

Efficacy and Safety of Anlotinib-Containing Regimens in Advanced Non-Small Cell Lung Cancer: A Real-World Study

Lei Sun^{1,*}, Qi Zhao^{2,*}, Yanning Wang^{3,*}, Yongsheng Wang², Ming Zheng², Xuansheng Ding⁴, Liyun Miao²

¹School of Basic Medicine and Clinical Pharmacy, China Pharmaceutical University Nanjing Drum Tower Hospital, Nanjing, People's Republic of China; ²Department of Respiratory and Critical Care Medicine, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, People's Republic of China; ³Clinical Stem Cell Center, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, People's Republic of China; ⁴School of Basic Medicine and Clinical Pharmacy, China Pharmaceutical University, Nanjing, People's Republic of China

*These authors contributed equally to this work

Correspondence: Liyun Miao, Department of Respiratory and Critical Care Medicine, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, 210008, People's Republic of China, Tel +86 13813920964, Fax +86-25-68183333, Email liyunmiao462@163.com

Purpose: Anlotinib is widely used in the clinical treatment of non-small cell lung cancer (NSCLC), alone or in combination with other anticancer drugs. The aim of this study was to investigate the real-world efficacy and safety of anlotinib-containing regimens.

Patients and Methods: Confirmed advanced NSCLC patients who had received anlotinib alone or in combination were enrolled. An overall analysis of the efficacy and safety of anlotinib was performed in all patients, and then subgroup analysis was used to further compare the efficacy between anlotinib monotherapy and combination therapy. The primary endpoint was progression-free survival (PFS), and the secondary endpoints were ADR, ORR, and DCR.

Results: A total of 240 patients were included. The overall median PFS was 8.5 months (95% confidence interval [CI]: 7.1–9.9 months). Anlotinib treatment regimens (monotherapy or combination therapy) and whether they received previous antiangiogenesis were associated with PFS. Anlotinib plus immunotherapy achieved longer PFS than anlotinib monotherapy (median PFS: 10.5 vs 6.5 months, $p=0.007$). Stratification analysis showed the PFS of anlotinib plus immunotherapy was significantly longer in male, adenocarcinoma, ≤ 65 years old, patients stage IV, EGFR wild type, with extrathoracic metastasis, performance status scores ≥ 2 , the first-line treatment, patients with a history of hypertension and no previous antiangiogenesis than anlotinib monotherapy. The median PFS of anlotinib plus chemotherapy, targeted therapy was slightly longer than anlotinib alone (respectively, 10.5 vs 6.5 months, $p=0.095$; 9.5 vs 6.5 months, $p=0.177$). Adverse reactions were mostly mild and acceptable, with hypertension being the most common.

Conclusion: Anlotinib is effective and tolerable in advanced NSCLC patients. Immunotherapy combination with anlotinib significantly improved PFS. The efficacy of anlotinib may be impaired by previous antiangiogenic therapy, which can be investigated in further studies.

Keywords: non-small cell lung cancer, anlotinib, combination therapy, immunotherapy, efficacy, safety

Introduction

Over the last decade, lung cancer has been the leading cause of cancer-related death among all malignancies. Lung cancer caused estimated 2.2 million new cases (11.7%) and 1.8 million deaths (18%) in 2020, as the second most commonly diagnosed cancer and the highest cancer-related mortality worldwide.¹ Non-small cell lung cancer (NSCLC) accounts for approximately 83% of lung cancer cases and tends to be diagnosed at advanced stages, causing a huge mortality burden.²

The processes of angiogenesis play crucial roles in cancer development, including NSCLC.^{3,4} Antiangiogenic agents, such as multi-target tyrosine kinase inhibitors (TKIs), bevacizumab (Avastin) and recombinant human endostatin

injection (endostar), can achieve therapeutic benefit in a wide range of solid tumors.⁵ Anlotinib, a novel TKI that inhibits tumor angiogenesis and proliferation, was approved for monotherapy as a third and further-line treatment for advanced or metastatic NSCLC in 2018.^{6–8} Anlotinib monotherapy in third and further-line NSCLC treatment showed significantly longer progression-free survival (PFS) (median PFS: 5.4 vs 1.4 months) than patients receiving placebo^{9,10} At the same time, with the rise of the concept of combination therapy in the field of tumors, anlotinib is being used more widely in clinical practice. Preclinical researches and case reports demonstrated that anlotinib may modulate the tumor micro-environment and act synergistically with immune checkpoint inhibitors.^{11–17} Current studies have shown encouraging efficacy of the clinical use of anlotinib combination therapy, and a case reported first-line pemetrexed and carboplatin plus anlotinib achieved CR.^{18–22} However, the sample size of current studies is small and more studies are still needed to confirm the efficacy and safety of anlotinib combination therapy in different patients with NSCLC.

Anlotinib is widely used in the field of NSCLC, including different treatment regimens and treatment timing, and there is still a lack of comprehensive and systematic description and analysis. Therefore, we conducted this retrospective study to describe the status, efficacy and safety of anlotinib in real-world clinical applications, and conducted subgroup analysis to compare the efficacy of anlotinib combination with immunotherapy/chemotherapy/targeted therapy and anlotinib alone. We performed comprehensive adjusted analysis and stratified analysis to explore factors associated with PFS and explore what kind of patients will benefit from anlotinib combination therapy.

Materials and Methods

Patients

Patients who visited the Department of Respiratory and Critical Care Medicine of Nanjing Drum Tower Hospital between June 2019 and March 2022 were retrospectively collected. The inclusion criteria were as follows: pathologically confirmed advanced NSCLC; with at least one measurable lesion; treated with anlotinib or anlotinib contained regimens. Patients who received anlotinib within two cycles or failed to obtain follow-up information were excluded. The clinical information system (Zesing software) was used to collect baseline characteristics, including gender, age, Eastern Cooperative Oncology Group performance status (ECOG PS) score, pathological type, stage, sites of metastasis, oncogenic driver mutations, history of hypertension, prior and subsequent treatment regimens.

Treatment

Anlotinib (Chia Tai Tianqing Pharmaceutical, China) was orally administered (12mg/day for 2 weeks on and 1 week off) in all patients, and the administration was adjusted by clinicians according to the patient's response during treatment. The combination regimens of anlotinib included the combination of immunotherapy drugs (programmed death-ligand 1 and programmed cell death protein 1 inhibitors intravenously administered: pembrolizumab [200mg] or sintilimab [200mg] or atezolizumab [1200mg] or camrelizumab [200mg] or tislelizumab [200mg] on day 1 of a 3-week cycle, nivolumab 3mg/kg or 240mg on day 1 of a 2-week cycle), combination of conventional chemotherapy drugs intravenously administered (pemetrexed [500mg/m²] or docetaxel [75mg/m²] on day 1 or gemcitabine [1250mg/m²] on day 1 and day 8 of a 21-day cycle), and combination of targeted therapy drugs orally administered, including epidermal growth factor receptor (EGFR) inhibitors, and anaplastic lymphoma kinase (ALK) inhibitors (gefitinib once daily at 250mg/day, erlotinib once daily at 150mg/day, osimertinib once daily at 80mg/day, crizotinib twice daily at 250mg/once). Previous antiangiogenic therapy included bevacizumab (Avastin) 400mg or 500mg, d1 or recombinant human endostatin injection (endostar) 30mg, d1-d7.

Efficacy and Safety

Therapeutic response was assessed based on Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 every 2 cycles, defined as complete response (CR), partial response (PR), stable disease (SD) and progression disease (PD). PFS was defined as the time firstly received anlotinib to the date of disease progression. OS was defined as the time anlotinib beginning to death from any cause. The safety of the treatment was evaluated by the occurrence of adverse events, and the severity of the adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE), version 5.0.

Statistical Analysis

The general analysis of efficacy and safety of anlotinib in all patients with advanced NSCLC were performed at first. The survival curves for OS and PFS were estimated with the Kaplan–Meier method and were compared between two groups using the Log rank test. Cox regression was used to analyze the statistically significant factors associated with PFS. Factors with p value less than 0.05 in univariate analysis were included in multivariate cox regression analysis. Adjusted or stratified analysis was conducted to reduce the risk of bias. Baseline characteristics, ORR and DCR between two groups were compared using chi-square test or Fisher's exact test when appropriate. All statistical tests were carried out on the basis of a 2-sided $\alpha=0.05$ and 95% confidence interval (CI). All analyses were performed using SPSS 25.0 software (IBM, Armonk, NY, USA) and GraphPad Prism 9.0.0 software (La Jolla, CA, USA).

Ethical Approval and Informed Consent

The study was approved by Medical Ethics Committee of Nanjing Drum Tower Hospital Affiliated to Nanjing University School of Medicine (GYHT20200023) and conducted according to the principles of the Declaration of Helsinki. Written consent was waived due to the retrospective nature of the review. We confirmed that the data was maintained with confidentiality.

Results

Baseline Characteristics of All Patients

Total 240 patients were included in our study, with a median age of 67 years (range, 33 to 91 years). There were 184(76.6%) males and 56(23.3%) females. While 63(26.2%) patients staged III, 177(73.7%) patients staged IV. Pathological types included adenocarcinoma (128, 53.3%) squamous cell carcinoma (91, 37.9%), and other type NSCLC (21, 8.7%). A total of 104 patients had extrathoracic metastases, including brain metastases (29, 12.0%), bone metastases (59, 24.5%), liver metastases (17, 7.0%) and kidney metastases (7, 2.9%). Fifty-two (21.6%) patients were diagnosed with EGFR mutation, and ALK rearrangement in 4/240 (1.6%) patients, ROS1 mutation in 2(0.8%), KRAS in 2(0.8%) and met14 mutation in 3(1.2%) patient. While 57 (23.7%) patients received anlotinib in first-line treatment, 76(31.6%) received anlotinib in second-line treatment, and 107(44.6%) patients received anlotinib in third or further-line treatment. Among all 240 patients, 82(34.2%) patients received anlotinib monotherapy and 158(65.8%) patients received anlotinib combination therapy, including anlotinib plus immunotherapy (74 cases)/chemotherapy (42 cases)/targeted therapy (33 cases) and anlotinib plus immune-chemotherapy (9cases). The characteristics information is shown in [Supplementary Table 1](#).

Overall Efficacy of Treatment

Of the 240 patients, CR was not achieved, PR was achieved in 58(24.1%) patients, SD was achieved in 147(61.2%) patients, and PD in 35(14.5%) patients. The ORR was 24.1% and DCR was 85.4%. The average follow-up duration was 10.7 months, and by the end of follow-up, 18 patients were declared dead. The overall median PFS was 8.5 months (95% CI: 7.1–9.9m) and the median OS was undefined ([Figure 1](#)). Univariate analysis revealed patients received anlotinib combination therapy, no previous antiangiogenic therapy and had hypertension history had significantly longer mPFS ([Figure 2A–C](#) and [Supplementary Table 1](#)). After adjusted for sex, age, histology, stage, ps scores, treatment line (first, second, third and further-line), EGFR gene state, previous antiangiogenic therapy (yes vs no HR:1.956, 95% CI: 1.257–3.044, $p=0.003$) and therapy regimen (combination vs monotherapy HR:0.675, 95% CI: 0.463–0.984, $p=0.041$) were associated with PFS ([Table 1](#)). The different combination treatment modalities (plus immunotherapy/chemotherapy/targeted therapy) were analyzed, which showed immunotherapy combination with anlotinib significantly improved PFS (after adjusted, HR:0.619, 95% CI:0.383–1.000, $P=0.05$), anlotinib plus chemotherapy and plus targeted therapy had a tendency to prolong survival, respectively ([Figure 2D](#) and [Supplementary Table 2](#)).

Anlotinib Combination Therapy vs Monotherapy

Baseline characteristics were comparable between the anlotinib combination therapy (n=158) with anlotinib alone (n=82) group ([Table 2](#)).

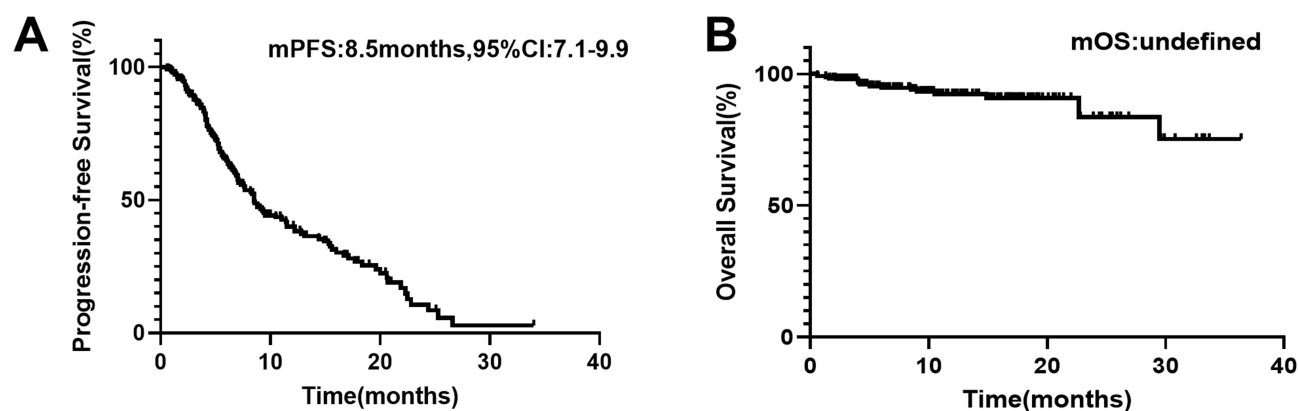


Figure 1 Kaplan-Meier curves of PFS and OS in all patients. (A) PFS: progression-free survival; (B) OS: overall survival.

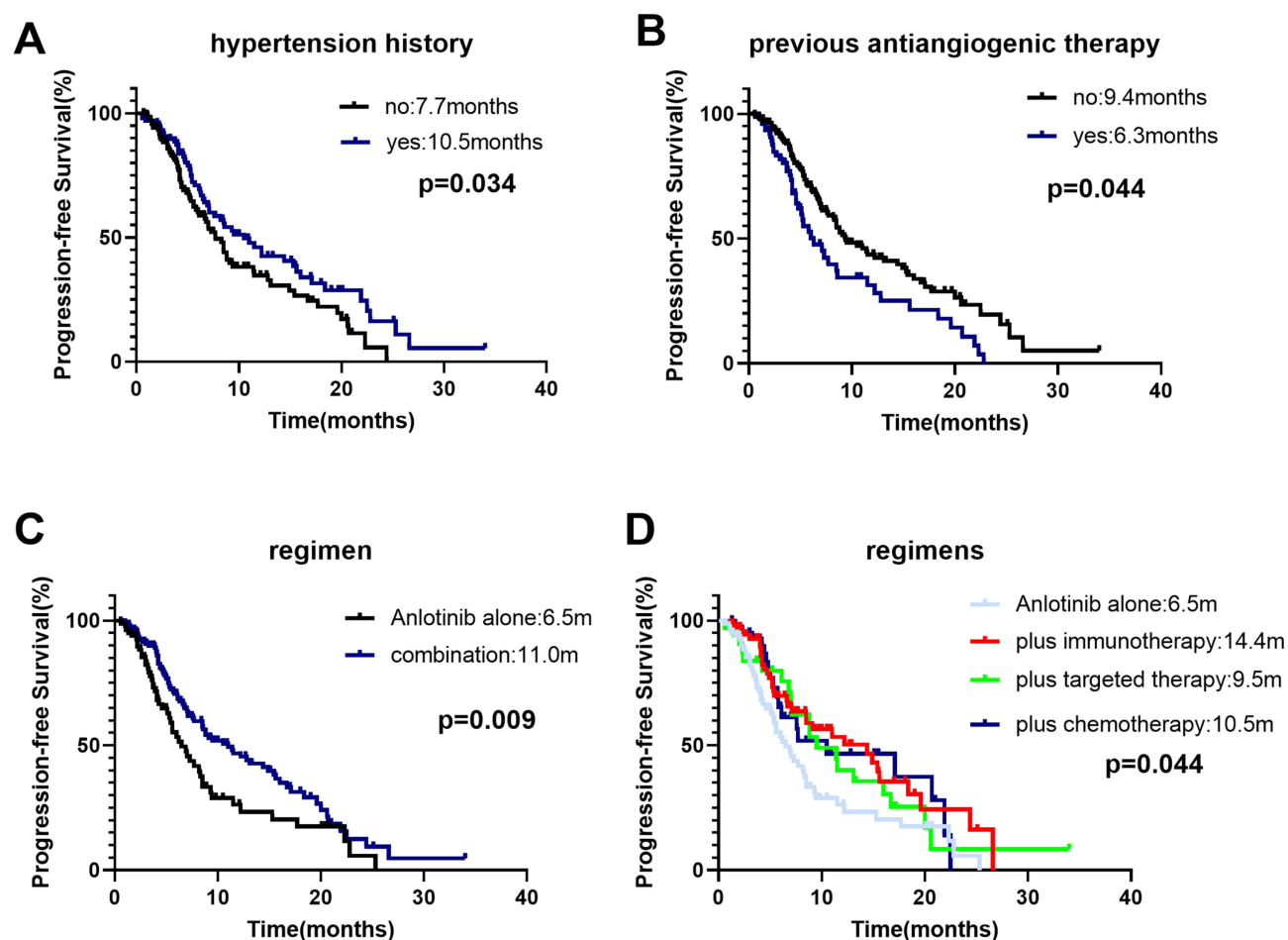


Figure 2 Univariate analysis of PFS in all patients. (A) Stratified by hypertension history; (B) stratified by previous antiangiogenesis; (C) stratified by therapy regimen (monotherapy vs combination therapy); (D) stratified by regimens (anlotinib alone, plus immunotherapy, plus chemotherapy, plus targeted therapy).

Efficacy of Anlotinib Combined with Immunotherapy

The basic characteristics were comparable between anlotinib combination and monotherapy group, except that patient EGFR-mutation status (Table 3). Patients receiving anlotinib plus immunotherapy ($n=74$) had a significantly longer median PFS than those receiving anlotinib alone ($n=82$), (14.4 vs 6.5 months; HR: 0.543, 95% CI: 0.348–0.846; $p=0.007$) (Figure 3A). The anlotinib alone group and plus immunotherapy group had similar ORR (14.6% vs 24.3%) and DCR (79.3% vs 90.5%) (Table 4). The median PFS was significantly longer with anlotinib plus immunotherapy than

Table 1 Cox Regression Analysis of Factors Associated with PFS in All Patients

Factors	Subgroups	Unadjusted		Adjusted ^a		Adjusted ^b	
		HR	95% CI	HR	95% CI	HR	95% CI
Regimen	Combination vs monotherapy	0.662	0.461–0.951	0.686	0.475–0.99	0.675	0.463–0.984
Previous antiangiogenic therapy	Yes vs no	1.698	1.172–2.459	1.967	1.276–3.032	1.956	1.257–3.044
Hypertension history	Yes vs no	0.68	0.474–0.975	0.673	0.467–0.969	0.688	0.469–1.009

Notes: Factors with p value less than 0.05 in univariate analysis were included in cox regression analysis. ^aAdjusted for histology, stage, ps scores, treatment line (first, second, third and further-line), EGFR gene state; ^bAdjusted for sex, age, histology, stage, ps scores, treatment line (first, second, third and further-line), EGFR gene state; bold font showed HR 95% CI range with statistical significance (p value less than 0.05).

Table 2 Comparison of Baseline Characteristics Between Anlotinib Monotherapy and Combination Therapy

Basic Characteristics	Monotherapy n=82(%)	Combination Therapy n=158(%)	P value
Age			
<=65	32(39.0)	69(43.7)	0.489
>65	50(61.0)	89(56.3)	
Sex			
Male	63(76.8)	121(76.6)	0.966
Female	19(23.2)	37(23.4)	
Histology			
Adenocarcinoma	41 (50.0)	87(55.1)	0.748
Squamous	33(40.2)	58(36.7)	
Other	8(9.8)	13(8.2)	
Clinical stage			
III	20(24.4)	43(27.2)	0.637
IV	62(75.6)	115(72.8)	
PS			
<=1	70(85.4)	142 (89.9)	0.302
≥2	12(14.6)	16(10.1)	
EGFR mutation			
Wild type	64(78.0)	124(78.5)	0.939
Mutant type	18(22.0)	34(21.5)	
Treatment line			
First-line	20(24.4)	37(23.4)	0.983
Second-line	26(31.7)	50(31.6)	
Third and further-line	36(43.9)	71(44.9)	
Tumor extrathoracic metastasis			
No	46(56.1)	90(57.0)	0.748
Metastasis	36(43.9)	68(43.0)	
Brain metastases			
No	72(87.8)	139(88.0)	0.969
Yes	10(12.2)	19(12.0)	
Bone metastases			
No	58(70.7)	123(77.8)	0.225
Yes	24(29.3)	35(22.2)	
Liver metastases			
No	76(92.7)	147(93.0)	0.919
Yes	6(9.3)	11(7.0)	

(Continued)

Table 2 (Continued).

Basic Characteristics	Monotherapy n=82(%)	Combination Therapy n=158(%)	P value
Kidney metastases			
No	80(97.6)	153(96.8)	1.000
Yes	2(2.4)	5(3.2)	
Previous antiangiogenesis			
No	53(64.6)	111(70.3)	0.375
Yes	29(35.4)	47(29.7)	
Hypertension history			
No	53(64.6)	88(55.7)	0.182
Yes	29(35.4)	70(44.3)	

Table 3 Comparison of Basic Characteristics of Patients Receiving Anlotinib Alone and Plus Immunotherapy, Chemotherapy, Targeted Therapy

Basic Characteristics	Anlotinib Alone n=82(%)	Plus Immunotherapy n=74(%)	p ^a	Plus Chemotherapy n=42	p ^b	Plus Targeted n=33	p ^c
Age							
≤65	32(39.0)	29(39.2)	0.983	18(42.9)	0.681	16(48.5)	0.352
>65	50(61.0)	45(60.8)		24(57.1)		17(51.5)	
Sex							
Male	63(76.8)	64(86.5)	0.122	37(88.1)	0.133	14(42.4)	0.000
Female	19(23.2)	10(13.5)		5(11.9)		19(57.6)	
Histology							
Adenocarcinoma	41 (50.0)	29(39.2)	0.355	21(50.0)	0.926	30(90.9)	0.000
Squamous	33(40.2)	38(51.4)		16(38.1)		3(9.1)	
Other	8(9.8)	7(9.5)		5(11.9)		0(0.0)	
Clinical stage							
III	20(24.4)	21(28.4)	0.572	7(23.3)	0.291	5(15.2)	0.277
IV	62(75.6)	53(71.6)		23(76.7)		28(84.8)	
PS							
≤1	70(85.4)	59(79.7)	0.353	41(97.6)	0.035	33(100)	0.020
≥2	12(14.6)	15(20.3)		1(2.3)		0(0.0)	
EGFR mutation							
Wild type	64(78.0)	70(94.6)	0.003	39(92.9)	0.037	8(24.2)	0.000
Mutant type	18(22.0)	4(5.4)		3(8.1)		25(75.8)	
Treat line							
Naive-treat	20(24.4)	19(25.7)	0.703	8(19.0)	0.501	9(27.3)	0.747
Second or later line	62(67.4)	45(74.3)		34(81.0)		24(72.7)	
Number of extrathoracic metastasis sites							
0	46(56.1)	41(55.4)	0.931	27(64.3)	0.381	14(42.4)	0.184
≥1	36(43.9)	33(44.6)		15(35.7)		19(57.6)	
Previous antiangiogenesis							
No	53(64.6)	53(71.6)	0.350	23(54.8)	0.285	29(87.9)	0.013
Yes	29(35.4)	21(28.4)		19(45.2)		4(12.1)	
Hypertension history							
No	53(64.4)	40(54.1)	0.179	22(52.4)	0.187	22(66.7)	0.836
Yes	29(35.4)	34(45.9)		20(47.6)		11(33.3)	

Notes: ^a(p value) comparison of basic characteristics of patients receiving anlotinib alone and plus immunotherapy; ^bComparison of basic characteristics of patients receiving anlotinib alone and plus chemotherapy; ^cComparison of basic characteristics of patients receiving anlotinib alone and plus targeted therapy; bold font showed p value less than 0.05.

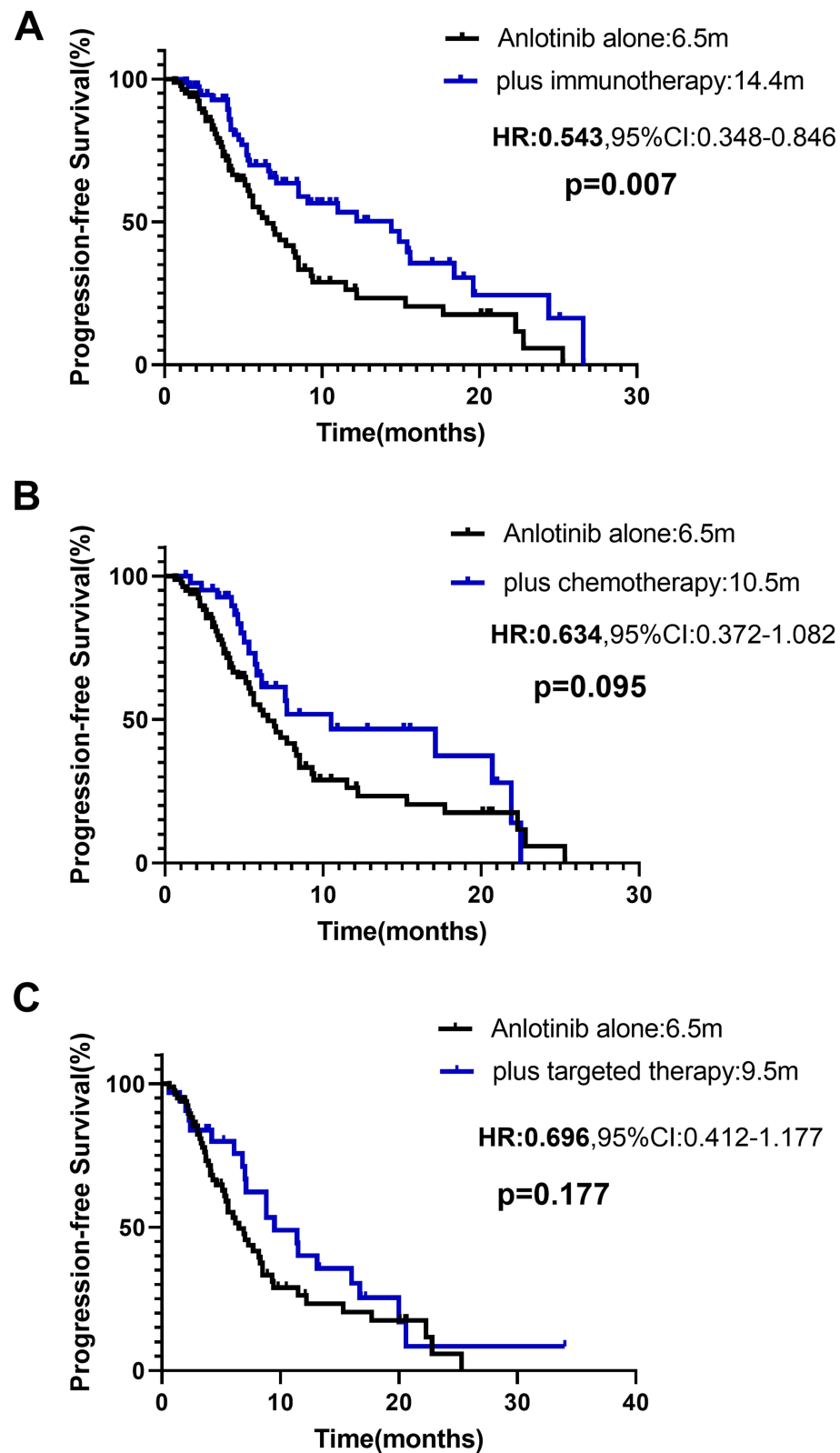


Figure 3 Kaplan-Meier curves of PFS between different combination regimens and anlotinib monotherapy. (A) Anlotinib alone and plus immunotherapy; (B) anlotinib alone and plus chemotherapy; (C) anlotinib alone and plus targeted-therapy.

Table 4 Response of All Patients and Subgroup Anlotinib Alone versus Combination Regimen

Efficacy	Total	Anlotinib Alone (n=82)	Plus Immunotherapy (n=74)	p ^a	Plus Chemotherapy (n=42)	p ^b	Plus Targeted Therapy (n=33)	p ^c
CR	0	0	0		0		0	
PR	58	12	18		13		11	
SD	147	53	49		24		17	
PD	35	17	7		5		5	
ORR (%)	58(24.1)	12(14.6)	18(24.3)	0.125	13(31.0)	0.032	11(33.3)	0.023
DCR (%)	205(85.4)	65(79.3)	67(90.5)	0.051	37(88.1)	0.223	28(84.8)	0.491

Notes: ^ap value between group anlotinib alone and group anlotinib plus immunotherapy; ^bp value between anlotinib alone and anlotinib plus chemotherapy; ^cp value between anlotinib alone and anlotinib plus targeted therapy; bold font showed p value less than 0.05.

with anlotinib alone in male, adenocarcinoma, ≤ 65 years old, patients stage IV, EGFR wild type, with or without extrathoracic metastasis, ps scores ≥ 2 , the first-line treatment, patients with a history of hypertension and no previous antiangiogenesis (Figure 4 and Table 5). Forest plot of the stratification analysis is shown in Figure 5A

Efficacy of Anlotinib Combined with Chemotherapy

Patients receiving anlotinib plus chemotherapy (n=42) had a longer median PFS than those receiving anlotinib alone (n=82); however, the difference was not statistically significant (10.5 vs 6.5 months; HR: 0.634, 95% CI: 0.372–1.082; p=0.095) (Figure 3B). The ORR of anlotinib plus chemotherapy was higher than anlotinib monotherapy (31.0% vs 14.6%, p=0.032) (Table 4). The basic characteristics were well balanced in two groups, except that patient EGFR-mutation status and ps scores (Table 3). Median PFS was significantly longer in patients ≤ 65 years (anlotinib plus chemotherapy vs anlotinib alone: 20.7 vs 3.7m, p=0.026) and first-line treatment (17.1 vs 6.9m, p=0.036). Although no statistically significant differences, median PFS of anlotinib plus chemotherapy tended to be longer in patients with adenocarcinoma, EGFR wild type, patients with a history of hypertension (Supplementary Table 3). Figure 5B present forest plot of the stratification analysis.

Efficacy of Anlotinib Combined with Targeted Therapy

Patients receiving anlotinib plus targeted therapy (n=33) had a slightly longer median PFS than those receiving anlotinib alone (9.5 vs 6.5 m; HR: 0.696, 95% CI: 0.412–1.177; p=0.177) (Figure 3C). The ORR of anlotinib plus targeted therapy was higher than monotherapy (33.3% vs 14.6%, p=0.023 (Table 4)). The basic characteristics were comparable between two groups, except patient histological type and status of EGFR mutation, sex and ps scores, which caused clinical differences in the choice of anlotinib therapy regimens (Table 3). Although not statistically significant, median PFS in patients receiving anlotinib plus targeted therapy tended to be longer in patients with adenocarcinoma, stage IV, ps scores ≤ 1 , first-line or second and further-line treatment, no previous antiangiogenic therapy, with EGFR mutation (Supplementary Table 4). Figure 5C present forest plot of the stratification analysis.

Safety Analysis

Anlotinib-related adverse reactions during the treatments with anlotinib alone or anlotinib combination therapy were well tolerable and there were no treatment-related deaths. The five most common adverse events were hypertension (64, 26.7%), fatigue (25, 10.4%), hand-foot syndrome (25, 10.4%), hemorrhinia (20, 8.3%) and cough (20, 8.3%), diarrhea (16, 6.7%), rash (16, 6.7%), oral mucositis (13, 5.4%). Grade 3–5 adverse events occurred in 34(14.2%) patients, including hypertension (21, 8.8%), hand-foot syndrome (6, 2.5%), hypothyroidism (3, 1.3%), hemorrhinia (2, 0.8%) and oral mucositis (1, 0.4%) (Table 6).

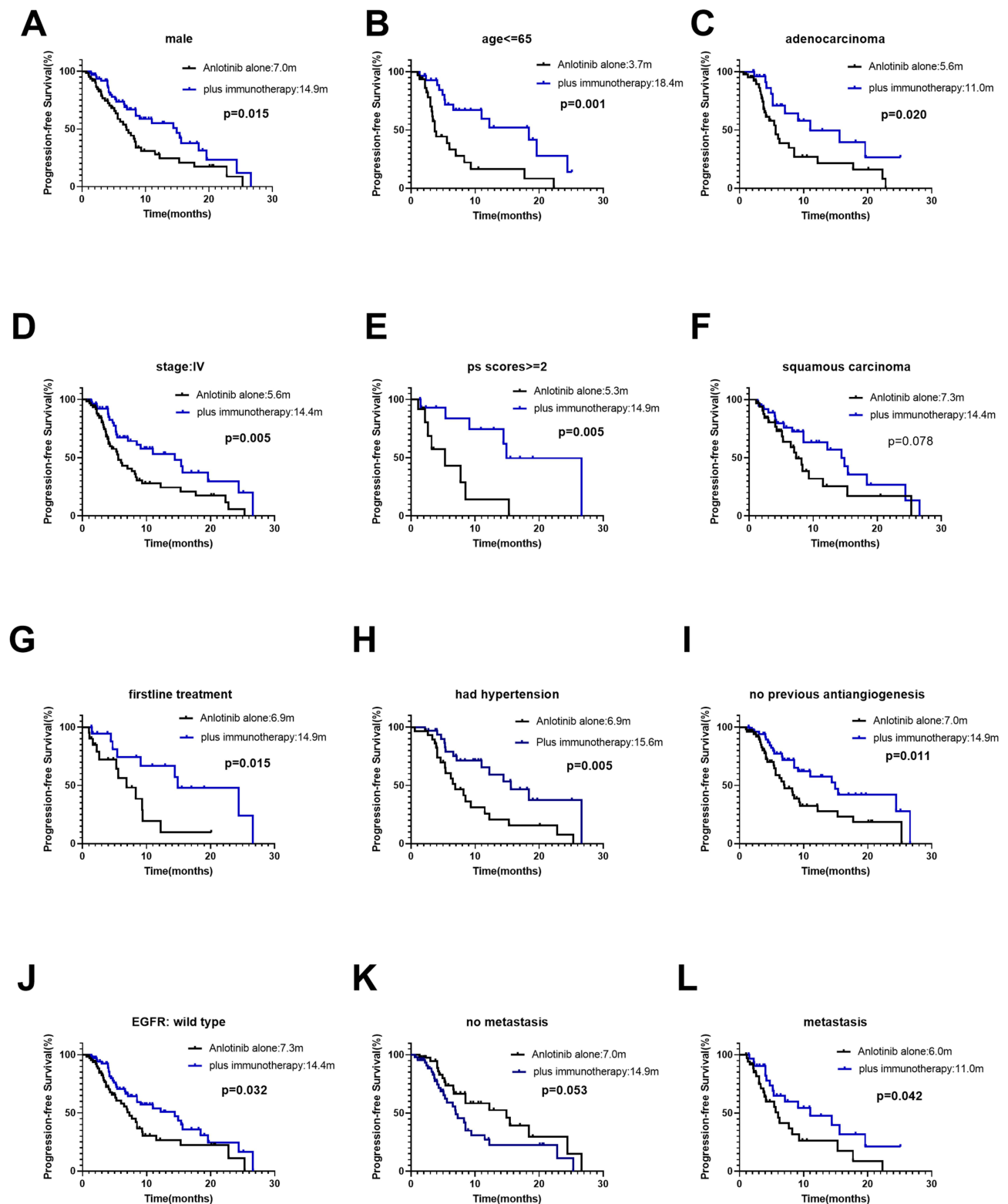


Figure 4 Stratification analysis of PFS between patients receiving anlotinib alone and plus immunotherapy. (A) stratified by sex: male; (B) stratified by age: ≤ 65 years old; (C) stratified by histological type: adenocarcinoma; (D) stratified by stage: IV; (E) stratified by performance state score: $ps \geq 2$ (F) squamous carcinoma (G) stratified by treatment line: first-line treatment; (H) hypertension history: had hypertension; (I) stratified by previous antiangiogenic therapy: no previous antiangiogenic therapy; (J) EGFR wild type; (K) stratified by tumor metastasis: no metastasis; (L) had metastasis.

Table 5 Stratification Analysis of PFS Between Patients Receiving Anlotinib Alone and Plus Immunotherapy

	Total N= 156	mPFS: Months (95% CI)		P value	HR (95% CI)
		Anlotinib Alone	Plus Immunotherapy		
Age					
<=65	61	3.7(3.0–4.4)	18.4(7.5–29.3)	0.001	0.299(0.145–0.618)
>65	95	7.7(6.1–9.3)	9.1(1.6–16.5)	0.295	0.738(0.415–1.311)
Sex					
Male	127	7.0(5.0–9.0)	14.9(8.4–21.4)	0.015	0.522(0.338–0.901)
Female	29	5.4(3.2–7.6)	5.3(5.0–5.6)	0.375	0.619(0.211–1.816)
Histology					
Adenocarcinoma	70	5.6(4.0–7.2)	11.0(2.2–19.8)	0.020	0.442(0.218–0.898)
Squamous	71	7.3(5.0–9.5)	14.4(10.2–18.5)	0.078	0.556(0.287–1.078)
Other	15	6.9(0–15.6)	4.7(3.6–5.8)	0.686	1.326(0.339–5.191)
Clinical stage					
III	41	8.5(6.2–10.8)	12.2(4.3–20.1)	0.543	0.754(0.302–1.885)
IV	115	5.6(4.5–6.7)	14.4(6.9–21.9)	0.005	0.486(0.29–0.814)
PS					
<=1	129	6.9(5.3–8.5)	11.0(5.7–16.3)	0.119	0.683(0.421–1.109)
≥2	27	5.3(0.3–10.3)	14.9(8.4–21.4)	0.005	0.211(0.066–0.675)
EGFR mutation					
Wild type	134	7.3(5.3–9.3)	14.4(7.9–20.9)	0.032	0.594(0.367–0.963)
Mutant type	22	5.6(3.9–7.2)	5.2	0.555	0.545(0.069–4.325)
Treat line					
Naive-treat	39	6.9(2.9–10.9)	14.9(9.6–20.2)	0.015	0.315(0.119–0.831)
Second or later line	117	6.5(4.5–8.5)	11.0(5.8–16.1)	0.112	0.663(0.397–1.107)
Tumor exthoracic metastasis					
No metastasis	87	7.0(4.8–9.2)	14.9(7.3–22.5)	0.053	0.56(0.308–1.02)
Metastasis	69	6.0(4.8–7.1)	11.0(3.0–18.9)	0.042	0.51(0.263–0.989)
Previous antiangiogenesis					
No	106	7.0(4.2–9.8)	14.9(9.6–20.2)	0.011	0.486(0.275–0.858)
Yes	50	5.1(2.2–7.9)	5.3(2.1–8.5)	0.574	0.813(0.393–1.682)
Hypertension history					
No	93	7.0(4.6–9.4)	8.5(5.4–11.6)	0.287	0.73(0.407–1.31)
Yes	63	6.5(4.6–8.4)	15.6(9.2–22.0)	0.005	0.381(0.19–0.766)

Notes: Bold font showed statistical significance (p value less than 0.05).

Discussion

This retrospective study is conducted to investigate the safety and efficacy of anlotinib in real world and the first to comprehensively compare the efficacy between different combination regimens of anlotinib and anlotinb monotherapy, respectively. In our study, anlotinib had a promising efficacy (mPFS:8.5 months) and achieved mPFS 11.0 months in combination therapy. Anlotinb-related adverse reactions were mostly mild and could be well controlled.

Previous studies have shown that the combination of antiangiogenic agents and other anti-tumor drugs including immunotherapy, chemotherapy, and targeted drugs had synergistic effects for tumor treatment.^{23–26} Phase III IMpower150 study showed that the atezolizumab plus bevacizumab plus carboplatin plus paclitaxel (ABevCPac) group significantly improved PFS (8.4 months vs 6.8 months) and OS (19.8 months vs 14.9 months) compared with the BevCPac group in intention-to-treat population.^{27,28} The phase III BEYOND trial confirmed the efficacy of first-line bevacizumab plus carboplatin/paclitaxel (PacC) in patients with advanced or recurrent non-squamous NSCLC, and both PFS (9.2 months vs 6.5 months) and OS (24.3 months vs 17.7 months) was prolonged with PacC + bevacizumab versus PacC + placebo.²⁹ Liu, H discovered that cisplatin-etoposide and bevacizumab combination, as the first-line treatment for ES-SCLC, can improve PFS.³⁰ In an open-label, random, multicentre, Phase 2 study aimed to compare the efficacy and

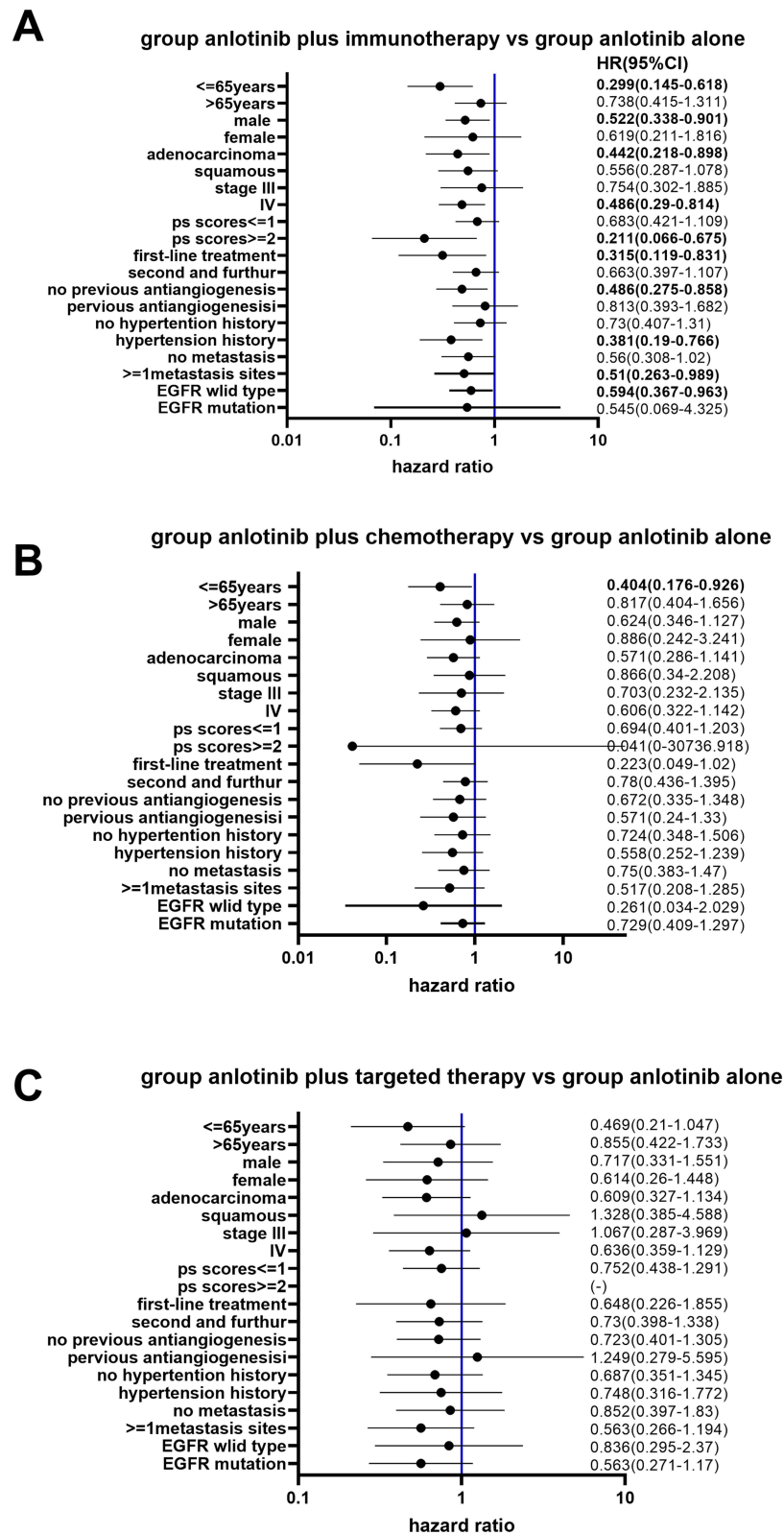


Figure 5 Forest plot of stratification analysis of PFS between patients in anlotinib alone and combined regimens. (A) Anlotinib plus immunotherapy vs anlotinib monotherapy; (B) Anlotinib plus chemotherapy vs monotherapy; (C) Anlotinib plus targeted therapy vs monotherapy.

Table 6 Anlotinib Treatment-Related Adverse Events in Patients Treated with Anlotinib Regimens

Adverse Events	Any Grade	%	≥3 Grade	%
Hypertension	64	26.7%	21	8.8%
Hand foot syndrome	25	10.4%	6	2.5%
Fatigue	25	10.4%	0	0.0%
Diarrhea	16	6.7%	0	0.0%
Cough	20	8.3%	0	0.0%
Hemorrhinia	20	8.3%	2	0.8%
Oral mucositis	13	5.4%	1	0.4%
Back soreness	13	5.4%	0	0.0%
Rash	16	6.7%	0	0.0%
Hypothyroidism	12	5.0%	3	1.3%
Hemoptysis	10	4.2%	0	0.0%
Thrombocytopenia	10	4.2%	0	0.0%
Hypertriglyceridemia	7	2.9%	0	0.0%
Neutropenia	7	2.9%	0	0.0%
Liver dysfunction	10	4.2%	1	0.4%
Hoarseness	9	3.8%	0	0.0%
Alimentary tract hemorrhage	7	2.9%	0	0.0%
UTI	4	1.7%	0	0.0%
Itchy skin	7	2.9%	0	0.0%
Erythema	4	1.7%	0	0.0%
Proteinuria	2	0.8%	0	0.0%
Hematuria	2	0.8%	0	0.0%
TOTAL	303	–	34	14.2%

Abbreviation: UTI, urinary tract infection.

safety of the combination of erlotinib and bevacizumab compared with erlotinib alone, mPFS was 16.0 months with erlotinib plus bevacizumab and 9.7 months with erlotinib alone (HR 0.54, 95% CI 0.36–0.79; Log rank test $p=0.0015$).³¹

Preclinical research demonstrated that anlotinib can modulate the tumor microenvironment and act synergistically with immune checkpoint inhibitors.^{11–13,32} Our study showed that anlotinib combined with immunotherapy significantly improved PFS in male, adenocarcinoma, ≤ 65 years old, patients stage IV, EGFR wild type, with metastasis, ps scores ≥ 2 , the first-line treatment, patients with a history of hypertension and no previous antiangiogenesis. Thus, anlotinib in combination with immunotherapy may be a reliable treatment option. Meanwhile, the good efficacy of first-line anlotinib in combination with immunotherapy was a noteworthy point. Our patients were driver gene-negative, but not all of them had detected PD-L1 expression (only 1 patient was detected PD-L1 $\geq 1\%$, the other 18 patients were unknown). The reason for choosing this regimen was based on the patients' own situation; some of them were unable to tolerate conventional first-line chemotherapy regimens because they had a lot of underlying disease, while others refused conventional chemotherapy because of fear of the adverse effects of chemotherapy or other reasons. Though in the context of evidence-based and precision medicine, it is not a conventional first-line treatment option, we made this choice of drug administration. Encouragingly, these patients benefited from first-line combination therapy even though they may not have PD-L1 indications for treatment, and we therefore recommend first-line immunotherapy combination with anlotinib as a treatment option to be chosen for patients who are driver gene-negative, have poor physical condition, are intolerable to chemo-regimen or do not want to receive chemotherapy.

Previous study indicated that anlotinib combined with chemotherapy had a significant longer median PFS than chemotherapy alone (5.0m vs 3.5m, $p=0.002$).³³ A case reported that first-line pemetrexed and carboplatin plus anlotinib achieved CR for the treatment of EGFR wild-type NSCLC.²² In our study, anlotinib plus chemotherapy had a tendency to prolong survival and had a significantly longer mPFS in patients ≤ 65 years and first-line treatment than anlotinib

monotherapy group. These studies suggest that anlotinib combined with chemotherapy may be an effective treatment for advanced NSCLC.

Mechanistic study showed anlotinib may overcome acquired resistance to EGFR-TKIs in NSCLC via FGFR1 signaling or c-MET/MYC/AXL axis, which indicated a synergistic anti-tumor effect of anlotinib and targeted drugs.^{34,35} In our study, the efficacy in the anlotinib combination targeted therapy group tended to be better than in the anlotinib monotherapy group, which suggested anlotinib combined with targeted therapy may be a promising regimen.

Previous studies have shown that the adverse reactions to anlotinib in advanced NSCLC were mostly mild and tolerable, with a low incidence of serious adverse reactions (11–61.9%).^{9,36,37} The most common adverse reactions were hypertension, hypothyroidism, hand-foot syndrome, diarrhea, anemia and fatigue.^{36,38} Our findings were similar to previous reports, and the incidence of serious adverse reactions (≥ 3 grade) was lower, which may be due to the fact that clinicians paid close attention to patients during treatment and promptly managed patients' adverse reactions, with management measures mainly being suspension of the drug and symptomatic treatment if necessary. Treatment-related adverse events could be controlled by early and appropriate intervention.

Bevacizumab and endostar are approved and undergoing clinical application in the systematic treatment of advanced NSCLC.⁵ A subgroup analysis of ALTER0303 trial reported previous antiangiogenic therapy had no impact on the efficacy of anlotinib (group yes vs group no, HR=1.588, 95% CI:0.802–3.145, $p=0.185$).³⁹ However, the number of patients received previous antiangiogenic therapy was very low (only 27 patients); thus, the findings need to be verified in further studies. Interestingly, our study, which included 76 patients who received previous antiangiogenic therapy, came to a different conclusion. Median PFS was significantly longer in patients who received no prior antiangiogenic therapy than in those who received prior antiangiogenic therapy (9.4 vs 6.3m, $p=0.004$). Cox regression suggested previous antiangiogenic therapy was associated with PFS. We searched the literature in the relevant field and found that the efficacy of anlotinib may be impaired by previous antiangiogenic therapy. One plausible mechanism is tumor hypoxia. It is proposed that the more proficient an antiangiogenic agent is, it will more efficiently prune tumor vessels and cause hypoxia. Hypoxia-tolerant tumor cell clones are selected and escape antiangiogenic treatment in hypoxic niches. Meanwhile, metabolic reprogramming to glucose addiction allows tumor cells to generate energy in hypoxic conditions. Hypoxia thus selects for more malignant metastatic cells, showing less sensitive to antiangiogenic treatment.^{40,41} The hypoxic environment caused by previous antiangiogenic therapy may select hypoxia-tolerant tumor cell clones, thus diminishing the effect of anlotinib. A research about anlotinib resistance showed that protecting mitochondria contributes to the resistance of anlotinib.⁴² This may be related to energy generation and the hypoxic environment, while other signaling systems (such as p53, IL-8), stromal cells and cytokine response may be associated with the resistance.^{43,44} A research proposed that antiangiogenic agents (especially broad-spectrum VEGF RTKIs, which also inhibit PDGFR β) or cytokine response they induce may inhibit the coverage of vessels by pericytes, which makes vessels more leaky and immature, facilitating intravasation of tumor cells and metastasis.⁴⁵ In conclusion, considering the partial overlap of antiangiogenic mechanisms and the complexity of angiogenesis, whether the efficacy of anlotinib in patients who had previous antiangiogenic therapy would be affected is still need to be verified in clinical practice.

We made the attempt to provide a comprehensive analysis of the current clinical use of anlotinib and to compare the efficacy of different combination regimens of anlotinib with that of anlotinib monotherapy. However, our study had some limitations. First, observational studies almost always have bias because prognostic factors are unequally distributed between patients, so we did adjusted and stratified analysis to reduce the risk of bias. Second, this study was from a single center, and the sample size was not sufficient, which caused population bias and made it difficult to show statistical differences in the stratification analysis. Third, due to the corona virus disease 2019 (COVID-19), for some patients, regular visits to the hospital were not possible, resulting in a lack of follow-up information, which also limited the number of patients we included. Fourth, we did not conduct comparisons of efficacy between different combination regimens, which may limit physician selection of different combination regimens. Further studies with larger sample size and longer follow-up are needed.

Conclusion

In conclusion, anlotinib has reliable efficacy and tolerable toxicity. Anlotinib in combination with immunotherapy showed promising efficacy and can be considered as an option for NSCLC treatment. Anlotinib plus chemotherapy and targeted therapy had a tendency to prolong survival, respectively. Previous antiangiogenic therapy may be an independent factor for the efficacy of anlotinib in patients, but more studies are needed to verify this. Treatment-related adverse events could be better controlled by early and appropriate intervention.

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Disclosure

The authors report no conflicts of interest in this work.

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