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Efficacy and Safety of Combined Anlotinib and Icotinib in EGFR-Mutant Advanced Non-Squamous NSCLC: Final Analysis of a Prospective Phase II Study

Anna B. Martinez[1]*, Laura Santos[1]

[1]Department of Clinical Oncology, School of Medicine, Medical University of Vienna, Vienna, Austria.

***E-mail** ✉ amartinez@outlook.com

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ABSTRACT

Individuals diagnosed with non-small cell lung cancer (NSCLC) who carry epidermal growth factor receptor (EGFR) mutations together with additional concurrent mutations generally face a worse outlook. This investigation aimed to assess the combination of anlotinib and icotinib as a potential first-line therapy for advanced NSCLC patients with EGFR mutations, regardless of the presence or absence of concomitant mutations. The phase 2, single-arm, multicentre study (ClinicalTrials.gov NCT03736837) took place in five Chinese hospitals from December 2018 through November 2020. Patients with non-squamous NSCLC exhibiting EGFR-sensitising mutations underwent treatment with anlotinib plus icotinib. The main outcome measure was progression-free survival (PFS). Additional outcomes encompassed objective response rate (ORR), disease control rate (DCR), overall survival (OS), and adverse effects. Enrollment included sixty patients, among whom 31 (52%) presented with concurrent mutations and 29 (48%) with pathogenic concurrent mutations. Median follow-up reached 26.9 (range, 15.0–38.9) months. The ORR stood at 68.5% and DCR at 98.2%. Median PFS reached 15.1 (95% CI: 12.6–17.6) months, satisfying the primary objective; median duration of response (DoR) was 13.5 (95% CI: 10.0–17.1) months, while median OS was 30.0 (95% CI: 25.5–34.5) months. For those with pathogenic concurrent mutations, median PFS was 15.6 (95% CI: 12.5–18.7) months and median OS had not been reached (95% CI: 17.46 months to not reached). Every participant experienced treatment-related adverse events (TRAEs), with 26 (43%) encountering grade ≥3 events and 1 (1.7%) facing serious TRAEs. Combining anlotinib with icotinib proved effective and generally well-tolerated when used as first-line therapy in advanced NSCLC cases positive for EGFR mutations, including those with or without additional concurrent mutations.

Keywords: Non-small-cell lung cancer, ErbB receptors, Prognosis, Safety, Anlotinib, Icotinib

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Introduction

Non-small cell lung cancer (NSCLC) accounts for the majority of fatalities (85–90%) from malignant lung tumours, often linked to tobacco use and occurring more frequently in older adults (aged 65 years and older). Certain driver mutations that fuel cancer development have been identified, including epidermal growth factor receptor (EGFR; present in 15–20% of NSCLCs) and anaplastic lymphoma kinase (ALK; 5% of NSCLCs), which drive cellular changes and tumour advancement [1]. Cases of NSCLC with EGFR-sensitising mutations typically respond well to EGFR tyrosine kinase inhibitors (TKIs) [2-5]. These EGFR-sensitising mutations are recognised as key indicators for recommending first-line EGFR-TKI treatment in affected patients. Outcomes can differ based on specific mutation patterns, however, and the advent of next-generation sequencing (NGS) has revealed many additional concurrent mutations in EGFR-mutant NSCLC, adding complexity to management [6]. Recent research [7-9] has demonstrated that such concurrent mutations significantly shorten progression-free survival (PFS) among patients treated with EGFR TKIs.

While the third-generation EGFR-TKI osimertinib showed strong first-line performance in the FLAURA trial, its impact on cases with concurrent mutations is not fully established. A recent LC-SCRUM analysis found that median PFS with osimertinib in EGFR-mutant NSCLC involving amplification of RTK-related or cell-cycle genes was roughly 8 months less than in the FLAURA results [10]. As a result, treatment results for NSCLC patients having both EGFR-sensitising and concurrent mutations remain inadequate, highlighting the need for innovative approaches.

Combining vascular endothelial growth factor (VEGF) inhibitors with EGFR inhibitors offers a mechanistic basis for enhanced antitumour activity and overcoming resistance in NSCLC. Major randomised trials such as JO25567 [11], NEJ026 [12], and RELAY [13-15] confirmed that incorporating anti-angiogenic agents with EGFR TKIs markedly extended PFS in previously untreated EGFR-mutant NSCLC patients. Those with co-existing TP53 mutations gained notable advantages from blocking both EGFR and angiogenesis pathways in the RELAY and ACTIVE studies [14-16]. In contrast to intravenous anti-angiogenic antibodies, oral options provide greater convenience.

Anlotinib targets VEGFR1/2/3, FGFR1/2/3, and PDGFRα/β with high selectivity and stands as the only approved single-agent anti-vascular therapy effective in advanced NSCLC [17]. In China, it is indicated for refractory advanced NSCLC after at least two prior systemic regimens [18], supported by the ALTER0303 trial where anlotinib (12 mg daily, 2 weeks on/1 week off) increased PFS by 4 months and overall survival by 3.3 months over placebo [19]. Therefore, pairing anlotinib with EGFR TKIs holds promise for superior efficacy and ease of use relative to earlier combinations.

This report details the efficacy and safety findings from a prospective multicentre study of anlotinib combined with icotinib as first-line treatment for advanced EGFR-mutant NSCLC. Of particular interest, the analysis examined how treatment outcomes relate to genetic characteristics in patients with EGFR-sensitising mutations plus concurrent alterations, to better pinpoint those most likely to gain from this dual approach.

Materials and Methods

Study design and patients

The phase 2, open-label, single-arm, multicentre study (ClinicalTrials.gov NCT03736837) took place across five sites in China from December 2018 to November 2020. Approval was obtained from the independent ethics committees or institutional review boards at each site. Every participant signed informed consent forms before entering the trial. Inclusion required histologically or cytologically proven stage IIIB/IV non-squamous NSCLC or postoperative recurrence, with confirmed EGFR-sensitising mutations (exon 19 deletion or exon 21 Leu858Arg). EGFR status was determined using CLIA- or CAP-certified next-generation sequencing (NGS) on tumour specimens. Participants had to be 18–75 years old and possess at least one target lesion measurable by RECIST 1.1. No prior systemic therapy for advanced disease was permitted, including EGFR-TKIs or chemotherapy (neoadjuvant/adjuvant excluded if completed >6 months before the last dose). Further requirements included ECOG performance status 0–1, adequate bone marrow, liver, and kidney function, controlled or asymptomatic brain metastases, and projected life expectancy of at least 3 months.

Principal exclusion factors were documented haemoptysis or blood-stained sputum, coagulopathies, poorly controlled severe hypertension, or tumours encroaching on or closely bordering large vessels.

Definition of concurrent mutations and pathogenic concurrent mutations

Concurrent mutations referred to any genetic variants detected via NGS apart from the classic EGFR exon 19 deletion or exon 21 L858R, but not including rare EGFR variants co-occurring with these, in accordance with earlier publications. Pathogenic concurrent mutations denoted variants outside the classic EGFR-sensitising sites that were forecasted as harmful or likely harmful by a minimum of two out of three prediction programs: PolyPhen2, PROVEAN, and Mutation Taster.

Treatment and follow-up

Subjects took icotinib orally at 125 mg three times daily and anlotinib orally at 12 mg once daily. Anlotinib followed a schedule of 2 weeks of administration and 1 week rest per cycle. Therapy persisted until radiographic progression, unacceptable adverse effects, or patient decision to stop. Investigators managed dose adjustments for treatment-emergent adverse events using NCI-CTCAE version 4.0 grading. Permitted anlotinib reductions were

to 10 mg/day or 8 mg/day per predefined rules. Dose re-escalation was forbidden after reduction, and permanent discontinuation occurred if 8 mg/day remained intolerable.

Imaging for response assessment occurred every 6 weeks according to RECIST 1.1. Ongoing monitoring tracked safety, treatment effects, and survival until death. Survival updates were collected every 12 weeks via in-person visits or phone contact.

Endpoints

Investigator-determined progression-free survival (PFS) served as the primary endpoint, calculated from the date of enrolment to objective progression or death from any cause (whichever earlier). Secondary measures were objective response rate (ORR), disease control rate (DCR), and overall survival (OS). ORR indicated the percentage of patients attaining complete response (CR) or partial response (PR). DCR indicated the percentage attaining CR, PR, or stable disease (SD). OS indicated the interval from enrolment to death from any cause, with censoring at the cutoff date if alive. All adverse events were captured and classified per National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.

NGS-based mutation profiling

Mutations were evaluated for pathogenicity using three algorithms: Mutation Taster, PolyPhen-2, and PROVEAN [20, 21]. Computational analysis integrated bioinformatics to explore how co-mutations affect TKI outcomes in patients with sensitising EGFR alterations. The PolyPhen-2 tool, created at Harvard University, applies physicochemical and evolutionary comparisons to estimate the consequences of amino acid changes on protein structure and activity [22]. Scores exceeding 0.800 identified missense variants as damaging, using this established threshold [23]. Mutation Taster predicts disease potential from sequence changes [24, 25]. PROVEAN assesses functional disruption from substitutions or indels and links this to oncogenic properties [26]. Here, variants classified as deleterious by ≥2 programs were labelled pathogenic.

TP53 destructive/non-destructive mutations

Past research has explored how TP53 alterations in particular exons affect outcomes with EGFR TKIs, but the findings have varied considerably [27-29]. Such variability indicates that simply noting the exon involved may not accurately predict influence on TKI response. Several reports have grouped TP53 variants into disruptive and non-disruptive groups [30, 31], helping to determine if the altered protein preserves any activity. For this reason, we applied the disruptive/non-disruptive TP53 mutation framework to assess their role in TKI effectiveness. Disruptive mutations consist of: (i) variants leading to premature termination (such as nonsense, frameshift, or splicing alterations); (ii) missense changes in the L2 or L3 domains that swap an amino acid for one with opposite polarity or charge; (iii) in-frame deletions involving the L2 or L3 domains. Non-disruptive mutations cover everything outside the disruptive category and consist of: (i) missense changes or in-frame deletions elsewhere than the L2-L3 domains; (ii) missense changes inside the L2-L3 domains that substitute an amino acid with one having comparable polarity or charge.

Statistical analysis

Based on earlier trials and results from related drugs, first-line icotinib was expected to deliver a median PFS of 9.9 months in EGFR-mutant NSCLC [32]. Taking into account data from the ALTER0302 and ALTER0303 studies plus routine clinical observations, we anticipated that adding anlotinib to icotinib in the first-line setting for EGFR-positive NSCLC would produce a median PFS of 15 months. We chose a one-sided Z-test for a single arm to provide 80% power with $\alpha = 0.05$, applying log-rank correction for sample size via PASS 15.0. With a projected 10% dropout, at least 58 enrollees were needed.

The full analysis set (FAS) followed the intention-to-treat (ITT) approach and covered everyone who took the investigational treatment at least once. The efficacy analysis set (EAS) included those with at least one tumour response assessment and was applied for all efficacy endpoints. The safety set (SS) encompassed all who received treatment at least once and had subsequent safety records; it formed the basis for toxicity reviews.

Analyses were run on SAS 9.1.3 (SAS Institute, Cary, NC, USA). Normally distributed continuous measures appeared as mean ± standard deviation; non-normal ones as median (minimum, maximum). Counts and proportions were shown as n (%) alongside 95% confidence intervals (CIs). Kaplan-Meier estimates were

generated for PFS and OS, whereas safety data relied mainly on descriptive summaries. Survival differences were tested with the log-rank method. A P value below 0.05 denoted significance.

Results and Discussion

Patients

Over the period from December 2018 to November 2020, 60 individuals entered the trial; every one received the anlotinib-icotinib regimen at least once and contributed to both the SS and FAS **(Table 1).** Median age stood at 62 years (range, 35–72), and 34 (43%) were men. Stage IV disease applied to 58 (98%) cases. Half the cohort (30, 50%) harboured EGFR exon 19 deletion, the other half (30, 50%) the L858R variant, while 31 (52%) showed any concurrent mutations and 29 (48%) pathogenic concurrent mutations. Prior to the initial response review, two subjects withdrew consent, and efficacy could not be evaluated in one further case because of toxicity; consequently, 57 patients made up the EAS.

Table 1. Baseline characteristics of the participants

Characteristic	ITT (n = 60)	ESA (n = 57)
Median age (range), years	62 (35–72)	62 (35–72)
Patients aged ≥60 years	35 (58.3%)	35 (59.6%)
Male sex	26 (43.3%)	25 (43.9%)
Clinical stage		
IIIB	1 (1.7%)	1 (1.8%)
IIIC	1 (1.7%)	1 (1.8%)
IV	58 (96.7%)	55 (96.5%)
Smoking history		
Ever smoked	11 (18.3%)	11 (19.3%)
Current smoker	9 (15.0%)	7 (12.3%)
Never smoked	40 (66.7%)	39 (68.4%)
ECOG performance status		
0	22 (36.7%)	22 (38.6%)
1	37 (61.7%)	34 (59.6%)
2	1 (1.7%)	1 (1.8%)
Recurrent NSCLC	2 (3.3%)	2 (3.5%)
Presence of brain metastases	21 (35.0%)	21 (36.8%)
EGFR mutation type		
Exon 19 deletion (19del)	30 (50.0%)	30 (52.6%)
L858R point mutation	30 (50.0%)	27 (47.4%)
Any concurrent mutations	31 (51.7%)	30 (52.6%)
Pathogenic concurrent mutations	29 (48.3%)	28 (49.1%)

Data are presented as median (range) or n (%).

ITT, intention-to-treat; EAS, efficacy analysis set; ECOG PS, Eastern Cooperative Oncology Group performance status; NSCLC, non-small-cell lung cancer; EGFR, epidermal growth factor receptor gene.

Efficacy

This trial achieved its planned statistical target (PFS = 15 months). By the cutoff date of October 26, 2022, follow-up lasted a median of 26.9 (range, 15.0–38.9) months. Investigator-assessed median PFS and OS reached 15.1 (95% CI: 12.6–17.6) months and 30.0 (95% CI: 25.5–34.5) months, respectively **(Figures 1a and 1b).** Within the Per Protocol Set (PPS), ORR attained 68.5% (39 out of 57 patients), with one case of complete response (CR). DCR hit 98.2% (56 out of 57) **(Figure 2).** Patterns of tumour response and duration of response (DoR) appear in **Figure 3**, where median DoR stood at 13.5 (95% CI: 10.0–17.1) months. By cutoff, 7 individuals remained on therapy, 22 had passed away, 38 stopped due to disease progression (PD), and 6 halted because of adverse events (AEs). For those carrying pathogenic concurrent mutations, median PFS was 15.6 (95% CI: 12.5–18.7) months, while median OS remained unreached (95% CI: 17.46 months to not estimable) **(Figures 1c and 1d).** Exploratory subgroup reviews showed no notable variations in median PFS related to concurrent mutation presence (P = 0.623), pathogenic concurrent status (P = 0.885), specific EGFR variant (P = 0.214), brain metastases (P = 0.417),

liver metastases (P = 0.428), bone metastases (P = 0.334), any dose pauses (P = 0.227), dose lowering (P = 0.970), or grade 3/4 adverse events (P = 0.464).

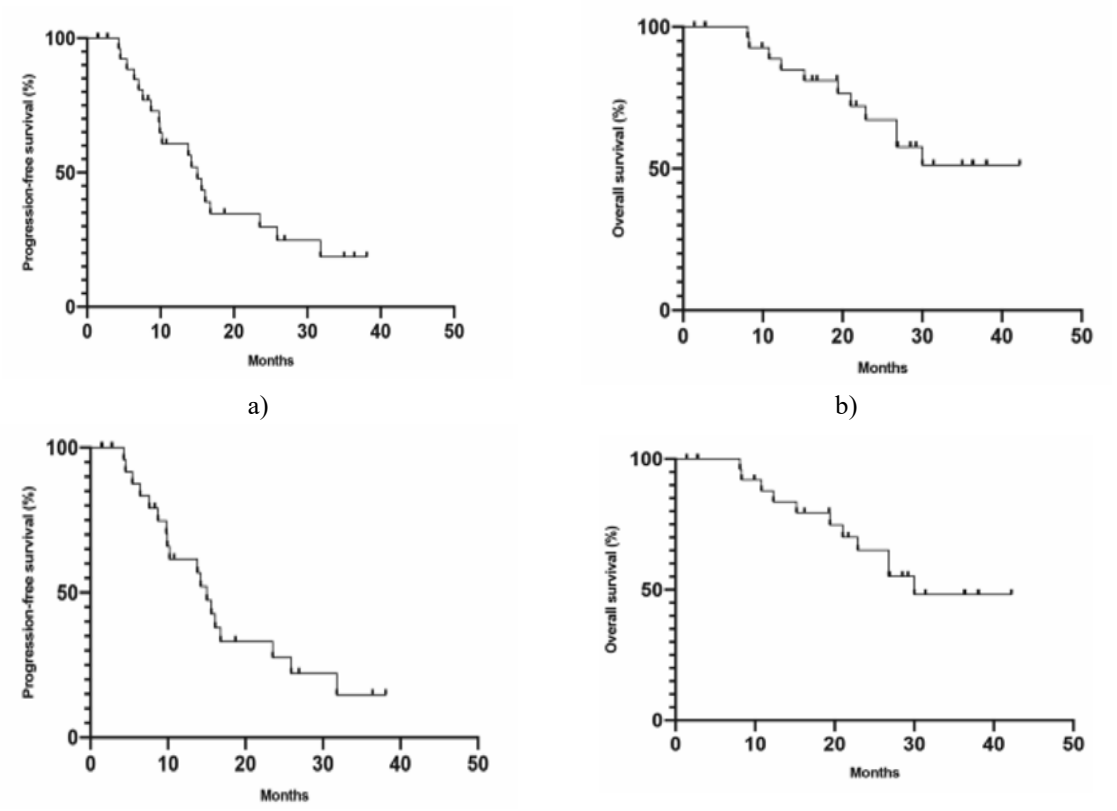


Figure 1. Activity from anlotinib plus icotinib therapy. Kaplan-Meier plots for PFS and OS across the intention-to-treat group (a, b) and subgroup with pathogenic concurrent mutations (c, d)

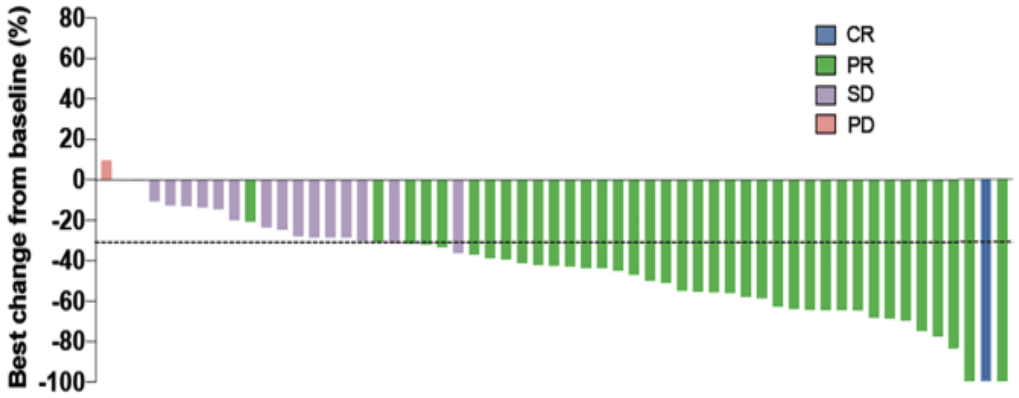


Figure 2. Maximum reduction percentages from baseline in sizes of target lesions among the efficacy-evaluable set. CR, complete response; PR, partial response; SD, stable disease; PD, disease progression

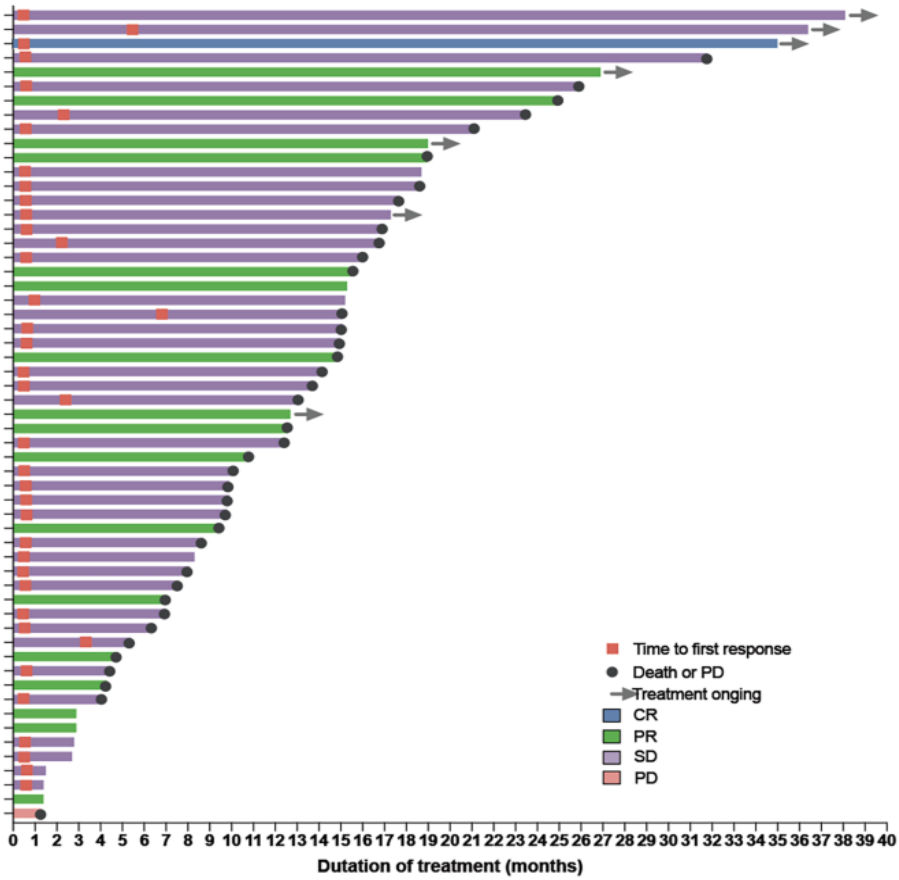
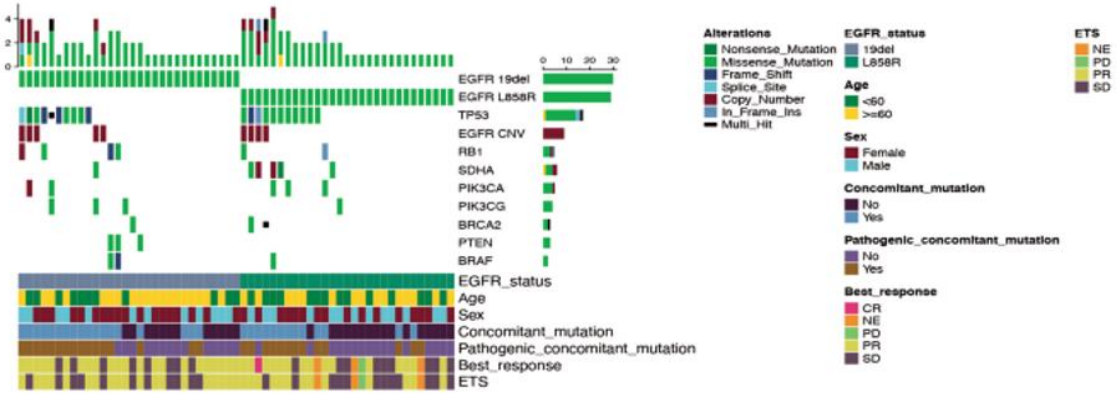


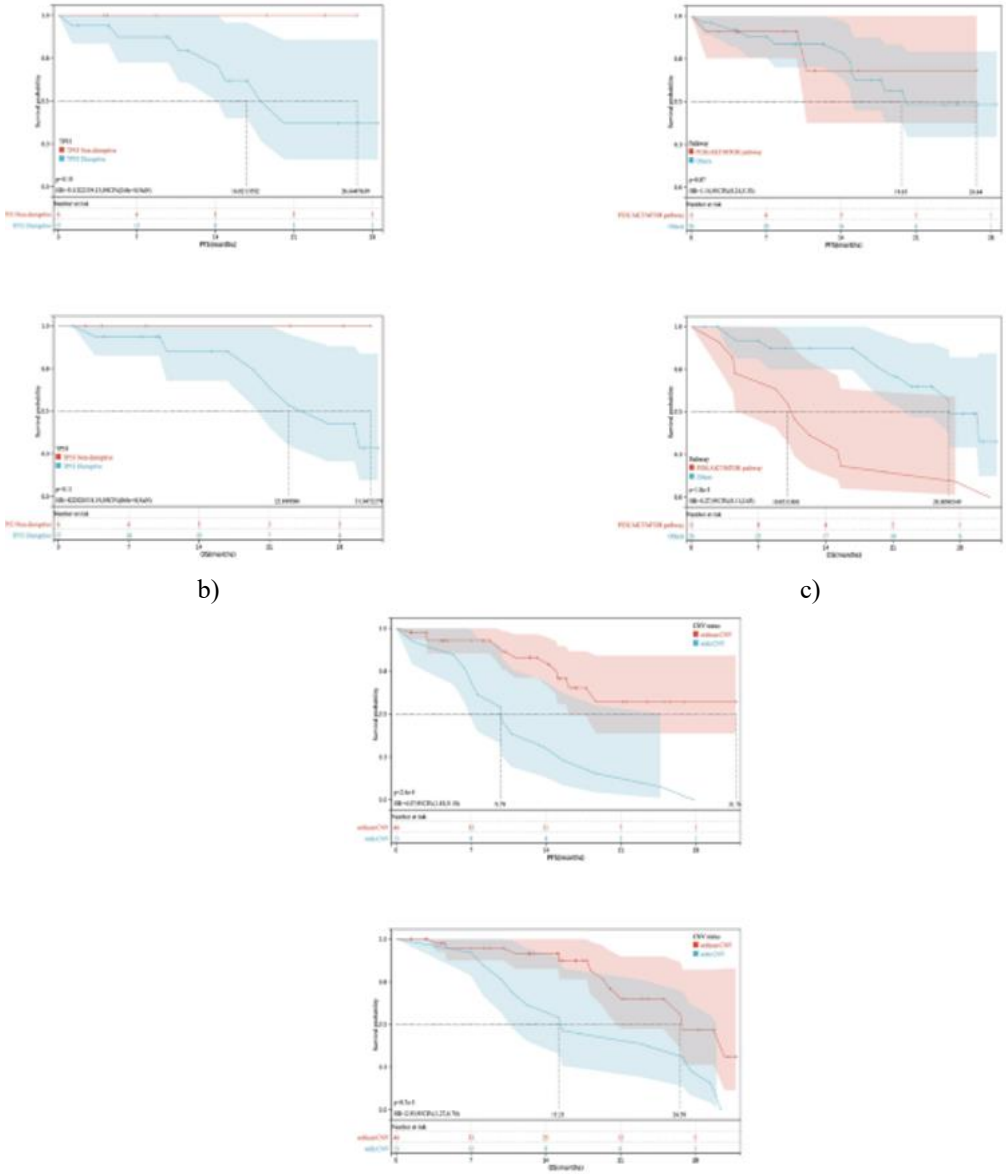
Figure 3. Patterns of tumour response and duration of response

Mutation frequencies and concurrent mutations

Notable variation emerged in ORR for patients with versus without pathogenic concurrent mutations (83% versus 43%), alongside DCR values of 100% versus 93%. Next-generation sequencing (NGS) on tissue from the full 60-patient cohort revealed the concurrent mutation profile at baseline in **Figure 4a**. Out of 37 cases, 23 featured TP53 alterations (including one with several TP53 changes). Six of those 23 involved non-disruptive TP53 variants. Associated survival patterns appear in **Figure 4b**. Sample size limited statistical power for differences in PFS or OS, yet non-disruptive TP53 cases trended toward better numeric PFS and OS. Eleven of the 37 harboured concurrent alterations in the PI3K/AKT/mTOR cascade, linking to markedly worse OS (P = 0.0018) **(Figure 4c).** Copy number variation (CNV) patterns at baseline aligned across groups. Thirteen among the 37 concurrent-mutation cases showed CNV, correlating with substantially reduced PFS (P = 0.0003) and OS (P = 0.0087) on dual therapy **(Figure 4d).**



a)



b) c)

d)

Figure 4. Profiles of mutation frequencies and concurrent alterations (a) Overview of concurrent mutations in the intention-to-treat cohort. (b) Progression-free survival (PFS) and overall survival (OS) by disruptive versus non-disruptive TP53 status. (c) PFS and OS by concurrent alterations in PI3K/AKT/mTOR versus alternative pathways. (d) PFS and OS by presence or absence of copy number variation (CNV).

Treatment-related adverse events (TRAEs) affected all participants, including 26 (43.7%) with grade ≥ 3 TRAEs and 1 (1.7%) with a serious TRAE **(Table 2).** TRAEs prompted interruptions in any agent, anlotinib reductions, or full discontinuation in 23 (38.3%), 15 (25.0%), and 5 (8.3%) cases, respectively. Typical drivers included elevated triglycerides, proteinuria, high blood pressure, elevated cholesterol, hand-foot skin reaction, and occult fecal blood. No interstitial lung disease arose. All-grade TRAEs at ≥ 15% incidence covered elevated triglycerides (65%), high blood pressure (57%), elevated cholesterol (52%), proteinuria (50%), loose stools (50%), hand-foot skin reaction (35%), low thyroid function (33%), raised TSH (28%), skin rash (27%), higher ALT (25%), raised LDL (22%), higher AST (20%), occult fecal blood (17%), gum bleeding (17%), mouth inflammation (15%), occult urine blood (15%), and visible blood in urine (15%). Grade ≥ 3 TRAEs exceeding 5% incidence involved high blood pressure (25%) and loose stools (5%). One grade 4 event occurred: elevated triglycerides (n = 1, 2%) **(Table 3).**

Table 2. Treatment-related adverse events

Adverse Events	Safety Population (n = 60)
Any treatment-related adverse event (TRAE)	60 (100.0%)
Grade ≥3 TRAEs	26 (43.3%)
Serious TRAEs	1 (1.7%)
TRAEs resulting in dose interruption (any drug)	23 (38.3%)
TRAEs resulting in dose interruption (anlotinib)	23 (38.3%)
TRAEs resulting in dose interruption (icotinib)	2 (3.3%)
TRAEs resulting in dose reduction (any drug)	15 (25.0%)
TRAEs resulting in dose reduction (anlotinib)	15 (25.0%)
TRAEs resulting in dose reduction (icotinib)	0
Discontinuation of combined therapy due to TRAEs	5 (8.3%)

TRAE, treatment-related adverse event.

Table 3. Preferred terms of the treatment-related adverse events (TRAEs)

Treatment-Related Adverse Event (TRAE)	Grade 1/2	Grade 3	Grade 4	Total
Hypertriglyceridemia	37 (62%)	1 (2%)	1 (2%)	39 (65%)
Hypertension	19 (32%)	15 (25%)	0 (0%)	34 (57%)
Hypercholesterolemia	31 (52%)	0 (0%)	0 (0%)	31 (52%)
Proteinuria	30 (50%)	0 (0%)	0 (0%)	30 (50%)
Diarrhea	27 (45%)	3 (5%)	0 (0%)	30 (50%)
Hand-foot syndrome	19 (32%)	2 (3%)	0 (0%)	21 (35%)
Hypothyroidism	20 (33%)	0 (0%)	0 (0%)	20 (33%)
Elevated thyroid stimulating hormone	17 (28%)	0 (0%)	0 (0%)	17 (28%)
Rash	16 (27%)	0 (0%)	0 (0%)	16 (27%)
Elevated alanine transaminase	13 (22%)	2 (3%)	0 (0%)	15 (25%)
Elevated low-density lipoprotein	13 (22%)	0 (0%)	0 (0%)	13 (22%)
Elevated aspartate aminotransferase	11 (18%)	1 (2%)	0 (0%)	12 (20%)
Fecal occult blood	10 (17%)	0 (0%)	0 (0%)	10 (17%)
Bleeding gums	9 (15%)	1 (2%)	0 (0%)	10 (17%)
Oral mucositis	8 (13%)	1 (2%)	0 (0%)	9 (15%)
Urine occult blood	9 (15%)	0 (0%)	0 (0%)	9 (15%)
Hematuria	9 (15%)	0 (0%)	0 (0%)	9 (15%)
Nasal bleeding	8 (13%)	0 (0%)	0 (0%)	8 (13%)
Hyperuricemia	6 (10%)	0 (0%)	0 (0%)	6 (10%)
Sinus bradycardia	2 (3%)	1 (2%)	0 (0%)	3 (5%)
Thrombocytopenia	3 (5%)	0 (0%)	0 (0%)	3 (5%)
Hyperbilirubinemia	1 (2%)	0 (0%)	0 (0%)	1 (2%)
Hemoptysis	1 (2%)	0 (0%)	0 (0%)	1 (2%)
Increased creatinine	1 (2%)	0 (0%)	0 (0%)	1 (2%)
Acute coronary syndrome	0 (0%)	1 (2%)	0 (0%)	1 (2%)
Intracranial hypertension	0 (0%)	1 (2%)	0 (0%)	1 (2%)
Myocardial infarction	0 (0%)	1 (2%)	0 (0%)	1 (2%)
Thromboembolic events	0 (0%)	1 (2%)	0 (0%)	1 (2%)

Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR TKIs) represent the established first-line therapy for non-small cell lung cancer (NSCLC) harbouring EGFR-sensitising mutations, with third-generation agents currently favoured. However, the Asian subgroup from the FLAURA trial indicated no significant overall survival (OS) advantage, with survival curves intersecting around 39 months (hazard ratio [HR] = 1.00, 95% CI: 0.75–1.32) [33]. Comparable findings emerged from the FLAURA China extension cohort (HR = 0.848, 95% CI: 0.557–1.291) [34]. The ALTER-L004 investigation, a multicentre phase 2 single-arm exploratory study, provided

robust evidence supporting the effectiveness and tolerability of anlotinib paired with icotinib in individuals with advanced NSCLC carrying EGFR mutations. As far as we are aware, this represents the initial thorough examination of how concurrent mutations relate to outcomes from combined angiogenesis and EGFR blockade in the first-line setting.

The median progression-free survival (PFS) observed here was 15.1 months, aligning with reports from trials combining EGFR TKIs and anti-angiogenic agents. For instance, Zhang *et al*. [35] documented a median PFS of 11.53 months with gefitinib plus anlotinib. The phase II JO25567 trial reported median PFS values of 9.7 months for erlotinib alone and 16 months for erlotinib combined with bevacizumab [11]. In the RELAY trial [13-15], PFS was markedly extended with ramucirumab plus erlotinib versus placebo plus erlotinib (median 19.4 vs. 12.4 months), yielding a stratified HR of 0.59 (95% CI: 0.46–0.76; $P < 0.0001$); exploratory subgroups indicated that TP53 co-mutations correlated with enhanced benefits from ramucirumab plus erlotinib across both exon 19 deletion and exon 21 L858R categories. This all-oral regimen of apatinib plus gefitinib offers a practical first-line alternative for EGFR-mutant disease. The phase 3 ACTIVE trial [16] revealed median PFS of 13.7 months with apatinib plus gefitinib versus 10.2 months with placebo plus gefitinib (HR = 0.71, $P = 0.0189$). Subsequent exploratory review suggested greater PFS gains with apatinib plus gefitinib among those with TP53 exon 8 mutations.

Research by Zhang *et al*. [9] highlighted that concomitant mutations in treatment-naïve cases were linked to lower objective response rate (ORR) (44% vs. 77%; $P = 0.01$), reduced median PFS (6.20 vs. 18.77 months, $P < 0.001$), and diminished median OS (22.70 vs. not reached, $P < 0.001$). The BENEFIT trial conducted by Wang *et al*. [8] reported median PFS of 13.2 months for patients with solely EGFR-sensitising mutations, dropping to 9.3 months with concomitant tumour-suppressor alterations and further to 4.6 months with concomitant oncogene changes.

Our findings indicate that anlotinib combined with icotinib yields favourable ORR and PFS even in the presence of concomitant mutations. Although osimertinib as first-line therapy may deliver superior PFS overall, individuals with concurrent alterations like TP53 exhibit shorter PFS compared to results in this study [36-38]. This implies that blocking a single pathway may fail to enhance survival when resistance mechanisms are remodelled by monotherapy escalation, underscoring the necessity for combined approaches. At the same time, PFS across the full cohort treated with anti-angiogenesis plus EGFR TKI did not surpass osimertinib outcomes, indicating the importance of patient selection for such dual regimens. Patients with multiple concurrent mutations might derive greater advantage. Higher ORR in the co-mutation cohort could stem from limited cohort size introducing bias. Alternatively, those with concurrent mutations may be better suited to this dual oral blockade strategy.

From available evidence, tumour behaviour is governed by genomic complexity rather than isolated driver targeting. Concomitant mutations probably alter core tumour biology via cooperative interactions, imparting novel traits and promoting resistance. Such alterations likely emerge progressively during therapy. While prior work has underscored the relevance of concomitant mutations, few have rigorously examined how particular sites influence cellular processes. Beyond frequent variants, changes in less common genes could substantially drive oncogenesis. Thus, deeper exploration is essential to uncover patterns in the emergence and evolution of concomitant mutations in NSCLC and their prognostic implications. Forecasting harmful variants is commonplace in modern oncology precision medicine. Here, we generated mutation profiles via next-generation sequencing (NGS), applied computational tools to forecast pathogenicity of non-canonical variants lacking established functional data, identified pertinent concomitant alterations, and assessed their influence on TKI response alongside resistance mechanisms. For missense changes in non-hotspot regions, we utilised three established algorithms (MutationTaster, PolyPhen-2, and PROVEAN), interpreting deleterious potential through sequence and protein-level impacts. In contemporary precision oncology, such predictive strategies for damaging mutations are routinely employed [39-43].

TP53, recognised as a key tumour suppressor extensively researched in oncogenesis, also exerts a substantial influence on the effectiveness of EGFR TKI therapy. Earlier investigations explored the impact of TP53 alterations in specific exons (such as exon 8) on TKI response, yet yielded conflicting outcomes. Consequently, the classification of disruptive versus non-disruptive mutations was adopted [18, 19]. Disruptive mutations lead to total or near-total loss of p53 protein activity. By comparison, non-disruptive mutations retain certain properties of wild-type p53, often described as conferring gain-of-function effects. Prior research has linked non-disruptive p53 mutations to poorer survival in advanced NSCLC [44]. In contrast, the current evaluation suggests that the anlotinib plus icotinib combination delivered better outcomes in patients with non-disruptive TP53 mutations

(despite lacking statistical significance, likely owing to the small cohort). As far as we know, this represents the first clinical trial to advance this observation, necessitating confirmation through larger investigations.

In this trial, five participants stopped the dual therapy because of treatment-related adverse events (TRAEs). No grade 5 adverse events were recorded, and no novel safety concerns emerged. Regarding anlotinib, TRAEs prompted dose interruptions in 23 patients and dose reductions in 15 patients, while two individuals initiated treatment at 8 mg. Optimising anlotinib dosing when combined with EGFR TKIs requires additional exploration.

Key limitations of this investigation encompass the modest cohort size, lack of a control arm, and absence of consistent repeat biopsies. Furthermore, variations in sequencing platforms, panel coverage, and depth could have resulted in under-detection of concomitant mutations. Relative to earlier reports, survival and response rates among patients lacking concurrent mutations did not appear elevated here, potentially explained by: (1) restricted sample numbers; (2) potential false-negative results from constrained sequencing depth and gene panel scope; (3) limited suitability of the dual blockade approach for cases without concurrent mutations; (4) utilisation of the first-generation TKI icotinib, which demonstrates inferior efficacy versus third-generation agents.

In conclusion, the combination of anlotinib and icotinib demonstrates efficacy and acceptable tolerability as first-line therapy for advanced NSCLC with EGFR mutations, irrespective of concurrent mutations. These data support the pursuit of larger randomised controlled trials. Based on the present results, individuals with pathogenic concurrent mutations appear particularly well-suited to the anlotinib plus icotinib approach. Our group is currently leading a multicentre phase III randomised controlled trial in EGFR-mutant NSCLC patients harbouring pathogenic concurrent mutations, evaluating anlotinib plus icotinib against icotinib monotherapy (NCT04797806). Future analyses are anticipated to deepen insights into concurrent mutations, aiming to optimise patient outcomes.

Conclusion

Overall, the dual blockade of anlotinib combined with icotinib proved effective and manageable as a first-line option for advanced NSCLC positive for EGFR mutations, with or without additional concurrent mutations. Recognising concurrent mutations as prognostic markers in EGFR-mutant NSCLC is essential for risk stratification and tailoring therapeutic choices. The current observations provide a basis for patient selection guided by concurrent mutation status. Additionally, non-disruptive TP53 mutations substantially affect the performance of EGFR TKI regimens.

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