Original Article

Comparative efficacy and safety of multitarget angiogenesis inhibitor combined with immune checkpoint inhibitor and nivolumab monotherapy as second-line or beyond for advanced lung adenocarcinoma in driver-negative patients: a retrospective comparative cohort study

Lian Yu[1], Jianlin Xu[1], Rong Qiao[1], Hua Zhong[1], Wolfgang M. Brueckl[2], Runbo Zhong[1]

[1]Department of Pulmonary Medicine, Shanghai Chest Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China; [2]Department of Respiratory Medicine, Allergology and Sleep Medicine, Paracelsus Medical University, General Hospital Nurnberg, Nuremberg, Germany

Contributions: (I) Conception and design: L Yu, R Zhong; (II) Administrative support: H Zhong, R Zhong; (III) Provision of study materials or patients: H Zhong, R Zhong; (IV) Collection and assembly of data: L Yu, J Xu, R Qiao; (V) Data analysis and interpretation: L Yu, WM Brueckl; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Runbo Zhong, MD, PhD. Department of Pulmonary Medicine, Shanghai Chest Hospital, Shanghai Jiao Tong University School of Medicine, Huaihai West Road No. 241, Shanghai 200030, China. Email: tonic_chung@139.com.

Background: The efficacy of immune monotherapy is not satisfactory in patients with advanced, treated non-small cell lung cancer (NSCLC). Combining antiangiogenic agents and immune checkpoint inhibitors (ICIs) can counteract the immunosuppression and confer synergistic therapeutic benefits. We explored the efficacy and safety of anlotinib and ICIs as a second- and subsequent-line treatment for advanced lung adenocarcinoma (LUAD) in patients without oncogenic driver alterations.

Methods: We reviewed patients with driver-negative LUAD who had received anlotinib, a multityrosine kinase inhibitor affecting vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR) and c-Kit, in combination with ICIs from October 2018 to July 2021 at Shanghai Chest Hospital as second- and subsequent-line treatment. Patients with advanced driver-negative LUAD who received nivolumab monotherapy as second-line treatment were included as a control group.

Results: In this study, 71 patients were included who had received anlotinib and programmed cell death-1 (PD-1) blockade combination therapy as second- and subsequent-line treatment, and 63 patients who had received nivolumab monotherapy as second-line therapy were included as controls, most of whom were male smokers at stage IV. The median progression-free survival (PFS) of the combination therapy and nivolumab monotherapy groups were 6.00 and 3.41 months, respectively (P<0.001). The median overall survival (OS) of the combination therapy and nivolumab monotherapy groups were 16.13 and 11.88 months, respectively (P=0.046). Twenty-nine patients (40.8%) in the combination group underwent previous immunotherapy (15 of whom were in first line), and they also achieved good survival (median OS: 25.67 months). The adverse reactions in the combination therapy group were mainly associated with either anlotinib or ICI administration, and there was a low incidence of grade 3 adverse events, all of which were resolved after intervention or discontinuation.

Conclusions: The combination of the multitargeting tyrosine kinase inhibitor anlotinib and PD-1 blockade demonstrated significant benefits as the second- and subsequent-line treatment in driver-negative patients with advanced LUAD, even in those who underwent previous immunotherapy.

Keywords: Non-small cell lung cancer (NSCLC); driver-negative; immunotherapy; antiangiogenics

Submitted Feb 09, 2023. Accepted for publication May 18, 2023. Published online May 24, 2023.
doi: 10.21037/tlcr-23-260
View this article at: https://dx.doi.org/10.21037/tlcr-23-260

Introduction

Lung cancer has become one of the leading causes of cancer-related death worldwide, with an increasing incidence and mortality rate (1). Genotype-directed targeted therapy is the standard of care for patients with advanced non-small cell lung cancer (NSCLC) (2), whereas immunotherapy has improved the treatment options for advanced NSCLC without actionable driver mutations. Antibodies targeting programmed cell death-1/programmed cell death-ligand 1 (PD-1/PD-L1) pathway could facilitate T cell activation and restore immune surveillance, thus significantly reshaping the landscape of tumor clearance. Depending on the PD-L1 expression level, PD-1/PD-L1 inhibitor monotherapy or in combination with chemotherapy in the first-line setting has significantly improved the response rate, prolonging survival with limited adverse effects in advanced driver mutation-negative NSCLC (3-7).

For second-line treatment of advanced NSCLC without oncogenic driver alteration, immune checkpoint inhibitor (ICI) monotherapy is one of the standard treatment options in patients who have not received previous immunotherapy. However, not all patients can benefit from ICI monotherapy, with an overall objective response rate (ORR) of less than 20% and a median overall survival (OS) of 12.0–13.8 months (8-10). To improve the efficacy of immunotherapy in second- and subsequent-line treatment, combination therapy should be considered. Research has shown that antiangiogenic agents promote vascular normalization and immune cell infiltration in the tumor microenvironment, while ICI relieves tumor suppression of immune cells (11). Therefore, combining antiangiogenic agents and ICIs could confer synergistic therapeutic benefits against malignancies, including NSCLC.

Combination strategy of anti-angiogenesis inhibitors have shown promising efficacy in NSCLC. Based on clinical trials REVEL and LUME-Lung 1, the combination of docetaxel with ramucirumab or nintedanib is approved as second-line standard of care in Europe (12,13). Anlotinib is a novel multitargeting tyrosine kinase inhibitor that targets vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), and c-Kit. Due its ability to suppress tumor angiogenesis and proliferation, anlotinib monotherapy was approved by the National Medical Products Administration (NMPA) as a third-line standard-of-care treatment for advanced NSCLC in China (14,15). Combination strategy of anti-angiogenesis inhibitor and ICIs could counteract the immunosuppression, exerting synergistic antitumor effects. The efficacy and safety of anlotinib and ICIs combination therapy has been validated in several solid tumors, including advanced NSCLC as first-line treatment (16-19). This retrospective study aimed to explore the efficacy and safety of anlotinib and ICIs as a second- and subsequent-line treatment for advanced lung adenocarcinoma (LUAD) in patients without oncogenic driver alterations. We present this article in accordance with the STROBE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-260/rc).

Highlight box

Key findings

- Median progression-free survival (PFS) and overall survival (OS) of multitarget angiogenesis inhibitor anlotinib and immune checkpoint inhibitor (ICI) combination therapy in second- and subsequent-line treatment of driver-negative LUAD were 6.00 and 16.13 months, respectively, demonstrating the superior efficacy of this therapy over standard immune monotherapy.

What is known and what is new?

- For second-line treatment of advanced non-small cell lung cancer (NSCLC) without oncogenic driver alteration, ICI monotherapy is one of the standard treatment options. However, not all patients can benefit from ICI monotherapy.
- It has been reported that combining antiangiogenic agents and ICIs could confer synergistic therapeutic benefits against certain malignancies.

What is the implication, and what should change now?

- To improve the efficacy of immunotherapy in second- and subsequent-line treatment, combination therapy, specifically the combination of multitarget angiogenesis inhibitor anlotinib and ICI, should be considered.

Methods

Patients

This study examined patients with driver-negative LUAD

who had received anlotinib in combination with ICI from October 2018 to July 2021 at Shanghai Chest Hospital as second- and subsequent-line treatment. The main inclusion criteria were the following: (I) with cytologically or histologically confirmed LUAD at clinical stage IIIB–IV, (II) driver gene-negative [without druggable genetic alterations in clinical practice, rat sarcoma (*RAS*) mutation and epidermal growth factor receptor (*EGFR*) exon 20 insertion included], and (III) disease progression after prior standard therapy and combination of anlotinib and ICIs as second- or subsequent-line treatment. The main exclusion criteria were patients with sensitive mutations of *EGFR*; fusions of anaplastic lymphoma kinase (*ALK*), c-ros oncogene 1 (*ROS1*), and rearranged during transfection (*RET*); mutations of v-Raf murine sarcoma viral oncogene homolog B (*BRAF*) *V600E*, and human epidermal growth factor receptor 2 (*HER2*); and mesenchymal-epithelial transition (*MET*) amplifications or *MET* exon 14 skipping. In addition, patients with advanced driver-negative LUAD who received nivolumab monotherapy as second-line treatment were included as a control group. Those with incomplete information were excluded (*Figure 1*). The number of cases in the area during the study period determined the sample size.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Shanghai Chest Hospital (No. IS22097) and individual consent for this retrospective analysis was waived.

Assessments

All patients were staged before the administration of the regimen according to the eighth edition of TNM classification. In the course of therapy, the treatment response was evaluated with chest computed tomography (CT) every 2 to 3 months, with additional abdominal ultrasound and cranial magnetic resonance imaging (MRI) and bone emission computed tomography (ECT) being conducted as necessary until disease progression or termination of the therapy or the last follow-up visit, whichever occurred first. Responses to therapy were classified as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) based on the Response Evaluation Criteria in Solid Tumors (RECIST; version 1.1).

The main assessment indicators included the ORR, disease control rate (DCR), progression-free survival (PFS), OS, and adverse events. ORR was defined as the proportion of patients with the best overall response, including CR or PR. DCR was the sum of the CR, PR, and SD rates. PFS was defined as the interval from the beginning treatment to the date of disease progression, treatment plan adjustment, or last follow-up visit, depending on which occurred first. OS was defined as time from start of treatment to death or last follow-up. Adverse events were categorized according to the Medical Dictionary for Regulatory Activities (MedDRA; version 20.0) and graded according to the Common Terminology Criteria for Adverse Events (CTCAE; version 5.0).

The last follow-up visit was on September 14, 2021.

Statistical analysis

SPSS 26.0 (IBM Corp., Armonk, NY, USA) was used for statistical analyses. Descriptive analyses were performed for all demographic, treatment, and response-related data for all patients. The frequencies and percentages were determined for categorical variables. Median and full range were additionally used for the continuous variable of age. χ^2 test was used to investigate significant differences in categorical variables, and the *t*-test was used for continuous variables. The survival curves for PFS and OS were estimated with the Kaplan-Meier method and were compared between the observation and control groups or subgroups using the log-rank test, the results of which are expressed in medians with corresponding 2-sided 95% confidence intervals (95% CIs). Multivariate Cox regression was used to calculate the hazard ratio (HR) and 95% CI to identify significantly different factors related to PFS and OS. Statistical significance was defined as a 2-sided P value <0.05.

Results

Baseline characteristics

This study included 71 patients who received anlotinib and PD-1 blockade combination therapy as second- and subsequent-line treatment in the observation group and 63 patients who received nivolumab monotherapy as second-line therapy in the control group (*Figure 1*). The demographic information and treatment of patients in observation and control groups are shown in *Table 1*.

In the anlotinib and PD-1 blockade combination therapy, the observation group comprised 51 males (71.8%) and 20 females (28.2%). The median age was 63 years

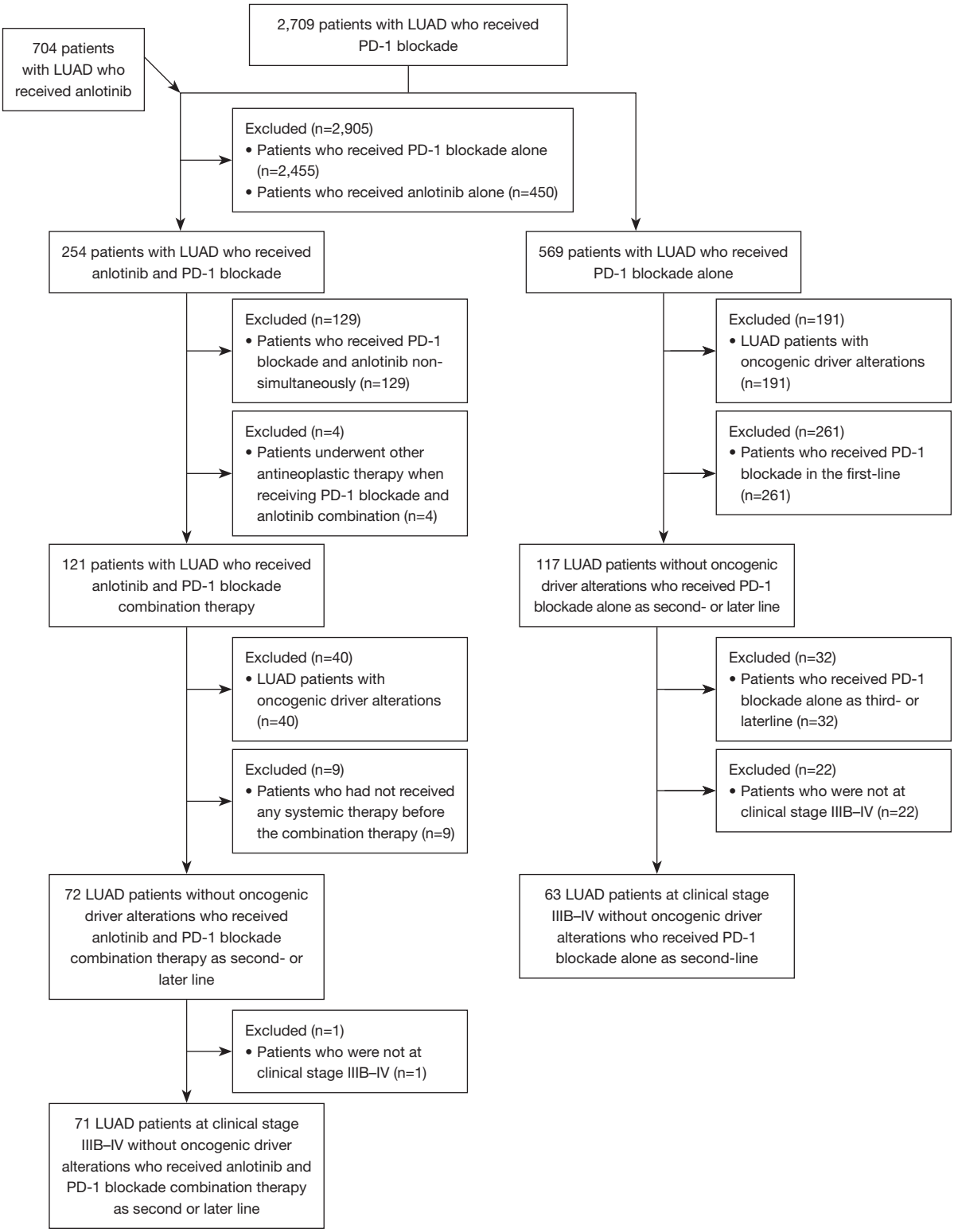

Figure 1 Flowchart of patient screening. LUAD, lung adenocarcinoma; PD-1, programmed cell death-1.

Table 1 Clinical characteristics and treatment of all included patients

Characteristics	Combination therapy (N=71), n (%)	ICI monotherapy (N=63), n (%)	P value
Age (years)			0.755
Median	63	61	
Range	35–80	33–82	
Gender			0.159
Male	51 (71.8)	38 (60.3)	
Female	20 (28.2)	25 (39.7)	
Smoking status			0.557
Never smoked	28 (39.4)	28 (44.4)	
Smoker	43 (60.6)	35 (55.6)	
Clinical T stage			0.899
1	11 (15.5)	7 (11.1)	
2	21 (29.6)	19 (30.2)	
3	12 (16.9)	12 (19.0)	
4	27 (38.0)	25 (39.7)	
Clinical N stage			0.093
0	9 (12.7)	8 (12.7)	
1	1 (1.4)	7 (11.1)	
2	29 (40.8)	25 (39.7)	
3	32 (45.1)	23 (36.5)	
Clinical M stage			0.066
0	5 (7.0)	9 (14.3)	
1a	27 (38.0)	13 (20.6)	
1b	11 (15.5)	17 (27.0)	
1c	28 (39.4)	24 (38.1)	
Clinical stage			0.549
IIIb	4 (5.6)	8 (12.7)	
IIIc	1 (1.4)	1 (1.6)	
IVa	38 (53.5)	30 (47.6)	
IVb	28 (39.4)	24 (38.1)	
PD-L1 TPS			
<1%	12 (16.9)	11 (17.5)	
≥1%, <50%	15 (21.1)	20 (31.7)	
≥50%	7 (9.9)	16 (25.4)	
Unknown	37 (52.1)	16 (25.4)	

Table 1 (*continued*)

Table 1 (*continued*)

Characteristics	Combination therapy (N=71), n (%)	ICI monotherapy (N=63), n (%)	P value
First-line treatment			
Chemotherapy	34 (47.9)	42 (66.7)	
Chemotherapy combined with PD-1 blockade	12 (16.9)	0	
Chemotherapy combined with bevacizumab	22 (31.0)	21 (33.3)	
PD-1 blockade monotherapy	3 (4.2)	0	
Received ICI before therapy			
First-line	15 (21.1)	0	
Second and subsequent-line	14 (19.7)	0	
No	42 (59.2)	63 (100.0)	
PD-1 blockade in the therapy			
Pembrolizumab	33 (46.5)	0	
Nivolumab	38 (53.5)	63 (100.0)	
Treatment line			
Second-line	32 (45.1)	63 (100.0)	
Third- and subsequent-line	39 (54.9)	0	

ICI, immune checkpoint inhibitor; PD-L1, programmed cell death-ligand 1; TPS, tumor proportion score; PD-1, programmed cell death-1.

(range, 35–80 years). In the combination therapy group, 43 of 71 patients (60.6%) were smokers. At the start of the treatment, only 5 patients were classified as c-III, while the rest of the patients were classified as c-IV (n=66). There were 39 patients with distant metastases (M1b-1c), including of the bone (26 cases), brain (13 cases), meninges (2 cases), adrenal gland (7 cases), kidney (1 case), liver (5 cases), and retroperitoneal lymph nodes (3 cases). Before administration of the combination regimen, the PD-L1 tumor proportion score (TPS) of tumor cells by immunohistochemistry (Dako 22C3 pharmDx, North America, Inc., Carpinteria, CA, USA) in 34 patients (47.9%) was assessed with repeat biopsy: 12 patients had a TPS <1%, 15 between 1% and 50%, and 7 ≥50%; PD-L1 expression levels were unknown in the other 37 patients (52.1%).

Oral anlotinib (10 mg/d) was administered from day 1 to day 14 of the 21-day cycle. Intravenous pembrolizumab (200 mg every 3 weeks) and intravenous nivolumab (3 mg/kg every 2 weeks) were administered.

Of the 71 patients in the combination therapy group, 32 (45.1%), 26 (36.6%), and 13 (18.3%) had received this combination regimen as second-, third-, and fourth-line-or-later treatment, respectively. In addition, 29 patients (40.8%) had received immunotherapy before administration of anlotinib and PD-1 inhibitor combination regimen. In addition, 34 patients (47.8%) had previously received bevacizumab for antiangiogenesis.

Treatment response

The median follow-up of all the patients studied was 10.05 months (95% CI: 6.65–13.44 months). The ORR of the combination therapy and nivolumab monotherapy groups were 7.0% and 3.2% (P=0.447), respectively. The DCR of the combination therapy group was significantly higher than that in the nivolumab monotherapy group (81.7% *vs.* 57.1%; P=0.002). The details of treatment efficacy are shown in *Table 2*.

In the combination therapy group, 39 patients (54.9%) discontinued the regimen, with 32 cases discontinuing due to disease progression and 7 due to adverse reactions (*Table 3*). The median PFS was 6.00 months (95% CI: 4.34–7.66 months), and the 6- and 12-month PFS rates were 49.9% and 32.2%, respectively. In the ICI monotherapy group, 58 patients (92.1%) discontinued the regimen and the median PFS was 3.41 months (95% CI: 2.16–4.67 months), with 6- and 12-month PFS rates of 27.4% and 12.0%, respectively. The P value in median PFS between the 2 groups was less

Table 2 Treatment responses to the combination therapy and nivolumab monotherapy groups

Treatment response	Combination therapy (N=71), n (%)	ICI monotherapy (N=63), n (%)	P value
CR	0	0	
PR	5 (7.0)	2 (3.2)	
SD	53 (74.6)	34 (54.0)	
PD	13 (18.3)	27 (42.9)	
ORR	5 (7.0)	2 (3.2)	0.447
DCR	58 (81.7)	36 (57.1)	0.002

ICI, immune checkpoint inhibitor; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate.

Table 3 Adverse events leading to the discontinuation of the anlotinib and PD-1 blockade combination regimen

Patient number	Adverse events leading to discontinuation of the regimen	Grade
1	Pulmonary embolism (considered anlotinib-related)	3
2	Cerebral infarction (considered anlotinib- or ICI-related)	3
3	Immune encephalitis (considered ICI-related)	2
4	Immune pneumonia (considered ICI-related)	2
5	Immune pneumonia (considered ICI-related)	3
6	Immune pneumonia (considered ICI-related)	3
7	Hematochezia (considered anlotinib-related)	3

PD-1, programmed cell death-1; ICI, immune checkpoint inhibitor.

than 0.001 (*Figure 2A*).

In this study, a total of 60 patients died: 25 of 71 patients (35.2%) in the observation group and 35 of 63 patients (55.6%) in the control group. The median OS of the combination therapy and nivolumab monotherapy groups were 16.13 months (95% CI: 10.48–21.79 months) and 11.88 months (95% CI: 8.88–14.88 months), respectively, with a P value of 0.046. The OS rates of the 2 groups were 60.2% and 47.5% at 12 months, 38.2% and 10.2% at 24 months, respectively (*Figure 2B*).

In the combination therapy group, 7 patients discontinued combination therapy due to adverse reactions (*Table 3*). Furthermore, 5 patients developed oral ulcers, gingivitis, immune pneumonia, and liver function abnormalities during treatment, all of which were grade 1–2 and manageable.

Subgroup analysis of the anlotinib and PD-1 blockade combination

In the anlotinib and PD-1 blockade combination therapy group (the observation group), the median PFS and OS of PD-L1-positive patients (TPS ≥1%) were 8.67 months (95% CI: 1.53–15.81 months) and 21.83 months (95% CI: 8.07–35.60 months), respectively. The median PFS and OS of PD-L1-negative (TPS <1%) patients or with unknown TPS were 5.20 months (95% CI: 3.40–7.01 months) and 14.60 months (95% CI: 9.98–19.22 months), respectively. The median OS of patients with PD-L1-positive status was longer than those with PD-L1-negative or unknown status (21.83 *vs.* 14.60 months). However, the difference was not statistically significant (P=0.769).

The median PFS of patients with brain metastases was 3.67 months (95% CI: 1.37–5.97 months), and the median OS was 5.33 months (95% CI: 3.42–7.25 months); meanwhile, the median PFS of patients without brain metastases was 6.80 months (95% CI: 3.52–10.08 months), and the median OS was 16.47 months (95% CI: 7.72–25.22 months). The median OS of patients with brain metastases was significantly shorter than that in those without brain metastases (5.33 *vs.* 16.47 months; P=0.007).

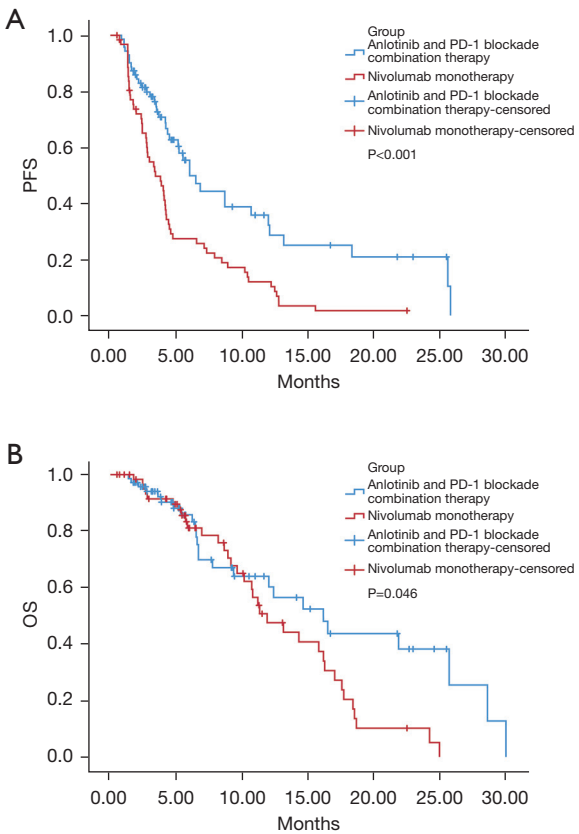

Figure 2 Kaplan-Meier curves for progression-free survival (A) and overall survival (B). PFS, progression-free survival; PD-1, programmed cell death-1; OS, overall survival.

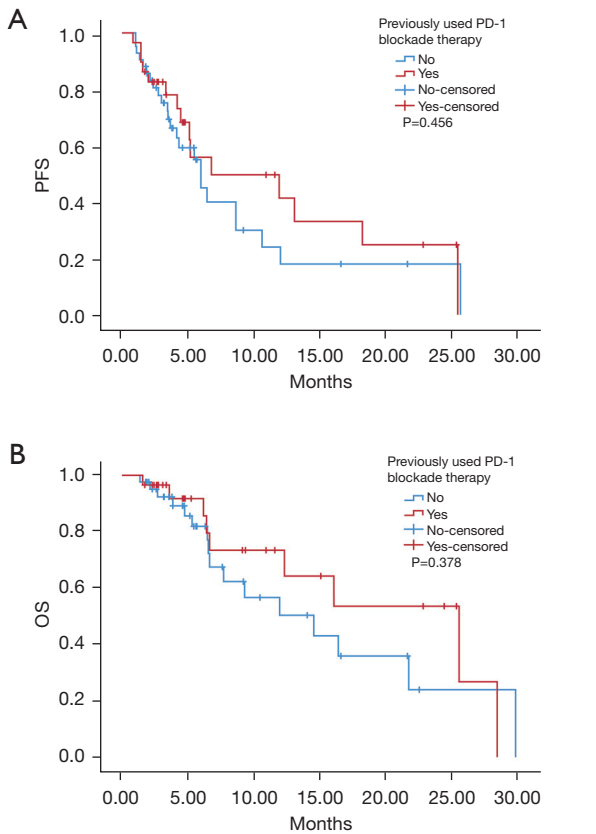

Figure 3 Kaplan-Meier curves for progression-free survival (A) and overall survival (B) of the combination therapy group who underwent previous immunotherapy or not. PFS, progression-free survival; PD-1, programmed cell death-1; OS, overall survival.

Sixteen patients (22.5%) developed cavities in their lung lesions during treatment, with a median PFS of 8.67 months (95% CI: 2.34–15.00 months) and a median OS of 14.60 months (95% CI: 6.16–23.05 months). In patients without tumor cavitation, the median PFS was 6.00 months (95% CI: 4.09–7.91 months), and the median OS was 16.47 months (95% CI: 1.60–31.34 months). The median OS did not differ significantly between those patients who did and did not develop cavities (P=0.564).

For patients who had undergone immunotherapy before initiating the combination therapy (29 patients, 40.8%), the median PFS was 6.80 months (95% CI: 0–17.33 months), and the OS was 25.67 months (95% CI: 11.59–39.75 months). In contrast, in patients who were not previously exposed to immunotherapy (42 patients, 59.2%), the median PFS was 6 months (95% CI: 4.79–7.21 months), and the median OS was 14.60 months (95% CI: 5.69–23.51 months). Patients who underwent previous immunotherapy could achieve good survival that was not inferior to the survival of those without previous immunotherapy (25.67 *vs.* 14.60 months; P=0.378) (*Figure 3*).

For patients who had undergone antiangiogenic therapy before initiating the combination therapy (34 patients, 47.8%), the median PFS was 5.50 months (95% CI: 4.06–6.94 months), and the median OS was 9.33 months (95% CI: 2.97–15.70 months). The median PFS and OS of patients who were not previously exposed to antiangiogenic therapy (37 patients, 52.2%) were 10.67 months (95% CI: 4.28–17.05 months) and 16.47 months (95% CI: 13.68–19.26 months), respectively. Patients who had undergone previous antiangiogenic therapy did not have a better median OS than did those who had not undergone this therapy (P=0.462).

Twenty patients were found to have RAS mutations and EGFR insertion mutations when they were first diagnosed including 2 cases with *EGFR* exon 20 insertion and 18 cases

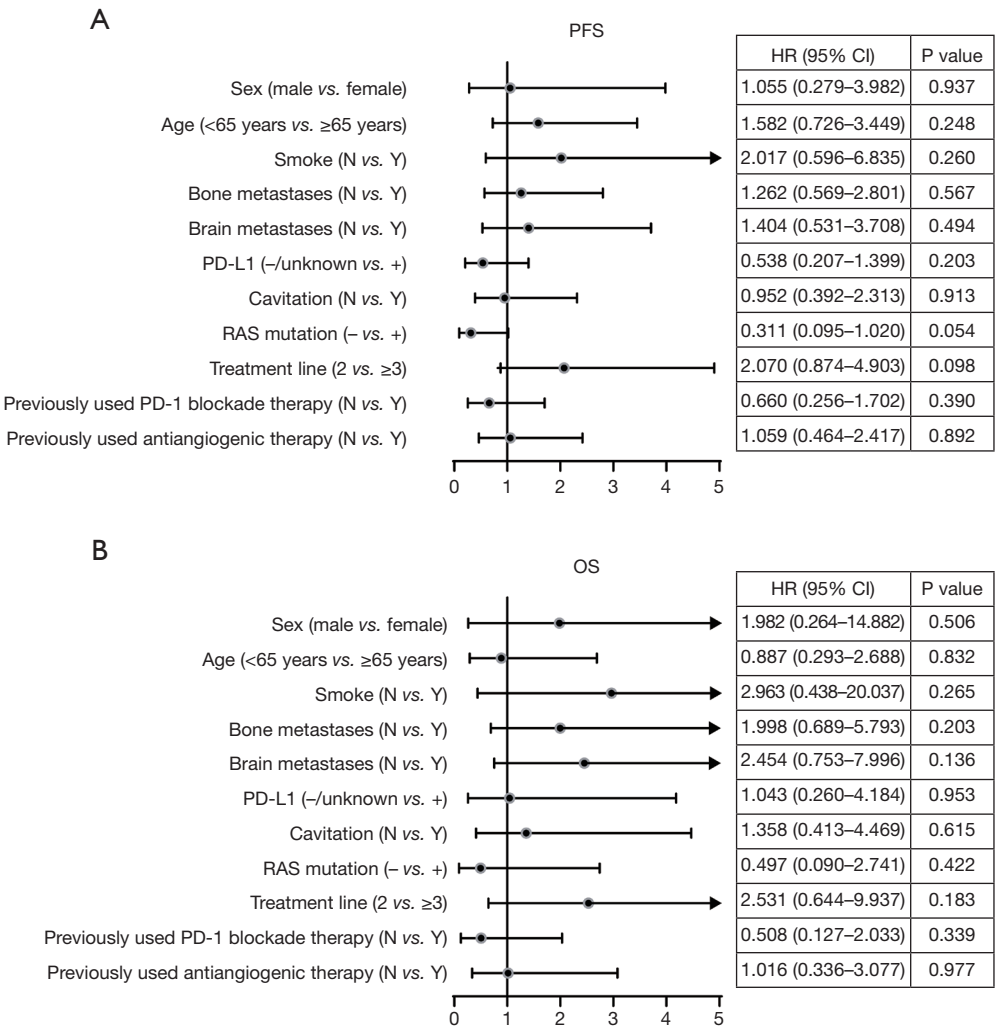

Figure 4 Subgroup analysis of progression-free survival (A) and overall survival (B) in the combination therapy group. PFS, progression-free survival; PD-L1, programmed cell death-ligand 1; RAS, rat sarcoma; PD-1, programmed cell death-1; OS, overall survival; HR, hazard ratio; CI, confidence interval; N, no; Y, yes.

with *RAS* mutations [including 15 cases of Kirsten-RAS (*KRAS*) and 3 cases of neuroblastoma-RAS (*NRAS*)] (Table S1). None of these 20 patients received molecular-targeted drugs before the combination therapy. The median PFS of patients with mutated *RAS* was 10.67 months (95% CI: not achieved), and the median PFS for patients with nonmutated *RAS* was 5.50 months (95% CI: 3.86–7.14 months); the median OS were 14.60 months (95% CI: 2.99–26.21 months) and 16.13 months (95% CI: 10.12–22.15 months), respectively. The OS between patients who harbored RAS or those who did not was not statistically significantly different (P=0.412).

The multivariate Cox regression related to the PFS and OS of the observation group is shown in *Figure 4*. Univariate analyses of the observation subgroup data related to the PFS and OS are shown in Table S2 and Table S3.

Discussion

This study investigated the survival of 71 patients with advanced driver gene–negative LUAD, revealing that patients treated with anlotinib and ICI combination therapy as second- or subsequent-line treatment (45.1% of them in the second-line and 54.9% in the third-line and beyond) achieved favorable clinical outcomes (DCR 81.7%, median PFS 6.00 months, median OS 16.13 months), irrespective of previous immunotherapy status. The combination showed

an advantage over the second-line immune monotherapy. No new adverse events were observed.

The current standard first-line treatment of patients with driver-negative advanced LUAD is immunotherapy or chemotherapy combined with immunotherapy, depending on the PD-L1 expression level. If first-line therapy is insufficient, pemetrexed or docetaxel monotherapy is the most common second-line treatment modality, with a relatively limited benefit (median PFS 2.8–3.5 months, median OS 6–9.6 months) (8,10,20,21). For patients receiving chemotherapy alone in the first line, single-agent immunotherapy in the second line may prolong survival compared to docetaxel. However, only a subset of patients benefits from immune monotherapy. This study thus investigated the ability of second- and later-line therapies to prolong patient survival.

Anti-angiogenesis treatment has evolved to achieve encouraging results in second-line therapy for NSCLC. The combination of ramucirumab and docetaxel could increase the efficacy compared with docetaxel alone in the second-line of late stage NSCLC (median OS 10.5 *vs.* 9.1 months, P=0.023) (12). The combination of nintedanib and docetaxel also showed superior efficacy over docetaxel monotherapy in second-line treatment of advanced NSCLC (median OS 10.1 *vs.* 9.1 months, P=0.2720), especially in LUAD (median OS 12.6 *vs.* 10.3 months, P=0.0359) (13). Relevant clinical outcomes reported are shown in Table S4. And in the ALTER0303 trial, multitargeting antiangiogenic agent anlotinib monotherapy was shown to benefit patients with advanced NSCLC, with a median PFS of 5.4 months (95% CI: 4.4–5.6 months) and a median OS of 9.6 months (95% CI: 8.2–10.6 months); with driver-positive patients (*EGFR*-sensitive mutations, *ALK* fusions) being excluded, the median OS was 8.9 months (95% CI: 4.7–15.6 months) for driver-negative patients (22).

Multitargeting antiangiogenic drugs may have additional functions worth exploring, such as significant synergistic therapeutic effects in combination with ICIs. A growing body of evidence indicates there to be a complex association between tumor angiogenesis and the tumor immune microenvironment. In the tumor microenvironment, the number of mature dendritic cells (DCs) is reduced, antigen-presenting functions are impaired, and the activation of T cells is inhibited through VEGF secreted by tumor cells. Moreover, the high level of VEGF can increase the number and proliferation of immunosuppressive cells, such as regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and M2-like tumor-associated macrophages (TAMs) (23). Anlotinib, a novel multitarget tyrosine kinase receptor inhibitor, significantly inhibits VEGFR, FGFR, PDGFR, and c-Kit, acting on the tumor microenvironment and the tumor itself. Anlotinib promotes infiltration of the innate immune cells and increases the number of DCs, increases tumor antigen presentation, decreases the percentage of M2-like TAMs, and induces the conversion of TAM to the M1 type (24). Furthermore, anlotinib promotes tumor vascular normalization, partly relying on $CD4^+$ T cells, thus alleviating immunosuppression in the tumor microenvironment (25). ICIs can reduce the activity of immunosuppressive cells and indirectly downregulate angiogenic factors (26). A combination of antiangiogenic drug with ICIs counteracts the immunosuppression caused by the upregulation of PD-L1, resulting in the prolongation of normalization of tumor vasculature. The incorporation of antiangiogenic drug into immunotherapy enhances the efficacy of both agents and converts the immune suppressive tumor microenvironment into a more immune permissive one (25). The efficacy and safety of anti-angiogenesis inhibitor combined with ICIs has been validated in clinical trials.

In the IMpower 150 trial, the combination of ICIs, bevacizumab, and chemotherapy showed an advantage as a first-line treatment for nonsquamous NSCLC (27). Some studies have found good results with the combination of anlotinib and ICIs in a variety of tumors, including lung cancer (16,19,28,29). Our study found superior efficacy of anlotinib and PD-1 blockade combination therapy as second- and subsequent-line treatment of patients with advanced driver-negative LUAD, a subset of patients that few studies have focused on.

Although immunotherapy has revolutionized the treatment of advanced NSCLC in patients without oncogenic driver alterations, the management of acquired resistance to immunotherapy is challenging. According to a randomized phase II Lung-MAP nonmatch substudy (S1800A), combination of ramucirumab and pembrolizumab demonstrated significantly improved OS (median 14.5 months) in patients with advanced NSCLC previously treated with ICIs and chemotherapy (30). Also, in our study, patients who had undergone previous immunotherapy received the combination of ICI and anlotinib as second- and subsequent-line therapy and benefited from superior efficacy of the treatment. This suggests that in immunotherapy-resistant patients, the addition of antiangiogenic therapy may restore immunotherapy sensitivity through potential mechanisms such as the normalization of tumor vasculature

and reduction of suppressive immune cells. However, the underlying mechanisms remain obscure. The combination of antiangiogenic therapy and immunotherapy may be a treatment option for immunotherapy rechallenge. Furthermore, patients in the combination group who underwent previous immunotherapy achieved good survival that was not inferior to that in patients without previous therapy. This indicates the importance of frontline use of ICIs and suggests that in patients who can benefit from immunotherapy, the addition of antiangiogenic agents may maximize the effects of immunotherapy, even after immunotherapy resistance occurs.

KRAS is one of the most common oncogenic drivers in NSCLC, occurring in approximately 30% of patients with LUAD (31). Sotorasib was evaluated for second-line use in patients with metastatic NSCLC and the *KRAS* G12C mutation (32), but the median PFS was only 6.3 months (33). Combination of docetaxel and nintedanib or ramucirumab did not confer significant benefits in patients with *KRAS* mutations in second-line after first□line immunochemotherapy (34,35) (Table S4). *KRAS* mutations are thought to be closely related to cigarette smoking (36) and are associated with higher PD-L1 expression in NSCLC through the activation of the downstream MEK signaling pathway (37,38). Previous studies have found that patients with *KRAS*-driven LUAD may benefit from immunotherapy (39,40). Patients with *KRAS* or *NRAS* mutations in this study who received anlotinib and PD-1 blockade combination therapy had good clinical outcomes (median PFS 10.67 months, median OS 14.60 months), and 44.4% of whom had received previous immunotherapy. Even as second- and subsequent-line treatment, immunotherapy combined with antiangiogenic therapy provided better benefit to patients with *KRAS*-mutant LUAD.

Bevacizumab plus chemotherapy was once a standard-of-care treatment in driver-negative advanced LUAD, but the AvaALL trial reported that the continuous use of bevacizumab after progression provided no improvement in survival (41). Our study found the effect of anlotinib and PD-1 blockade combination therapy was not satisfactory in patients who had undergone previous antiangiogenic therapy (median PFS 5.50 months, median OS 9.33 months). Anlotinib is a VEGF pathway inhibitor that acts on its receptor, VEGFR, in a manner similar to the action of bevacizumab (competing with the VEGF-A factor to block its binding to VEGFR). The result further confirmed that the use of antivascular drugs beyond disease progression is less effective.

The expression of PD-L1 in NSCLC is important in selecting patients to treat with ICIs, not only in late stage but also in neoadjuvant treatment (3-6,42). In this study, patients with PD-L1 positive appeared to benefit more from combination therapy than patients with PD-L1-negative or unknown (21.83 *vs.* 14.60 months), but this was not statistically supported. In addition, this combination did not demonstrate a benefit in survival for patients who had brain metastases or patients with lesion cavitation. This suggests that biomarkers are needed to better define populations who will benefit the most from this combination treatment.

The adverse reactions in the combination therapy group were essentially associated with either anlotinib or ICI use, with a low incidence of grade 3 adverse events, all of which were resolved after intervention or discontinuation. This is in accordance with the conclusions reported in previous studies: the combination of antiangiogenic agents and ICIs did not lead to an increase in side reactions (43,44).

Combinations of anti-PD-1/PD-L1 antibodies with other therapeutic options are being explored as potentially synergistic therapeutic strategies, such as the combination of lag-3 monoclonal antibodies and PD-1/PD-L1 inhibitors, which suggests that the "chemotherapy-free" approach may be feasible.

The shortfall of our study is its retrospective nature and, as a result, subject to some inherent selection bias, which needs to be taken into account when interpreting the results. Some patients in the observation and control groups had not died at the last follow-up, contributing to the immaturity of OS; moreover, there was some degree of heterogeneity in the observation group because some patients received combination therapy in third-line or beyond and some patients received previous immunotherapy. Moreover, biomarkers for this combination therapy still need to be identified.

Conclusions

In summary, the combination of the multitargeting tyrosine kinase inhibitor anlotinib and PD-1 blockade demonstrated significant benefits in the second- and subsequent-line treatment of driver-negative patients with advanced LUAD. This combination warrants further clinical verification.

Acknowledgments

We would like to thank all of the investigators for

their involvement in this study. The authors appreciate the academic support from the AME Lung Cancer Collaborative Group.
Funding: This work was supported by the Science and Technology Innovation Program of Shanghai (No. 20Y11913800) and the Nurture Projects for Basic Research of Shanghai Chest Hospital (No. 2020YNJCM04). The funders did not participate in the study design, data collection, analysis, decision to publish, or preparation of the manuscript.

Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-260/rc

Data Sharing Statement: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-260/dss

Peer Review File: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-260/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-260/coif). WMB received honoraria for lectures or educational events from AstraZeneca, Boehringer, Novartis, MSD, BMS, Lilly, Pfizer and Roche; support for attending meetings and/or travel from Boehringer, Roche Pharma and AstraZeneca; received equipment, material, drugs, medical writing, gifts or other services from Boehringer for medical writing; served on advisory board of Astra Zeneca, Boehringer, Novartis, MSD, Lilly Pharma, BMS, and Roche, and patent application EP21183549.1 (method for predicting a clinical response towards an immune checkpoint inhibitor based on pretreatment therewith) was filed with regard to the results of this study. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Shanghai Chest Hospital (No. IS22097) and individual consent for this retrospective analysis was waived.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209-49.
2. Kris MG, Johnson BE, Berry LD, et al. Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. JAMA 2014;311:1998-2006.
3. Mok TSK, Wu YL, Kudaba I, 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;393:1819-30.
4. Herbst RS, Giaccone G, de Marinis F, et al. Atezolizumab for First-Line Treatment of PD-L1-Selected Patients with NSCLC. N Engl J Med 2020;383:1328-39.
5. Gettinger S, Rizvi NA, Chow LQ, et al. Nivolumab Monotherapy for First-Line Treatment of Advanced Non-Small-Cell Lung Cancer. J Clin Oncol 2016;34:2980-7.
6. Yang Y, Sun J, Wang Z, et al. Updated Overall Survival Data and Predictive Biomarkers of Sintilimab Plus Pemetrexed and Platinum as First-Line Treatment for Locally Advanced or Metastatic Nonsquamous NSCLC in the Phase 3 ORIENT-11 Study. J Thorac Oncol 2021;16:2109-20.
7. Liang J, Li M, Sui Q, et al. Compare the efficacy and safety of programmed cell death-1 (PD-1) and programmed cell death ligand-1 (PD-L1) inhibitors for advanced non-small cell lung cancer: a Bayesian analysis. Transl Lung Cancer Res 2020;9:1302-23.
8. Wu YL, Lu S, Cheng Y, et al. Nivolumab Versus Docetaxel in a Predominantly Chinese Patient Population With Previously Treated Advanced NSCLC: CheckMate 078 Randomized Phase III Clinical Trial. J Thorac Oncol 2019;14:867-75.

9. Rittmeyer A, Barlesi F, Waterkamp D, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. Lancet 2017;389:255-65.
10. Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet 2016;387:1540-50.
11. Huinen ZR, Huijbers EJM, van Beijnum JR, et al. Anti-angiogenic agents - overcoming tumour endothelial cell anergy and improving immunotherapy outcomes. Nat Rev Clin Oncol 2021;18:527-40.
12. Garon EB, Ciuleanu TE, Arrieta O, et al. Ramucirumab plus docetaxel versus placebo plus docetaxel for second-line treatment of stage IV non-small-cell lung cancer after disease progression on platinum-based therapy (REVEL): a multicentre, double-blind, randomised phase 3 trial. Lancet 2014;384:665-73.
13. Reck M, Kaiser R, Mellemgaard A, et al. Docetaxel plus nintedanib versus docetaxel plus placebo in patients with previously treated non-small-cell lung cancer (LUME-Lung 1): a phase 3, double-blind, randomised controlled trial. Lancet Oncol 2014;15:143-55.
14. Shen G, Zheng F, Ren D, et al. Anlotinib: a novel multi-targeting tyrosine kinase inhibitor in clinical development. J Hematol Oncol 2018;11:120.
15. Syed YY. Anlotinib: First Global Approval. Drugs 2018;78:1057-62.
16. Chu T, Zhong R, Zhong H, et al. Phase 1b Study of Sintilimab Plus Anlotinib as First-line Therapy in Patients With Advanced NSCLC. J Thorac Oncol 2021;16:643-52.
17. Han C, Ye S, Hu C, et al. Clinical Activity and Safety of Penpulimab (Anti-PD-1) With Anlotinib as First-Line Therapy for Unresectable Hepatocellular Carcinoma: An Open-Label, Multicenter, Phase Ib/II Trial (AK105-203). Front Oncol 2021;11:684867.
18. Yuan M, Zhu Z, Mao W, et al. Anlotinib Combined With Anti-PD-1 Antibodies Therapy in Patients With Advanced Refractory Solid Tumors: A Single-Center, Observational, Prospective Study. Front Oncol 2021;11:683502.
19. Yu L, Hu Y, Xu J, et al. Multi-target angiogenesis inhibitor combined with PD-1 inhibitors may benefit advanced non-small cell lung cancer patients in late line after failure of EGFR-TKI therapy. Int J Cancer 2023. [Epub ahead of print]. doi: 10.1002/ijc.34536.
20. Borghaei H, Gettinger S, Vokes EE, et al. Five-Year Outcomes From the Randomized, Phase III Trials CheckMate 017 and 057: Nivolumab Versus Docetaxel in Previously Treated Non-Small-Cell Lung Cancer. J Clin Oncol 2021;39:723-33.
21. Herbst RS, Garon EB, Kim DW, et al. Long-Term Outcomes and Retreatment Among Patients With Previously Treated, Programmed Death-Ligand 1–Positive, Advanced Non–Small-Cell Lung Cancer in the KEYNOTE-010 Study. J Clin Oncol 2020;38:1580-90.
22. Han B, Li K, Wang Q, 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;4:1569-75.
23. Yang J, Yan J, Liu B. Targeting VEGF/VEGFR to Modulate Antitumor Immunity. Front Immunol 2018;9:978.
24. Yang Y, Li L, Jiang Z, et al. Anlotinib optimizes anti-tumor innate immunity to potentiate the therapeutic effect of PD-1 blockade in lung cancer. Cancer Immunol Immunother 2020;69:2523-32.
25. Su Y, Luo B, Lu Y, et al. Anlotinib Induces a T Cell-Inflamed Tumor Microenvironment by Facilitating Vessel Normalization and Enhances the Efficacy of PD-1 Checkpoint Blockade in Neuroblastoma. Clin Cancer Res 2022;28:793-809.
26. Rahma OE, Hodi FS. The Intersection between Tumor Angiogenesis and Immune Suppression. Clin Cancer Res 2019;25:5449-57.
27. Halmos B, Burke T, Kalyvas C, et al. Pembrolizumab+chemotherapy versus atezolizumab+chemotherapy+/-bevacizumab for the first-line treatment of non-squamous NSCLC: A matching-adjusted indirect comparison. Lung Cancer 2021;155:175-82.
28. Zhou N, Jiang M, Li T, et al. Anlotinib combined with anti-PD-1 antibody, camrelizumab for advanced NSCLCs after multiple lines treatment: An open-label, dose escalation and expansion study. Lung Cancer 2021;160:111-7.
29. Zhang X, Zeng L, Li Y, 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;70:2517-28.
30. Reckamp KL, Redman MW, Dragnev KH, et al. Phase II Randomized Study of Ramucirumab and Pembrolizumab Versus Standard of Care in Advanced Non-Small-Cell Lung Cancer Previously Treated With Immunotherapy-Lung-MAP S1800A. J Clin Oncol 2022;40:2295-306.

31. Veluswamy R, Mack PC, Houldsworth J, et al. KRAS G12C-Mutant Non-Small Cell Lung Cancer: Biology, Developmental Therapeutics, and Molecular Testing. J Mol Diagn 2021;23:507-20.
32. Nakajima EC, Drezner N, Li X, et al. FDA Approval Summary: Sotorasib for KRAS G12C-Mutated Metastatic NSCLC. Clin Cancer Res 2022;28:1482-6.
33. Hong DS, Fakih MG, Strickler JH, et al. KRAS(G12C) Inhibition with Sotorasib in Advanced Solid Tumors. N Engl J Med 2020;383:1207-17.
34. Grohé C, Wehler T, Dechow T, et al. Nintedanib plus docetaxel after progression on first-line immunochemotherapy in patients with lung adenocarcinoma: Cohort C of the non-interventional study, VARGADO. Transl Lung Cancer Res 2022;11:2010-21.
35. Brueckl WM, Reck M, Rittmeyer A, et al. Efficacy of docetaxel plus ramucirumab as palliative second-line therapy following first-line chemotherapy plus immune-checkpoint-inhibitor combination treatment in patients with non-small cell lung cancer (NSCLC) UICC stage IV. Transl Lung Cancer Res 2021;10:3093-105.
36. Chapman AM, Sun KY, Ruestow P, et al. Lung cancer mutation profile of EGFR, ALK, and KRAS: Meta-analysis and comparison of never and ever smokers. Lung Cancer 2016;102:122-34.
37. Coelho MA, de Carné Trécesson S, Rana S, et al. Oncogenic RAS Signaling Promotes Tumor Immunoresistance by Stabilizing PD-L1 mRNA. Immunity 2017;47:1083-1099.e6.
38. Chen N, Fang W, Lin Z, et al. KRAS mutation-induced upregulation of PD-L1 mediates immune escape in human lung adenocarcinoma. Cancer Immunol Immunother 2017;66:1175-87.
39. Dong ZY, Zhong WZ, Zhang XC, et al. Potential Predictive Value of TP53 and KRAS Mutation Status for Response to PD-1 Blockade Immunotherapy in Lung Adenocarcinoma. Clin Cancer Res 2017;23:3012-24.
40. Cinausero M, Laprovitera N, De Maglio G, et al. KRAS and ERBB-family genetic alterations affect response to PD-1 inhibitors in metastatic nonsquamous NSCLC. Ther Adv Med Oncol 2019;11:1758835919885540.
41. Gridelli C, de Castro Carpeno J, Dingemans AC, et al. Safety and Efficacy of Bevacizumab Plus Standard-of-Care Treatment Beyond Disease Progression in Patients With Advanced Non-Small Cell Lung Cancer: The AvaALL Randomized Clinical Trial. JAMA Oncol 2018;4:e183486.
42. Forde PM, Spicer J, Lu S, et al. Neoadjuvant Nivolumab plus Chemotherapy in Resectable Lung Cancer. N Engl J Med 2022;386:1973-85.
43. Fukumura D, Kloepper J, Amoozgar Z, et al. Enhancing cancer immunotherapy using antiangiogenics: opportunities and challenges. Nat Rev Clin Oncol 2018;15:325-40.
44. Manegold C, Dingemans AC, Gray JE, et al. The Potential of Combined Immunotherapy and Antiangiogenesis for the Synergistic Treatment of Advanced NSCLC. J Thorac Oncol 2017;12:194-207.

(English Language Editor: J. Gray)

Cite this article as: Yu L, Xu J, Qiao R, Zhong H, Brueckl WM, Zhong R. Comparative efficacy and safety of multitarget angiogenesis inhibitor combined with immune checkpoint inhibitor and nivolumab monotherapy as second-line or beyond for advanced lung adenocarcinoma in driver-negative patients: a retrospective comparative cohort study. Transl Lung Cancer Res 2023;12(5):1108-1121. doi: 10.21037/tlcr-23-260

Supplementary

Table S1 EGFR and RAS mutations in the combination therapy group

Patient number	Mutations
1	NRAS Q61K
2	KRAS G12C
3	NRAS Q61H
4	KRAS G12V
5	KRAS G12L
6	KRAS G12C, copy number amplification
7	EGFR 20ins
8	KRAS G12V
9	KRAS G12C
10	KRAS G12A
11	KRAS G12V
12	KRAS G13D
13	KRAS G12A
14	KRAS G12A
15	NRAS Q61L
16	KRAS G12C
17	KRAS G12V
18	KRAS 2G12S
19	EGFR 20ins
20	KRAS Q61H

EGFR, epidermal growth factor receptor; RAS, rat sarcoma; KRAS, Kirsten-RAS; NRAS, neuroblastoma-RAS.

https://dx.doi.org/10.21037/tlcr-23-260

Table S2 Univariate analyses of the observation subgroup data related to progression-free survival

Characteristic	DCR (%)	Median PFS (months) (95% CI)	P value
Sex (male *vs.* female)	80.4 *vs.* 85.0	6.47 (4.73-8.20) *vs.* 6.00 (3.01-8.99)	0.920
Age (<65 years *vs.* ≥65 years)	76.3 *vs.* 87.9	5.50 (3.91-7.09) *vs.* 8.67 (2.63-14.71)	0.888
Smoke (No *vs.* Yes)	85.7 *vs.* 79.1	6.80 (0-13.73) *vs.* 6.00 (4.34-7.66)	0.561
Bone metastases (No *vs.* Yes)	86.7 *vs.* 73.1	8.67 (5.02-12.31) *vs.* 5.20 (3.89-6.51)	0.191
Brain metastases (No *vs.* Yes)	82.8 *vs.* 76.9	6.80 (3.52-10.08) *vs.* 3.67 (1.37-5.97)	0.259
PD-L1(-/unknown *vs.* +)	77.6 *vs.* 90.9	5.20 (3.40-7.01) *vs.* 8.67 (1.53-15.81)	0.337
Cavitation (No *vs.* Yes)	79.6 *vs.* 87.5	6.00 (4.09-7.91) *vs.* 8.67 (2.34-15.00)	0.683
RAS mutation (- *vs.* +)	77.4 *vs.* 94.4	5.50 (3.86-7.14) *vs.* 10.67 (not achieved)	0.082
Treatment line (2 *vs.* ≥ 3)	84.4 *vs.* 79.5	10.67 (3.95-17.38) *vs.* 5.13 (3.45-6.82)	0.077
Previously used PD-1 blockade therapy (No *vs.* Yes)	78.6 *vs.* 86.2	6.00 (4.79-7.21) *vs.* 6.80 (0-17.33)	0.456
Previously used antiangiogenic therapy (No *vs.* Yes)	86.5 *vs.* 76.5	10.67 (4.28-17.05) *vs.* 5.50 (4.06-6.94)	0.171

DCR, disease control rate; PFS, progression-free survival; 95%CI, 95% confidence interval; PD-L1, programmed cell death-ligand 1; RAS, rat sarcoma; PD-1, programmed cell death-1.

Table S3 Univariate analyses related to overall survival of the combination therapy group

Characteristic	Median OS (months) (95% CI)	P value
Sex (male *vs.* female)	14.60 (8.23-20.98) *vs.* 16.47 (6.44-26.50)	0.671
Age (<65 years *vs.* ≥65 years)	12.37 (4.92-19.81) *vs.* 25.67 (12.25-39.09)	0.105
Smoke (No *vs.* Yes)	16.47 (5.25-27.69) *vs.* 14.60 (4.42-24.78)	0.220
Bone metastases (No *vs.* Yes)	21.83 (12.61-31.06) *vs.* 12.00 (3.64-20.36)	0.052
Brain metastases (No *vs.* Yes)	16.47 (7.72-25.22) *vs.* 5.33 (3.42-7.25)	0.007
PD-L1(-/unknown *vs.* +)	14.60 (9.98-19.22) *vs.* 21.83 (8.07-35.60)	0.769
Cavitation (No *vs.* Yes)	16.47 (1.60–31.34) *vs.* 14.60 (6.16–23.05)	0.564
RAS mutation (- *vs.* +)	16.13 (10.12-22.15) *vs.* 14.60 (2.99-26.21)	0.412
Treatment line (2 *vs.* ≥ 3)	21.83 (12.17-31.49) *vs.* 12.37 (4.88-19.86)	0.077
Previously used PD-1 blockade therapy (No *vs.* Yes)	14.60 (5.69-23.51) *vs.* 25.67 (11.59-39.75)	0.378
Previously used antiangiogenic therapy (No *vs.* Yes)	16.47 (13.68-19.26) *vs.* 9.33 (2.97-15.70)	0.462

OS, overall survival; 95% CI, 95% confidence interval; PD-L1, programmed cell death-ligand 1; RAS, rat sarcoma; PD-1, programmed cell death-1.

https://dx.doi.org/10.21037/tlcr-23-260

Table S4 Relevant clinical outcomes reported in the literatures and our study

Study	Number of patients	PFS	OS
Yu L *et al.* (this study, advanced recurrent driver-negative LUAD, second-line and later)	anlotinib+PD-1 inhibitor (n=71) vs nivolumab (n=63)	median 6.00 months (95%CI 4.34-7.66) (ORR 7.0%, DCR 81.7%) vs 3.41 months (95%CI 2.16-4.67), P<0.001; patients with and without RAS mutations: median 10.67 months (95%CI not achieved) vs 5.50 months (95%CI 3.86-7.14), HR 0.444 (95%CI 0.172-1.143), P=0.082	median 16.13 months (95%CI 10.48-21.79) vs 11.88 months (95%CI 8.88-14.88), P=0.046; patients with and without RAS mutations: median 14.60 months (95%CI 2.99-26.21) vs 16.13 months (95%CI 10.12-22.15), HR 0.603 (95%CI 0.177-2.050), P= 0.412
LUME-Lung 1 (13) (stage IIIB/IV recurrent NSCLC progressing after first-line chemotherapy, second-line)	docetaxel+nintedanib (n=655, LUAD: n=322) vs docetaxel+placebo (n=659, LUAD: n=336)	median 3.4 months (95%CI 2.9-3.9) vs 2.7 months (95%CI 2.6-2.8), HR 0.79 (95%CI 0.68-0.92), P=0.0019	median 10.1 months (95%CI 8.8-11.2) vs 9.1 months (95%CI 8.4-10.4), HR 0.94 (95%CI 0.83-1.05), P=0.2720; LUAD: median 12.6 months (95%CI 10.6-15.1) vs 10.3 months (95%CI 8.6-12.2), HR 0.83 (95%CI 0.70-0.99), P=0.0359
REVEL (12) (stage IV NSCLC progressed during or after first-line platinum-based chemotherapy, second-line)	docetaxel+ramucirumab (n=628) vs docetaxel+placebo (n=625)	median 4.5 months (IQR 2.3-8.3) vs 3.0 months (IQR 1.4-6.9), HR 0.76 (95%CI 0.68-0.86), p<0.0001	median 10.5 months (IQR 5.1-21.2) vs 9.1 months (IQR 4.2-18.0), HR 0.86 (95%CI 0.75-0.98), P=0.023
VARGADO Cohort C (34) (locally advanced, metastatic, or locally recurrent LUAD following first line chemotherapy with ICIs, second-line)	docetaxel+nintedanib (n=137)	median 4.8 months (95%CI 3.7-6.6) (DCR 72.5%); patients with and without KRAS mutations: median 4.8 months (95%CI 2.2–not estimable) vs 6.4 months (95%CI 2.5–9.9), P=0.4784	immature
Brueckl WM *et al.* (35) (stage IV NSCLC following first-line chemotherapy plus ICI, second-line)	docetaxel+ramucirumab (n=77)	median 3.9 months (95%CI 3.1-4.6) (ORR 32.5%, DCR 62.4%); patients with and without KRAS mutations: median 2.8 months (95%CI 1.7-3.9) vs 4.5 months (95%CI 2.6-6.4), P=0.021	median 7.5 months (95%CI 5.1-10.0)
Brueckl WM *et al.* (45) (stage IV NSCLC following second-line ICI, third-line)	docetaxel+ramucirumab (n=67)	median 6.8 months (95%CI 4.6-9.0) (ORR 36%, DCR 69%)	median 11.0 months (95%CI 7.1-14.9)
Lung-MAP S1800A (30) (advanced NSCLC previously treated with ICI and platinum-based chemotherapy)	ramucirumab+pembrolizumab (n=69) vs standard of care (docetaxel/ramucirumab,docetaxel,gemcitabine, and pemetrexed) (n=67)	median 4.5 months (80%CI 4.2-6.1) vs 5.2 months (80%CI 4.2-5.7), HR 0.86 (80%CI 0.66-1.14), P=0.25 (one-sided, standard log-rank test), P=0.14 (one-sided, weighted log-rank test)	median 14.5 months (80%CI 13.9-16.1) vs 11.6 months (80%CI 9.9-13.0), HR 0.69 (80%CI 0.51-0.92), P=0.05 (one-sided, standard log-rank test), P=0.15 (one-sided, weighted log-rank test)

References

45. Brueckl WM, Reck M, Rittmeyer A, et al. Efficacy of Docetaxel Plus Ramucirumab as Palliative Third-Line Therapy Following Second-Line Immune-Checkpoint-Inhibitor Treatment in Patients With Non-Small-Cell Lung Cancer Stage IV. Clin Med Insights Oncol 2020; 14:1179554920951358.

https://dx.doi.org/10.21037/tlcr-23-260