Research Square

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Effect of PD-1 inhibitor combined with chemotherapy on cytokines and immune cell function in patients with advanced lung cancer

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Research Article

Keywords: PD-1 inhibitor, chemotherapy, advanced lung cancer, cytokines, immune cell function

Posted Date: March 31st, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9020342/v1

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Additional Declarations: No competing interests reported.

Title:

Effect of PD-1 inhibitor combined with chemotherapy on cytokines and immune cell function in patients with advanced lung cancer

Running title:

PD-1 inhibitor plus chemotherapy in lung cancer

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Keywords:

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Acknowledgements:

None.

Funding:

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest:

The authors declare no conflict of interest.

Authors' contributions:

Chenxin Zhang: Conceptualization, Data curation, Formal analysis, Writing – original draft. Jiqing Hao: Conceptualization, Supervision, Writing – review & editing.

Data availability statement:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. The data are not publicly available due to privacy restrictions.

Ethics approval and consent to participate:

This study was approved by the Medical Ethics Committee of The First Affiliated Hospital of Anhui Medical University (Approval No. 2022437). All procedures were performed in accordance with the Declaration of Helsinki. Patient data were anonymized.

Consent for publication:

Not applicable.

Abstract

Objective: To investigate the regulatory effects of PD-1 inhibitor combined with chemotherapy on peripheral blood cytokine levels and immune cell function in patients with advanced lung cancer.

Methods: A retrospective study was conducted on patients with advanced lung cancer admitted to our hospital from January 2022 to May 2025, including 68 cases in the combination therapy group (PD-1 inhibitor combined with pemetrexed/paclitaxel-based chemotherapy) and 34 cases in the chemotherapy group. Among the combination therapy group, there were 46 cases of lung adenocarcinoma and 22 cases of lung squamous cell carcinoma; in the chemotherapy group, there were 24 cases of lung adenocarcinoma and 10 cases of lung squamous cell carcinoma. The combination therapy group received a chemotherapy regimen based on pemetrexed/paclitaxel, combined with sintilimab, camrelizumab, or tislelizumab (all at 200 mg per cycle), while the chemotherapy group received only chemotherapy. Both groups were treated for more than 2 cycles, with 3 weeks as one cycle. Peripheral blood T-cell subsets, NK cell-related indicators, and serum cytokine levels were measured before treatment and after 2 cycles of treatment in both groups, and changes and intergroup differences were analyzed.

Results: After treatment, immune cell-related indicators (CD3+%, CD3+CD8+%, NK cell proportion, etc.) and cytokine levels (e.g., elevated IL-2, decreased IL-6 and IL-8) in both groups showed significant improvement compared to pre-treatment levels ($P<0.05$), but the improvement in the combination therapy group was significantly

greater than that in the chemotherapy group (P<0.05). Subgroup analysis revealed that the increase in CD3+CD8+% was higher in lung squamous cell carcinoma patients than in lung adenocarcinoma patients in the combination therapy group (P<0.05), and EGFR-TKI resistance and driver gene negativity were also observed.There was no significant difference in the decline of IL-6 levels among patients (P>0.05).

Conclusion: The combination of PD-1 inhibitors and chemotherapy improves immune cell function and positively regulates cytokines in advanced lung cancer patients more effectively than chemotherapy alone. This approach demonstrates favorable immunomodulatory effects across different pathological types and EGFR-TKI-resistant patients, potentially providing a basis for precision therapy. Relevant immune and cytokine markers may serve as potential predictive biomarkers for treatment efficacy and prognosis.

Keywords:PD-1 inhibitor; chemotherapy; advanced lung cancer; cytokine; immune cell; T cell subset; NK cell; efficacy

Lung cancer, as the malignant tumor with the highest incidence and mortality rates globally, is estimated to account for approximately 2.2 million new cases and over 1.8 million deaths annually worldwide in 2024, according to global cancer statistics. Among these cases, patients with advanced lung cancer (TNM stage III B-IV) constitute more than 60%, with a 5-year survival rate of less than 15% [1]. Although traditional chemotherapy can control the disease by directly killing tumor cells, long-term use may

lead to immune suppression, such as reduced T-cell activity and decreased NK cell counts, thereby accelerating tumor immune evasion [2].

Immunotherapy, represented by PD-1 inhibitors, has become a core treatment for advanced lung cancer by blocking the PD-1/PD-L1 signaling pathway, thereby relieving the "brake" inhibition of immune cells by tumors and activating the antitumor functions of effector cells such as T cells and NK cells. However, the response rate of PD-1 inhibitors as monotherapy is only 20%-30%, and approximately 40% of patients develop primary or secondary resistance [3]. In recent years, the synergistic effect of PD-1 inhibitors combined with chemotherapy has been widely demonstrated: chemotherapeutic agents can release tumor antigens and disrupt the immunosuppressive barrier in the tumor microenvironment, creating conditions for immune cells to "recognize tumors"; PD-1 inhibitors further amplify the immune response and prolong the duration of effector cell-mediated killing [4]. This study retrospectively analyzed clinical data from patients with advanced lung cancer who received PD-1 inhibitors combined with chemotherapy versus chemotherapy alone, aiming to explore the effects of different regimens on cytokines (IL-2, IL-6, IL-8, INF-γ) and immune cells (T cell subsets, NK cells), providing a reference for optimizing clinical treatment regimens. Additionally, the predictive value of these indicators for efficacy and prognosis was investigated.

II. Materials and Methods

(1) Research Subjects

A retrospective study enrolled 102 patients with advanced lung cancer treated at our hospital from January 2022 to May 2025. Patients were divided into a combination therapy group (68 cases) and a single chemotherapy group (34 cases) based on their treatment regimens. All patients were pathologically or cytologically diagnosed with advanced lung cancer (TNM stage III B-IV, according to the International Association for the Study of Lung Cancers 8th edition staging criteria) and met the following inclusion criteria: ① Age 18–80 years, ECOG performance status score 0–2; ② Presence of at least one measurable lesion (RECIST 1.1 criteria); ③ No prior use of PD-1/PD-L1 inhibitors, CTLA-4 inhibitors, or other immunotherapies in the combination therapy group; no prior immunotherapy in the single chemotherapy group; ④ Normal pre-treatment blood counts (white blood cells $\geq 3.0\times10^9$/L, platelets $\geq 100\times10^9$/L) and liver/kidney function (ALT, AST $\leq 2.5\times$ULN, creatinine $\leq 1.5\times$ULN), with no history of severe immune-related diseases (e.g., systemic lupus erythematosus, rheumatoid arthritis); ⑤ Complete clinical data, including treatment regimens, laboratory test results, and follow-up records. The study was conducted with informed consent from patients and approved by the hospital ethics committee.

Exclusion criteria: ① Presence of other malignancies (excluding basal cell carcinoma of the skin and cervical carcinoma in situ); ② Active infections (e.g.,

pneumonia, sepsis), active tuberculosis, or history of organ transplantation; ③ Development of grade ≥3 immune-related adverse reactions (e.g., immune-mediated pneumonia, myocarditis) during combination therapy leading to protocol discontinuation; ④ Follow-up duration less than 3 treatment cycles.

(II) Treatment Protocol

All patients in the combination therapy group received PD-1 inhibitor combined with chemotherapy, as detailed below:

Selection of PD-1 inhibitors: Sintilimab (Innovent Biologics, National Drug Approval No. S20180016) 200 mg, intravenous infusion, once every 3 weeks (29 cases); Camrelizumab (Sintilimab, Sintilimab Pharmaceuticals, National Drug Approval No. S20190027) 200 mg, intravenous infusion, once every 3 weeks (21 cases); Tislelizumab (BeiGene, National Drug Approval No. S20 190045) 200 mg, intravenous infusion, once every 3 weeks (18 cases).

Chemotherapy regimen selection: Pemetrexed (Huiyu Pharmaceutical, National Drug Approval No. H 20173301) 0.5 g/m², intravenous infusion, day 1, combined with Carboplatin (Qilu Pharmaceutical, National Drug Approval No. H20020181) AUC=5-6, intravenous infusion, day 1 (38 cases, all lung adenocarcinoma); Paclitaxel liposome (Nanjing Luye Sike, National Drug Approval No. H20030357) 135-175 mg/m², intravenous infusion, day 1, combined with Cisplatin (Hausen Pharmaceutical, National

Drug Approval No. H 20040813) 25 mg/m², intravenous infusion, days 1-3 (22 cases, all lung squamous cell carcinoma); Gemcitabine (Qilu Pharmaceutical, National Drug Approval No. H20 113286) 1.2 g/m², intravenous infusion, days 1 and 8, combined with Cisplatin 25 mg/m², intravenous infusion, days 1-3 (8 cases, all lung squamous cell carcinoma).

Management of adverse reactions: During treatment, grade 1-2 nausea and vomiting were managed with ondansetron for antiemesis, grade 1-2 rash with topical corticosteroid ointment, and grade 3 thrombocytopenia with platelet growth factor therapy. No patients withdrew from the study due to adverse reactions. Monitoring and management of immune-related adverse reactions were conducted in accordance with the latest clinical guidelines, with regular follow-up of thyroid function, cardiac enzyme levels, and other indicators to enable early intervention [7].

Patients in the single chemotherapy group received only chemotherapy, with the same regimen as the combination therapy group, as detailed below:

Pemetrexed 0.5 g/m², intravenous infusion, day 1, combined with carboplatin AUC=5-6, intravenous infusion, day 1 (18 cases, all with lung adenocarcinoma);

-Paclitaxel liposome 135-175 mg/m², intravenous infusion, day 1, combined with cisplatin 25 mg/m², intravenous infusion, days 1-3 (10 cases, all with lung squamous cell carcinoma);

-Gemcitabine 1.2 g/m², intravenous infusion, on days 1 and 8, in combination with

cisplatin 25 mg/m², intravenous infusion, on days 1-3 (6 cases, all with lung squamous cell carcinoma);

-Adverse reaction management was the same as in the combination therapy group.

(3) Detection Indicators and Methods

Peripheral venous blood samples of 5 mL were collected from patients in both groups at two time points: before treatment (within 1 week prior to the first dose) and after 2 treatment cycles (within 1 week after the last dose). The following methods were employed for parameter determination:

1. Immune cell parameters: T-cell subsets (CD3+%, CD3+CD4+%, CD3+CD8+%, CD3+PD-1+%, CD4+PD-1+%, CD8+PD-1+) and NK cell-related parameters (NK cell proportion, granulase B-positive NK cells, interferon-gamma (IFN-γ)-positive NK cells) were detected using a flow cytometer (BD FACSCanto II, BD, USA). The detection reagents were BDs matching antibodies (CD3-PE-Cy7, CD4-FITC, CD8-APC, PD-1-PE, granulase B-FITC, IFN-γ-APC). The procedure was strictly performed according to the instrument manual, with 1×10^6 cells per sample.

2. Cytokine markers: Serum levels of IL-2, IL-6, and IL-8 were detected using enzyme-linked immunosorbent assay (ELISA) with kits purchased from Shanghai Enzyme Biotechnology Co., Ltd. (product codes: ELISA-IL2-001, ELISA-IL6-001, ELISA-IL8-001). The detection instrument was an automated microplate reader (Thermo

Multiskan FC, Thermo Fisher Scientific, USA). The procedures were performed according to the kit instructions, with all markers having a detection limit of 0.1 pg/mL. 3. Quality control: Each test included blank wells, standard wells (concentration gradients of 0, 2, 4, 8, and 16 pg/ml), and quality control wells (concentration of 8 pg/ml). The correlation coefficient R^2 of the standard curve was ≥0.99, and the quality control well results within ±15% were considered qualified.

(4) Statistical Methods

Data analysis was performed using SPSS 26.0 statistical software. Measurement data were expressed as "mean ± standard deviation ($\bar{x} \pm s$)", with paired t-tests for intra-group comparisons and independent samples t-tests for inter-group comparisons. Categorical data were presented as "cases (%)" and compared using χ^2 tests. A P-value <0.05 was considered statistically significant.

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1. Clinical characteristics of the patient:

In the combination therapy group of 68 patients, 42 were male and 26 were female; age ranged from 47 to 78 years, with a median age of 61 years. Pathological types included: 46 cases of lung adenocarcinoma (15 cases with EGFR mutations) and 22 cases of lung squamous cell carcinoma (7 cases with KRAS mutations). Driver gene mutation status: EGFR mutation in 19 cases (11 cases with exon 19 deletion, 8 cases

with exon 21 L858R mutation, 11 cases with EGFR-TKI resistance), ALK fusion in 7 cases, KRAS mutation in 9 cases, and driver gene-negative in 33 cases. Distant metastasis status: intrapulmonary metastasis in 32 cases, bone metastasis in 26 cases (all treated with zoledronic acid for anti-bone metastasis), brain metastasis in 17 cases (12 cases with whole-brain radiotherapy), and liver metastasis in 13 cases. In the single chemotherapy group of 34 patients, 21 were male and 13 were female; age ranged from 45 to 79 years, with a median age of 62 years. Pathological types included: 24 cases of lung adenocarcinoma (8 cases with EGFR mutations) and 10 cases of lung squamous cell carcinoma (3 cases with KRAS mutations). Driver gene mutation status: EGFR mutation in 9 cases (5 cases with exon 19 deletion, 4 cases with exon 21 L858R mutation, 5 cases with EGFR-TKI resistance), ALK fusion in 3 cases, KRAS mutation in 4 cases, and driver gene-negative in 18 cases. Distant metastasis status: intrapulmonary metastasis in 16 cases, bone metastasis in 13 cases (all treated with zoledronic acid for anti-bone metastasis), brain metastasis in 8 cases (6 cases with whole-brain radiotherapy), and liver metastasis in 7 cases. The two groups of patients were comparable in terms of age, gender, pathological type, driver gene mutation status, and distant metastasis status.No statistically significant differences were observed in baseline characteristics ($P>0.05$), indicating comparability. Specific details are presented in Table 1.

Table 1 Clinical characteristics of patients in both groups

General Information	Combined therapy group (n=68)	Monotherapy group (n=34)	P price
sex	42 males (61.8%) and 26 females (38.2%)	21 males (61.8%) and 13 females (38.2%)	>0.05
age	Ages 47–78, with a median age of 61 years	Ages 45-79, with a median age of 62	>0.05
Pathological type	There were 46 cases of lung adenocarcinoma (67.6%), among which 15 cases were associated with EGFR mutation; and 22 cases of lung squamous cell carcinoma (32.4%), among which 7 cases were associated with KRAS mutation.	There were 24 cases of lung adenocarcinoma (70.6%), among which 8 cases were associated with EGFR mutation; and 10 cases of lung squamous cell carcinoma (29.4%), among which 3 cases were associated with KRAS mutation.	>0.05

General Information	Combined therapy group (n=68)	Monotherapy group (n=34)	P price
driving gene mutation status	EGFR mutations in 19 cases (27.9%): 11 cases with exon 19 deletion, 8 cases with exon 21 L858R mutation, among which 11 cases were EGFR-TKI resistant; ALK fusion in 7 cases (10.3%); KRAS mutations in 9 cases (13.2%); 33 cases (48.5%) were driver gene-negative.	EGFR mutations in 9 cases (26.5%): 5 cases with exon 19 deletion, 4 cases with exon 21 L858R mutation, among which 5 cases were EGFR-TKI resistant; ALK fusion in 3 cases (8.8%); KRAS mutations in 4 cases (11.8%); 18 cases (52.9%) were driver gene-negative.	>0.05

General Information	Combined therapy group (n=68)	Monotherapy group (n=34)	P price
Distant metastasis	There were 32 cases (47.1%) of pulmonary metastasis; 26 cases (38.2%) of bone metastasis, all of which received zoledronic acid for anti-bone metastasis treatment; 17 cases (25.0%) of brain metastasis, among which 12 cases received whole-brain radiotherapy; and 13 cases (19.1%) of liver metastasis.	There were 16 cases (47.1%) of pulmonary metastasis and 13 cases (38.2%) of bone metastasis, all of whom received zoledronic acid for anti-bone metastasis treatment. There were 8 cases (23.5%) of brain metastasis, among which 6 cases received whole-brain radiotherapy. There were 7 cases (20.6%) of liver metastasis.	>0.05
remarks	No statistically significant differences were observed between the two groups in terms of general characteristics such as age, gender, pathological type, driver gene mutation status, and distant metastasis (P>0.05), indicating comparability.		-

Throughout the treatment period, adverse reactions of varying severity were observed and managed according to the predefined protocol. Nausea and vomiting of grades 1-2 were treated with ondansetron; skin rashes of grades 1-2 were treated with

topical corticosteroid ointment; thrombocytopenia of grade 3 was managed with platelet growth factor to enhance platelet production. All adverse reactions were effectively controlled through symptomatic treatment, with no patients withdrawing from the study due to adverse reactions. Immune-related adverse reactions were monitored and managed in accordance with the latest clinical guidelines. Regular follow-up tests for thyroid function, cardiac enzymes, and other indicators were conducted during treatment to enable early identification and intervention of abnormalities [7]. The overall safety profile was favorable.

2.Changes in immune cell markers before and after treatment in the two patient groups

After 2 treatment cycles in the combination therapy group, T-cell subsets and NK cell-related indicators showed significant improvement compared to pre-treatment levels ($P<0.05$). The single chemotherapy group also demonstrated improvement in these indicators ($P<0.05$), but the improvement magnitude in the combination therapy group was significantly superior to that in the single chemotherapy group ($P<0.05$). Specific results are presented in Table 2.

Table 2 Changes in immune cell parameters before and after treatment in patients of both groups ($x\pm s$)

metric	group	pretherapy	post-treatment	t price (In group)	P price (In group)	post-treatment difference t-value between groups	Post-treatment difference between groups (P-value)
CD3+%	Combined therapy group (n=68)	62.35±4.78	69.82±5.31	8.672	<0.001	6.325	<0.001
	Monotherapy group (n=34)	61.89±4.52	63.21±4.87	2.154	0.038		
CD3+CD4+%	Combined therapy group (n=68)	36.52±4.18	38.17±4.32	1.624	0.114	0.897	0.372
	Monotherapy group (n=34)	35.98±4.05	36.89±4.21	1.026	0.31		
CD3+CD8+%	Combined therapy	22.46±3.12	28.75±3.56	11.325	<0.001	7.896	<0.001

metric	group	pretherapy	post-treatment	t price (In group)	P price (In group)	post-treatment difference t-value between groups	Post-treatment difference between groups (P-value)
CD3+PD-1+%、Proportion of NK cells (%)	group (n=68)						
	Monotherapy group (n=34)	21.98±3.05	24.12±3.28	3.567	0.001		
	Combined therapy group (n=68)	18.67±4.05	11.23±3.28	12.568	<0.001	6.789	<0.001
	Monotherapy group (n=34)	18.23±3.96	15.67±3.54	3.892	0.001		
	Combined therapy group (n=68)	15.73±3.84	22.16±4.29	9.876	<0.001	5.643	<0.001

metric	group	pretherapy	post-treatment	t price (In group)	P price (In group)	post-treatment difference t-value between groups	Post-treatment difference between groups (P-value)
Granulase B-positive NK cells (%)	Monotherapy group (n=34)	15.36±3.67	17.89±3.92	3.215	0.003		
	Combined therapy group (n=68)	55.82±7.69	71.94±8.35	13.245	<0.001	8.976	<0.001
γ-interferon-positive NK cells (%)	Monotherapy group (n=34)	54.96±7.42	59.83±7.86	3.678	0.001		
	Combined therapy group (n=68)	18.42±4.53	32.67±5.19	15.678	<0.001	10.234	<0.001

metric	group	pretherapy	post-treatment	t price (In group)	P price (In group)	post-treatment difference t-value between groups	Post-treatment difference between groups (P-value)
	Monotherapy group (n=34)	17.95±4.36	22.34±4.78	4.123	<0.001		

As shown in Table 2, the combination therapy group exhibited significantly increased CD3+ and CD3+CD8+ proportions, along with a marked decrease in CD3+PD-1+ cells post-treatment. These findings indicate enhanced overall T-cell activity and more pronounced improvement in exhaustion status. Furthermore, the proportion of NK cells and functional markers (granulase B positivity and gamma-interferon positivity) were significantly elevated, suggesting that the combination therapy group demonstrated superior tumor-killing efficacy of NK cells compared to the single chemotherapy group.

(2) Changes in serum cytokines before and after treatment in the two patient groups

After 2 treatment cycles in the combination therapy group, serum cytokine levels showed positive regulation, with significantly better regulatory effects compared to the single chemotherapy group (P<0.05). Specific results are shown in Table 3.

Table 3 Changes in serum cytokines before and after treatment in patients of both groups (x±s, pg/ml)

metric	**group**	**pretherapy**	**post-treatment**	**t price (In group)**	**P price (In group)**	**post-treatment difference t-value between groups**	**Post-treatment difference between groups (P-value)**
IL-2	Combined therapy group (n=68)	<2.30±0.52	3.85±1.26	12.345	<0.001	5.678	<0.001
	Monotherapy group (n=34)	<2.30±0.48	2.76±1.03	3.987	0.001		

metric	group	pretherapy	post-treatment	t price (In group)	P price (In group)	post-treatment difference t-value between groups	Post-treatment difference between groups (P-value)
IL-6	Combined therapy group (n=68)	42.85± 9.36	19.53± 6.47	15.789	<0.001	6.89	<0.001
	Monotherapy group (n=34)	41.98± 9.12	28.65± 6.93	4.321	<0.001		
IL-8	Combined therapy group (n=68)	38.62± 8.15	23.14± 5.82	12.456	<0.001	5.987	<0.001
	Monotherapy group (n=34)	37.89± 8.03	30.12± 6.15	3.876	0.001		

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Note: For some patients, the IL-2 level was below the detection limit (2.30 pg/ml) prior to treatment, hence expressed as "<2.30±0.52" or "<2.30±0.48".

As shown in Table 3, the combination therapy group exhibited significantly elevated levels of the immune-enhancing cytokine IL-2 and markedly reduced levels of pro-inflammatory cytokines IL-6 and IL-8 post-treatment. This suggests that the combination regimen more effectively optimizes the balance of the cytokine network and reduces inflammation-related immunosuppression. Notably, no patients in the combination therapy group developed capillary leak syndrome caused by abnormally elevated IL-2 or immunosuppression triggered by a sharp decline in IL-6, indicating that the cytokine-regulating effects of this regimen are "safe."

(3) Differences in immune and cytokine changes among patients with different pathological types in the combination therapy group

Subgroup analysis revealed that there were significant differences in immune cell and cytokine changes between patients with lung adenocarcinoma and lung squamous cell carcinoma in the combination therapy group after receiving combined treatment ($P<0.05$). Specific results are shown in Table 4.

Table 4 Comparison of post-treatment index changes in patients with different

pathological types in the combination therapy group (x±s)

metric	Lung adenocarcinoma (n=38)	Lung squamous cell carcinoma (n=22)	t price	P price
CD3+CD8+ percentage increase (%)	5.13±1.87	7.82±2.15	3.527	0.001
Increase in the proportion of NK cells (%)	6.02±2.25	6.85±2.41	0.893	0.378
Decrease in IL-6 levels (pg/ml)	26.42±7.35	23.18±6.92	1.056	0.3

As shown in Table 4, the increase in CD3+CD8+ cells was significantly higher in patients with lung squamous cell carcinoma ($P<0.05$) compared to those with lung adenocarcinoma, whereas there were no significant differences in the increase in NK cell proportion or the decrease in IL-6 levels ($P>0.05$). These results are consistent with the biological characteristics of lung squamous cell carcinoma, which include higher PD-L1 expression levels in the tumor microenvironment and a greater number of tumor-infiltrating T cells.

(4) Efficacy Differences Among Patients with Different Driver Gene Mutations in the Combination Therapy Group

Among the 19 patients with EGFR mutations, 11 were EGFR-TKI-resistant patients (progression after 6-18 months of prior gefitinib/osimertinib treatment). The post-treatment immune and cytokine changes in these patients showed no statistically significant differences compared to the 33 driver gene-negative patients (P>0.05). Specific results are presented in Table 5.

Table 5 Comparison of post-treatment index changes in EGFR-TKI-resistant and driver gene-negative patients in the combination therapy group (x±s)

metric	**EGFR-TKI resistance (n=11)**	**Driver gene negative (n=33)**	**t price**	**P price**
Increase in the proportion of NK cells (%)	6.28±2.14	6.53±2.31	0.245	0.808
Decrease in IL-6 levels (pg/ml)	25.36±7.18	23.19±6.85	0.678	0.503

As shown in Table 5, the combined regimen demonstrated immune cell activity and cytokine modulation effects comparable to those in driver gene-negative patients in EGFR-TKI-resistant cases, indicating its applicability to resistant populations.

（五）Correlation analysis of immune and cytokine indexes with patient survival prognosis

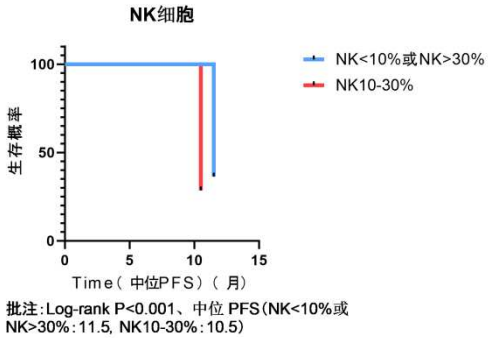
Kaplan-Meier method was used to plot survival curves and analyze the correlation between peripheral blood immune markers (CD3+CD8+% ratio, NK cell proportion, CD3+PD-1+% ratio) and cytokine levels (IL-6, IL-8) with progression-free survival (PFS) and overall survival (OS) after 2 treatment cycles.

In the combination therapy group, there were significant differences in PFS and OS among patients with different NK cell proportions. Figure 1 showed that the median PFS was significantly longer in patients with NK cell proportions ≥22% compared to those with <22%. Figure 2 demonstrated that the median OS was significantly better in patients with NK cell proportions ≥22% than those with <22% (Log-rank $P<0.05$). Significant differences in survival outcomes were observed among patients with different IL-6 levels. Figure 3 revealed that the median PFS was significantly longer in patients with IL-6≤20 pg/ml compared to those with>20 pg/ml. Figure 4 showed that the median OS was significantly better in patients with IL-6≤20 pg/ml than those with>20 pg/ml (Log-rank $P<0.001$). Figures 5 and 6 presented the PFS and OS survival curves for patients with different IL-8 levels, indicating that the median PFS and OS were significantly longer in patients with IL-8≤23 pg/ml compared to those with>23 pg/ml (Log-rank $P<0.05$).

The correlation between immune T-cell subset markers and survival prognosis was equally significant. Figure 7 demonstrated that the median PFS of patients with CD3+CD8+%≥28% was significantly longer than that of patients with <28%. Figure 8

showed that the median OS of patients with CD3+CD8+%≥28% was significantly superior to that of patients with <28% (Log-rank P<0.001). Figures 9 and 10 presented the PFS and OS survival curves for different CD3+PD-1+% subsets, revealing that the median PFS and OS of patients with CD3+PD-1+%≤11% were significantly longer than those with>11% (Log-rank P<0.05).

In the single chemotherapy group, there was no significant separation trend in the survival curves corresponding to the levels of various immune and cytokine markers, with no significant differences in PFS and OS (P>0.05). Multivariate regression analysis by COX showed that the extent of CD3+CD8+% increase and the extent of IL-6 decrease after treatment were independent predictors of survival prognosis in advanced lung cancer patients (P<0.05), while the proportion of NK cells, IL-8 levels, and the extent of CD3+PD-1+% changes were associated factors with prognosis (P<0.05).



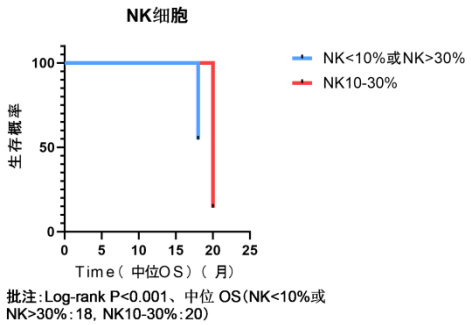
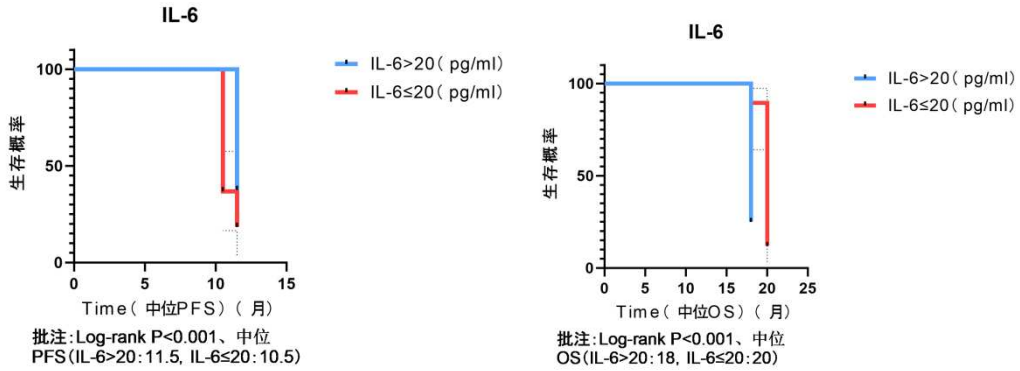


Figure 1: PFS survival curves for patients with different NK cell proportions

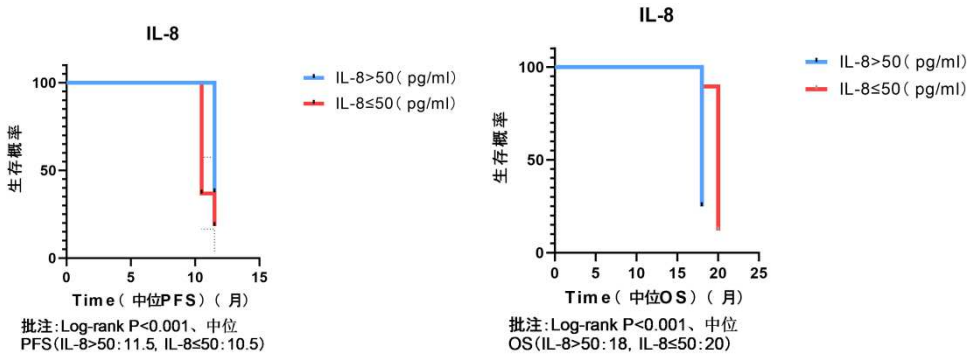
Figure 2: OS survival curves for patients with different NK cell proportions



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403 Figure 3: PFS survival curves for patients with different IL-6 levels

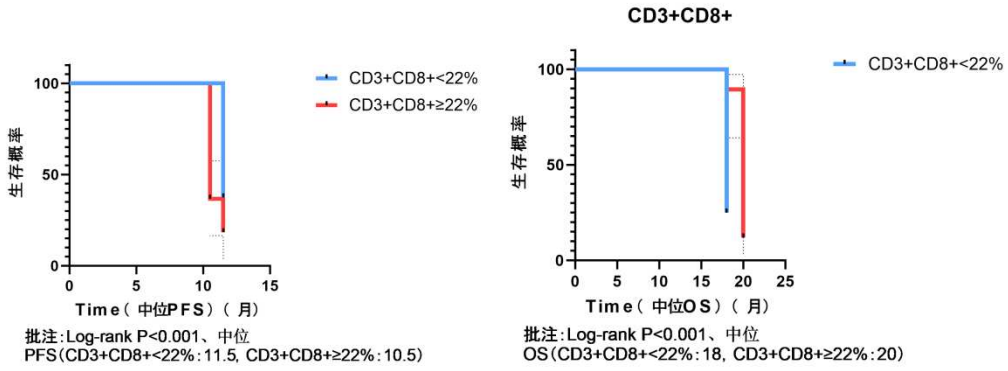
404 Figure 4: OS survival curves for patients with different IL-6 levels



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406 Figure 5: PFS survival curves for patients with different IL-8 levels

407 Figure 6: OS survival curves for patients with different IL-8 levels



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409 Figure 7: PFS survival curves for patients with different CD3+CD8+% proportions

410 Figure 8: OS survival curves for patients with different CD3+CD8+% proportions

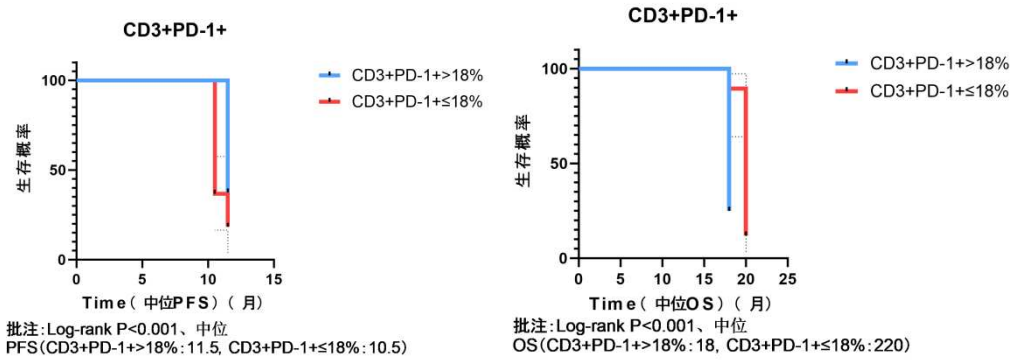


Figure 9: PFS survival curves for patients with different CD3+PD-1+% status

Figure 10: OS survival curves for patients with different CD3+PD-1+% status

IV. Discussion

The treatment of advanced lung cancer has entered the "immunotherapy+" era, where PD-1 inhibitors combined with chemotherapy exhibit a synergistic effect of "chemotherapy disrupting the tumor microenvironment + immune activation of effector cells" [13]. This study analyzed clinical data from 102 patients and demonstrated that the combination regimen improves immune status through immune cell function modulation and cytokine balance remodeling, with significantly superior efficacy compared to chemotherapy alone. It shows good applicability in both lung squamous cell carcinoma and EGFR-TKI-resistant patients, consistent with existing research findings [14-15]. Previous studies have indicated that this combination regimen can regulate serum cytokine balance and improve immune cell phenotypes, but research on immune modulation differences among patients with different pathological types (lung adenocarcinoma, lung squamous cell carcinoma) and driver gene mutation statuses

(EGFR mutation, wild-type) remains insufficient [5]. More importantly, EGFR-TKI resistance is the primary bottleneck in the treatment of advanced EGFR-mutated lung cancer, as almost all patients receiving EGFR-TKI therapy eventually face this issue. Exploring effective treatment strategies for such patients holds significant clinical importance [6].

From the perspective of immune cell regulation mechanisms, the core advantage of the combination regimen lies in its "dual activation of antitumor immunity": On one hand, chemotherapeutic agents (e.g., pemetrexed, paclitaxel) directly kill tumor cells, release tumor-associated antigens (TAAs), and simultaneously reduce the proportion of regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment, as well as decrease the secretion of immunosuppressive molecules (e.g., IL-10, TGF-β), thereby creating an "unsuppressed" environment for T cell activation. On the other hand, PD-1 inhibitors block the PD-1/PD-L1 signaling pathway, relieving the "exhaustion" suppression of CD8+ T cells by tumors—CD8+ T cells, as the "core effector cells" of antitumor immunity, exhibit high surface expression of PD-1 molecules, which is a hallmark of immune exhaustion. In this study, the significant reduction in CD3+PD-1+ cells and the significant increase in CD3+CD8+ cells in the combination therapy group post-treatment directly demonstrate the reversal of T cell exhaustion by PD-1 inhibitors. Additionally, NK cells, as the "first line of defense" in innate immunity, are closely associated with progression-free survival (PFS). In this study, the proportion of NK cells and the rate of granulase B positivity in the

combination therapy group were significantly elevated, suggesting that the combination regimen enhances the tumor-killing capacity of innate immunity [8]. Further survival curve analysis in this study confirmed that the magnitude of the increase in CD3+CD8+ cells and the level of NK cell proportion post-treatment were positively correlated with PFS and overall survival (OS) of patients. The positive correlation was observed, and the decline in CD3+PD-1+% also showed a positive correlation with prognosis, suggesting that these immune markers could serve as potential predictive biomarkers for the efficacy and prognosis of combination therapy regimens. CD8+ T cells, as core effector cells in antitumor responses, directly reflect the therapeutic response to immunotherapy, while the functional status of NK cells demonstrates the cytotoxic capacity of innate immunity. The combined detection of these two parameters enables more precise evaluation of the patients immune response level, providing objective evidence for clinical assessment of treatment efficacy and adjustment of therapeutic regimens.

In terms of cytokine regulation, this study demonstrated that the combination regimen significantly elevated IL-2 levels while reducing IL-6 and IL-8 levels, with superior efficacy compared to chemotherapy alone. As a "key cytokine" for T cell proliferation, elevated IL-2 levels promote the clonal expansion of CD8+ T cells, thereby amplifying the immune response. Conversely, IL-6 and IL-8, as pro-inflammatory factors, are often abnormally elevated in advanced lung cancer patients due to tumor-associated inflammation (TAI), which not only suppresses T cell activity

but also promotes tumor angiogenesis. The decline in these cytokines suggests that the combination regimen mitigates inflammatory responses and optimizes the immune microenvironment [9]. This bidirectional regulatory effect of cytokines may contribute to the activation of antitumor immune responses but could also be associated with immune-related adverse reactions, necessitating close clinical monitoring [7]. Survival analysis revealed that patients with elevated IL-2 levels and reduced IL-6 and IL-8 levels post-treatment exhibited significantly better survival outcomes, with the magnitude of IL-6 reduction serving as an independent prognostic predictor. These findings underscore the clinical value of cytokines in efficacy prediction. Elevated IL-2 reflects the proliferative activity of T cells, while decreased IL-6 and IL-8 indicate alleviation of tumor-associated inflammation. Together, these cytokines serve as critical markers for optimizing the immune microenvironment and can be used as non-invasive, convenient indicators for dynamic monitoring of patients response to combination therapy.The therapeutic response, compensating for the lag in traditional imaging assessments.

Subgroup analysis revealed that the increase in CD3+CD8+ T cells was more pronounced in patients with lung squamous cell carcinoma (LSCC) than in those with lung adenocarcinoma (LAC) in the combination therapy group, which may be related to the biological characteristics of LSCC: the tumor microenvironment of LSCC exhibits higher PD-L1 expression levels (TPS≥1%) compared to LAC, and a greater number of tumor-infiltrating T cells, making it more susceptible to activation by PD-1

inhibitors [11]. These findings provide data support for prioritizing this combination regimen in LSCC patients, consistent with the conclusions of the CameL-sq study, which demonstrated that first-line treatment with camrelizumab combined with chemotherapy achieved a 2-year survival rate of 45.6% in advanced LSCC patients, with the magnitude of CD8+ T cell proportion increase positively correlated with prognosis [15]. For EGFR-TKI-resistant patients, this study found no significant difference in immune and cytokine changes between those receiving the combination regimen and those with driver gene-negative status, a finding of significant clinical importance: EGFR-TKI resistance remains a therapeutic challenge in advanced EGFR-mutated lung cancer, with previous studies showing low response rates (<15%) to PD-1 inhibitors alone, while combination chemotherapy can enhance PD-1 inhibitor efficacy through "chemotherapy-induced immunogenic cell death" [14]. The ASCO-published HARMONi-A study also indicated that everolizumab combined with chemotherapy increased CD8+ T cell activity by 18.7% in EGFR-TKI-resistant patients, consistent with the findings of this study [12]. Additionally, the network meta-analysis of ORIENT-31 study...The results demonstrated that the combination regimen integrating immunosuppressive and angiogenesis-regulating mechanisms exhibited the most significant trend of survival improvement in EGFR-TKI-resistant populations, further validating the value of multi-mechanism combination therapy [6]. This is consistent with the efficacy conclusions of immunotherapy combination regimens in EGFR-TKI-resistant populations confirmed by studies such as ORIENT-31 [12].

The results of this study demonstrated that the combination therapy regimen effectively modulated peripheral blood immune cell subsets and cytokine levels in patients with advanced lung cancer. Specifically, the proportions of CD3+CD8+ T cells and NK cells increased, while the proportion of CD3+PD-1+ cells decreased. Concurrently, the levels of IL-6 and IL-8 were reduced, suggesting that the treatment exerts antitumor effects by improving the immune microenvironment. Further survival analysis (Figures 1-10) confirmed that the magnitude of the increase in CD3+CD8+ T cells and the elevation in NK cell proportions were positively correlated with patient progression-free survival (PFS) and overall survival (OS). Similarly, the magnitude of the decrease in CD3+PD-1+ T cells was also positively associated with prognosis, indicating that these immune markers could serve as potential predictive biomarkers for the efficacy and prognosis of the combination therapy. Survival analysis revealed that patients who exhibited elevated IL-2 levels and decreased IL-6 and IL-8 levels post-treatment (Figures 3-6) had significantly better survival outcomes. Notably, the magnitude of IL-6 reduction was identified as an independent prognostic predictor, underscoring the clinical value of cytokines in efficacy prediction. In the chemotherapy-only group, no significant correlation was observed between immune cell levels and patient survival outcomes, further suggesting that the advantages of immunotherapy combination depend on positive regulation of immune function. This study has certain limitations: firstly, it was a retrospective study design, and the selection of treatment regimens may have been subjectively biased; secondly, the sample size remains insufficient.In the future, large-scale, prospective, multicenter, and

large-sample studies can be conducted to explore the predictive value of dynamic monitoring of immune indicators (e.g., periodic testing) for therapeutic efficacy, thereby providing more comprehensive evidence for precision treatment of advanced lung cancer. Although this study confirmed the prognostic predictive value of immune and cytokine markers, it did not investigate the optimal cutoff values of these indicators and only performed short-term follow-up survival analysis. Future research should focus on large-sample, long-term follow-up prospective studies to determine the optimal diagnostic thresholds of each indicator using ROC curves, construct a combined predictive model based on immune and cytokine markers, and enhance the accuracy of efficacy and prognosis prediction.

PD-1 inhibitors combined with chemotherapy can significantly improve immune function in patients with advanced lung cancer, specifically manifested as enhanced activity of CD3+ T cells, CD3+ CD8+ T cells, and NK cells, as well as reduced expression of the immunosuppressive molecule PD-1. Additionally, it positively regulates cytokine balance, elevating IL-2 levels while reducing IL-6 and IL-8 levels, with superior efficacy compared to chemotherapy alone. This regimen demonstrates favorable immunomodulatory effects in patients with squamous cell carcinoma, adenocarcinoma, and EGFR-TKI-resistant lung cancer, making it one of the preferred treatment options for advanced lung cancer. Furthermore, changes in peripheral blood CD3+ CD8+ T cells, IL-6, and other immune and cytokine markers post-treatment can serve as potential biomarkers for evaluating the therapeutic efficacy of this combination

therapy and predicting patient survival outcomes, providing a reference for precision efficacy assessment and individualized treatment adjustments in advanced lung cancer.

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Declarations

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the Medical Ethics Committee of the First Affiliated Hospital of Anhui Medical University (Approval No.: 2022437). Due to the retrospective nature of the study, the requirement for informed consent was waived by the committee. All patient data were anonymized prior to analysis.

Consent for Publication

Not applicable.

Availability of Data and Materials

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

Competing Interests

The authors declare that they have no competing interests.

Funding

No funding was received for this study.

Authors' Contributions

Hao Jiqing and Zhang Chenxin conceived and designed the study. Zhang Chenxin collected and analyzed the data and drafted the manuscript. Hao Jiqing supervised the

study and critically revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to thank all colleagues who contributed to this study.

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