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Low-dose anlotinib plus immune checkpoint inhibitors offers better efficacy and safety in advanced non-small cell lung cancer treatment

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The combination of anlotinib with immune checkpoint inhibitors (ICIs) has become a common treatment modality in clinical practice. However, the optimal dose of anlotinib to use remains unclear. We collected patients with advanced non-small cell lung cancer (NSCLC) who received programmed cell death-1 blockade combined with different dose of anlotinib as second-line or later line therapy. Subsequently, the efficacy and safety of the combination therapy as well as subgroup analyses of different doses of anlotinib were analyzed. Cox regression was performed to analyze significant factors correlated with progression-free survival (PFS) and overall survival (OS). A total of 50 eligible patients with NSCLC who received anlotinib combined with ICIs therapy were included, of which 27 received low-dose anlotinib (8 mg), and 23 were administered high-dose anlotinib (12 mg). The median PFS (mPFS) and the median OS (mOS) for all patients were 8.3 months [95% confidence interval (CI): 6.3–10.3] and 17.6 months (95% CI: 16.5–18.7), respectively. Subgroup analyses showed that patients treated with 8 mg of anlotinib plus ICIs had significantly longer mPFS than those treated with 12 mg of anlotinib plus ICIs (8.7 vs 6.7 months, $P = 0.016$). The overall incidence of adverse events was 68.0%, and the most common adverse events of all grades were hypertension. Meanwhile, the incidence of adverse events was higher for 12 mg of anlotinib plus ICIs than that of 8 mg of anlotinib plus ICIs (82.6 vs 55.6%, $P = 0.041$). Low-dose anlotinib in combination with ICIs for advanced NSCLC may be an effective and well-tolerated option. *Anti-Cancer Drugs* 36: 408–414

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Introduction

The Global Cancer Report 2020 showed that there were approximately 2.2 million new cases of lung cancer and 1.8 million deaths, accounting for approximately 11.4% of newly diagnosed cancers and 18.0% of deaths [1]. Lung cancer is the leading cause of cancer-related deaths globally, and non-small cell lung cancer (NSCLC) accounts for about 83% of lung cancer cases, most of which are locally advanced or metastatic at the time of diagnosis as well as have a poor prognosis [2]. The advent of immune checkpoint inhibitors (ICIs) has provided new strategies for the treatment of advanced NSCLC. A growing body of evidence suggests that ICIs, such as antibodies against programmed cell death-1, significantly improve outcomes and prolong overall survival (OS) in patients with advanced NSCLC [3–5]. However, not all patients benefit from ICIs alone. Some patients even over-progress after treatment with single ICIs [6]. Therefore, it is important to explore combination therapy regimens with ICIs for the benefit of patients with advanced NSCLC.

Angiogenesis plays a very important role in cancer progression [7,8]. Tumor growth and metastasis depend on abundant blood vessels, so blocking the pathways of tumor angiogenesis is an effective therapeutic strategy. Anlotinib is a tyrosine kinase inhibitor (TKI) that targets a wide range of tumor vascular and proliferative signaling receptors [9,10]. Currently, anlotinib has been approved by China National Medical Products Administration for use in two types of advanced NSCLC patients who have progressed after chemotherapy [11]. Some studies have shown that the combination of anlotinib and ICIs has a synergistic effect. For patients with advanced NSCLC without epidermal growth factor receptor (EGFR) mutations, the combination of

anlotinib and ICIs has good efficacy and tolerance as first-line therapy [12]. In second-line and beyond, anlotinib plus ICIs produced higher objective effectiveness rates (ORR) and longer progression-free survival (PFS) compared to ICIs monotherapy [13]. These studies confirm that the combination of anlotinib with ICIs can improve the treatment outcome of NSCLC patients.

Based on the concept of a positive correlation between efficacy and dose, the 12 mg dose of anlotinib was frequently combined with ICIs in most studies [14]. Interestingly, a series of studies have presented that using a lower dose results in a more homogeneous distribution of tumor vasculature compared to higher doses of antiangiogenic drugs [15–18]. Specifically, apatinib (a TKI drug similar to anlotinib) is better at normalizing tumor blood vessels when used at low doses. When combined with ICIs, the synergistic effect of low-dose apatinib was significantly higher than that of high dose [18]. In addition, low-dose apatinib better reprogrammed the tumor microenvironment from an immunosuppressive to an immunologically permissive microenvironment [19]. Therefore, the application of low-dose combination of antiangiogenic drugs and ICIs may have better antitumor synergistic effects than the high-dose combination group.

Anlotinib in combination with ICIs as a therapeutic strategy is becoming more widely used in patients with NSCLC. However, the optimal combined dose of anlotinib remains to be determined. Here, we conducted this retrospective study to look at the status of anlotinib plus ICIs in the real world. Subgroups of results were also performed to compare the efficacy and safety of ICIs in combination with high dose (12 mg) or low dose (8 mg) of anlotinib. This study provides a reference for the clinical application of anlotinib combined with ICIs.

Methods

Study design and patients

Patients diagnosed with advanced NSCLC who received 8 or 12 mg anlotinib in combination with ICIs as second-line or later therapy between February 2021 and August 2022 at the First Affiliated Hospital of Anhui Medical University in China were included. Further, the inclusion criteria were as follows: age ≥18 years; advanced stage (IIIB–IV) NSCLC were pathologically identified; at least one measurable lesion; Eastern Cooperative Oncology Group (ECOG) ≤ 3. While exclusion criteria were: mixed tumors of small cell and NSCLC; active bleeding or serious systemic diseases; any adjust of the dosage of anlotinib during treatment; any have not received follow-up information within two cycles after taking anlotinib. The clinical information system (Dong Hua software, DHC Software Co., Ltd, Beijing, China) was performed to collect baseline characteristics, including gender, age, dose, ECOG score, pathological type, clinical stage, smoking history, gene mutation, treat line, hypertension, prior targeted therapy, prior antiangiogenesis treatment, and prior thoracic radiotherapy.

Treatment

Anlotinib was administered once daily (12 or 8 mg) on days 1–14 of a 21-day cycle, and ICIs (Camrelizumab) was administered 200 mg every 3 weeks. The initial dose of drugs was determined by the oncologist according to the patients' status. Follow-up data were collected up to 30 October 2023. This study was approved by the First Affiliated Hospital of Anhui Medical University (No. Quick-PJ2019-14-15) and conducted according to the principles of the Declaration of Helsinki. Given the retrospective analysis, the requirement for individual consent was waived.

Efficacy and safety assessments

Therapeutic responses were assessed based on Response Evaluation Criteria in Solid Tumors version 1.1 every two cycles, defined as complete response (CR), partial response (PR), stable disease, and progression disease. PFS was characterized as the time between the date of the start of treatment with both anlotinib and ICIs, and the documented disease progression or death from any cause. OS was defined as the time from initiation of treatment to death or last follow-up. ORR referred to the proportion of patients who have complete or a PR to the therapy. Disease control rate (DCR) was defined as the proportion of patients with PR, CR, and stable disease. Adverse reactions were graded using the Common Terminology Criteria for Adverse Events version 5.0.

Statistical analysis

The baseline clinical features of all patients were summarized as categorical (percentage) and continuous data (mean and standard). Pearson Chi-square test was used to compare categorical variables and tumor responses between two groups. Comparison between groups for continuous variables were performed by an independent-sample *t*-test. The median PFS (mPFS), OS, and 95% confidence interval (CI) were estimated using the Kaplan–Meier method. Cox proportional hazards regression was carried out by the univariable and multivariable analyses and to calculate the hazard ratios with 95% CIs. All statistical analyses were performed using Statistical Products and Services Solutions, version 26 (SPSS 26.0, IBM, Armonk, New York, USA).

Results

Baseline clinical characteristics of patients

A total of 50 patients were included in the analysis, of whom 31 (62.0%) were male and 19 (38.0%) were female. Among the patients, the mean age was 60.93 ± 10.15. And there were 27 patients (54.0%) taking 8 mg of anlotinib, and 23 patients (46.0%) taking 12 mg of anlotinib. Besides, a total of 9 patients (18.0%) had a smoking

Table 1 Baseline clinical characteristics of patients

Basic characteristics	*N* (%)
Gender	
Male	31 (62.0)
Female	19 (38.0)
Age	
Mean ± SD	60.93 ± 10.15
Doses of anlotinib	
8 mg	27 (54.0)
12 mg	23 (46.0)
ECOG score	
0–1	45 (90.0)
2–3	5 (10.0)
Pathological type	
Squamous carcinoma	22 (44.0)
Adenocarcinoma	28 (56.0)
Clinical stage	
<IV	8 (16.0)
IV	42 (84.0)
Smoking history	
Yes	9 (18.0)
No	41 (82.0)
Gene mutation	
Yes	15 (30.0)
No	35 (70.0)
Treat line	
2	25 (50.0)
≥3	25 (50.0)
Prior targeted therapy	
Yes	11 (22.0)
No	39 (78.0)
Prior antiangiogenesis treatment	
Yes	18 (36.0)
No	32 (64.0)
Prior thoracic radiotherapy	
Yes	15 (30.0)
No	35 (70.0)

ECOG, Eastern Cooperative Oncology Group.

history and 45 patients (90.0%) had an ECOG of 0–1. The pathological diagnosis of the patient included squamous carcinoma (44.0%, 22/50) and adenocarcinoma (56.0%, 28/50). Of those, 25 (25.0%) patients received 3rd line and above treatment with anlotinib. Regarding treatment, 11 (22.0%) patients received prior targeted therapy; 18 patients (36.0%) were treated with prior antiangiogenesis therapy. Additionally, 15 patients (30.0%) had previously undergone thoracic radiotherapy. The detailed baseline clinical characteristics of patients are listed in Table 1.

Overall efficacy of treatment

In the entire cohort, the mPFS of patients was 8.3 months (95% CI: 6.3–10.3), and the median OS (mOS) was 17.6 months (95% CI: 16.5–18.7) (Fig. 1a and b). Moreover, univariate analysis was performed to identify potential factors that associated with PFS and OS in all patients (Table 2), meanwhile the statistically significant factors ($P < 0.05$) were included into multivariate Cox regression analysis. Data revealed that gender (male vs female: hazard ratio = 3.288, 95% CI: 1.712–6.313, $P = 0.000$), and ICIs combined with different dose anlotinib (8 vs 12 mg: hazard ratio = 2.110, 95% CI: 1.154–3.858, $P = 0.015$) were identified as the independent influencing factors of PFS. Similarly, the independent factors influencing OS were gender (male vs female: hazard ratio = 2.449, 95% CI: 1.305–4.597, $P = 0.005$), and ICIs combined with anlotinib (8 vs 12 mg: hazard ratio = 1.843, 95% CI: 1.029–3.300, $P = 0.040$) as well (Table 3).

Efficacy of low-dose anlotinib plus immune checkpoint inhibitors vs high-dose anlotinib plus immune checkpoint inhibitors

Baseline characteristics were comparable between 8 mg of anlotinib plus ICIs and 12 mg of anlotinib plus ICIs (Table 4). Further data showed that patients receiving 8 mg of anlotinib + ICIs ($n = 27$, 8.7 months) had significantly longer mPFS compared to those receiving 12 mg of anlotinib + ICIs ($n = 23$, 6.7 months, $P = 0.016$; Fig. 2a). In addition, patients treated with 8 mg of anlotinib in combination with ICIs was associated with longer mOS than patients treated with 12 mg of anlotinib in combination with ICIs, although the difference was not statistically significant (18.5 vs 14.3 months, $P = 0.065$; Fig. 2b). However, 8 mg of anlotinib plus ICIs and 12 mg of anlotinib plus ICIs were similar in terms of ORR (18.5% vs 13.0%, $P = 0.889$) and DCR (85.2% vs 82.6%, $P = 1.000$; Table 5). Overall, patients treated with low-dose anlotinib in combination with ICIs had longer survival than those treated with high-dose anlotinib.

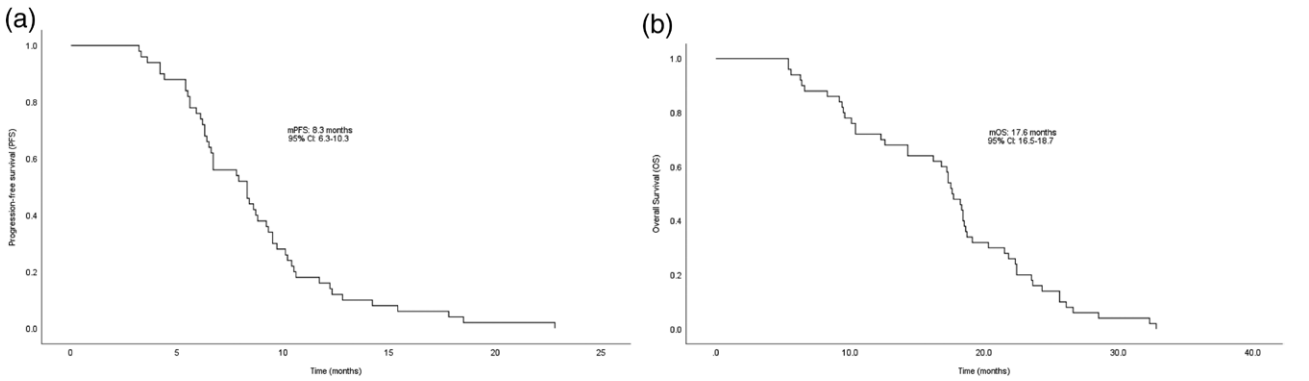
Safety

In this analysis, treatment-related adverse events occurred in 34 of the 50 patients. The five most common adverse events (all grades) were hypertension (24.0%), hand-foot syndrome (16.0%), fatigue (16.0%), thyroid dysfunction (12.0%), and hoarseness (12.0%). The five most common ≥3 grade adverse events were hypertension (6.0%) and hand-foot syndrome (2.0%). There were no treatment-related deaths or life-threatening adverse events. Furthermore, the most common adverse reaction was hypertension, followed by hand-foot syndrome and fatigue, regardless of whether ICIs was combined with 8 or 12 mg of anlotinib. Notably, compared with low-dose anlotinib plus ICIs, high-dose anlotinib plus ICIs resulted in an increased incidence of adverse events of all grades, although no new adverse reactions were observed (55.6 vs 82.6%, $P = 0.041$). The incidence of grade 3 or above adverse reactions was similar between the two groups (13.0 vs 3.7%, $P = 0.49$). The adverse reactions are listed in Table 6.

Discussion

The combination of antiangiogenic agents and ICIs has shown definite efficacy in a variety of solid tumors [20]. Similarly, the results of retrospective studies of anlotinib plus ICIs are promising treatment. In one hand, the mPFS for third-line treatment of NSCLC with anlotinib alone was 5.4 months and the mOS was 9.6 months [21]. Surprisingly, the combination therapy in our study had mPFS and mOS of 8.3 months and 17.6 months, and 16% ORR respectively, which provided an additional

Fig. 1



Efficacy of anlotinib in all patients. (a) The progression-free survival of all patients. (b) The overall survival of all patients.

Table 2 Univariate cox regression analyses of PFS and OS

	PFS			OS		
Variable	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Gender (male vs female)	3.169	1.667–6.022	<0.01	2.298	1.231–4.289	<0.01
Age (<65 vs ≥65 years)	0.684	0.384–1.219	0.198	0.823	0.454–1.491	0.521
ICIs combined with anlotinib (8 vs 12 mg)	2.033	1.120–3.690	0.020	1.708	0.958–3.044	0.069
ECOG score (0–1 vs ≥2)	1.866	0.728–4.785	0.194	1.108	0.435–2.823	0.830
Pathological type (squamous carcinoma vs adenocarcinoma)	0.701	0.387–1.270	0.241	0.703	0.386–1.282	0.250
Clinical stage (<IV vs IV)	0.565	0.260–1.231	0.151	0.556	0.255–1.215	0.141
Smoking history (yes vs no)	0.944	0.468–1.904	0.873	0.950	0.471–1.915	0.885
Gene mutation (yes vs no)	1.533	0.820–2.867	0.181	1.200	0.640–2.252	0.569
Treat line (2 vs ≥3)	1.155	0.657–2.031	0.617	1.114	0.634–1.957	0.708
Previous targeted therapy (yes vs no)	1.972	0.979–3.973	0.058	1.404	0.703–2.804	0.336
Previous antiangiogenesis therapy (yes vs no)	0.939	0.512–1.722	0.839	0.944	0.524–1.699	0.847
Previous thoracic radiotherapy (yes vs no)	0.630	0.320–1.239	0.181	0.626	0.321–1.223	0.171

CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; ICIs, immune checkpoint inhibitors; OS, overall survival; PFS, progression-free survival.

Table 3 Multivariate cox regression analysis of PFS and OS

	PFS			OS		
Variable	HR	95% CI	*P*-value	HR	96% CI	*P*-value
Gender (male vs female)	3.288	1.712–6.313	<0.01	2.449	1.305–4.597	<0.01
ICIs combined with anlotinib (8 vs 12 mg)	2.110	1.154–3.858	0.015	1.843	1.029–3.300	0.040

CI, confidence interval; HR, hazard ratio; ICIs, immune checkpoint inhibitors; OS, overall survival; PFS, progression-free survival.

2.9 months of PFS and 8.0 months of OS benefit compared with anlotinib alone. In the other hand, favorable efficacy was obtained with combination therapy compared to programmed cell death-1 blockade alone. In the programmed cell death-1 blockade alone trial (KEYNOTE-001), ORR, mPFS, and mOS values were 19.4%, 3.7 months, and 12.0 months, respectively [22]. The comparison of our data shows that the combination therapy is highly effective. Consistent with our conclusion, others have also confirmed that anlotinib in combination with ICIs has a favorable therapeutic effect in NSCLC [23]. In terms of the mechanism, the combination of anlotinib and ICIs has a synergistic effect to regulate the tumor immune microenvironment, such as promoting the infiltration of natural killer cells, M1-like tumor-associated macrophages (TAMs), and dendritic cells, while decreasing the infiltration of M2-like TAMs [24]. In addition, anlotinib is able to inactivate the AKT pathway reducing PD-L1 expression on vascular endothelial cells, leading to an elevation in the CD8/FoxP3, thus activating the immune response [25].

Antiangiogenic agents work by pruning tumor blood vessels and limiting nutrient supply to tumor cells [26]. In vivo and in vitro studies demonstrated that relatively low-dose anlotinib significantly reduced tumor vessel density, whereas rising the dose of anlotinib did not improve the ability to prune the number of vessels. An effective low dose of anlotinib is sufficient to inhibit tumor growth with fewer side effects compared to higher doses [27].

Table 4 Comparison of baseline characteristics between 8 mg of anlotinib plus ICIs and 12 mg of anlotinib plus ICIs

Basic characteristics	8 mg of anlotinib plus ICIs ($n = 27$)	12 mg of anlotinib plus ICIs ($n = 23$)	t/X^2	P-value
Gender				
Male	17 (63.0)	14 (60.9)	0.023	0.879
Female	10 (37.0)	9 (39.1)		
Age				
Mean ± SD	63.43 ± 7.95	60.93 ± 11.73	0.869	0.389
<65	14 (51.9)	14 (60.9)	0.410	0.522
≥65	13 (48.1)	9 (39.1)		
ECOG score				
0–1	23 (85.2)	22 (95.7)	0.573	0.449
≥2	4 (14.8)	1 (4.3)		
Pathological type				
Squamous carcinoma	12 (44.4)	10 (56.5)	0.005	0.945
Adenocarcinoma	15 (55.6)	13 (43.5)		
Clinical stage				
<IV	4 (14.8)	4 (17.4)	0.000	1.000
IV	23 (85.2)	19 (82.6)		
Smoking history				
Yes	5 (18.5)	4 (17.4)	0.000	1.000
No	22 (81.5)	19 (82.6)		
Gene mutation				
Yes	8 (29.6)	7 (30.4)	0.004	0.951
No	19 (70.4)	16 (69.6)		
Treat line				
2	15 (55.6)	10 (43.5)	0.725	0.395
≥3	12 (44.4)	13 (56.5)		
Prior targeted therapy				
Yes	6 (22.2)	5 (21.7)	0.002	0.967
No	21 (77.8)	18 (78.3)		
Prior antiangiogenesis treatment				
Yes	13 (48.1)	5 (21.7)	3.76	0.053
No	14 (51.9)	18 (78.3)		
Prior thoracic radiotherapy, *n* (%)				
Yes	5 (18.5)	10 (43.5)	3.685	0.055
No	22 (81.5)	13 (56.5)		

ECOG, Eastern Cooperative Oncology Group; ICIs, immune checkpoint inhibitors.

Table 5 Response of 8 mg of anlotinib plus ICIs and 12 mg of anlotinib plus ICIs

Response	8 mg of anlotinib plus ICIs (n = 27)	12 mg of anlotinib plus ICIs (n = 23)	X^2	P-value
CR	0	1		
PR	5	2		
SD	18	16		
PD	4	4		
ORR (%)	5 (18.5%)	3 (13.0%)	0.019	0.889
DCR (%)	23 (85.2%)	19 (82.6%)	0.000	1.000

CR, complete response; DCR, disease control rate; ICIs, immune checkpoint inhibitors; ORR, objective response rate; PD, progression disease; PR, partial response; SD, stable disease.

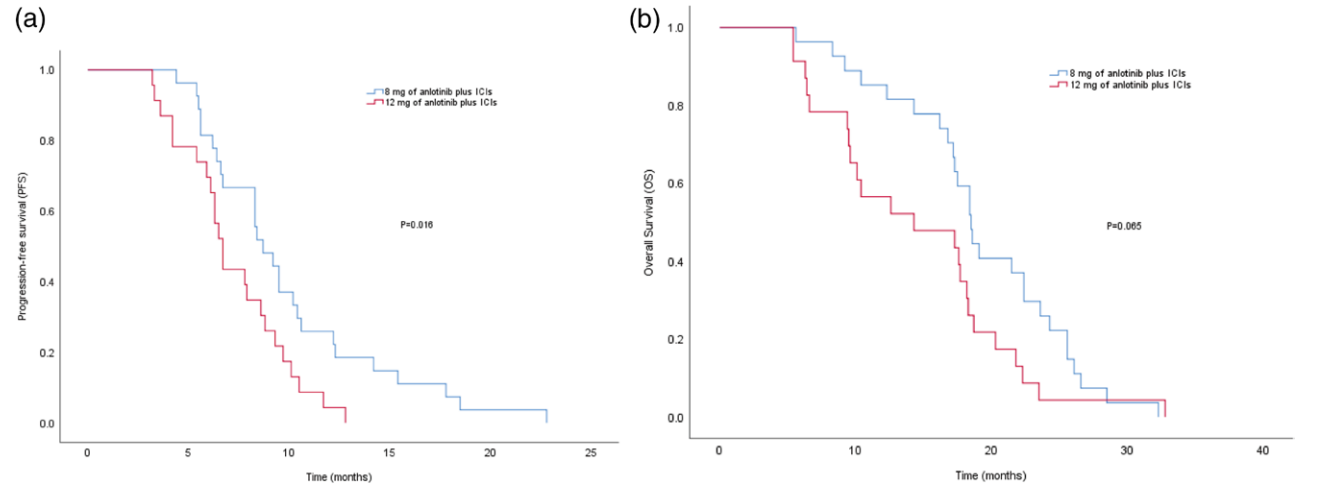
Recently, Yuan *et al.* demonstrated that low-dose anlotinib is a good antiangiogenic partner for combination therapy with ICIs in advanced NSCLC treatment. Forty patients with anlotinib administered at a dose of 8 and 10 mg were included in this study, but different doses were not stratified and analyzed [28]. In our study, we enrolled widely varying doses of anlotinib, 8 and 12 mg to explore the effects of different doses of anlotinib on patients. The analysis confirmed longer PFS with ICIs in combination with 8 mg of anlotinib than that with 12 mg (8.7 vs 6.7 months, $P = 0.016$). Meanwhile, OS was longer in patients treated with ICIs + anlotinib (8 mg) than those treated with 12 mg, although the difference was not statistically significant (18.5 vs 14.3 months, $P = 0.065$). This may be due to the fact that the relatively low dose of anlotinib resulted in a more sustained normalization of tumor vasculature. Besides, some studies have reported longer mPFS in patients without prior antiangiogenic therapy. Regretly, the data was not displayed in our study ($P = 0.839$). In our findings, males treated with immunotherapy plus anlotinib had longer PFS and OS compared to females. It is well known that sex differences are important in both the development and treatment of cancer [29]. There are also differences between men and women in immunotherapy, with one study reported to show that the efficacy of ICIs in men is twice that of women [30]. The possible reasons for this are the result of a multifactorial complex of factors such as a stronger immune response in women than in men, a higher mutational load of tumors in all men than in women, and hormone levels. Therefore, the better prognosis of men than women in NSCLC patients treated with anlotinib in combination with ICIs can be explained, in addition to the fact that studies achieving the same results as ours have also been reported [31].

In this study, the overall incidence of adverse events with anlotinib in combination with ICIs was 68.0%. The most common adverse events were hypertension, with a 24% incidence (≥3 grade = 3). Four patients (8.0%) experienced grade 3 or higher adverse events. In addition, our data showed that the incidence of adverse events was higher with 12 mg of anlotinib plus ICIs than with 8 mg of anlotinib plus ICIs (82.6 vs 55.6%, $P = 0.041$), indicating that low-dose anlotinib combined with ICIs has lower adverse events. It is worth mentioning that the incidence of adverse events in this study was significantly lower than that reported by others. For example, Wang *et al.* [23] reported that the overall incidence of adverse events was 85.0%. The incidence of grade 3–4 treatment-related adverse events was about 40.0%. Zhai *et al.* [32] found that the most common adverse events were hypertension (45.5%), and the incidence of grade 3–4 hypertension was 9.1%. Our study also has some limitations. First, this is a retrospective analysis from a small sample of a single center, and bias is inevitable. Therefore, further large-scale prospective studies are needed to confirm our findings, which would benefit patients using anlotinib combination immunotherapy. Second, PD-L1 expression is considered a biomarker for treatment with anti-programmed cell death-1 drugs, but the levels of PD-L1 in patients was not available in this study [33,34].

Conclusion

In summary, the efficacy and safety of 8 and 12 mg of anlotinib in combination with ICIs in patients with advanced

Fig. 2



Kaplan–Meier curves for progression-free survival (a) and overall survival (b) for different doses of anlotinib combined with immune checkpoint inhibitors.

Table 6 Adverse events in patients treated with 8 mg of anlotinib plus ICIs and 12 mg of anlotinib plus ICIs

	All patients ($n = 50$)		8 mg of anlotinib plus ICIs ($n = 27$)		12 mg of anlotinib plus ICIs ($n = 23$)	
Adverse event	Any grade (%)	≥3 grade (%)	Any grade (%)	≥3 grade (%)	Any grade (%)	≥3 grade (%)
Hypertension	12 (24.0)	3 (6.0)	6 (22.2)	1 (3.7)	6 (26.1)	2 (8.7)
Hand-foot syndrome	8 (16.0)	1 (2.0)	2 (7.4)	0 (0.0)	6 (26.1)	1 (4.3)
Fatigue	8 (16.0)	0 (0.0)	3 (11.1)	0 (0.0)	5 (21.7)	0 (0.0)
Gastrointestinal reaction	4 (8.0)	0 (0.0)	1 (3.7)	0 (0.0)	3 (13.0)	0 (0.0)
Thyroid dysfunction	6 (12.0)	0 (0.0)	1 (3.7)	0 (0.0)	5 (21.7)	0 (0.0)
Proteinuria	4 (8.0)	0 (0.0)	1 (3.7)	0 (0.0)	3 (13.0)	0 (0.0)
Hoarseness	6 (12.0)	0 (0.0)	2 (7.4)	0 (0.0)	4 (17.4)	0 (0.0)
Rash	2 (4.0)	0 (0.0)	1 (3.7)	0 (0.0)	1 (4.3)	0 (0.0)
Liver dysfunction	3 (6.0)	0 (0.0)	1 (3.7)	0 (0.0)	2 (8.7)	0 (0.0)
Hemoptysis	2 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (8.7)	0 (0.0)
Hypertriglyceridemia	1 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)	0 (0.0)

ICIs, immune checkpoint inhibitors.

NSCLC were explored. Patients with advanced NSCLC treated with the combination of 8 mg of anlotinib plus ICIs had better efficacy and lower toxicity. Therefore, our results add to the growing body of evidence supporting the benefits of combining immunotherapy with low-dose anlotinib.

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J.W. and T.T. conceptualized and supervised the project. T.T., S.Y., and J.J. carried out the study and interpreted the results. T.T. and S.Y. drafted the initial version of the manuscript. W.C. and M.C. searched the literature and collected data. J.W. and Q.X. modified the revised manuscript. All authors have read and agreed to the published version of the article.

The approval for this retrospective study was obtained from the Ethics Committee of the First Affiliated Hospital of Anhui Medical University, and the need to obtain informed consent was waived.

All data generated or analyzed during this study are included in this published article. The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of interest

There are no conflicts of interest.

References

1 Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021; **71**:209–249.

2 Miller KD, Nogueira L, Mariotto AB, Rowland JH, Yabroff KR, Alfano CM, *et al.* Cancer treatment and survivorship statistics, 2019. *CA Cancer J Clin* 2019; **69**:363–385.

3 Brahmer J, Reckamp KL, Baas P, Crino L, Eberhardt WE, Poddubskaya E, *et al.* Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med* 2015; **373**:123–135.

4 Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, *et al.* Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med* 2015; **373**:1627–1639.

5 Garassino MC, Cho BC, Kim JH, Mazieres J, Vansteenkiste J, Lena H, *et al.* Final overall survival and safety update for durvalumab in third- or later-line advanced NSCLC: the phase II ATLANTIC study. *Lung Cancer* 2020; **147**:137–142.

6 Kim JY, Lee KH, Kang J, Borcoman E, Saada-Bouzid E, Kronbichler A, *et al*. Hyperprogressive disease during anti-PD-1 (PDCD1)/PD-L1 (CD274) therapy: a systematic review and meta-analysis. *Cancers (Basel)* 2019; **11**:1699.
7 Claesson-Welsh L, Welsh M. VEGFA and tumour angiogenesis. *J Intern Med* 2013; **273**:114–127.
8 Casanovas O, Hicklin DJ, Bergers G, Hanahan D. Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. *Cancer Cell* 2005; **8**:299–309.
9 Shen G, Zheng F, Ren D, Du F, Dong Q, Wang Z, *et al*. Anlotinib: a novel multi-targeting tyrosine kinase inhibitor in clinical development. *J Hematol Oncol* 2018; **11**:120.
10 Lin B, Song X, Yang D, Bai D, Yao Y, Lu N. Anlotinib inhibits angiogenesis via suppressing the activation of VEGFR2, PDGFRbeta AND FGFR1. *Gene* 2018; **654**:77–86.
11 Syed YY. Anlotinib: first global approval. *Drugs* 2018; **78**:1057–1062.
12 Chu T, Zhong R, Zhong H, Zhang B, Zhang W, Shi C, *et al*. Phase 1b study of sintilimab plus anlotinib as first-line therapy in patients with advanced NSCLC. *J Thorac Oncol* 2021; **16**:643–652.
13 Zhang X, Zeng L, Li Y, Xu Q, Yang H, Lizaso A, *et al*. Anlotinib combined with PD-1 blockade for the treatment of lung cancer: a real-world retrospective study in China. *Cancer Immunol Immunother* 2021; **70**:2517–2528.
14 Sun Y, Niu W, Du F, Du C, Li S, Wang J, *et al*. Safety, pharmacokinetics, and antitumor properties of anlotinib, an oral multi-target tyrosine kinase inhibitor, in patients with advanced refractory solid tumors. *J Hematol Oncol* 2016; **9**:105.
15 Huang Y, Yuan J, Righi E, Kamoun WS, Ancukiewicz M, Nezivar J, *et al*. Vascular normalizing doses of antiangiogenic treatment reprogram the immunosuppressive tumor microenvironment and enhance immunotherapy. *Proc Natl Acad Sci U S A* 2012; **109**:17561–17566.
16 Van der Veldt AA, Lubberink M, Bahce I, Walraven M, de Boer MP, Greuter HN, *et al*. Rapid decrease in delivery of chemotherapy to tumors after anti-VEGF therapy: implications for scheduling of anti-angiogenic drugs. *Cancer Cell* 2012; **21**:82–91.
17 Huang Y, Goel S, Duda DG, Fukumura D, Jain RK. Vascular normalization as an emerging strategy to enhance cancer immunotherapy. *Cancer Res* 2013; **73**:2943–2948.
18 Zhao S, Ren S, Jiang T, Zhu B, Li X, Zhao C, *et al*. Low-dose apatinib optimizes tumor microenvironment and potentiates antitumor effect of PD-1/PD-L1 blockade in lung cancer. *Cancer Immunol Res* 2019; **7**:630–643.
19 Fukumura D, Kloepper J, Amoozgar Z, Duda DG, Jain RK. Enhancing cancer immunotherapy using antiangiogenics: opportunities and challenges. *Nat Rev Clin Oncol* 2018; **15**:325–340.
20 Manegold C, Dingemans AC, Gray JE, Nakagawa K, Nicolson M, Peters S, *et al*. The potential of combined immunotherapy and antiangiogenesis for the synergistic treatment of advanced NSCLC. *J Thorac Oncol* 2017; **12**:194–207.
21 Han B, Li K, Wang Q, Zhang L, Shi J, Wang Z, *et al*. Effect of anlotinib as a third-line or further treatment on overall survival of patients with advanced non-small cell lung cancer: the ALTER 0303 phase 3 randomized clinical trial. *JAMA Oncol* 2018; **4**:1569–1575.
22 Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, *et al*. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med* 2015; **372**:2018–2028.
23 Wang P, Fang X, Yin T, Tian H, Yu J, Teng F. Efficacy and safety of anti-PD-1 plus anlotinib in patients with advanced non-small-cell lung cancer after previous systemic treatment failure-a retrospective study. *Front Oncol* 2021; **11**:628124.
24 Yang Y, Li L, Jiang Z, Wang B, Pan Z. Anlotinib optimizes anti-tumor innate immunity to potentiate the therapeutic effect of PD-1 blockade in lung cancer. *Cancer Immunol Immunother* 2020; **69**:2523–2532.
25 Liu S, Qin T, Liu Z, Wang J, Jia Y, Feng Y, *et al*. Anlotinib alters tumor immune microenvironment by downregulating PD-L1 expression on vascular endothelial cells. *Cell Death Dis* 2020; **11**:309.
26 Eelen G, Treps L, Li X, Carmeliet P. Basic and therapeutic aspects of angiogenesis updated. *Circ Res* 2020; **127**:310–329.
27 Fan P, Qiang H, Liu Z, Zhao Q, Wang Y, Liu T, *et al*. Effective low-dose anlotinib induces long-term tumor vascular normalization and improves anti-PD-1 therapy. *Front Immunol* 2022; **13**:937924.
28 Yuan S, Peng L, Liu Y, Till BG, Yan X, Zhang J, *et al*. Low-dose anlotinib confers improved survival in combination with immune checkpoint inhibitor in advanced non-small cell lung cancer patients. *Cancer Immunol Immunother* 2023; **72**:437–448.
29 Haupt S, Caramia F, Klein SL, Rubin JB, Haupt Y. Sex disparities matter in cancer development and therapy. *Nat Rev Cancer* 2021; **21**:393–407.
30 Conforti F, Pala L, Bagnardi V, De Pas T, Martinetti M, Viale G, *et al*. Cancer immunotherapy efficacy and patients' sex: a systematic review and meta-analysis. *Lancet Oncol* 2018; **19**:737–746.
31 Sun L, Zhao Q, Wang Y, Wang Y, Zheng M, Ding X, Miao L. Efficacy and safety of anlotinib-containing regimens in advanced non-small cell lung cancer: a real-world study. *Int J Gen Med* 2023; **16**:4165–4179.
32 Zhai C, Zhang X, Ren L, You L, Pan Q, Pan H, Han W. The efficacy and safety of anlotinib combined with PD-1 antibody for third-line or further-line treatment of patients with advanced non-small-cell lung cancer. *Front Oncol* 2020; **10**:619010.
33 Mok TSK, Wu YL, Kudaba I, Kowalski DM, Cho BC, Turna HZ, *et al*. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (Keynote-042): a randomised, open-label, controlled, phase 3 trial. *Lancet* 2019; **393**:1819–1830.
34 Li H, Xu Y, Wan B, Song Y, Zhan P, Hu Y, *et al*. The clinicopathological and prognostic significance of PD-L1 expression assessed by immunohistochemistry in lung cancer: a meta-analysis of 50 studies with 11,383 patients. *Transl Lung Cancer Res* 2019; **8**:429–449.