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Original Article

Effects of PD-1 inhibitor combined with anti-angiogenic drugs on efficacy and immune function of non-small cell lung cancer

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Abstract: Objective: To investigate the effect of PD-1 inhibitor combined with anti-angiogenic drugs on the therapeutic efficacy and immune function of patients with non-small cell lung cancer (NSCLC). Methods: Clinical data of 60 NSCLC patients who admitted to a regional Hospital of Traditional Chinese Medicine from May 2020 to August 2021 were analyzed retrospectively. Among them, 23 patients who received sintilimab and anlotinib were in group A, 20 patients treated with sintilimab were in group B, and 17 patients intervened by anlotinib alone were in group C. The changes of clinical efficacy, objective remission rate (ORR) and disease control rate (DCR) among the three groups were compared. The levels of cluster of differentiation 4 $(CD4)^+$, cluster of differentiation 8 $(CD8)^+$ and $CD4^+/CD8^+$ were assessed before and 6 weeks after treatment. The progression-free survival (PFS) was calculated and the prognostic factors were analyzed by Cox regression. The adverse reactions of immunotherapy in three groups were evaluated. Results: There was no obvious difference in ORR among the three groups ($P>0.05$). The proportion of DCR in group A was dramatically higher than that in group B and C ($P<0.05$). After treatment, the $CD4^+$ and $CD4^+/CD8^+$ levels were markedly higher, while the $CD8^+$ level in group A was lower in group A than those in the other two groups ($P<0.05$). There was no obvious difference in the incidence of immune-related adverse reactions among the three groups ($P>0.05$). The median PFS of patients was 6.03 months. Cox regression analysis revealed that Eastern Cooperative Oncology Group score, tumor metastasis and treatment regimen were independent prognostic factors affecting PFS. Conclusion: Sintilimab combined with anlotinib can effectively improve DCR and prolong PFS in NSCLC patients, and this regimen does not increase immune-related adverse reactions during treatment.

Keywords: PD-1 inhibitor, anti-angiogenic drugs, non-small cell lung cancer, immunity

Introduction

Lung cancer (LC), a common malignancy of respiratory system, is the main cause of cancer death all over the world [1]. The latest epidemiological statistics revealed that there were 4.3 million new cancer cases and 2.9 million new cancer deaths in China in 2018, of which 770,000 new cases and 690,000 deaths were because of LC [2]. Non-small cell lung cancer (NSCLC) accounts for about 85% of all LC and is the leading cause of cancer-related death [3]. At present, surgery is still the primary treatment method. The onset of LC is relatively hidden without obvious initial clinical manifestations, and there is a lack of promising clinical diagnostic indicators. When patients are admitted to the hospital, the course of the disease is usually at the middle or late stages, and thus, the patients missed the best operation time [4]. Besides, the treatment time of middle and advanced LC is long, and the prognosis is poor, which increases the pressure of patients and financial burden of the family [5].

Recently, the research progress of NSCLC is mainly reflected in the following two aspects. First, patients with clear driving genes have been receiving individualized precision therapy: small molecule inhibitors driving gene muta-

tion/fusion corresponding targets such as EGFR, ALK, ROS-1 and c-MET have become the first choice [6, 7]. Second, the immunotherapy of checkpoint inhibitors: cell programmed death receptor 1 (PD-1) and its ligand (PD-L1) inhibitors have been approved by FDA for first-line treatment of patients without driving gene mutation/fusion (wild type) PD-L1 expression ≥50% [8, 9]. The combination of chemotherapy and immune checkpoint inhibitors has become the standard first-line treatment for patients without driving gene mutation/fusion (wild type) PD-L1 expression <50% [10]. As a recombinant human immunoglobulin G4 monoclonal antibody, sintilimab can bind to PD-1, block the interaction with ligands PD-L1 and PD-L2, restore endogenous anti-tumor T cell response [11, 12]. Anlotinib hydrochloride, as a targeted antineoplastic drug, is a small molecular tyrosine kinase inhibitor, which inhibits vascular endothelial growth factor-related kinases and cell proliferation-related kinases [13]. Recent research has shown that both sintilimab and anlotinib hydrochloride can control the disease in advanced NSCLC patients [14]. However, it's vague whether the combination can improve the condition of NSCLC patients.

The purpose of this research was to analyze the efficacy, immune function and survival in NSCLC patients after the combined therapy, and to provide new ideas for clinical treatment.

Methods and materials

Clinical data of 60 NSCLC patients who admitted to Zhenhai Hospital of Traditional Chinese Medicine from May 2020 to August 2021 were analyzed retrospectively. Among them, 23 patients who received sintilimab and anlotinib were in group A, 20 patients treated with sintilimab were in group B, and 17 patients intervened by anlotinib alone were in group C. This research was approved by the Medical Ethics Committee of Zhenhai Hospital of Traditional Chinese Medicine (Ethical approval number: LL2020-064).

Inclusion and exclusion criteria

Inclusion criteria: Patients diagnosed with non-squamous NSCLC patients [15]; in patients with negative EGFR/ALK driving gene or positive EGFR/ALK driving gene, PD-L1 expression was more than 1 in those who failed or could not tolerate TKI therapy; patients with LC TNM stage IIIb-IV, and there was no operative indication.

Exclusion criteria: Patients were complicated with other tumors; patients were allergic to the experimental drugs; patients received immunotherapy or anti-angiogenic drugs; patients had incomplete clinical data.

Treatment schemes

Anlotinib monotherapy (group C): Altogether 12 mg/d (CHIA TAI TIANQING (CTTQ) Pharmaceutical Co., Ltd., SFDA Approval No. H20180002) was taken orally for 2 weeks and then stopped for 1 week. The treatment cycle was 21 days. If patients cannot tolerate it, the dose can be reduced to 10 or 8 mg/d. If they are still unable to tolerate it, the treatment was terminated.

Sintilimab monotherapy (group B): Sintilimab 200 mg (Innovent Biologics, Inc., SFDA Approval No. S20180016) was dissolved in 0.9% sodium chloride solution, and the concentration was 1.5-4.0 mg/mL according to the tolerance of patients. Intravenous infusion (lasting 30-60 min) of the solution was given to patients for 3 weeks. Twenty-one days was a treatment cycle.

Sintilimab combined with anlotinib (group A): The injection 200 mg was dissolved in 0.9% sodium chloride solution, and the concentration was matched to 1.5-4.0 mg/mL based on patients' tolerance. Intravenous infusion (lasting 30-60 min) of the solution was given to patients for 3 weeks. On the basis of the above, oral treatment of anlotinib hydrochloride was combined, with an initial dose of 12 mg, once a day for continuous 2 weeks, then a withdrawal for 1 week. A course of treatment was 21 days.

When patients developed poor tolerance, the dose of 10 mg/d that could not be tolerated was reduced to 8 mg/d. The treatment of patients in the three groups was stopped if they were intolerable, developed adverse events or disease progression.

Immune index detection

Whole venous blood was collected and mixed in a purple blood tube of 2 mL containing EDTA

anticoagulant. After numbering, T cell subsets were detected by Flow cytometry (BD FACS Canto II). For relative counting, we use normal flow sampling tubes (12×75 mm) marked with letters or numbers to distinguish. For absolute counting, we use a BD Tru count tubes also marked with letters or numbers. Then, 20 ul BD Tritest cluster of differentiation 4 (CD4)/cluster of differentiation 8 (CD8)/cluster of differentiation 3 (CD3) reagent was transferred to the bottom of the flow sampling tube/BD Tru count tubes. The anticoagulant whole blood fully mixed with 50 uL was removed to the bottom of each tube. The tube was covered, gently mixed and incubated at room temperature (20-25°C) for 15 min. BDFACS hemolysin of 1 mL of 450 uL was added to each tube. Afterwards, the tube was incubated again in the same condition. Finally, the samples were analyzed by Flow cytometry.

Outcome measures

Main outcome measures: The clinical efficacy were evaluated by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 and compared among the three groups [16]. Complete remission (CR) referred to disappearance of targeted lesions and no new lesions for at least 4 weeks. Partial remission (PR) referred that the sum of the maximum diameter of the targeted focus was reduced by more than 30%, and the maintenance time was ≥4 weeks. Disease stabilization (SD) referred to no new lesions or progress, and the maximum diameter of targeted lesions decreased by less than 30% or increased by less than 20%. Disease progression (PD) referred to the maximum diameter of new lesions or lesions increased by over 20%. Objective remission rate (ORR) = cases of (CR+PR)/total number of cases; disease control rate (DCR) = cases of (CR+PR+SD)/total number of cases. The levels of $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ were compared before treatment and 6 weeks after treatment. The overall survival (OS) of patients was calculated. OS refers to the time from the start of immunotherapy to death due to any cause. The progression-free survival (PFS) of patients was counted, and it was defined as the time from the start of immunotherapy to disease progression or death. Loss to follow-up or events that had not occurred by the end of follow-up were defined as censoring.

Secondary outcome measures: The clinical data of the three groups were compared. The adverse reactions after immunotherapy were compared, including 14 types of skin toxicity, endocrine toxicity and cardiac toxicity, and were divided into 5 grades: mild toxicity; G2: moderate toxicity; G3: severe toxicity; G4: life-threatening toxicity; G5: toxicity-related deaths. The clinical symptoms include skin toxicity, thyroid dysfunction, hematotoxicity, cardiotoxicity, pulmonary toxicity, gastrointestinal toxicity, hepatotoxicity, nephrotoxicity, weakness and neurotoxicity, etc.

Statistical analysis

SPSS19.0 was used for statistical analysis. The measurement data were tested by t-test and the counting data by chi-square test. PFS and OS curves were drawn by Kaplan-Meier method, and Log-rank test was conducted to compare both groups. The prognostic factors of PFS were assessed through Cox regression. All statistical tests were bilateral tests, and the difference was considered to be statistically significant when $P<0.05$.

Results

Comparison of baseline data

We found no marked difference in age, sex, Eastern Cooperative Oncology Group (ECOG) score, driving gene mutation, smoking history and TNM staging among the three groups ($P>0.05$, **Table 1**).

Evaluation of clinical efficacy of patients after treatment

The clinical efficacy of the three groups were evaluated. We found that there was no marked difference in ORR among the groups ($P>0.05$). However, the proportion of DCR in group A was dramatically higher than that in group B and C ($P<0.05$), but there was no difference in the proportion of DCR between group A and B ($P>0.05$, **Table 2**).

Changes of immune indexes in patients after treatment

After treatment, the $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ levels were markedly higher while the $CD8^+$ level was dramatically lower in group A than those in group B and C, ($P<0.05$). In addi-

Table 1. Comparison of baseline data

Factor	Group A (n=23)	Group B (n=20)	Group C (n=17)	*P* value
Age				0.518
≥65 years old	11	7	9	
<65 years old	12	13	8	
Sex				0.678
Male	12	8	7	
Female	11	12	10	
ECOG score				0.951
0 points	3	2	1	
1 point	10	10	8	
2 points	10	8	8	
Degree of differentiation				0.919
Well-differentiated	10	7	7	
Moderately differentiated	7	8	7	
Poorly differentiated	6	5	3	
Driver gene mutation				0.757
mutation	3	2	1	
No mutation	20	18	16	
Smoking history				0.722
Yes	13	9	8	
No	10	11	9	
Tumor metastasis				0.631
Yes	13	10	7	
No	10	10	10	
TNM stage				0.460
IIIb	14	10	7	
IV	9	10	10	

Note: Eastern Cooperative Oncology Group (ECOG); tumor node metastasis classification (TNM).

Table 2. Clinical efficacy

Group	CR	PR	SD	PD	ORR	DCR
Group A	0	10	11	2	10 (43.47)	21 (91.30)
Group B	0	6	5	9	6 (30.00)	11 (55.00)
Group C	0	5	5	7	5 (29.41)	10 (58.82)
χ^2					1.180	8.152
P value					0.554	0.017

Note: complete remission (CR); partial remission (PR); disease stabilization (SD); disease progression (PD); objective remission rate (ORR); disease control rate (DCR).

tion, after treatment, the levels of $CD4^+$ and $CD4^+/CD8^+$ were higher and the $CD8^+$ level was lower in group B than those in group C, while (P<0.05). Furthermore, the $CD4^+$ and $CD4^+/CD8^+$ levels were higher and the $CD8^+$ level was lower after treatment than those before treatment in the three groups (P<0.05, **Figures 1**, **2**).

Adverse immune reactions in patients

The immune adverse reactions in the three groups were statistically analyzed (**Table 3**). The overall incidence of adverse immune reactions in 60 patients was 60.005% (36/60). The adverse immune reactions with an incidence over 10% were gastrointestinal toxicity, blood toxicity, pulmonary toxicity, thyroid dysfunction and hepatotoxicity. The incidence of ≥G3 adverse reactions was 17.39% in group A, 20.00% in group B and 17.64% in group C (**Table 4**, P>0.05).

Prognostic survival analysis

During the follow-up up to March 1, 2022, 15 patients died and the survival rate was 75.00%. Besides, 48 patients developed to PD. The median PFS was 6.03 months. In the first case, the PFS of patients in group A were 6.76 months, in group B was 5.85 months, and in group C was 4.36 months. The medium survival time of patients with OS was not mature. Cox regression analysis of PFS revealed that ECOG score, tumor metastasis and treatment regimen were independent prognostic factors of PFS (**Table 5**; **Figure 3**, P<0.05). Nevertheless, we did not find that any index was independently tied to the prognostic factors of OS (**Table 6**, P<0.05).

Discussion

There are more than 2 million new cases worldwide every year, and the number of deaths from LC has reached 1.7 million every year, accounting for 11.6% and 18.4% of all cancer morbidity and mortality respectively [17, 18]. Surgery is the main clinical treatment method for LC. However, patients have reached the middle and advanced stages of the disease are unable to undergo surgical treatment [19]. Chemotherapy has become an essential treatment for advanced LC patients. For most advanced LC patients, with or without maintenance therapy, the median overall survival time (mOS) is only 1

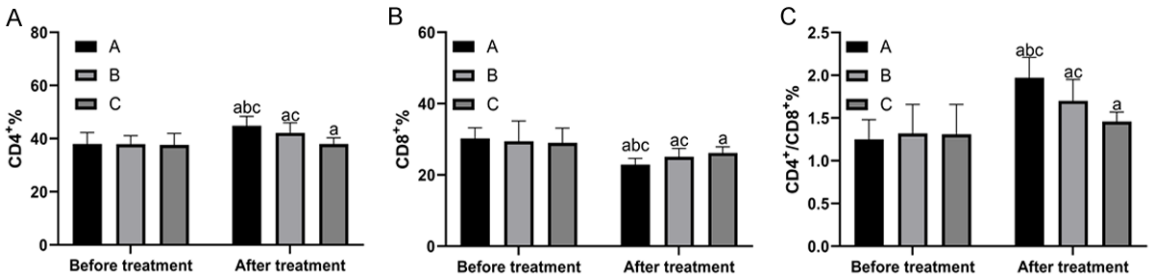

Figure 1. Changes of percentage of $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ in the peripheral blood of patients before and after treatment. A. Changes of $CD4^+$ in peripheral blood before and after treatment. B. Changes of $CD8^+$ in peripheral blood before and after treatment. C. Changes of $CD4^+/CD8^+$ in peripheral blood before and after treatment. Compared with before treatment, $^aP<0.05$; compared with group B, $^bP<0.05$; compared with group C, $^cP<0.05$. Cluster of differentiation 4 (CD4); Cluster of differentiation 8 (CD8).

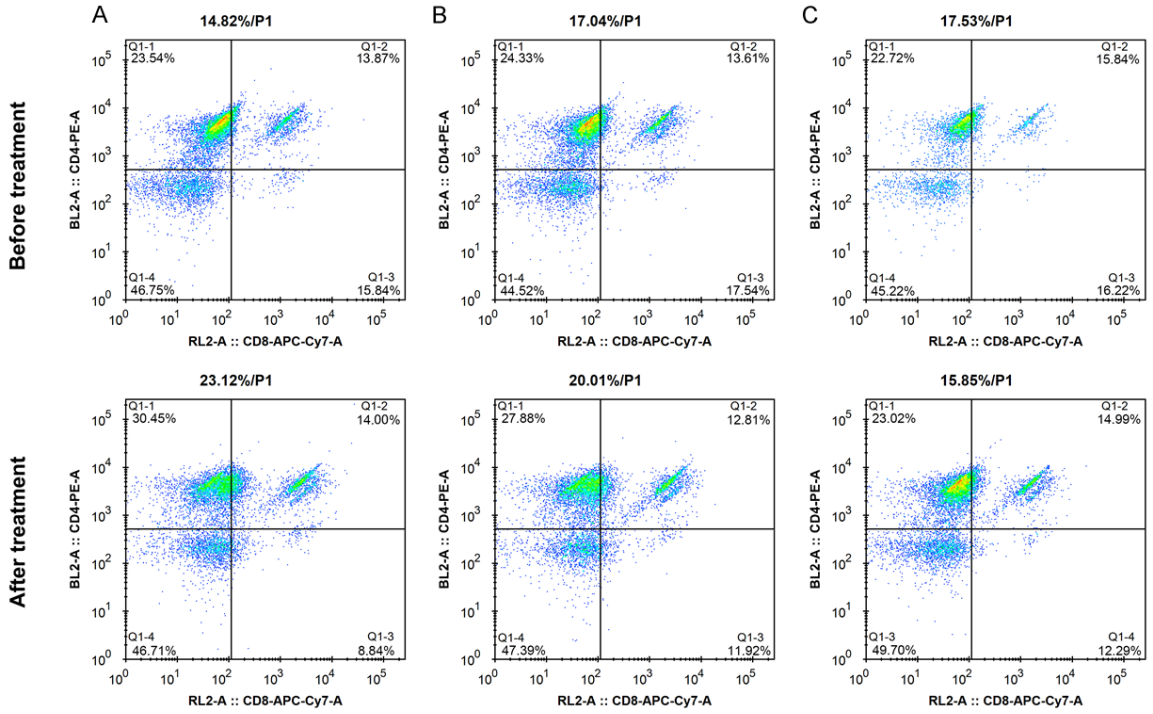

Figure 2. Flow cytometry. A. Changes of $CD4^+$ in peripheral blood before and after treatment. B. Changes of $CD8^+$ in peripheral blood before and after treatment. C. Changes of $CD4^+/CD8^+$ in peripheral blood before and after treatment. Cluster of differentiation 4 (CD4); Cluster of differentiation 8 (CD8).

year [20]. Compared with chemotherapy, immunotherapy can restore patients' anti-tumor immune response and can indirectly kill tumor cells, which may produce a strong and lasting clinical response [21, 22].

In this research, we retrospectively analyzed the clinical efficacy of sintilimab combined with anlotinib in NSCLC patients. There was no difference in ORR among the three groups after treatment, but the DCR in group A was dramatically higher than that in group B and C. It indicates that the combination of drugs can improve the condition of patients. This is mainly due to the inhibition of platelet-derived growth factor receptor, fibroblast growth factor receptor, vascular endothelial growth factor receptor, c-Kit and other kinases, which can inhibit tumor angiogenesis and tumor growth [23, 24]. Sintilimab is a kind of PD-1 inhibitor. By binding to PD-1, it blocks the interaction between PD-1 and PD-L1 and PD-L2, thereby blocking the

Table 3. Adverse immune reactions of patients

Type	G1	G2	G3	G4	G5	Total (incidence)
Skin toxicity	2	1	1	0	0	4 (6.67)
Thyroid dysfunction	7	2	2	0	0	11 (18.33)
Hematotoxicity	5	5	3	0	0	13 (21.67)
Cardiotoxicity	4	0	1	0	0	5 (8.33)
Pulmonary toxicity	6	3	1	1	0	11 (18.33)
Gastrointestinal toxicity	11	3	0	0	0	14 (23.33)
Hepatotoxicity	3	3	2	0	0	8 (13.33)
Nephrotoxicity	0	0	0	1	0	1 (1.67)
Weakness	3	1	1	0	0	5 (8.33)
Neurotoxicity	3	0	0	0	0	3 (5.00)
Others	5	0	0	0	0	5 (8.33)

Table 4. Comparison of immune adverse reactions among three groups of patients

Type	Group A		Group B		Group C		*P* value
	G1-G2	≥G3	G1-G2	≥G3	G1-G2	≥G3	
Skin toxicity	1	1	1	0	1	0	0.879
Thyroid dysfunction	4	1	2	1	3	0	0.847
Hematotoxicity	3	1	4	1	3	1	0.813
Cardiotoxicity	2	0	1	1	1	0	0.901
Pulmonary toxicity	4	1	2	0	3	1	0.493
Gastrointestinal toxicity	4	0	5	0	5	0	0.658
Hepatotoxicity	2	1	1	1	3	0	0.791
Nephrotoxicity	0	1	0	0	0	0	0.441
Weakness	2	0	1	0	1	1	0.757
Neurotoxicity	1	0	1	0	1	0	0.976
Others	2	0	3	0	0	0	0.257

PD-1/PD-L1 pathway that leads to tumor immune tolerance. Also, it activates the antitumor activity of cells and plays a role in tumor treatment [25, 26]. The combination of the two drugs can better inhibit tumor growth. Moreover, Liang et al. [27] found that sintilimab combined with GP regimen effectively improved ORR in advanced NSCLC patients. However, our study did not find that sintilimab combined with anlotinib could improve ORR in the patients. We believe that this may be related to the sample type and patient staging, but the pathological type and the specific staging of patients were not clearly stated in their study, so it is difficult for us to discuss further. In any case, our results demonstrated that the combined therapy effectively improved the DCR of patients.

In this research, we detected CD cells in the peripheral blood of patients before and after treatment. $CD4^+$ assists or induces T cell, which assists humoral immunity and cellular immunity [28]. $CD8^+$ suppresses or kills T cell, and its effector cell $CD8^+$ mediates cytotoxicity and kills tumor cells. The ratio of them dynamically shows the changes of immune function in patients [29]. We found that the $CD4^+$ and $CD4^+/CD8^+$ levels were higher while the $CD8^+$ level was lower after treatment than those before treatment in all three groups, which indicated that all the three treatments improved the immune function of patients. We also discovered that the $CD4^+$ and $CD4^+/CD8^+$ levels were higher and the $CD8^+$ level was lower in group A than those in the other two groups. It is suggested that sintilimab combined with anlotinib could improve and regulate cellular immune function, kill tumor cells. inhibit disease progression and obtain satisfactory clinical effect. In addition, there was no difference in immune adverse reactions among the three groups, which suggested that the combined treatment did not increase the postoperative adverse reactions, and it was safe.

We then counted the PFS of patients. The medium PFS was 6.03 months, which is consistent with previous studies. Then, Cox regression analysis found that ECOG score, tumor metastasis and treatment regimen were independent prognostic factors affecting PFS. Early studies found that compared with patients with an ECOG PS score of 0-1, those with a score greater than 2 were less likely to receive radiotherapy or chemotherapy and had a poor prognosis. We also found that the incidence of PD in patients with ECOG score greater than 2 was 2.014 times higher than that in those with a score of 0-1. Hence, when receiving anti-PD-1

Table 5. Prognostic analysis of PFS

Factor	Univariate analysis			Multivariate analysis		
	P value	HR value	95% CI	*P* value	HR value	95% CI
Age (≥65 years old vs. <65 years old)	0.930	0.975	0.551-1.724			
Sex (male vs. female)	0.393	0.780	0.441-1.379			
ECOG score (0-1 vs. 2)	0.004	2.345	1.308-4.205	0.028	2.014	1.08-3.755
Degree of differentiation (well- and moderately differentiated vs. poorly differentiated)	0.203	0.633	0.314-1.279			
Driver gene mutation (Yes vs. No)	0.915	0.951	0.376-2.405			
Smoking history (Yes vs. No)	0.660	0.880	0.498-1.554			
Tumor metastasis (Yes vs. No)	0.009	0.455	0.252-0.820	0.044	0.524	0.279-0.984
Treatment plan (A vs. B vs. C)	0.013	1.554	1.096-2.202	0.003	1.721	1.203-2.463

Note: progression-free survival (PFS); Eastern Cooperative Oncology Group (ECOG); tumor node metastasis classification (TNM).

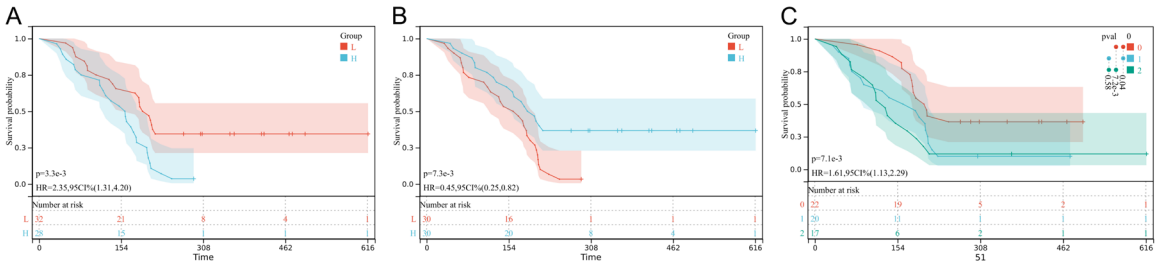

Figure 3. Survival curve of independent factors affecting prognosis of patients. A. Relationship between ECOG score and patient survival; B. Relationship between tumor metastasis and patient survival; C. Relationship between treatment plan and patient survival. Note: Eastern Cooperative Oncology Group (ECOG).

Table 6. Prognostic analysis of OS

Factor	Univariate analysis		
	P value	HR value	95% CI
Age (≥65 years old vs. <65 years old)	0.124	2.458	0.782-7.723
Sex (male vs. female)	0.118	2.491	0.792-7.832
ECOG score (0-1 vs. 2)	0.858	1.097	0.398-3.026
Degree of differentiation (well- and moderately differentiated vs. poorly differentiated)	0.699	0.779	0.220-2.761
Driver gene mutation (Yes vs. No)	0.618	0.684	0.154-3.034
Smoking history (Yes vs. No)	0.644	1.270	0.461-3.504
Tumor metastasis (Yes vs. No)	0.350	0.611	0.217-1.717
Treatment plan (A vs. B vs. C)	0.058	1.855	0.980-3.511

Note: overall survival (OS); Eastern Cooperative Oncology Group (ECOG).

antibody combined with anlotinib treatment, patients with an ECOG score of 0-1 benefited more, and those with 0-1 had better physical fitness and were able to complete the treatment cycle. Patients with ECOG score of 2 or more have poor physical condition and are difficult to tolerate the toxicity of related drugs, or the disease progressed rapidly, and patients failed to survive for an effective time. So, the efficacy was unsatisfied. The effect of tumor metastasis on PFS has been reported in many studies [30, 31]. We found that the incidence of PD in non-metastatic patients was 0.524 times higher than that in metastatic patients, indicating that the former ones were more likely to suffer PD. Finally, we found that the PFS of NSCLC patients treated with combination therapy was higher than that of those in the other two groups, indicating that combination therapy could improve the PFS. We speculate that the combined therapy can play a multi-target mechanism and block multiple signal transduction pathways/signal networks in tumor cells, so as to improve disease progression and ultimately improve PFS in patients.

Nevertheless, this research still has some limitations. Firstly, as a retrospective study, we did

not collect complete OS data of patients, so we did not analyze the effect of combination drugs on OS. Secondly, the sample size is small, and the results may not be representative. Thirdly, our study samples are all non-squamous NSCLC patients, it's unclear whether the combination of drugs has an effect on patients with squamous cell carcinoma. Thus, we hope to carry out randomized controlled trials in follow-up studies and collect more clinical samples to improve our conclusions.

To sum up, sintilimab combined with anlotinib can effectively improve DCR and prolong PFS in NSCLC patients, and this regimen does not increase immune-related adverse reactions during treatment.

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Disclosure of conflict of interest

None.

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