months (95% CI 3.8–50.1). At data cutoff (September 4, 2023), the median follow-up time was 17.9 months (95% CI 14.8–19.8).

**Table 1** Baseline patients characteristics

| Characteristic | | *N* = 120 |
|---|---|---|
| Age | Median (range) | 57.5 (29.0–75.0) |
| | ≥ 65 | 35 (29.2) |
| | < 65 | 85 (70.8) |
| Sex, n (%) | Male | 49 (40.8) |
| | Female | 71 (59.2) |
| ECOG PS, n (%) | 0 | 24 (20.0) |
| | 1 | 96 (80.0) |
| Smoking history, n (%) | Never smoker | 89 (74.2) |
| | Former smoker | 27 (22.5) |
| | Current smoker | 4 (3.3) |
| Histologic type, n (%) | Adenocarcinoma | 116 (96.7) |
| | Other | 4 (3.3) |
| EGFR mutation type, n (%) | 19del | 65 (54.2) |
| | L858R | 52 (43.3) |
| | Other | 3 (2.5) |
| Brain metastases, n (%) | Yes | 22 (18.3) |
| | No | 98 (81.7) |
| Previous EGFR-TKIs treatment, n (%) | 1st /2nd | 86 (71.7) |
| | 3rd | 32 (26.7) |
| | 1st and 3rd | 1 (0.8) |
| | 2nd and 3rd | 1 (0.8) |
| Previous EGFR-TKIs response, n (%) | PR | 56 (46.7) |
| | SD | 43 (35.8) |
| | NE | 18 (15.0) |
| | NA | 3 (2.5) |
| EGFR-TKIs progression modes, n (%) | Gradual progression | 109 (90.8) |
| | Oligo progression | 6 (5.0) |
| | Potential progression | 5 (4.2) |

NA, not assessed; NE, not evaluable; PR, partial response; SD, stable disease

### Efficacy

At the time of data cutoff, 72 of 120 patients had disease progression or death. The mPFS was 9.1 months (95% CI 6.8–11.7). The PFS rate at 6 and 12 months were 68.5% (95% CI 58.7–76.4) and 38.8% (95% CI, 28.4–49.0), respectively (Fig. 1A). The subgroup analysis is shown in Fig. 1B. The mPFS of first-line treated with 1st /2nd and 3rd generation EGFR-TKIs was 9.2 months (95% CI 6.7–12.6) and 10.3 months (95% CI 6.1–13.3), respectively (Fig. 1C). The mPFS was 6.8 months (95% CI 3.5–21.7) and 9.2 months (95% CI 6.8–11.7) in patients with or without brain metastases (Fig. 1D). After progression, 61 patients (50.8%) started a first subsequent antitumor therapy (FST), further details can be found in Supplemental Fig. 2. Overall survival was immature with 33 (27.5%) events. The 12-month OS rate was 81.1% (95% CI 71.8–87.5) (Supplemental Fig. 3).

As shown in Table 2; Fig. 2, overall ORR and DCR were 6.7% (95% CI 2.9–12.7) and 87.5% (95% CI 80.2–92.8), respectively. 76 (63.3%) of 120 patients experienced a reduction from baseline in target lesion size.

### Safety

At data cutoff, the median duration of exposure to anlotinib, irrespective of dose interruption, was 6.1 months (range 0.0–28.7). Treatment-emergent adverse events (TEAEs) were reported in 116 patients (96.7%), and TEAEs of grade 3 or higher were reported in 63 patients (52.5%). 49 (40.8%) patients experienced TEAEs leading to dose interruption or reduction, and 15 (12.5%) patients treatment was discontinued (Supplemental Table 1). Most common TEAEs were diarrhea (53.3%), hypertension (50.0%) and proteinuria (39.2%) (Supplemental Table 2).

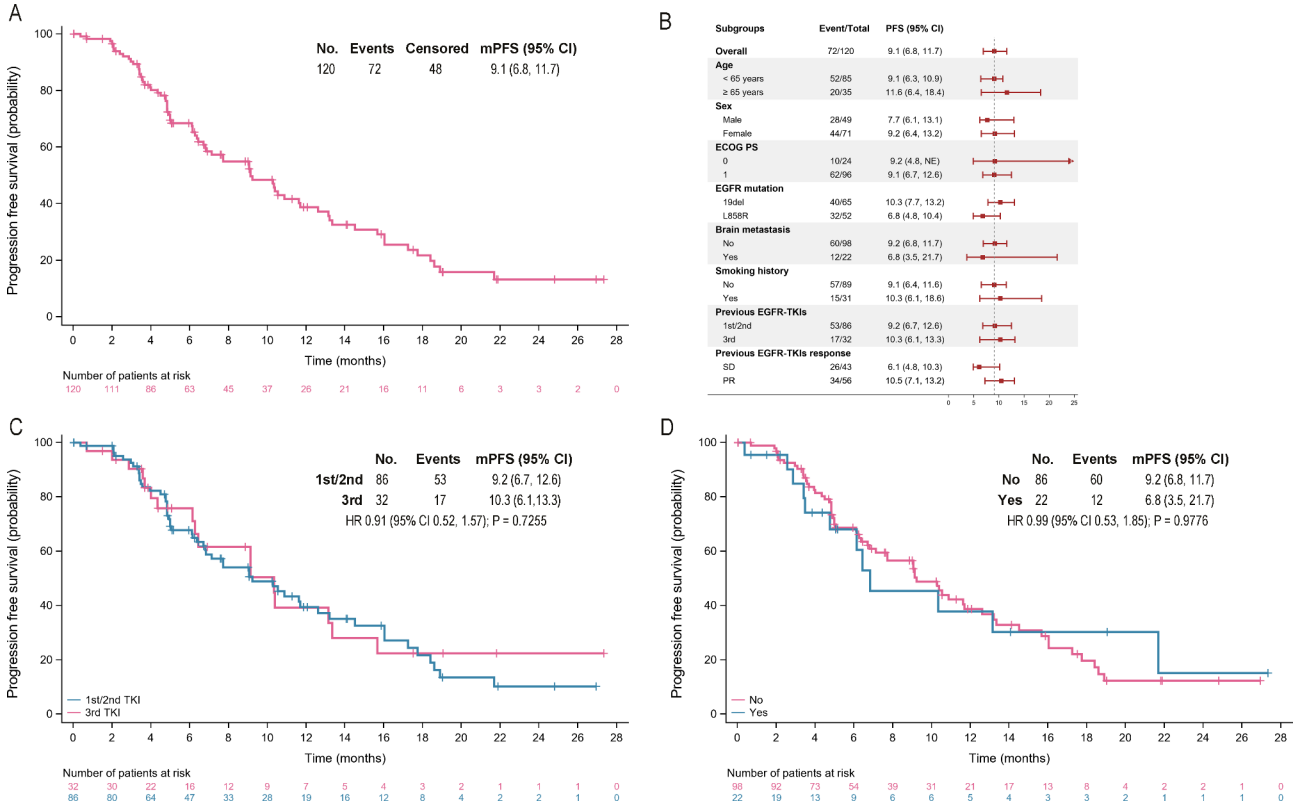


**Fig. 1** (**A**) Kaplan-Meier estimates of progression-free survival in all patients. (**B**) Subgroup analysis of progression-free survival. (**C**) Kaplan-Meier estimates of progression-free survival in patients treated with first-line 1st /2nd and 3rd generation EGFR-TKIs. (**D**) Kaplan-Meier estimates of progression-free survival in patients with and without brain metastases. Tick marks indicate censored data. CI, confidence interval; HR, hazard ratio; mPFS, median PFS

**Table 2** Summary of efficacy endpoints

| Efficacy | *N* = 120 |
|---|---|
| Best overall response, n (%) | |
| PR | 8 (6.7) |
| SD | 97 (80.8) |
| PD | 9 (7.5) |
| NE | 6 (5.0) |
| ORR, % (95% CI) | 6.7 (2.9, 12.7) |
| DCR, % (95% CI) | 87.5 (80.2, 92.8) |
| PFS | |
| Median, months (95% CI) | 9.1 (6.8, 11.7) |
| 6 months, % (95% CI) | 68.5 (58.7, 76.4) |
| 12 months, % (95% CI) | 38.8 (28.4, 49.0) |
| OS | |
| Median, months (95% CI) | NR (22.9, NR) |
| 12 months, % (95% CI) | 81.1 (71.8, 87.5) |

CI, confidence interval; DCR, disease control rate; NE, not evaluable; NR, not reached; ORR, objective response rate; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease

### Biomarker analysis

For the exploratory endpoint, 31 paired ctDNA samples were collected at baseline, the third cycle of treatment, and progression. After removing unqualified samples, mutation analyses were conducted on 25 patients at baseline, 27 at the third cycle, and 28 at progression.

Baseline plasma-based next-generation sequencing (NGS) analyses revealed potential resistance mechanisms at gradual, oligo, or potential progression (Fig. 3A). Among patients treated with 1st /2nd generation EGFR-TKIs, 23.8% (5/21) patients had a known resistance mechanism, all of which were EGFR T790M mutations, and two had concurrent TP53 mutations (Fig. 3C). No other EGFR on-target or off-target resistance mechanisms were identified, 76.2% (16/21) had an unknown resistance mechanism. For patients treated with 3rd generation EGFR-TKIs, one patient had concurrent EGFR D1012Y and TP53 mutations, two patients had concurrent SMARCA4 mutations.

Plasma-based NGS analyses at progression revealed potential resistance mechanisms after combination with anlotinib (Fig. 3B). Among patients treated with 1st /2nd generation EGFR-TKIs, compared with baseline, 60% (3/5) of patients retained T790M and three had newly emerged T790M. 73.9% (17/23) of patients had an unknown resistance mechanism. For patients treated with anlotinib and 3rd generation EGFR-TKIs, The patient with EGFR D1012Y at baseline continued retained but TP53 disappeared at progression, achieving a 5.1% shrinkage of target lesions as stable disease with

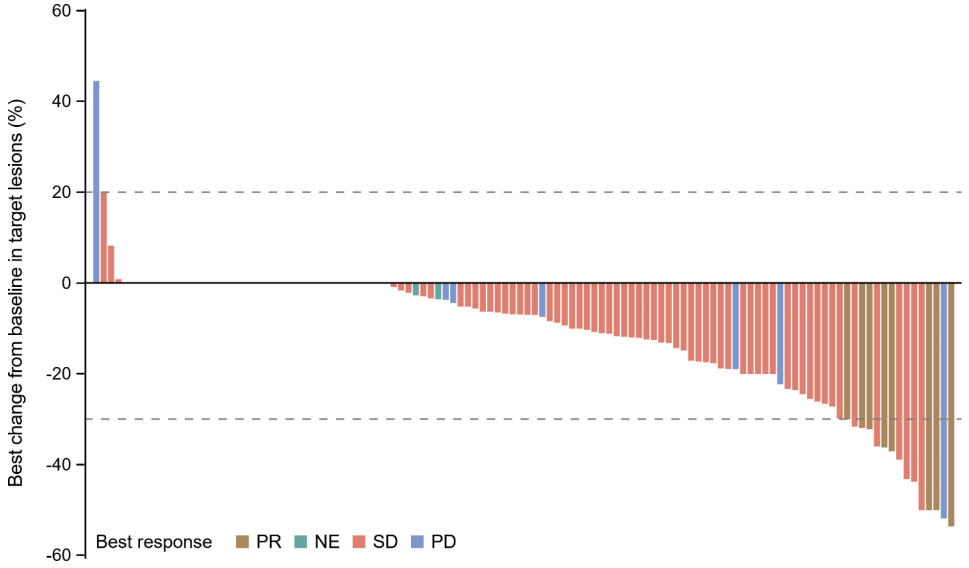


**Fig. 2** Waterfall plot of best percentage change in target lesion size in all patients. PD, progressive disease; PR, partial response; SD, stable disease; NE, not evaluable

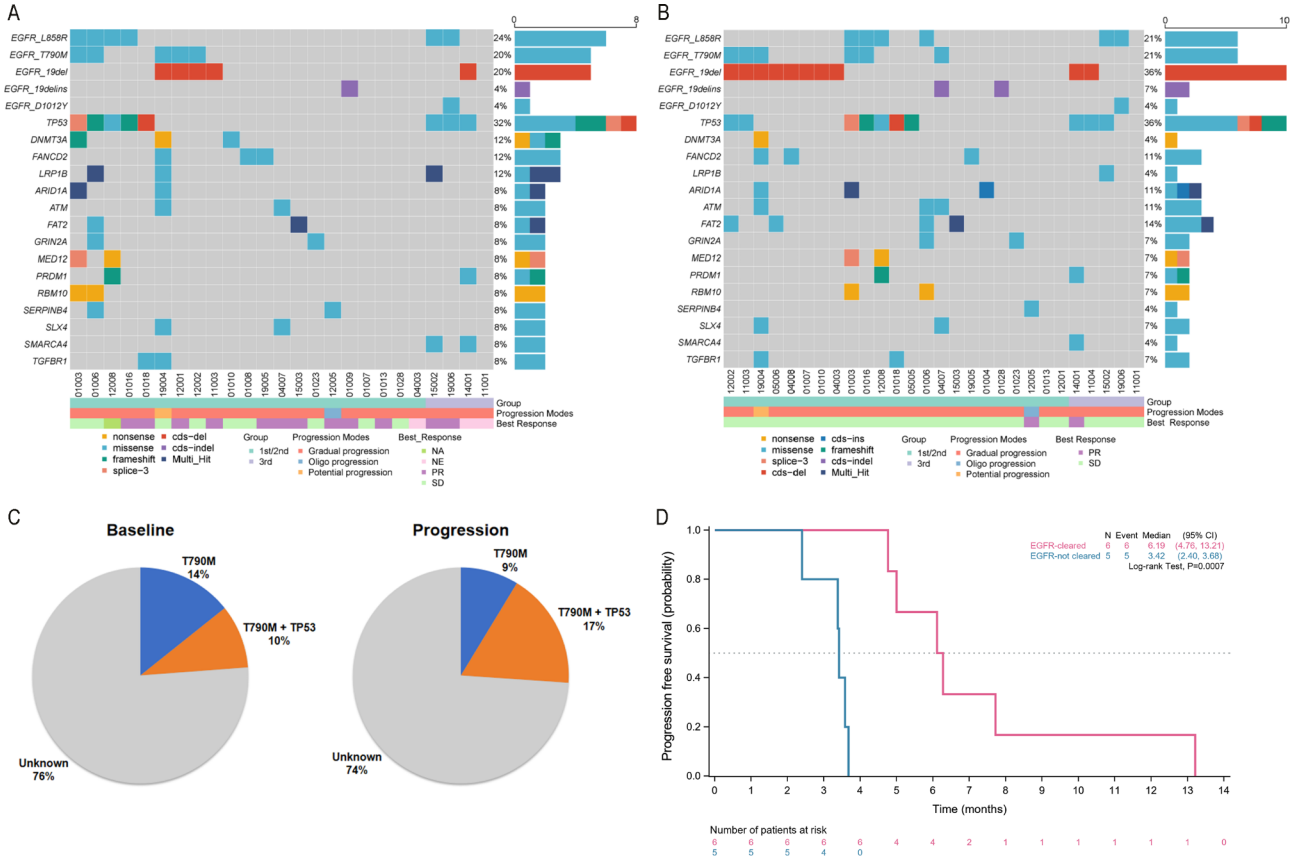


**Fig. 3** Analysis of gene mutations, potential resistance mechanisms, and molecular dynamics of EGFR-sensitive mutations. The oncoplot showing the mutation frequencies of EGFR and other genes in ctDNA of patients treated with 1st /2nd or 3rd generation EGFR-TKIs at (**A**) baseline and (**B**) progression. (**C**) The pie chart displaying the distribution of acquired resistance mechanisms in patients treated with 1st /2nd generation EGFR-TKIs at baseline (left) and progression (right). (**D**) Kaplan-Meier estimates of progression-free survival in EGFR cleared/not-cleared patients. PR, partial response; SD, stable disease; NA, not applicable; NE, not evaluable

a PFS of 3.7 months. One patient experienced the disappearance of SMARCA4.

We also analyzed the molecular dynamics of EGFR-sensitive mutations (L858R and 19del) in ctDNA samples during treatment. EGFR-sensitive mutations were reduced or not detected at the third cycle and increased or reappeared at progression. Notably, 54.5% (6/11) of patients had EGFR-sensitive mutations cleared. The mPFS of EGFR cleared/not-cleared patients was 6.2 months (95% CI 4.8–13.2) and 3.4 months (95% CI 2.4–3.7), respectively ($p=0.0007$) (Fig. 3D). Swimmer plot of PFS (months) of individual EGFR cleared/not-cleared patients was shown in Supplemental Fig. 4.

## Discussion

To our knowledge, CTONG-1803/ALTER-L001 is the first prospective study to investigate antiangiogenic small molecule plus EGFR-TKIs in specific EGFR-TKIs progression modes. This study met the primary endpont, anlotinib plus EGFR-TKIs achieved a PFS of 9.1 months in NSCLC patients with gradual, oligo, or potential progression after previous EGFR-TKIs. The mPFS was 10.3 months for patients treated with first-line 3rd generation EGFR-TKIs, which is similar to the patients treated with first-line 1st /2nd generation EGFR-TKIs (9.2 months). It is common for cases administered 1st or 2nd generation EGFR-TKIs to check for the T790M after progression. However, this study screened patients based on clinical characteristics, so the presence or absence of T790M did not affect enrollment. There were one case in each of 1st and 3rd, and 2nd and 3rd generation in previous EGFR-TKIs treatment. Our study enrolled patients with potential progression for the first time, details of these patients are provided in Supplemental Table 3. In subgroups analysis all patients could benefit from this combination even smoker or brain metastasis. Potential benefits in continuing EGFR-TKIs therapy after progression have been reported in ASPIRATION [21] and LUX-Lung 5 [27]. The IMPRESS study indicated that continuing gefitinib upon gefitinib progression did not improve PFS and OS in patients who received platinum-based doublet chemotherapy as subsequent line of treatment [28, 29]. However, none of these studies considered progression modes nor included 3rd generation EGFR-TKIs. Continuing osimertinib in patients with slow disease progression and no deterioration in clinical symptoms is mentioned in the European Society for Medical Oncology (ESMO) expert consensus statements [30], and our results provide further supporting evidence for this viewpoint. Recent studies demonstrated that EGFR-TKIs combined with bevacizumab in gradual progression NSCLC patients after first-line EGFR-TKIs has a PFS of 5 ~ 11.4 months [9–11], but all were retrospective with small sample sizes ($n=15 \sim 48$). A recent FLAURA2 showed that osimertinib plus platinum-pemetrexed as first-line treatment for EGFR mutant advanced NSCLC led to significantly longer PFS (25.5 months) than osimertinib monotherapy [31]. In the MARIPOSA study, amivantamab plus lazertinib showed superior PFS (23.7 months) to osimertinib as first-line treatment in EGFR mutant advanced NSCLC [32]. In our study, the time from initiation of first-line EGFR-TKIs to systemic progression may have exceeded 20 months, including 12.9 months for time to gradual, oligo, or potential progression after previous EGFR-TKIs and 9.1 months for mPFS after anlotinib in combination with EGFR-TKIs (Supplemental Fig. 5). However, since the front-line outcomes were analyzed retrospectively, the overall PFS benefit needs to be further validated.

CTONG-1803/ALTER-L001 explored the resistance mechanisms at gradual, oligo, or potential progression and after the combination of anlotinib for the first time. EGFR-TKI acquired resistance can be classified as on-target (EGFR dependent), off-target (EGFR independent), and unknown [33]. 1st /2nd generation EGFR-TKIs acquired resistance is mainly on-target, with T790M mutation being the most common, with an incidence of 50–60%. For these patients, 3rd generation EGFR-TKIs have become the standard of care [34]. On-target acquired resistance to 3rd generation EGFR-TKIs is relatively rare, occurring in approximately 10–20% of patients treated with first-line osimertinib [35], with C797S being the most common, with an incidence of only 7% in a small cohort from the FLAURA study ($n=91$) [2, 36]. Several 4th generation EGFR-TKIs targeting C797S are in development but not yet approved. Off-target acquired resistance involves a variety of genes other than EGFR, and the known mechanisms include amplifications (MET, HER2, PIK3CA amplification, etc.), oncogenic fusions (ALK, BRAF, MET, ROS1, RET fusion, etc.), MAPK/PI3K alterations (BRAF, KRAS, PIK3CA, etc.), cell cycle gene alterations (CDKN2A, CDKN2B, CDK4, etc.), and histological transformation [2, 35, 37–39]. In the baseline analyses of our study, the resistant mechanism seems relatively simple and uncomplex. The incidence of T790M mutations was only 23.8% in patients treated with 1st /2nd generation TKIs, while no on-target resistance mechanism could be detected in the four patients treated with 3rd generation TKIs. Off-target resistance mechanism was not detected in either 1st /2nd or 3rd generation TKIs, and most of the resistance mechanisms are still unknown, suggesting that the resistance mechanisms at gradual, oligo, and potential progression may differ from previously reported acquired resistance mechanisms to EGFR-TKIs and lack of targetable alternations [31, 33]. We also found that 54.5% of patients achieved EGFR-sensitive mutations clearance in the third cycle of treatment, including 5 patients treated with 1st /2nd generation EGFR-TKIs and one patient

treated with 3rd generation EGFR-TKIs, and the mPFS of cleared patients was significantly longer than that of not-cleared patients, suggesting that early clearance of EGFR as a predictor of response to EGFR-TKIs plus anlotinib [40, 41]. The ARTEMIS/CTONG1509 study has demonstrated that bevacizumab plus erlotinib had no effect on the acquired EGFR mutation profile [42], among patients treated with 1st /2nd generation TKIs in our study, 60% retained T790M at progression, along with three patients with newly emerged T790M, who remained eligible for subsequent treatment with 3rd generation TKIs. All of the above conclusions still need to be confirmed in further biomarker analyses with larger sample sizes.

In terms of safety profile, most of the AEs in this study were Grade 1–2. Grade≥3 TEAEs were mainly hypertension (19.2%), diarrhea (5.0%), weight loss (4.2%), hypertriglyceridemia (4.2%), and palmar-plantar erythrodysaesthesia syndrome (4.2%), which were often managed through dose interruption or reduction. There were four instances of treatment-related hemoptysis and one case of treatment-related interstitial lung disease. Compared with ARTEMIS/CTONG1509 [8] and WJOG9717L [43], the incidence of proteinuria, rash, transaminase elevation, thrombocytopenia, and anemia were low in this study, possibly due to shorter exposure time to antiangiogenic agents.

This study has several limitations. It was a single-arm study, an additional arm of EGFR-TKIs alone was not set, preventing the determination of the contribution of each component in the combination strategy. A prospective study comparing the efficacy of combined EGFR-TKIs with anlotinib to EGFR-TKIs alone may provide more insights. Many patients who met the definition of oligo progression were more suitable for local therapy and were not enrolled in our study. The relatively small number of patients with oligo or potential progression, and patients treated with osimertinib in this study suggests that a larger sample size might yield more reliable results. More studies on the clinical significance of these EGFR-TKI progression modes are needed.

In conclusion, this study suggested the feasibility of the combination of EGFR-TKIs and anlotinib. The strategy not only extends the clinical benefit of original EGFR-TKIs, but also presents an effective, convenient, and well-tolerated option for patients with specific progression modes, such as gradual progression. Further research with larger sample sizes and randomized controlled designs may provide additional valuable insights into this treatment approach.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13045-024-01656-0.

Supplementary Material 1

Supplementary Material 2

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**Author contributions**
Yi-Long Wu conceived and designed the study. All authors contributed to patient recruitment. Hua-Jun Chen and Hai-Yan Tu collected the data, directed the statistical analysis and prepared the original draft. All authors were involved in data interpretation, writing, revision, and critical review of the article. All authors approved the final version for submission.

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**Data availability**
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request (Yi-Long Wu, syylwu@live.cn).

## Declarations

**Ethics approval and consent to participate**
The study protocol was approved by the institutional review board (IRB) of each participating center. The IRB approval number in leading site Guangdong Lung Cancer Institute was 2018–375 H, and all patients provided written informed consent prior to participation. The study was conducted according to the principles of the Declaration of Helsinki and Good Clinical Practice requirements.

**Consent for publication**
Not applicable.

**Competing interests**
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