The peripheral CD4+ T cells predict efficacy in non-small cell lung cancer (NSCLC) patients with the anti-PD-1 treatment

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Background: Programmed cell death protein 1 (PD-1) inhibitor therapy has become a routine treatment for advanced non-small cell lung cancer (NSCLC). However, only some NSCLC patients would benefit from anti-PD-1 therapy. We urgently need to identify biomarkers associated with clinical response to change treatment strategies promptly for patients who fail to benefit from anti-PD-1 treatment. This study was aimed to explore whether circulating CD4+ T cells and CD8+ T cells could be biomarkers for predicting anti-PD-1 efficacy.

Methods: In this study, 118 NSCLC patients who received anti-PD-1 therapy were enrolled. The percentages of circulating CD4+ T cells and CD8+ T cells before and after anti-PD-1 treatment were determined by flow cytometry. The programmed cell death ligand 1 (PD-L1) expression of tumor tissues was detected by immunocytochemistry. The anti-PD-1 treatment efficacy was assessed by immune response evaluation criteria in solid tumors (iRECIST).

Results: The percentage of CD4+ T cells and CD4+/CD8+ ratio in the peripheral blood (PB) was significantly elevated after anti-PD-1 treatment. In contrast, the percentage of CD8+ T cells in the PB was significantly decreased after anti-PD-1 treatment. Furthermore, we found that the percentages of CD4+ T cells and CD4+/CD8+ ratios considerably increased, and the percentages of CD8+ T cells significantly reduced in the effective group. On the contrary, the patients in the ineffective group showed no significant differences in the biomarkers. Multivariate logistic revealed that the percentage of CD4+ T cells at baseline was an independent predictor of anti-PD-1 treatment. The area under the curve (AUC) of the CD4+ T cells percentage was 0.7834 with a cut-off value of 28.53% (sensitivity =82.5%, specificity =66.23%).

Conclusions: The percentage of CD4+ T cells at baseline could predict anti-PD-1 efficacy in NSCLC patients.

Keywords: Programmed cell death protein 1 inhibitor (PD-1 inhibitor); non-small cell lung cancer (NSCLC); CD4+ T cells; CD4+/CD8+ ratio

Introduction

Non-small cell lung cancer (NSCLC) accounts for about 80% of primary lung cancer, the leading cause of cancer mortality (1). Immune checkpoint inhibitor (ICI) therapy could significantly improve the prognosis of NSCLC patients and has been a promising treatment strategy (2). However, some patients still could not benefit from ICIs therapy. Some researchers demonstrate that a small proportion of NSCLC patients receiving mono-immunotherapy do not benefit from it but experienced hyper-progression (3). Therefore, it is urgent to explore novel biomarkers for predicting the efficacy of ICI therapy in NSCLC patients to adjust treatment strategies on time for those unable to benefit from ICI therapy.

Programmed cell death protein 1/programmed cell death ligand 1 (PD-1/PD-L1) inhibitors show promising efficiency in some solid tumors, such as NSCLC (2,4). Although the PD-L1 tumor percentage score (TPS) of tumor tissue is a good predictor for the efficacy of ICIs therapy in NSCLC patients, the accuracy of PD-L1 TPS still needs to be improved. Some groups demonstrated that the objective response rate of NSCLC patients with PD-L1 TPS ≥50% was only 39.1% (5). Tumor-infiltrating immune cells, gene mutation, and immune gene signatures are also related to efficacy for anti-PD-1 treatment (6). Additionally, PD-L1 single nucleotide polymorphisms rs822336 could induce of PD-L1 expression and act as a novel biomarker for predicting PD-1 inhibitors immunotherapy in advanced NSCLC patients (7). However, their utility is still limited

due to the difficulties in obtaining tumor tissues and the high cost (8,9). Therefore, exploring novel non-invasive biomarkers that could predict the PD-1/PD-L1 inhibitor's efficacy is urgent. As a non-invasive and dynamic method of predicting and evaluating ICIs treatment outcomes, the liquid biopsy is considered an appropriate method.

Activating lymphocytes is critical for ICIs treatment. Thus, ICIs treatment is therefore closely associated with lymphocytes. T cells mainly comprise CD4^{+} T cells and CD8^{+} T cells, the central effector cells of immunotherapy, and constitute the primary effector cells involved in anti-PD-1 treatment response (10,11). CD8^{+} T cells play an essential role in anti-tumor immunity since they can directly kill tumor cells and migrate into tumor tissues from peripheral blood (PB) (12). CD4^{+} T cells act as an immune-modulatory role in anti-tumor response in contrast to cytotoxic CD8^{+} T cells (13). CD4^{+} T cells could promote initial CD8^{+} T cells differentiation into cytotoxic T cells by the CD70-CD27 pathway and secreting some cytokines, such as interleukin-2 and interferon γ (14). Recently, some literature has demonstrated that the peripheral CD4^{+} T cells may be related to response to anti-PD-1 treatment (15,16). Several studies have revealed lymphocyte subsets in PB that could predict anti-PD-1 treatment efficacy in NSCLC patients (17,18). In this study, we sought to determine whether peripheral CD4^{+} T cells and CD8^{+} T cells could be potential biomarkers for predicting anti-PD-1 treatment efficacy in NSCLC patients. We present this article in accordance with the TRIPOD reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-405/rc).

Methods

Patients

NSCLC patients receiving anti-PD-1 treatment were enrolled in this study from September 2021 to July 2022 at The First Affiliated Hospital of Fujian Medical University. We determined the percentages of peripheral CD4^{+} T cells and CD8^{+} T cells before and after anti-PD-1 therapy. Clinicopathological data such as age, smoking history, distant metastasis, and pathological type were collected retrospectively by reviewing the medical records. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This research was approved by the Ethical Committee of The First Affiliated Hospital of Fujian Medical University (No. MRCTA, ECFAH of FMU [2021] 378). All patients gave their informed consent

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Highlight box

Key findings

- The percentage of CD4^{+} T cells at baseline could predict anti-programmed cell death protein 1 (anti-PD-1) efficacy in non-small cell lung cancer (NSCLC) patients.

What is known and what is new?

- T cell subsets have been reported to be associated with the clinical outcomes of anti-PD-1 treatment in some tumors.
- The percentage of circulating CD4^{+} T cells at baseline could predict anti-PD-1 efficacy in NSCLC patients and the cutoff is 28.53% which sensitivity and specificity were 82.5% and 66.23%, respectively.

What is the implication, and what should change now?

- CD4^{+} T cells have the potential to be a prognostic marker of anti-PD-1 treatment in NSCLC patients. More studies should be conducted to verify our result.

Table 1 Clinical characteristics of NSCLC patients

Characteristics	Data, n (%)
Gender	
Male	86 (72.9)
Female	32 (27.1)
Age (years)	
≥65	61 (51.7)
<65	57 (48.3)
Smoker	
Yes	35 (29.7)
No	83 (70.3)
Histology	
Adenocarcinoma	66 (55.9)
Squamous cell carcinoma	52 (44.1)
Distant metastasis	
Yes	39 (33.1)
No	79 (66.9)
EGFR mutation	
Yes	47 (39.8)
No	71 (60.2)
PD-L1 expression	
≥50%	84 (71.2)
<50%	34 (28.8)

NSCLC, non-small cell lung cancer; EGFR, epidermal growth factor receptor; PD-L1, programmed cell death ligand 1.

to participate.

Response assessment

The anti-PD-1 treatment efficacy of NSCLC patients was assessed after 4 cycles of anti-PD-1 treatment. The NSCLC patients were divided into effective and ineffective groups according to short-term clinical efficacy. Stable disease (SD), partial response (PR), and complete response (CR) were classified into the effective group. Progressive disease (PD) was classified into the ineffective group.

The expression levels of PD-L1 in tumor tissues

Tumor tissues were obtained by biopsy and then stained with anti-PD-L1 antibodies on the automatic immunohistochemical staining apparatus. The PD-L1 TPS is the ratio of tumor cells that are partial or complete membrane staining.

PB collection and flow cytometry

PB (about 1mL) was obtained from NSCLC patients at baseline and after 4 cycles of PD-1 inhibitors treatment. The CD3+ T cells, CD4+ T cells, and CD8+ T cells in the PB were determined by BD Canto II flow cytometry. The samples were stained by CD3-FITC (Biolegend HIT3a), CD4-APC-Cy7 (BD Pharmingen RPA-T4) and CD8-APC (BD RPA-T8).

Statistical analysis

Statistic analysis was performed using GraphPad Prism 7.0 and SPSS 19.0 software. Tests for the differences between the two groups were performed using a t-test. Multivariate analyses of the factors associated with anti-PD-1 treatment efficacy were tested by logistic. Receiver operator characteristic (ROC) curves were performed to determine the predictor's area under the curve (AUC), sensitivity and specificity. P values <0.05 were considered statistically significant.

Results

The clinical characteristics of NSCLC patients

A total of 118 NSCLC patients were enrolled in this study. The clinical characteristics of NSCLC patients at baseline are listed in *Table 1*. All NSCLC patients were PD-L1 TPS positive. About 71.2% (84/118) of NSCLC patients had TPS ≥50%.

The changes in percentages of circulating CD4+ T cell and CD8+ T cell after anti-PD-1 treatment

The flow gates strategy of CD4+ T cells and CD8+ T cells are shown in *Figure 1*. The percentages of CD4+ T cells and CD8+ T cells were determined before and after anti-PD-1 treatment (*Figure 2A*). The percentage of peripheral CD4+ T cells in PB from NSCLC patients were significantly increased (*Figure 2B*). In contrast, the percentage of peripheral CD8+ T cells in PB from NSCLC patients were significantly decreased compared to that at baseline (*Figure 2C*). Consequently, the CD4+/CD8+ ratios were significantly elevated after anti-PD-1
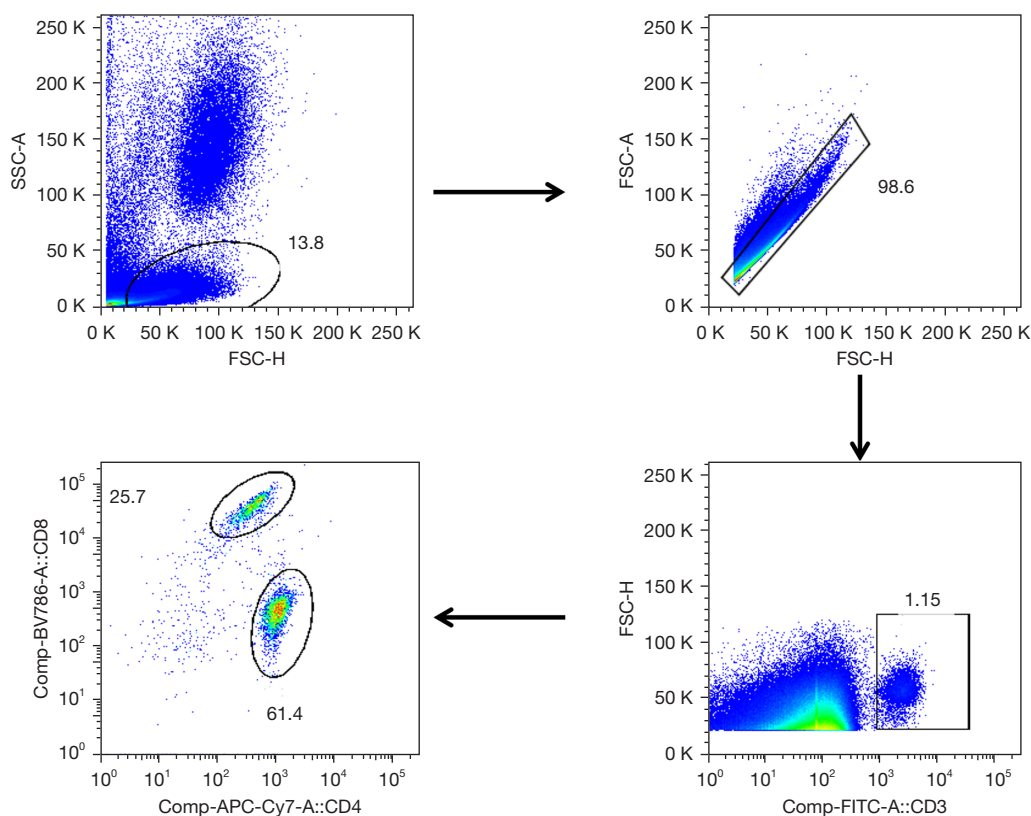
Figure 1 Gating strategy for flow cytometry identification of CD4^{+} T cells and CD8^{+} T cells. SSC-A, side scatter area; FSC-A, forward scatter area; FSC-H, forward scatter height; FITC, fluorescein isothiocyanate; APC, allophycocyanin.

treatment compared to that at baseline (*Figure 2D*).

The changes in percentages of circulating CD4^{+} T cells and CD8^{+} T cells in NSCLC patients from different efficacy groups after anti-PD-1 treatment

After anti-PD-1 treatment, 78 NSCLC patients and 40 NSCLC patients were classified as the effective and ineffective groups, respectively. The efficacy of anti-PD-1 treatment was 73.7%. As shown in *Figure 3A*, we compared the percentages of circulating CD4^{+} T cells and CD8^{+} T cells in NSCLC patients from the effective and ineffective groups. We found that the percentages of CD4^{+} T cells in the PB were significantly elevated after immunotherapy in the effective group (*Figure 3B*). The percentages of CD8^{+} T cells decreased considerably after immunotherapy in the effective group (*Figure 3C*). The CD4^{+}/CD8^{+} ratios were dramatically increased after immunotherapy in the effective group (*Figure 3D*). However, there was no significant change in the percentages of CD4^{+} T cells and CD8^{+} T cells in the PB from patients classified as an ineffective group compared to the baseline (*Figure 3E,3F*).

The percentage of peripheral CD4^{+} T cells and CD8^{+} T cells at baseline between the effective group and the ineffective group

We found significant differences between the effective and ineffective groups in PD-L1 TPS (P=0.005), CD4^{+} T cells (P<0.0001), CD8^{+} T cells (P=0.03), and CD4^{+}/CD8^{+} ratios (P=0.003). The ratio of PD-L1 TPS ≥50% was higher in the effective group than the ineffective group (*Figure 4A*). The percentages of circulating CD4^{+} T cells (*Figure 4B*) at baseline were higher in the effective group compared to that in the ineffective group. The percentages of baseline CD8^{+} T cells (*Figure 4C*) were also higher in the effective group than in the ineffective group. The CD4^{+}/CD8^{+} ratios (*Figure 4D*) at baseline were higher in the effective group compared to that in the ineffective group.
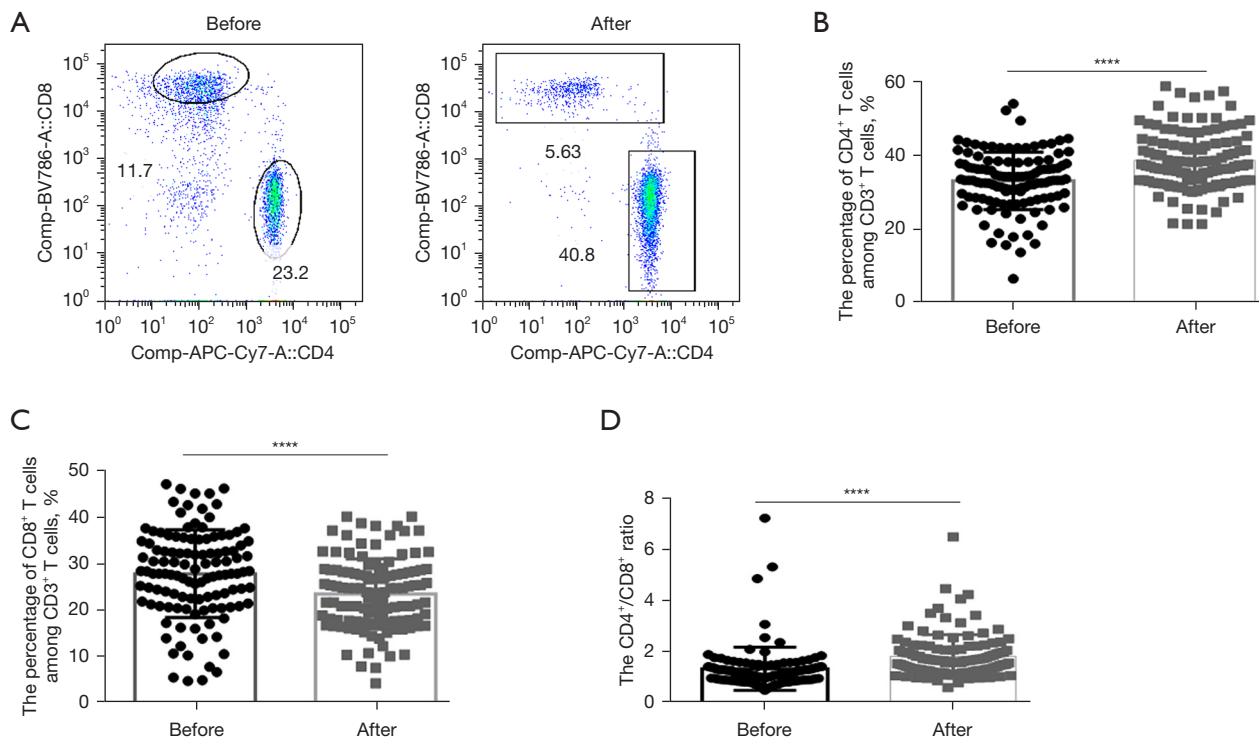
Figure 2 The changes in percentages of circulating CD4⁺ T cells and CD8⁺ T cells after anti-PD-1 treatment. (A) Flow cytometric analysis of the percentages of CD4⁺ T cells and CD8⁺ T cells among CD3⁺ T cells in the PB from NSCLC patients before and after treatment. (B,C) The quantification of the percentages of CD4⁺ T cell subset (B) and CD8⁺ T cell subset (C) in the PB from NSCLC patients before treatment (n=118) and after treatment (n=118). (D) The quantification of the peripheral CD4⁺/CD8⁺ ratio before treatment (n=118) and after treatment (n=118). Each symbol indicates a person. The results were performed by Student's non-paired *t*-test (B-D) (****, P<0.0001). APC, allophycocyanin; NSCLC, non-small cell lung cancer; PD-1, programmed cell death protein 1; PB, peripheral blood.

### The percentage of peripheral CD4+ T cells could predict anti-PD-1 efficacy in NSCLC patients

Then, we constructed the logistic model that included the above parameters and found that the percentage of peripheral CD4⁺ T cells (odds ratio: 0.872, 95% confidence interval: 0.862–0.935, P=0.02) was an independent predictor of anti-PD-1 treatment efficacy (*Table 2*). ROC curves revealed that the AUC area of the percentage of peripheral CD4⁺ T cells at baseline for predicting the efficacy of anti-PD-1 treatment in NSCLC patients was 0.7834 (*Figure 5*). The cut-off value was 28.53 for the percentage of peripheral CD4⁺ T cells (sensitivity =82.5%, specificity =66.23%) with maximum AUC.

Discussion

There is still an urgent need to explore novel and reliable predictors for anti-PD-1 efficacy despite some researchers having identified some biomarkers for predicting the effectiveness of anti-PD-1 treatment in NSCLC patients (19). We found that the percentage of peripheral CD4⁺ T cells and CD4⁺/CD8⁺ ratios were significantly elevated, while the percentage of peripheral CD8⁺ T cells decreased considerably after anti-PD-1 treatment in the effective group. However, there was no significant change in the ineffective group's percentages of peripheral CD4⁺ T cells, peripheral CD8⁺ T cells, and CD4⁺/CD8⁺ ratio before and after anti-PD-1 treatment. Moreover, we found that the percentage of peripheral CD4⁺ T cells at the baseline could be an independent predictor for the efficacy of anti-PD-1 therapy. The ROC curves verified the value of the percentage of peripheral CD4⁺ T cells for predicting anti-PD-1 efficacy.

The CD4⁺ T cells in the PB is necessary for anti-tumor immunity, regulating and promoting the activation, migration, and cytotoxicity ability of CD8⁺ T cells (20,21). Our results revealed that the percentages of peripheral
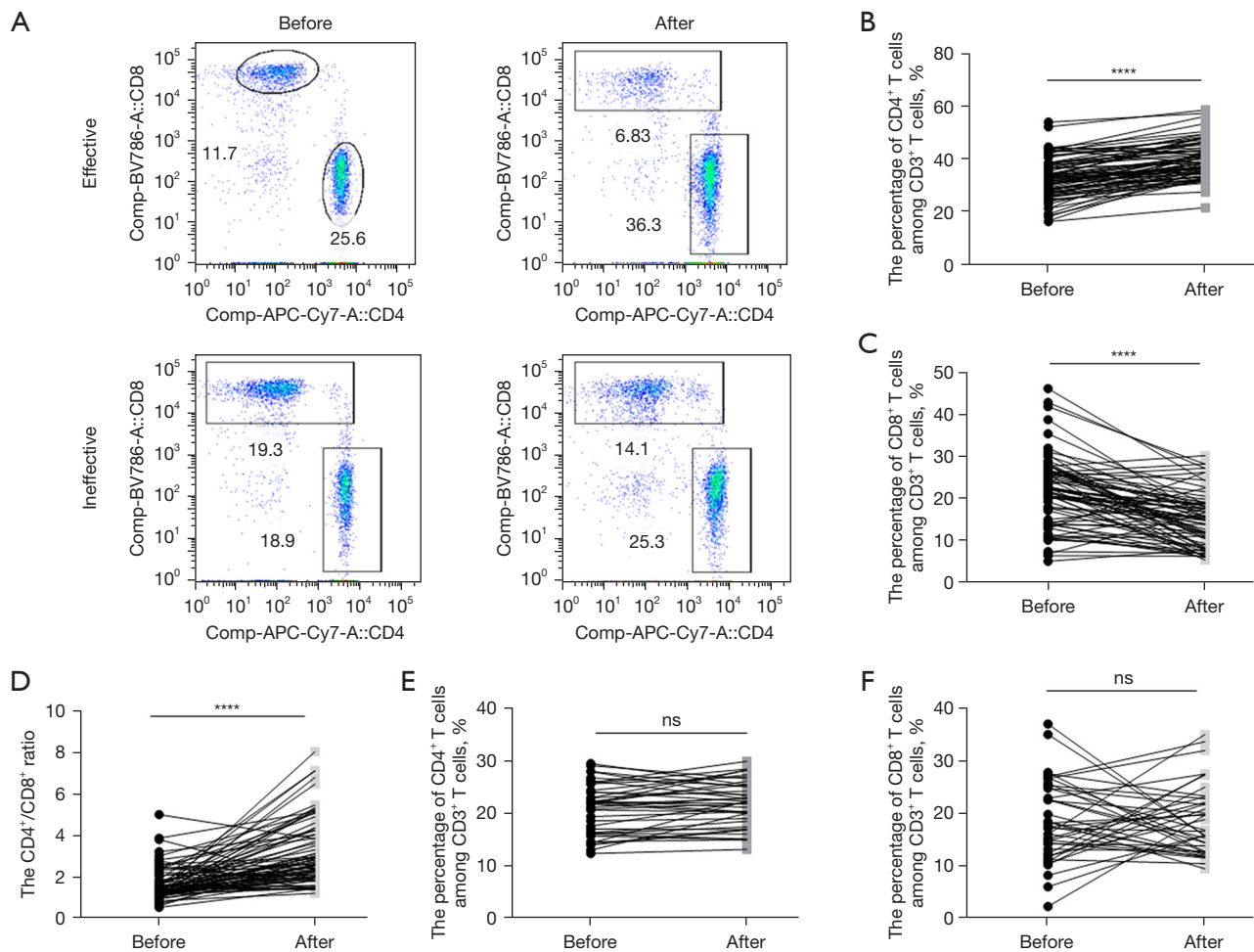
Figure 3 The changes in percentages of circulating CD4+ T cells and CD8+ T cells in NSCLC patients from different efficacy groups after anti-PD-1 treatment. (A) Flow cytometric analysis of the percentages of CD4+ T cells and CD8+ T cells among CD3+ T cells in the PB from NSCLC patients in effective group and ineffective group. (B) The percentages of CD4+ T cells in the PB from NSCLC patients in the effective group were quantified before and after treatment (n=78). (C) The quantification of the percentages of CD8+ T cells in the PB from NSCLC patients in the effective group before and after treatment (n=78). (D) The CD4+/CD8+ ratios were increased after immunotherapy in the effective group (n=78). (E) The quantification of the percentages of CD4+ T cells in the PB from NSCLC patients in the ineffective group before and after treatment (n=40). (F) The quantification of the percentages of CD8+ T cells in the PB from NSCLC patients in the ineffective group before and after treatment (n=40). Each symbol indicates a person. The results were performed by Student's paired *t*-test (ns: no significance; ****, P<0.0001). APC, allophycocyanin; NSCLC, non-small cell lung cancer; PD-1, programmed cell death protein 1; PB, peripheral blood.

CD4+ T cells were elevated compared to the baseline in the effective group. In comparison, there was no alteration in the percentages of CD4+ T cells after anti-PD-1 treatment in the ineffective group. These results suggested that NSCLC patients in the effective group had a durable anti-tumor response and may continuously recruit anti-tumor T cells from the PB into tumor tissue. The high percentage of CD4+ T cells at baseline in NSCLC patients who received

anti-PD-1 inhibitor treatment was related to a better prognosis (22,23). Some researchers reported that CD4+ T cells subsets could predict the efficacy of anti-PD-1 therapy and the survival of patients, and the high proportion of memory CD4+ T cell subsets at baseline indicated the clinical beneficiaries (24,25). Some researchers revealed the rate of CD4+T effector memory subsets in the responders before anti-PD-1 treatment was significantly higher (26).

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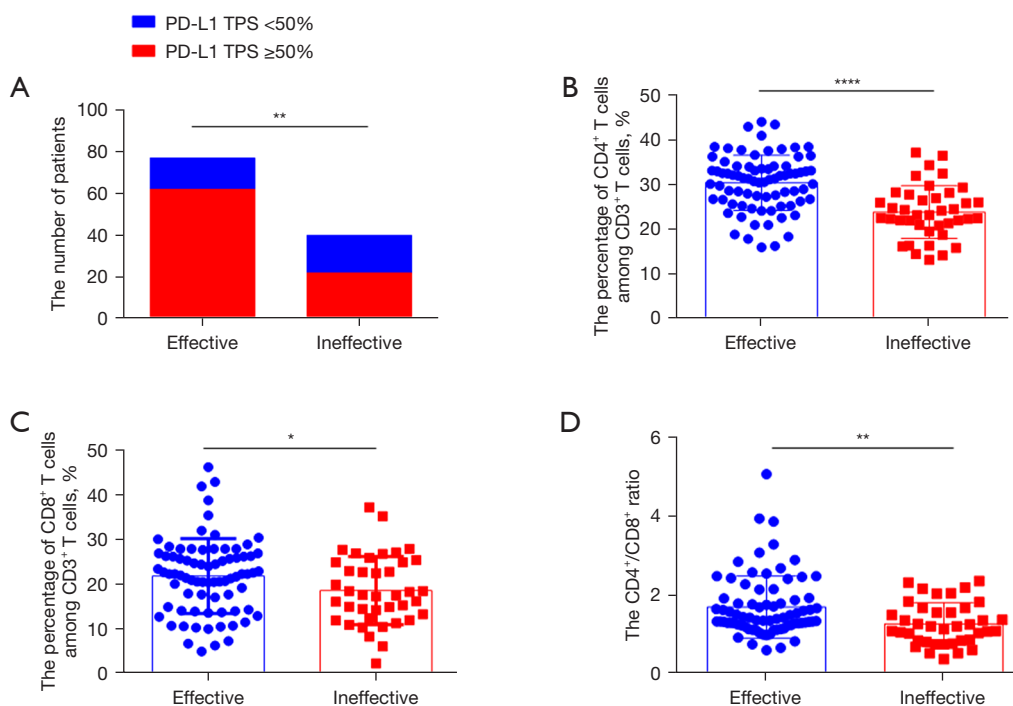
Figure 4 The percentage of peripheral CD4+ T cells and CD8+ T cells at baseline between the effective group and the ineffective group. (A) The quantification of the ratio of PD-L1 TPS ≥50% between the effective group (n=78) and the ineffective group (n=40). (B-D) The quantification of the percentages of circulating CD4+ T cells (B), CD8+ T cells (C), and CD4+/CD8+ ratio (D) between the effective group (n=78) and the ineffective group (n=40). The results were performed by Student's non-paired t-test (*, P<0.05; **, P<0.01; ****, P<0.0001). PD-L1, programmed cell death ligand 1; TPS, tumor percentage score.

Table 2 Logistic analysis for independent predictors for anti-PD-1 treatment efficacy in NSCLC patients

| Variable	B	SD	Odds ratio (95% CI)	P value
PD-L1 TPS	−5.17	2.21	0.568 (0.321–0.958)	0.06
CD4+ T cells	−6.13	3.127	0.872 (0.862–0.935)	0.02
CD8+ T cells	−0.203	0.265	0.817 (0.486–1.373)	0.44
CD4+/CD8+ ratio	−5.288	2.811	0.005 (0.000–1.247)	0.26

PD-1, programmed cell death protein 1; NSCLC, non-small cell lung cancer; B, coefficient values; SD, standard deviation; CI, confidence interval; PD-L1, programmed cell death ligand 1; TPS, tumor percentage score.

Another study verified that higher proportions of central memory and effector CD4+ T cell subsets were related to immunotherapy benefits in NSCLC patients receiving nivolumab treatment (27).

Thus, CD4+ T cells play an essential role in anti-tumor response. Monitoring circulating CD4+ T cells during anti-PD-1 treatment could help to evaluate the efficacy of immunotherapy and identify the patients who could not benefit from immunotherapy. Some researchers found that

dysfunctional CD4+ T cells were also associated with the anti-PD-1 treatment efficacy (28,29). High expression of CD28 is a hallmark of T cells. A substantial expansion of the CD28−CD4+ T cell subset is significantly related to disease progression (28). PD-1/lymphocyte activation gene-3 (LAG-3) co-signaling could promote systemic CD4+ T cells dysfunction, and a combination of PD-1 and LAG-3 blockade may improve the efficacy of immunotherapy (29).

Although this study demonstrated that the percentage
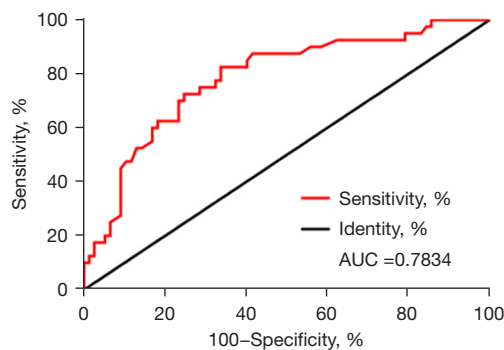
Figure 5 The ROC curves analysis of the percentage of peripheral CD4$^+$ T cells to predict anti-PD-1 efficacy in NSCLC patients. AUC, area under the curve; ROC, receiver operator characteristic; PD-1, programmed cell death protein 1; NSCLC, non-small cell lung cancer.

of CD4$^+$ T cells at baseline could predict the efficacy of anti-PD-1 treatment in NSCLC patients, we just focused on the percentages of CD4$^+$ T cells before and after anti-PD-1 treatment. We did not analyze the alteration in subgroups and function of CD4$^+$ T cells. Thus, we should focus on the dysfunctional CD4$^+$ T cells and the role of CD4$^+$ T cells subsets in anti-PD-1 treatment. Furthermore, some literature demonstrated that the soluble form of PD-L1 could act as a noninvasive marker that correlates over time with lymphocyte markers (30), as also demonstrated in other fields other than cancer (31). Unfortunately, we did not determine the soluble form of PD-L1 in PB from NSCLC patients before and after PD-1 treatment.

CD8$^+$ T cells could differentiate into cytotoxic T cells and then migrate into tumor tissues from PB to directly kill tumor cells (32). In this study, the percentage of peripheral CD8$^+$ T cells was decreased compared to the baseline in the effective group. Accordingly, the CD4$^+$/CD8$^+$ ratio in PB was higher after anti-PD-1 treatment than at the baseline in the effective group. The CD4$^+$/CD8$^+$ ratios in patients could reflect cell-mediated immunity, and a low CD4$^+$/CD8$^+$ ratio indicates immunosuppression (33). However, in this study, although we found that CD8$^+$ T cells and CD4$^+$/CD8$^+$ ratio had significantly changed after anti-PD-1 treatment, they were not independent predictors for anti-PD-1 treatment efficacy.

There are some limitations in our study. We determined the percentages of peripheral CD4$^+$ T cells and CD8$^+$ T cells and did not detect the absolute counts of CD4$^+$ T cells and CD8$^+$ T cells. Prospective collecting samples to analyze

the percentage and total count would be more valuable in future studies. Furthermore, considering that the primary goal of anti-PD-1 treatment for NSCLC patients is to prolong overall survival, the following research should extend the duration of follow-up and monitor the changes in CD4$^+$ T cells and CD8$^+$ T cells longitudinally to explore their correlation with overall survival.

Conclusions

CD4$^+$ T cells play a critical role in the immunotherapy in NSCLC patients. The percentage of CD4$^+$ T cells at baseline could predict anti-PD-1 efficacy in NSCLC patients.

Acknowledgments

Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-405/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-405/dss

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-405/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-405/coif). The 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). This research was approved by the Ethical Committee of The First Affiliated Hospital of Fujian Medical University (No. MRCTA, ECFAH of FMU [2021] 378). All patients gave their informed consent to participate.

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