Low-dose anlotinib combined with EGFR-TKI can be used as an alternative for EGFR-TKI-resistant non-small cell lung cancer in elderly patients

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Abstract. The current treatment options for epidermal growth factor receptor (EGFR) mutation-positive lung cancer in the elderly with tyrosine kinase inhibitor (TKI) resistance are limited. Although chemotherapy combined with vascular endothelial growth factor inhibitors significantly improves progression-free survival (PFS) in TKI-resistant patients, it often cannot be tolerated in elderly patients, leading to treatment failure. Anlotinib is a small molecule inhibitor made in China. The application of low-dose anlotinib in elderly patients with TKI-resistant lung cancer deserves further investigation. A total of 48 elderly patients with non-small cell lung cancer (NSCLC) were enrolled to evaluate the efficacy of anlotinib combined with continuous EGFR-TKI vs. anlotinib monotherapy in patients with acquired EGFR-TKI resistance. Anlotinib was administered at a dose of 6-8 mg per day, lower than the normal dose and known as a low dose, which is well tolerated in elderly patients. There were 25 cases in the combination group and 23 cases in the anlotinib monotherapy group. The primary endpoint of the present study was PFS, and the secondary endpoints were overall survival (OS), response rate and toxicity. The median PFS (mPFS) was significantly longer in the combination group than that in the anlotinib monotherapy group: 6.0 months [95% confidence interval (CI), 4.35-7.65] compared with 4.0 months (95% CI, 3.38-4.62) (P=0.002). Analysis of the subgroups showed similar trends in results. The median OS was 32 months (95% CI, 22.04-41.96) in the combination group and 28 months (95% CI, 27.13-28.87) in the anlotinib monotherapy group (P=0.217). According to stratification analysis, second-line treatment with anlotinib combined with EGFR-TKI resulted in a better mPFS than third-line treatment (7.5 vs. 3.7 months, HR=3.477; 95% CI, 1.117-10.820; P=0.031). In the combination group, patients with gradual/local progression after EGFR-TKI failure had a longer mPFS than those with dramatic progression (7.5 vs. 6.0 months, HR=5.875; 95% CI, 1.414-10.460; P=0.015). Multivariate analyses showed that continuous EGFR-TKI combined with anlotinib after EGFR-TKI resistance was associated with longer PFS (P=0.019), whereas dramatic progression (P=0.014) had a detrimental effect on follow-up treatment. Grade 2 adverse events (AEs) were reported in four patients (17.39%) in the anlotinib monotherapy group and eight patients (32.00%) in the combination group. Of these, the most common grade 2 AEs were hypertension, fatigue, diarrhea, paronychia, mucositis and transaminase elevation. There were no grade 3/4/5 AEs. In conclusion, the present study demonstrated that low-dose anlotinib combined with EGFR-TKI is superior to anlotinib alone following EGFR-TKI failure, making it the preferred regimen for elderly patients with acquired EGFR-TKI resistance.

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Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide and non-small cell lung cancer (NSCLC) accounts for 80-85% of lung cancer cases (1). Among them, lung adenocarcinoma comprises ~40% of all NSCLC cases. NSCLC has a poor prognosis, with a 5-year overall survival (OS) rate of <10% (2). Notably, ~50% of patients with NSCLC are positive

for epidermal growth factor receptor (EGFR) mutations, and benefit from targeted therapy with first- and second-generation tyrosine kinase inhibitors (TKIs), resulting in disease-free survival of 10-14 months (3,4). Third-generation EGFR-TKIs, such as osimertinib, aumolertinib and furmonertinib, have been reported to achieve a median progression-free survival (mPFS) time of 18.9-20.8 months (5-7). EGFR mutations are responsible for 51.4% of cases of advanced lung adenocarcinoma in patients of Asian origin, and are strictly concentrated in four exons (exons 18-21) (8). The most common mutations include exon 19 deletion (mutation frequency 45%; DEL) and exon 21 point mutation (mutation frequency 40-45%; L858R) (8,9); these two mutations account for 85-90% of all EGFR mutations (8,9). The National Comprehensive Cancer Network guidelines recommend first-line EGFR-TKI treatment for patients with NSCLC and EGFR mutations, such as gefitinib and erlotinib (first-generation EGFR-TKI), afatinib and dacomitinib (second-generation EGFR-TKI), or osimertinib (third-generation EGFR-TKI) (2). Although EGFR-TKIs have significantly prolonged survival in patients with EGFR-mutant NSCLC compared with traditional chemotherapy (10-12), acquired EGFR-TKI resistance is inevitable. In addition to the EGFR T790M mutation (13), a high level of vascular endothelial growth factor (VEGF) is also involved in EGFR-TKI resistance (14,15). Activation of the EGFR signaling pathway can upregulate VEGF production in human cancer cells (16-18). Conversely, inhibition of EGFR has been shown to inhibit the secretion of VEGF (16-18). Notably, the level of VEGF has been shown to increase after EGFR-TKI resistance (14). Therefore, the combination of anti-angiogenic therapy with TKI or chemotherapy after EGFR-TKI failure can theoretically control tumor proliferation.

Anlotinib is an oral small-molecule multi-targeted TKI, which not only hinders tumor angiogenesis by inhibiting VEGF receptor (VEGFR)2/3, platelet-derived growth factor receptor-β, fibroblast growth factor receptor (FGFR) and other signaling pathways, but also directly suppresses tumor cell proliferation by inhibiting c-kit gene expression (19,20). Compared with other receptor TKIs, such as sorafenib, sunitinib and pazopanib, anlotinib has more inhibitory targets and better antitumor effects (21), and it has been approved for third-line treatment of advanced NSCLC (22).

The present study retrospectively evaluated the efficacy of anlotinib, alone or in combination with EGFR-TKI, as second- or third-line treatment for patients with EGFR mutation-positive advanced lung adenocarcinoma.

Patients and methods

Study population. Between March 1, 2018 and December 31, 2021, 48 patients were enrolled from the First Affiliated Hospital of Nanjing Medical University. Patients histologically and cytologically diagnosed with advanced lung adenocarcinoma (stage III/IV), who experienced disease progression after EGFR-TKI treatment, were retrospectively screened. Of the 48 patients enrolled, 64.58% of patients were female, median age was 70 years (range, 60-85), and 62.5% of patients had never smoked. All patients enrolled were assessed to be positive for EGFR mutations (exon 19 deletion or exon 21 L858R mutation), Eastern Cooperative Oncology Group (ECOG) performance status (PS) (23) of 0-2, and had measurable disease based on Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (24). After EGFR-TKI resistance, patients were divided into two groups: One group received anlotinib monotherapy and the other received treatment with anlotinib plus EGFR-TKI. Gene mutation status was determined by next-generation sequencing.

Anlotinib and EGFR-TKI regimens. Low-dose anlotinib (Chia Tai Tianqing Pharmaceutical Group Co., Ltd.) was taken orally once daily (6 or 8 mg) on days 1-14 of a 21-day cycle. First-generation EGFR-TKIs, such as gefitinib (250 mg/day) and icotinib (125 mg three times per day), or third-generation EGFR-TKIs, such as osimertinib (80 mg/day) and almonertinib (110 mg/day), were combined with low-dose anlotinib. The choice of different regimens was based on objective factors, such as the regimens formulated by different doctors, the degree of TKI side effects, and the physical and economic conditions of the patient.

Of note, based on clinical trials showing that anlotinib brings survival benefits to patients with NSCLC in third-line or further treatment (22), two patients with adencarcinoma and EGFR mutations chose the trial package of anlotinib for treatment before anlotinib went on the market in May 2018. These two patients were also enrolled in the present study.

Ethics approval and patient consent. The present retrospective study was approved by the Ethics Committee of First Affiliated Hospital of Nanjing Medical University (Nanjing, China, approval no. 2020-SR-279) and was conducted according to the principles of The Declaration of Helsinki as revised in 2013. The present study is a retrospective study without intervention in clinical treatment. The study collected basic information, treatment options, adverse reactions and follow-up information of patients. Written informed consent was obtained from the patients for the collection/analysis of their personal information. If the patient was dead at the time of signing, the consent form was signed by their immediate family. The next of kin of the patient whose images are displayed provided written informed consent for the publication of their data and images.

Genetic sequencing method. The mutation status of ERFR was obtained from NGS next-generation sequencing detection. Tissue samples were fixed with 10% formalin at room temperature for 4 h. DNA was extracted from paraffin-embedded tissue (FFPE) with QIAamp DNA FFPE Tissue kit (Qiagen; cat. no. 56404), and the quality of the DNA was ensured by a NanoDrop DNA analyzer (Thermo Fisher Scientific, Inc.). The length of the sequencing was 150 bp and paired end. The sequencing kit was NovaSeq 6000 S4 Rgt Kit v1.5 (300 cycles; cat. no. 20028312; Illumina Inc.). The loading concentration of the final library was 200-400pM QPCR. The software used for reference sequence alignment, post-alignment processing, and variation detection were BWA v0.7.17 and lofreq v2.1.3a, respectively.

Specific modified primers were used for PCR amplification to accurately identify the target sequence. The PCR cycle was completed by Pfu DNA Polymerase (Promega Corporation). The specific steps included pre-denaturation at 98˚C for 1 min,

denaturation at 98˚C for 10 sec, annealing at 65˚C for 30 sec and extension at 72˚C for 30 sec for 38 cycles then extension at 72˚C for 2 min. The amplified products were purified and enriched by 108 μl magnetic beads (Beckman Coulter, Inc.), followed by DNA fragment repair and terminal modification, and then DNA fragments ligated to form a library for sequencing on the Illumina platform (Illumina, Inc.). Finally, the data software was used to analyze the gene variation information and obtain the genetic test report. The primers for EGFR exon 19 were: Forward 5'-CACTGGGCAGCATGTGGCA-3' and reverse 5'-CAGCTGCCAGACATGAGAA-3', and the primers for EGFR exon 21 were: Forward 5'-ATTCGGATGCAGAGCTTCT-3' and reverse 5'-CTGGTGTCAGGAAAATGCT-3'. The sequencing data are not publicly available to protect patient privacy.

Efficacy and safety evaluation. EGFR-TKI treatment failure was classified into dramatic progression (disease control ≥3 months; rapid increase of tumor burden compared with the previous assessment; symptom score, 2), gradual progression (disease control ≥6 months; minor increase of tumor burden compared with the previous assessment; symptom score f1), and local progression (disease control ≥3 months; isolated extracranial or intracranial progression; symptom score r1) according to the criteria of Yang *et al* (25). The primary endpoint of the present study was PFS, and the secondary endpoints included OS, response rate and toxicity. PFS was defined as the time from the start of treatment to disease progression or the last follow-up, and OS was defined as the time from the start of treatment to death or the last follow-up. Tumor response was evaluated using RECIST 1.1. Objective tumor responses included complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). Objective response rate (ORR) was the sum of CR and PR. Disease control rate (DCR) is the sum of CR, PR and SD. Adverse events (AEs) were assessed according to Common Terminology Criteria for Adverse Events of the National Cancer Institute 4.0 (https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03/Archive/CTCAE_4.0_2009-05-29_QuickReference_8.5x11.pdf).

Statistical analyses. Statistical analysis was performed using SPSS 23.0 (IBM) and GraphPad Prism 8.3.0 (Dotmatics). To assess the between-group differences, clinical characteristics and treatment efficacy were compared using the χ^2 test or the Fisher's exact test when the expected count was ≤5 in >20% of cells in a contingency table. Survival analyses were performed using the Kaplan-Meier method and the survival time was compared using the log-rank test. Both univariate and multivariate analyses were conducted using the Cox proportional hazards model to analyze factors associated with treatment response and survival. Covariates with P<0.05 in univariate analyses were incorporated in the multivariate model constructed using the enter method. P<0.05 was considered to indicate a statistically significant difference.

Results

Patients and clinical characteristics. A total of 48 elderly patients with EGFR mutation-positive advanced lung adenocarcinoma were enrolled after first- or second-line EGFR-TKI treatment failure between March 2018 and December 2021. Patients subsequently received anlotinib or anlotinib combined with EGFR-TKI. Among them, 25 patients received anlotinib plus EGFR-TKI, whereas the remaining 23 patients received anlotinib alone. Baseline demographic and clinical characteristics are listed in Table I, including age, sex, ECOG PS, smoking history, clinical stage, EGFR mutation type, brain metastasis status, EGFR-TKI generation resistance, line of treatment and mode of EGFR-TKI failure. There were 17 men (35.42%) and 31 women (64.58%) aged 60-85 years, and the median age was 70 years. A total of 24 patients (50%) were treated with first-generation EGFR-TKI and the remaining patients were treated with third-generation EGFR-TKI. There was no significant difference in the mode of EGFR-TKI failure between the two groups. Second- and third-line treatments were each given to 50% of patients.

Efficacy outcomes and subgroup analysis. After two cycles/2 months of treatment, the response rate was evaluated. One (4.35%) of the 23 patients in the anlotinib monotherapy group and three (12.00%) of the 25 patients in the combination group experienced PR (P=0.663). A total of 13 (56.52%) of the 23 patients in the anlotinib monotherapy group and 19 (76.00%) of the 25 patients in the combination group experienced DCR (P=0.153). No statistical difference in ORR and DCR between the two groups was observed (Table II).

On December 31, 2021, 42 patients (87.50%) had reached the endpoint of disease progression or death, and the median follow-up time was 14.75 months. The mPFS was 4.0 months [95% confidence interval (CI), 3.38-4.62] in the anlotinib monotherapy group and 6.0 months (95% CI, 4.35-7.65) in the combination group (HR=0.425; 95% CI, 0.224-0.805; Fig. 1A), and the difference was statistically significant (P=0.002). The median OS (mOS) was 28 months (95% CI, 27.13-28.87) in the anlotinib monotherapy group and 32 months (95% CI, 22.04-41.96) in the combination group, and there was no statistically significant difference between the two groups (HR=0.506; 95% CI, 0.132-1.935; P=0.217; Fig. 1B). However, mOS in the combination group had a prolonged trend compared with that in the anlotinib monotherapy group.

At the time of data cutoff, anlotinib combined with EGFR-TKI had a significant benefit on mPFS as second-line treatment compared with as third-line treatment (7.5 vs. 3.7 months; HR=3.477; 95% CI, 1.117-10.820; P=0.031; Fig. 2A), whereas there was no significant difference in mPFS between second- and third-line treatment with anlotinib alone (4.3 vs. 3.65 months; HR=1.477; 95% CI, 0.652-3.346; P=0.323; Fig. 2B). Moreover, the difference between anlotinib combined with first- or third-generation TKIs was statistically compared; the results revealed that anlotinib combined with first-generation TKIs could more significantly prolong the PFS of TKI-resistant patients (8.0 vs. 6.0 months; HR=0.314; 95% CI, 0.113-0.877; P=0.003; Fig. 2C). Further stratification analysis was performed based on EGFR-TKI failure modes. In the combination group, patients with gradual/local progression had longer mPFS than those with dramatic progression (7.5 vs. 6.0 months; HR=5.875; 95% CI, 1.414-10.460; P=0.015; Fig. 3A). In the anlotinib monotherapy group, mPFS was not statistically

Table I. Baseline characteristics of the study population.

Characteristic	Anlotinib (n=23)	Anlotinib + EGFR-TKI (n=25)	χ^2	P-value
Median age, years (range)	69 (63-84)	71 (60-85)		
Sex			0.479	0.489
Male	7 (30.43%)	10 (40.00%)		
Female	16 (69.57%)	15 (60.00%)		
ECOG PS			0.280	0.597
0-1	13 (56.52%)	16 (64.00%)		
2	10 (43.48%)	9 (36.00%)		
Smoking history			0.941	0.332
Yes	7 (30.43%)	11 (44.00%)		
No	16 (69.57)	14 (56.00%)		
Clinical stage			-	0.407[a]
III	4 (17.39%)	2 (8.00%)		
IV	19 (82.61%)	23 (92.00%)		
Brain metastases			0	0.990
Yes	11 (47.83%)	12 (48.00%)		
No	12 (52.17%)	13 (52.00%)		
EGFR mutation type			0.004	0.951
Exon 19 deletion	14 (60.87%)	15 (60.00%)		
L858R	9 (39.13%)	10 (40.00%)		
T790M mutation			0.117	0.732
Yes	9 (39.13%)	11 (44.00%)		
No	14 (60.87%)	14 (56.00%)		
EGFR-TKI generation resistance			0.751	0.386
First-generation	10 (43.48%)	14 (56.00%)		
Third-generation	13 (56.52%)	11 (44.00%)		
Mode of EGFR-TKI failure			0.034	0.853
Gradual/Local progression	16 (69.57%)	18 (72.00%)		
Dramatic progression	7 (30.43%)	7 (28.00%)		
Treatment-line			2.087	0.149
Second-line	9 (39.13%)	15 (60.00%)		
Third-line	14 (60.87%)	10 (40.00%)		

[a]Analyzed using Fisher's exact test. ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

different between patients with gradual/local progression and those with dramatic progression (4.5 vs. 3.7 months; HR=2.124; 95% CI, 0.717-6.294; P=0.063; Fig. 3B).

The factors in subgroup analysis included age, sex, smoking history, tumor stage, ECOG PS, brain metastasis status, EGFR mutation type, EGFR-TKI generation resistance, line of treatment and mode of EGFR-TKI failure. As shown in Fig. 4 (log-rank test), combination therapy could markedly reduce the risk of PD in patients ≥70 years old (HR=0.339; 95% CI, 0.150-0.767; P=0.005), in female patients (HR=0.386; 95% CI, 0.175-0.853; P=0.009), in patients with an ECOG PS of 2 (HR=0.218; 95% CI, 0.072-0.655; P=0.001), in patients without brain metastasis (HR=0.297; 95% CI, 0.115-0.766; P=0.003) and in patients with EGFR L858R mutation (HR=0.281; 95% CI, 0.092-0.851; P=0.002). Significant differences were also detected in subgroups of patients with first-generation EGFR-TKI resistance (HR=0.275; 95% CI, 0.095-0.795; P=0.001) and gradual/local EGFR-TKI progression (HR=0.376; 95%CI, 0.172-0.822; P=0.012).

Exploratory analyses were also performed to determine whether any clinical or pathological features were associated with PFS. In the Cox univariate analysis, EGFR-TKI generation resistance, mode of EGFR-TKI failure, treatment group and line of treatment were associated with PFS (P=0.028, P=0.005, P=0.004 and P=0.005, respectively; Table III). Notably, tumor stage, ECOG PS, EGFR mutation type and brain metastasis status were not found to be associated with any predictive effects. In multivariate analysis, combination therapy of anlotinib and EGFR-TKI was identified as an independent predictor for better PFS as compared with anlotinib

Table II. Efficacy evaluation.

Treatment outcome	Total	Anlotinib (n=23)	Anlotinib + EGFR-TKI (n=25)	χ^2	P-value
CR	0	0	0		
PR	4	1 (4.35%)	3 (12%)		
SD	28	12 (52.17%)	16 (64%)		
PD	16	10 (43.48%)	6 (24%)		
ORR	13	1 (4.35%)	3 (12%)	-	0.610[a]
DCR	32	13 (56.52%)	19 (76%)	2.045	0.153

[a]Analyzed using Fisher's exact test. CR, complete response; DCR (CR + PR +SD), disease control rate; EGFR, epidermal growth factor receptor; ORR (CR + PR), overall response rate; PD, progressive disease; PR, partial response; SD, stable disease; TKI, tyrosine kinase inhibitor.

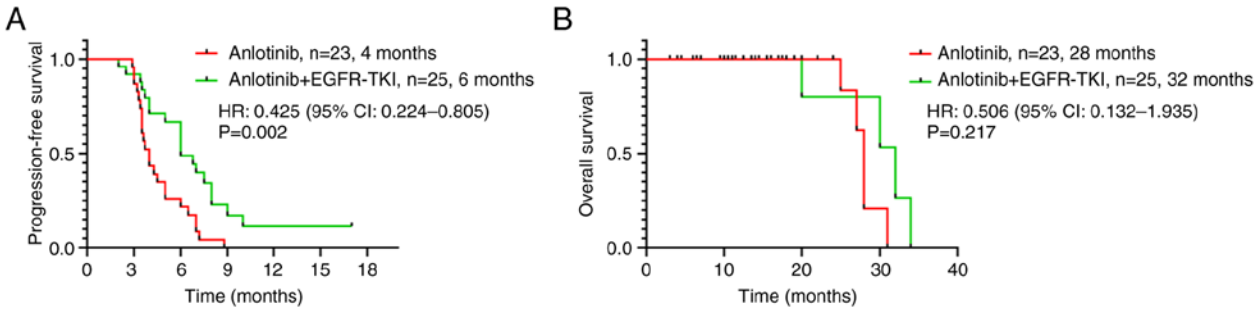

Figure 1. Comparison of mPFS and mOS in TKI-resistant patients treated with anlotinib alone vs. anlotinib in combination with EGFR-TKI. (A) mPFS and (B) mOS were statistically analyzed in the anlotinib monotherapy group and the combination group. CI, confidence interval; EGFR, epidermal growth factor receptor; HR, hazard ratio; mOS, median overall survival; mPFS, median progression-free survival; TKI, tyrosine kinase inhibitor.

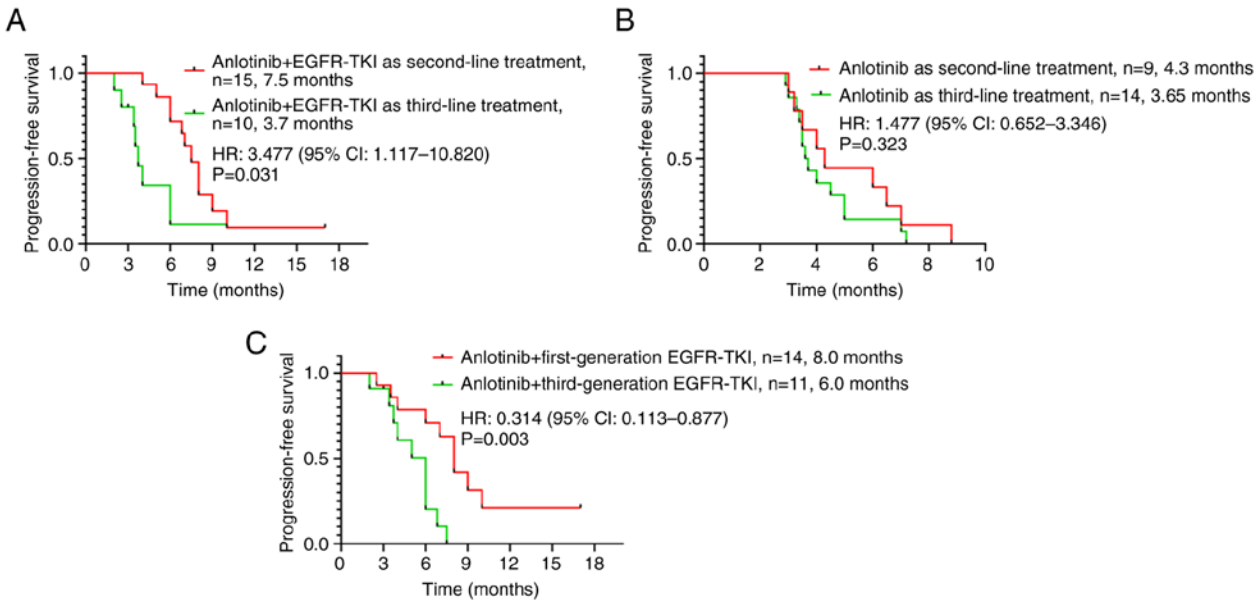

Figure 2. Comparison of anlotinib alone vs. anlotinib in combination with EGFR-TKI in TKI-resistant patients with different treatment lines. (A) mPFS of patients treated with anlotinib combined with EGFR-TKI as second-line or third-line treatment was statistically analyzed. (B) mPFS of patients treated with anlotinib alone as second-line or third-line treatment was statistically analyzed. (C) mPFS of patients treated with anlotinib combined with first-generation or third-generation TKI was statistically analyzed. CI, confidence interval; EGFR, epidermal growth factor receptor; HR, hazard ratio; mPFS, median progression-free survival; TKI, tyrosine kinase inhibitor.

monotherapy (HR=0.438; 95% CI, 0.220-0.871; P=0.019). Dramatic progression was also revealed to be an independent risk factor for poor prognosis (HR=2.637; 95% CI, 1.218-5.706; P=0.014) (Table III).

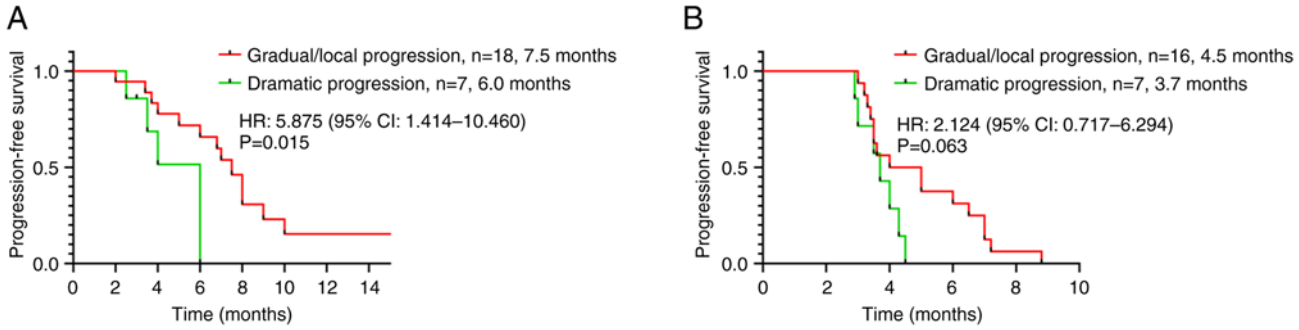

Figure 3. mPFS of patients with gradual/local progression or dramatic progression, and treated with anlotinib combined with TKI or anlotinib alone, was compared. (A) mPFS of patients with gradual/local progression or dramatic progression treated with anlotinib combined with EGFR-TKI was statistically analyzed. (B) mPFS of patients with gradual/local progression or dramatic progression treated with anlotinib monotherapy was statistically analyzed. CI, confidence interval; EGFR, epidermal growth factor receptor; HR, hazard ratio; mPFS, median progression-free survival; TKI, tyrosine kinase inhibitor.

Characteristics	No. of patients	Hazard ratio (95% CI)	P
Overall	48	0.425 (0.224–0.805)	0.002
Age (years)			
<70	19	0.541 (0.188–1.558)	0156
≥70	29	0.339 (0.150–0.767)	0.005
Sex			
Male	17	0.502 (0.169–1.493)	0.128
Female	31	0.386 (0.175–0.853)	0.009
Smoking history			
Yes	18	0.390 (0.122–1.244)	0.078
No	30	0.469 (0.215–1.019)	0.075
Tumor stage			
III	6	0.181 (0.031–1.069)	0.074
IV	42	0.503 (0.256–0.990)	0.075
ECOG PS			
0-1	19	0.656 (0.301–1.428)	0.631
2	29	0.218 (0.072–0.655)	0.001
Brain metastasis			
Yes	23	0.663 (0.279–1.577)	0.574
No	25	0.297 (0.115–0.766)	0.003
EGFR mutation type			
19 del	29	0.579 (0.264–1.268)	0.131
21 L858R	19	0.281 (0.092–0.851)	0.002
EGFR-TKI generation resistance			
First-generation	24	0.275 (0.095–0.795)	0.001
Third-generation	24	0.867 (0.356–2.109)	0.753
Treatment-line			
Second-line	24	0.341 (0.118–0.985)	0.005
Third-line	24	0.809 (0.346–1.890)	0.976
Mode of EGFR-TKI failure			
Gradual/local progression	34	0.376 (0.172–0.822)	0.012
Dramatic progression	14	0.461 (0.147–1.441)	0.277

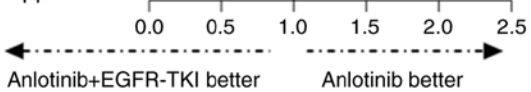

Figure 4. Effects of different factors on the prognosis of patients with TKI treatment failure were analyzed in subgroups, including age, sex, smoking history, tumor stage, ECOG PS, brain metastasis, EGFR mutation type, EGFR-TKI generation resistance, treatment-line and mode. CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

AEs. None of the patients stopped treatment due to severe AEs. During the treatment, two patients (8.69%) in the anlotinib monotherapy group and five patients (20.00%) in the combination group had their anlotinib dose reduced. The patients experienced grade 3 adverse reactions after taking anlotinib, including hypertension, fatigue and mucositis. The

Table III. Univariate and multivariate Cox regression analyses of factors associated with progression-free survival.

	Univariate analysis			Multivariate analysis		
Characteristic	HR	95% CI	P-value	HR	95% CI	P-value
Age (<70 vs. ≥70 years)	0.390	0.406-1.422	0.456			NI
Sex (Male vs. Female)	0.786	0.417-1.480	0.456			NI
Smoking (No vs. Yes)	0.862	0.461-1.611	0.642			
ECOG PS (0-1 vs. 2)	1.064	0.564-2.009	0.848			NI
Tumor stage (III vs. IV)	0.794	0.306-2.063	0.637			NI
Brain metastases (No vs. Yes)	0.849	0.578-1.946	1.061			NI
EGFR mutation type (Exon 19 deletion vs. L858R)	1.169	0.625-2.186	0.624			NI
EGFR-TKI generation resistance (First-generation vs. Third-generation)	2.060	1.083-3.918	0.028[a]	1.269	0.587-2.741	0.545
Mode of EGFR-TKI failure (Gradual/Local progression vs. Dramatic progression)	2.831	1.361-5.891	0.005[a]	2.637	1.218-5.706	0.014[a]
Treatment group (Anlotinib vs. Anlotinib + EGFR-TKI)	0.390	0.205-0.744	0.004[a]	0.438	0.220-0.871	0.019[a]
Treatment-line (Second-line vs. Third-line)	2.465	1.319-4.607	0.005[a]	1.512	0.697-3.283	0.296

ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; HR, hazard ratio; CI, confidence interval; NI, not included in multivariate model. [a]P<0.05.

Table IV. Adverse events.

	Anlotinib group (n=23)		Anlotinib + EGFR-TKI group (n=25)	
Toxicity	All grades (%)	Grade 2	All grades (%)	Grade 2
Hypertension	12 (52.17)	2 (8.70%)	15 (60.00)	2 (8.00%)
Fatigue	10 (43.48)	1 (4.35%)	11 (44.00)	3 (12.00%)
Diarrhea	6 (26.09)	1 (4.35%)	6 (24.00)	0
Mucositis	4 (17.39)	0	4 (16.00)	1 (4.00%)
Hoarseness	2 (8.70)	0	3 (12.00)	0
Rash	7 (30.43)	0	10 (40.00)	0
Bleeding	3 (13.04)	0	3 (12.00)	0
Proteinuria	5 (21.74)	0	6 (24.00)	0
Paronychia	2 (8.70)	0	3 (12.00)	1 (4.00%)
Leukopenia	1 (4.35)	0	1 (4.00)	0
Thrombocytopenia	4 (17.39)	0	3 (12.00)	0
Transaminase elevation	2 (8.70)	0	5 (20.00)	1 (4.00%)

Data are presented as n (%). There were no grade 3/4/5 adverse events.

elderly patients had poor tolerance to adverse reactions, so the dosage of anlotinib was reduced. After the dose reduction, the patient had no adverse reactions of grade 3 or above. No new or unexpected AEs were observed in the present study. The most common AEs included hypertension, fatigue, diarrhea, mucositis, hoarseness, rash, bleeding, proteinuria, paronychia, leukopenia, thrombocytopenia and transaminase elevation (Table IV). AEs of grade 2 were reported in four patients (17.39%) in the anlotinib monotherapy group and eight patients (32.00%) in the combination group. Of these, the most common grade 2 AEs were hypertension, fatigue, diarrhea, paronychia, mucositis and transaminase elevation. There were no grade 3-5 AEs.

Typical case. A 74-year-old woman was pathologically diagnosed with right lower lobe adenocarcinoma with pericardial effusion and bone metastasis in Jiangsu Provincial Hospital of Traditional Chinese Medicine in January 2015 (Fig. 5A and B).

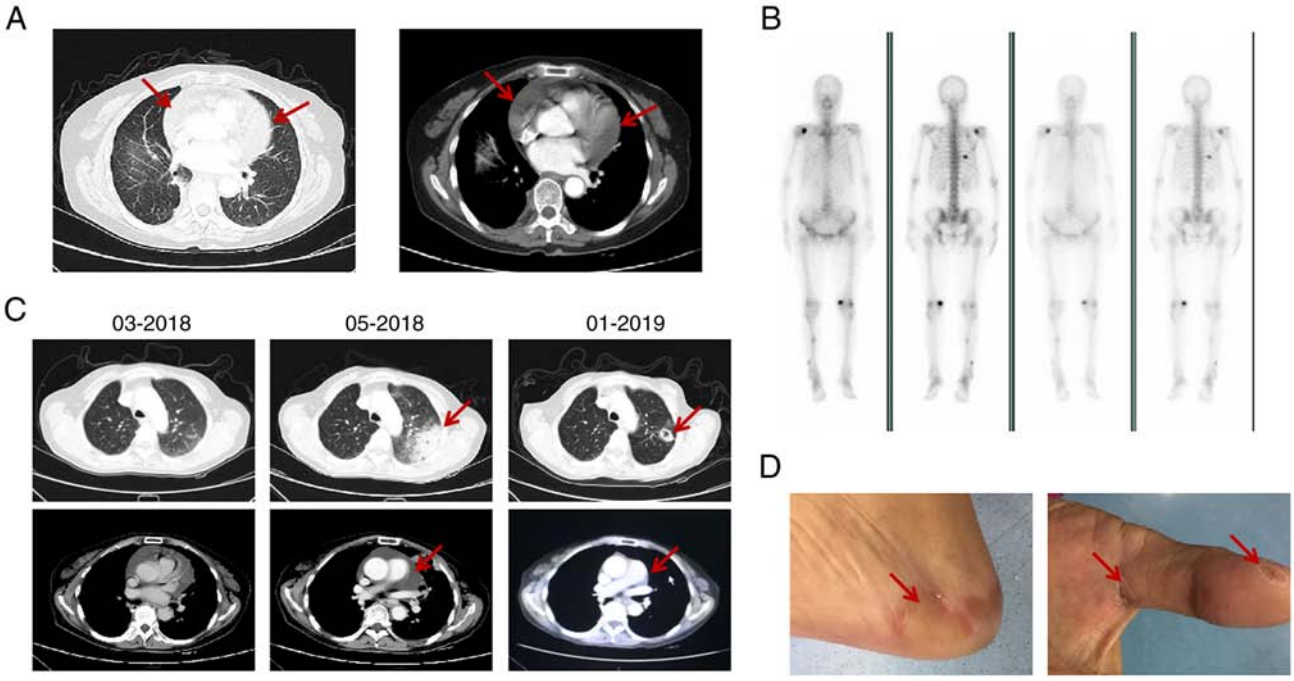

Figure 5. Relevant clinical data during treatment for a typical patient in the enrolled study group. (A) CT showed pericardial effusion following disease progression, as indicated by arrows. (B) CT showed multiple bone metastases. (C) CT showed that anlotinib therapy did not control the tumor well, and subsequently, anlotinib combined with EGFR-TKI therapy significantly reduced the tumor size. (D) Hand-foot syndrome in the course of treatment; the adverse reactions improved after reducing the dose of anlotinib, as shown at arrow. CT, computerized tomography; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

The EGFR L858R mutation was confirmed. From January 2015, icotinib (125 mg, three times daily) was taken orally as first-line treatment, during which hand, foot skin and oral mucosa reactions occurred. In March 2018, computerized tomography (CT) and echocardiography showed enlargement of the lesion and PD was reached, suggesting acquired resistance to icotinib. In addition, genetic testing results again showed that T790M had no mutations.

After she had refused second-line chemotherapy, the patient began oral antitumor therapy with anlotinib in April 2018. A CT scan was performed in May, which showed progression (Fig. 5C). After written informed consent was obtained, the treatment was switched to anlotinib in combination with icotinib. The treatment was well tolerated, and the clinical symptoms, such as decreased physical strength and chest tightness, were gradually relieved. In addition, repeated chest CT showed lesions with cavitation (Fig. 5C) Solid tumor lesions show internal cavitation, which is also a sign of tumor improvement. The dose of anlotinib was reduced during treatment, due to hand and foot syndrome, and was gradually increased to normal after symptom remission (Fig. 5D). Unfortunately, cryptococcal infection developed in February 2019 and the treatment was abandoned, leading to tumor progression. Overall, the PFS of this patient achieved 9 months as a result of the anlotinib combined with EGFR-TKI in the third treatment line.

Discussion

EGFR is a transmembrane receptor tyrosine kinase often upregulated in NSCLC (26). This protein can lead to cell proliferation and survival, and may inhibit apoptosis and activate angiogenesis (9,27). Oral EGFR-TKIs have shown initial clinical efficacy, significantly prolonging PFS in patients with EGFR mutations (4,11); however, drug resistance is the biggest barrier to EGFR-TKI treatment for patients with NSCLC (13). It has been reported that the mechanism underlying EGFR-TKI resistance is complex, with the T790M mutation and mesenchymal-epithelial transition factor amplification being the most common causes, accounting for 50 and 20% of resistance, respectively (13). Although osimertinib has achieved outstanding efficacy in patients with EGFR T790M-mutant NSCLC in terms of PFS and OS (28), most people inevitably develop resistance, which presents another challenge in the treatment of NSCLC. For T790M-negative patients with third- or first-generation TKI resistance, chemotherapy with or without antiangiogenic inhibitors is often used sequentially, but the side effects are severe and the efficacy is unsatisfactory (29). There is an urgent need to find a new treatment mode for elderly patients with cancer who cannot tolerate chemotherapy. The present study suggested that anlotinib combined with TKI may be an effective and tolerable new treatment mode.

Anlotinib is a novel oral multi-targeted TKI, which is characterized by a broad spectrum of inhibitory action on tumor angiogenesis and growth (20). The effect of anlotinib has been revealed in ALTER-0303, a multi-center, double-blind, phase 3 randomized clinical trial (22). The results of this trial showed that anlotinib improved OS compared with a placebo (9.6 vs. 6.3 months; P=0.002), and the primary PFS was longer in the anlotinib group (4.8 months; 95% CI, 3.5-6.4) compared with that in the placebo group (1.2 months, 95% CI, 0.7-1.6);

furthermore, the overall response rate (ORR) was 10.0% and the disease control rate (DCR) was 83.3% in the anlotinib group. Moreover, a subgroup analysis reported that anlotinib improved the survival of patients with adenocarcinoma treated with at least two lines of chemotherapy or TKIs (22). Anlotinib has been approved in China for the third-line treatment of patients with locally advanced or metastatic NSCLC, and is well tolerated, especially in elderly patients (19).

VEGF serves an important role in the formation of new blood vessels (30), and inhibition of VEGF is a key therapeutic strategy for cancer treatment (31). EGFR-TKI resistance is often accompanied by increased levels of VEGF (14), and dual inhibition of EGFR and VEGF in NSCLC with EGFR mutations is theoretically a promising strategy. Several studies have reported long-term clinical benefits from continued use of original EGFR-TKIs and anti-angiogenic inhibitors, such as bevacizumab and apatinib (VEGFR2 inhibitors), after EGFR-TKI resistance (32-35). In addition, preclinical data showed that anlotinib can overcome the acquired EGFR-TKI resistance by inhibiting the FGFR1 signaling pathway, or by downregulating the ERK and AKT signaling pathway (36-38). These data suggested that anlotinib combined with TKIs may be considered a new treatment mode, which provides a basis for the treatment of elderly patients with TKI-resistant NSCLC.

In the present study, the efficacy of anlotinib combined with EGFR-TKI was compared with that of anlotinib monotherapy in elderly patients with acquired EGFR-TKI resistance. Low-dose anlotinib ensured tolerance and compliance in elderly patients. Although there was no statistical difference in ORR and DCR between the two groups, the mPFS in the combination group was longer than that in the anlotinib monotherapy group (6 vs. 4 months, HR=0.425; 95% CI, 0.224-0.805; P=0.002), suggesting that the addition of anlotinib after EGFR-TKI treatment failure can reverse drug resistance to some extent and gain survival benefits. It has recently been reported that anlotinib combined with TKIs or immune checkpoint inhibitors, compared with anlotinib alone, can prolong PFS in elderly patients with lung cancer and EGFR mutations (39). In this study, the research population was not all patients with lung adenocarcinoma, 20% of patients had rare EGFR mutations (not 19Del or L858R mutation), and the combination therapy included anlotinib combined with immune checkpoint inhibitors (4/13 patients); these confounding factors contributed to the bias of the study results (39). However, the present study focused on a population of elderly patients with lung adenocarcinoma, with exon 19 deletion or L858R mutation, and explored the efficacy of anlotinib alone or in combination with TKIs as second- or third-line therapy, which differs from previous studies (39,40). The present study revealed that anlotinib combined with EGFR-TKI can benefit the PFS of TKI-resistant elderly patients with lung cancer; however, this benefit could not be translated into prolonged OS. This finding may be due to the retrospective nature of the study, which failed to achieve randomization of patients, which inevitably had a slight impact on the statistical results.

Further stratification analysis showed that EGFR-TKI combined with anlotinib as second-line treatment had a significant benefit on PFS than as third-line treatment (7.5 vs. 3.7 months; HR=3.477; 95% CI, 1.117-10.820; P=0.031), indicating that early application of EGFR-TKI and anlotinib may lead to better survival. Stratification analysis based on the EGFR-TKI failure mode demonstrated that patients with gradual/local progression were more likely to benefit from this combination strategy than those with dramatic progression (7.5 vs. 6.0 months; HR=5.875; 95% CI, 1.414-10.460; P=0.015), consistent with previous findings (41). As for PFS, multivariate Cox regression suggested that combination therapy with anlotinib and EGFR-TKI (HR=0.438; 95% CI, 0.220-0.871; P=0.019) was considered a protective factor for prognosis, whereas dramatic progression (HR=2.637; 95% CI, 1.218-5.706; P=0.014) had adverse effects on subsequent treatment (Table III).

Subgroup analysis showed that the combination therapy was superior to anlotinib monotherapy for the majority of patients, and was better in young, female, non-smoking patients, and in those without brain metastasis and with gradual/local progression. Significant differences were also observed in subgroups of patients with first-generation EGFR-TKI resistance and third-generation EGFR-TKI resistance, indicating that the combination strategy of EGFR-TKI and anlotinib will be more beneficial to the first-generation EGFR-TKI in improving the PFS of drug-resistant patients. The advantage of combination therapy was more obvious for patients with the EGFR L858R mutation than those with the 19Del mutation, implying a potential mechanism of sensitivity that requires further study. Several clinical trials have reported that bevacizumab or apatinib combined with first-generation EGFR-TKI can be used as a treatment option for TKI resistance in EGFR-mutant lung adenocarcinoma (35,42). Another retrospective study suggested the superiority of osimertinib + bevacizumab over chemotherapy + bevacizumab after the failure of osimertinib (43). The present study suggested that anlotinib combined with EGFR-TKI was more advantageous than anlotinib alone, especially anlotinib combined with first-generation EGFR-TKI. These findings demonstrated that EGFR-TKI combined with anti-tumor angiogenesis drugs, including bevacizumab, apatinib and anlotinib can be effective after EGFR-TKI resistance. In the present study, a novel treatment mode of anlotinib combined with EGFR-TKI was proposed for EGFR-TKI-resistant patients with EGFR-mutant lung adenocarcinoma, especially for elderly patients.

In view of the efficacy of drugs, a number of previous studies (44,45) did not consider the dosage of drugs as the main obstacle to the prognosis of elderly patients with cancer. However, the tolerance of elderly lung cancer patients to anticancer drugs was specifically considered in the present study. The potential for different people to tolerate different drugs may also attract more attention in further studies. Due to the low dose of anlotinib used in the present study, there were no AEs of grade 3 or higher. Elderly patients with lung cancer have a poor constitution and often suffer from a variety of complications, including pulmonary heart disease, chronic obstructive pulmonary disease and atelectasis. In view of this, based on genetic testing, elderly patients are more willing to choose targeted drug therapy (46,47). Anlotinib, as a small-molecule multi-targeted anti-angiogenic drug, also has the same side effects as other targeted drugs, including high blood pressure, fatigue, diarrhea and elevated transaminase. In the present study, low-dose anlotinib was used, which could better control

the AEs of patients, and ensure the persistence and compliance of treatment. With the advent of precision cancer treatment, real-time gene detection is performed throughout lung cancer treatment, which can provide more elderly patients with individualized treatment. TKI resistance remains an issue to be solved, and it is imperative to understand the mechanism of drug resistance and seek new treatment options.

In conclusion, low-dose anlotinib in combination with EGFR-TKI provides an effective and well-tolerated treatment mode for elderly patients with TKI-resistant EGFR-mutant NSCLC, with significant improvements in disease burden and time to progression.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

RG conceived the idea for the study and supervised it. YiC and NJ were responsible for designing and writing the manuscript. XL and NC performed the collection and analysis of clinical data. YuC and CZ were responsible for interpretation of data and revising the manuscript critically for important intellectual content. JS performed data interpretation, editing the manuscript and guaranteed the integrity of the study. YiC and RG confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present retrospective study was approved by the Ethics Committee of First Affiliated Hospital of Nanjing Medical University (Nanjing, China; Approval no. 2020-SR-279) and was conducted according to the principles of The Declaration of Helsinki as revised in 2013. Written informed consent was obtained from the patients for the collection/analysis of their personal information. If patients had succumbed at the time of signing, the consent form was signed by their immediate family.

Patient consent for publication

The next of kin of the patient whose images are displayed provided written informed consent for the publication of their data and images.

Competing interests

The authors declare that they have no competing interests.

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