TCF-1^{+} PD-1^{+} CD8^{+}T Cells Are Associated With The Response To PD-1 Blockade In Non-Small Cell Lung Cancer Patients

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Research Article

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Abstract

Purpose: To determine whether TCF-1$^+$ PD-1$^+$ CD8$^+$T cells are associated with the response to PD-1 blockade in non-small cell lung cancer (NSCLC) patients.

Methods: We investigated the expression of TCF-1$^+$ PD-1$^+$ CD8$^+$T cells and elucidated their predictive role in NSCLC patients. Pretreatment specimens from fifteen advanced NSCLC patients who underwent PD-1 immunotherapy or combined with chemotherapies were analyzed. The frequency of TCF-1$^+$ cells in PD-1$^+$ CD8$^+$T cells were determined in these biospecimens by using multi-label immunofluorescence staining and multi-spectral acquisition technology. The clinical role of TCF-1$^+$PD-1$^+$CD8$^+$T cells were evaluated via analyzing our patients' clinic parameters and public NSCLC database.

Results: A high frequency of TCF-1$^+$ PD-1$^+$ CD8$^+$T cells were identified in responders compared with non-responders (p=0.0427), and the patients with high expression of this cell subset had durable clinical benefit of anti-PD-1 therapy. There were no significant association between the expression of TCF-1$^+$ PD-1$^+$ CD8$^+$T cells and patients' age, gender, smoking history, pathologic type and genetic status. In univariate logistic regression analysis, high frequency of TCF-1$^+$ PD-1$^+$ CD8$^+$T cells were significantly correlated with patients' benefit of PD-1 blockade (p=0.035).

Conclusion: Our study indicated that TCF-1$^+$ PD-1$^+$ CD8$^+$T cells are associated with the response to PD-1 blockade, and may be a predictor of anti-PD-1 therapy.

Introduction

PD-1/L1 immunotherapy, the checkpoint inhibitor-based treatment, has favorable efficiency in virous cancers, including melanoma [1], colorectal cancer [2], renal cell carcinoma [3], prostate cancer and lung cancer, etc [4–5]. Programmed cell death ligand-1 (PD-L1) expression in tumor cells is widely used as a predictor for anti-PD-1/L1 therapy. In non-small cell lung cancer (NSCLC), the objective response rate of PD-1/L1 immunotherapy is about 20% with mainly rely on the expression of PD-L1 [6]. But in fact, some NSCLC patients with negative expression of PD-L1 can also benefit from anti-PD-1/L1 therapy combined with other treatments [7]. Thus, PD-L1 expression may not an absolute predictor of therapeutic response, other accurate biomarker need to be explored.

It is well known that PD-1/L1 antibodies enhance T cells immune response via relieving the inhibited state of terminal exhausted T cells (Tex) [8], so the status of T cells is critical for the response and efficacy of PD-1/L1 immunotherapy. Recently, few studies have showed that there are precursor cells of Tex, namely "progenitor exhausted" cells. This subpopulation was identified as high expressing T cell factor-1 (TCF-1, TCF-1$^+$CD8$^+$PD-1$^+$T cells), and has similar molecular characteristics to memory T cells [9–10]. Intriguingly, this subpopulation can expand and differentiate into Tex to maintain the therapeutic effect of PD-1 blockade treatment in melanoma mouse model [10]. However, the mechanism by which this

subpopulation responds to immunotherapy, and whether it has predictive value for anti-PD-1 treatment in NSCLC patients remain largely unknown.

The present study was conducted to determine the expression and predicted value of TCF-1$^+$ PD-1$^+$ CD8$^+$T cells in NSCLC patients who received anti-PD-1 treatment or combined with chemotherapies.

Materials And Methods

Patient cohort

This is a retrospective analysis, we reviewed patients who definitely diagnosed NSCLC by pathologist and received anti-PD-1 therapy or combined with chemotherapies between Jan 2019 to Oct 2020 in the Department of Respiration and Critical care medicine at Tongji Hospital. Finally, fifteen patients were enrolled in our study. Inclusion criteria: 1)Patients was definitely diagnosed as NSCLC by pathological test; 2) Patients received anti-PD-1 treatment as first-line therapy or as second-line therapy after relapse to conventional chemotherapy or targeted therapy; 3) PD-L1 expression in tumor cells and gene mutation were detected; 4) Patients must have paraffin-embedded tissue samples and integrated follow-up data. Exclusion criteria: 1) Patients who have received tumor vaccine or have been treated with other immunotherapy before; 2) Patients had no paraffin-embedded tissue samples and incomplete follow-up data. The clinical data, including patients' individual information, radiological data and follow-up data were obtained by medical record system, outpatient service system and telephone follow-up records, respectively. This study was approved by the ethic committee of Tongji Hospital, Tongji University School of Medicine. The informed consents were obtained from all patients.

Quantitative multiplex immunofluorescence

For quantitative multiple immunofluorescences, 4 μm-thick formalin-fixed and paraffin embedded tissues from tumors were obtained from department of pathology, Tongji Hospital. The tissue sections were treated with antigen repair and blocking endogenous peroxidase. After blocking with serum, each section was incubated overnight with primary antibodies in 4℃ (CD8, Fuzhou Maixin RMA-0514, 1:5, Opal 520, China; TCF-1 CST 2203, 1:1000, Opal 570, USA; PD-1 CST 86163, 1:500, Opal 650, USA), then the sections were incubated with secondary antibodies for 30 minutes at 37℃. Finally, the sections were incubated with TSA-Fluorescein for 30 minutes at 37℃, and labeled nuclei with DAPI. After incubation, the sections were analyzed on the PE Vectra automated multi-spectral histopathology quantitative analysis system. At least 10 random visual fields were collected from each section.

Deconvolution

The single-cell transcriptome from GSE131907 was used to identify TCF-1$^+$PD-1$^+$CD8$^+$T cells. To identify TCF-1$^+$PD-1$^+$CD8$^+$T cells, we evaluated average expression levels of TCF-7 (the protein encoding TCF-1) and PDCD1 mRNA on single cell level in all CD8$^+$ cell groups, including CD8$^+$/CD4$^+$ Mixed Th, Cytotoxic CD8$^+$ T cell, Exhausted CD8$^+$ T cell, Naïve CD8$^+$ T cell, by AddModuleScore function in R package Seurat

(v3.2.3). Cells with average expression score > 0 were identified as TCF-1$^+$PD-1$^+$CD8$^+$T cells. In order to comply with the CIBERSORTx requirements, we concentrated all TILs (120876 cells in 14 cell types with more than 2000 cells: 22527 of Naïve CD4$^+$ T cell, 22089 of Follicular B cells, 13788 of mono-Mac, 13761 of Alveolar Mac, 8945 of CD4$^+$ Th, 7748 of NK, 6743 of TCF-1$^+$CD8$^+$T cell, 5166 of Treg, 4926 of CD8 low T cell, 4311 of monocyte, 3396 of MAST, 2979 of Pleural Mac, 2420 of MALT B cell, and 2077 of CD8$^+$/CD4$^+$ Mixed Th) 20 times into a pseudo cells expression matrix which by average of 20 cells randomly selected from each cell types (PMID: 29724907). The pseudo cells expression matrix then submitted to CIBERSORTx as a single-cell reference to deconvolute the RNA-seq results from GSE145896.

Statistical analysis

Student's t-test and Chi-square test were used to compare variables between subgroups of patients, and the association between TCF-1$^+$PD-1$^+$CD8$^+$T cells and patients' clinical parameters. The probability of the benefit with anti-PD-1 therapy or combined with chemotherapies based on clinical variables was determined by univariate logistic regression analyses. For survival analyses, Kaplan-Meier curves were used to estimate time to event outcome parameters, and different groups were compared using a log-rank test. All tests were two-sided, and *p*-values < 0.05 were considered statistically significant. The data were analyzed using SPSS version 19.0 software (SPSS Inc., Chicago, IL).

Results

Clinical characteristics of non-small cell lung cancer patients

Detailed patient characteristics were summarized in Table.1. Briefly, the median age of patients was 68.5 years (range: 50–86), with fourteen male and one female. There were ten cases with negative expression of PD-L1 (66.6%), and five cases with positive expression of PD-L1 (33.4%). Regarding the guideline of NCCN, majority of patients (73%) received anti-PD-1 treatment combined with chemotherapies, and the remaining patients received anti-PD-1 monotherapy. Among them, most patients were treated as second or third-line therapy after relapse to conventional chemotherapy or targeted therapy, and five patients received as first-line therapy. According to RECIST guideline version 1.1, eight patients (53.3%) were identified responders, including six durable clinical benefit patients (DCB) and two non-durable clinical benefit patients (NDB), while remaining seven patients (46.7%) were considered non-responders, including one hyperprogressive patient. Among these cases, one EGFR-mutated and one ALK-mutated patient received tyrosine kinase inhibitor therapy before PD-1 blockade treatment.

Table.1 Clinical characteristics of patients

Characteristics at diagnosis	Number of patients (n, %)
Age (year)	
≤65	6 (40.0%)
> 65	9 (60.0%)
Gender	
Male	14 (93.3%)
Female	1 (6.7%)
Smoking history	
Never	11 (73.3%)
Ever	4 (26.7%)
Histologic subtype	
ADC	6 (40.0%)
SqCC	6 (40.0%)
ADC with ND	2 (13.3%)
NSCLC, PD	1 (6.7%)
Genetic status	
EGFR-mutated	1 (6.7%)
ALK-mutated	1 (6.7%)
Wild type	13 (86.6%)
Primary vs. metastasis	
Primary	10 (66.7%)
Metastasis	5 (33.3%)
Type of PD-1 blockade	
Nivolumab	1 (6.7%)
Pembrolizumab	3 (20.0%)
More than two drugs	11 (73.3%)

PD-L1 expression	
Positive (≥1%)	2 (13.4%)
Positive (≥50%)	3 (20.0%)
Negative	10 (66.6%)
Response to PD-1 blockade	
Clinical benefit	8 (53.3%)
Non-responder	6 (40.0%)
HPD	1 (6.7%)

ADC: adenocarcinoma, SqCC: squamous cell carcinoma, ADC with ND: adenocarcinoma with non-differentiated, NSCLC, PD: non-small cell lung cancer, poorly differentiated, HP: Hyperprogressive disease.

Identification of TCF-1-expressing PD-1-positve CD8-positve TILs in NSCLC

Recently, few studies have shown that intra-tumoral TCF-1$^+$PD-1$^+$CD8$^+$T cells display stem-like properties, which can expand and differentiate into exhaust T cells, and promote tumor control in response to checkpoint blockade immunotherapy. To elucidate the predict value of TCF-1$^+$PD-1$^+$CD8$^+$T cells in NSCLC with anti-PD-1 therapy or combined with chemotherapies, we performed quantitative multiplex immunofluorescence of pre-treatment biopsies from 15 patients with NSCLC. As shown in Fig.1, although the frequency of TCF-1$^+$ cells in PD-1$^+$CD8$^+$T cells were varied among these patients, range from 0–86%, this cell subset was identified in almost all biopsies. Besides, we analyzed the relationship between the expression of TCF-1$^+$ PD-1$^+$CD8$^+$T cells and patients' clinical parameters. This cell subset was no associated with patients' age, gender, smoking history, pathological types and genetic status.

TCF-1$^+$PD-1$^+$CD8$^+$T cells are associated with the response to PD-1 blockade treatment

To evaluate the predictive role of TCF-1$^+$PD-1$^+$CD8$^+$T cells in response to PD-1 blockade or combined with chemotherapies, we analyzed its expression in responders and non-responders, respectively. As shown in Fig. 2a, high frequency of TCF-1$^+$ cells in PD-1$^+$CD8$^+$T cells could be identified in responders compared with those in non-responders, the average frequency of TCF-1$^+$ cells in PD-1$^+$CD8$^+$T cells was 41% in responders and 14% in non-responders ($p = 0.0427$). To further validate its role in the response to PD-1 immunotherapy, we analyzed human NSCLC data from public databases. Firstly, we identified this cell subset based on expression of TCF-1 and PD-1 mRNA at single cell level in all CD8$^+$ cell groups, including CD8$^+$/CD4$^+$ Mixed Th, Cytotoxic CD8$^+$ T cell, Exhausted CD8$^+$ T cell, Naïve CD8$^+$ T cell, in human LUAD TILs (PMID: 32385277) (Figure. 2b). As a result, we acquired 6743 TCF-1$^+$PD-1$^+$ cells from 13103 CD8$^+$T cells which annotated by Kim (PMID:32385277). The expression matrix of TILs (tumor infiltrating lymphocytes) then submitted to CIBERSORTx as a single-cell reference to perform deconvolution analysis

of transcriptomes from human NSCLC with different response to PD-1 blockade (GSE145896) (Table. S1). The abundance of TCF-1$^+$PD-1$^+$CD8$^+$T cells exhibit a pro-PD-1 blockade trend (p = 0.075, Student's T test) (Fig. 2c), which partially supported it may contribute to the response of PD-1 blockade.

Relevance between TCF-1$^+$PD-1$^+$CD8$^+$T cells and the prognosis of NSCLC patients with anti-PD-1 therapy

Interestingly enough, we found that the patients with high expression of TCF-1$^+$PD-1$^+$CD8$^+$T cells achieved a progression-free survival of more than 1 year (Fig. 3), indicating that TCF-1$^+$PD-1$^+$CD8$^+$T cells may be related to the favorable progression of PD-1 immunotherapy or combined with chemotherapies. The average frequency of this cell subset was 51% in patients with durable clinical benefit (DCB) and 16% in patients with non-durable clinical benefit (NDB), respectively. In univariate analysis, high frequency of TCF-1$^+$PD-1$^+$CD8$^+$T cells was found to be a predictor of clinical benefit from anti-PD-1 therapy or combined with chemotherapies (Table 2, p = 0.035). Clinical parameters, including age, gender, smoking history, EGFR/ALK (epidermal growth factor receptor/anaplastic lymphoma kinase) mutation and PD-L1 expression were not correlated with the benefit of monotherapy or combinatory chemotherapies of PD-1 blockade. Since the small sample size, the prediction of this cell subset in the durable benefit of PD-1 immunotherapy or combinatory treatment needs further study.

Table.2 Univariate logistic regression analysis for predicting clinical benefit group with PD-1 blockade or combined with chemotherapy

Variables		Univariate		
		HR	95% CI	*P* value
Age	≥65 VS <65	0.319	0.058-1.754	0.189
Ever/ current smoker	No VS Yes	4.912	0.965-24.988	0.055
Presence of driver mutation	No VS Yes	0.040	0.000-15.170	0.550
PD-L1 expression	(-) VS (+)	5.396	0.629-46.293	0.124
Strong positive	≥50% VS <50%	4.912	0.965-24.988	0.055
TCF-1+ ratio	≥34% VS <34%	0.089	0.009-0.839	0.035
Type of PD-1 blockade	Monotherapy VS combination	1.017	0.185-5.590	0.985

PD-L1: programmed-cell death ligand-1, PD-1: programmed-cell death 1, TCF-1: T cell factor-1.

Discussion

In this study, we found that the expression of TCF-1$^+$PD-1$^+$CD8$^+$T cells were positively correlated with the response to monotherapy or combinatory chemotherapies of PD-1 blockade. We also demonstrated that

the patients with high ratio of this cell subset possessed longer survival time, indicating that TCF-1$^+$PD-1$^+$CD8$^+$T cells may also contribute to the benefit of PD-1 immunotherapy or combined with chemotherapies.

As well known, PD-1/L1 blockade are widely applicated in the treatment of various solid tumors based on the principle of preventing exhaustion of T cells and maintaining its lethality. Thus, the status and function of T cells are very crucial for the effect of anti-PD-1/L1 immunotherapy, and examination the subset of TILs in patients treated with immune checkpoint inhibitors (ICIs) has revealed as potent predictive biomarkers for anti-PD-1 therapy. For instance, patient with NSCLC possess PD-L1$^+$CD4$^+$CD25$^+$Tregs serves as a diagnostic biomarker for response to ICIs [11]. The density of intramural CD3$^+$CD45RO$^+$CD8$^+$T cells in melanoma patients was associated with the duration for anti-PD-1 therapy [12]. Due to the crucial role of TILs in response to ICIs, many researches have deeply studied the evolution of TILs subsets and its functions during immunotherapy. Recently, a subpopulation of exhausted CD8$^+$ TILs was identified as stem-like property that expressed transcription factor TCF-1 and can persist long term and differentiate into 'terminally exhausted' TILs, that is, progenitor exhausted cells [13]. Intriguingly, these studies found that the frequency of TCF-1$^+$PD-1$^+$CD8$^+$ T cells was significantly increased and gave rise to terminal exhausted T cells following PD-1 blockade in melanoma mouse model or under chronic antigen stimulation [10]. Thus, these results indicated that this cell subset is not only 'stem cell pool' for exhausted TILs, but also a target cells of PD-1 treatment. Consistent with basic researches, the relatively high expression of TCF-1$^+$PD-1$^+$CD8$^+$ T cells correlates with improved duration of response to ICIs treatment in patients with advanced melanoma [10]. In our cases, as well as above studies, the expression of TCF-1$^+$PD-1$^+$CD8$^+$ T cells was identified in almost all patients with NSCLC, and the high frequency of this subset was detected in patients with long-term remission for PD-1 blockade or combined with chemotherapies, indicating that the favorable therapeutic effect of monotherapy or combinatory treatment of PD-1 blockade may depend on the more durable TCF-1$^+$PD-1$^+$CD8$^+$ T cells in the pool of exhausted cells. Importantly, unlike previous studies that demonstrated the frequency of this subpopulation has no different between responders and non-responders, we identified the predictive value of TCF-1$^+$PD-1$^+$CD8$^+$ T cells for the response to PD-1 blockade or combined with chemotherapies in NSCLC by analyzing our cases and public databases. Therefore, our study showed that TCF-1$^+$PD-1$^+$CD8$^+$ T cells may will improved the selection of patients who will best response to monotherapy or combinatory treatments of PD-1 immunotherapy, and is a potent biomarker for predicting the prognosis of NSCLC patients with PD-1 blockade.

Hyperprogressive disease (HPD) is an acknowledged pattern of rapid tumor progression after the initiation of PD-L1 blockade treatment [14–16], and it is associated with patients' poor survival. At present, many molecules and signaling pathways have been proved to be involved in the underlying mechanisms of HPD with PD-1/L1 immunotherapy, such as the tumor suppressor genes of TSC2 and VHL [17], and the oncogenic pathways that include IGF-1 [17], ERK/MAPK [17], PI3K/AKT [18] and TGF-β [19–20]. Recently, PD-1$^+$ regulatory T cells were identified to promote the hyperprogression after PD-1 blockade treatment [17],

indicating the tumor infiltrating T lymphocytes may contributed the development of hyperprogression during anti-PD-1/L1 treatment. In our study, we found that the frequency of TCF-1^+PD-$1^+$$CD8^+$ T cells was lower in patient with hyperprogression. Although we only observed in one patient, our study suggests that the status of T cells may be associated with the hyperprogression, which needs to be further verified in more patients in the future.

In conclusion, we evaluated the clinical role of TCF-1^+PD-$1^+$$CD8^+$ T cells in the response and benefit of monotherapy and combinatory treatment of PD-1 blockade. A higher frequency of this cell subset is associated with the response to PD-1 blockade or combined with chemotherapies, and may be a predictive factor of therapeutic effect of above treatments. Because the small sample size, the correlation between TCF-1^+PD-$1^+$$CD8^+$ T cells and the prognosis of patients with monotherapy or combination treatment of anti-PD-1 therapy need further studies, as well as whether this cell subset could be used as a predictor for discriminating hyperprogression from immunotherapy.

Declarations

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Conflicts of interest:

The authors declare no conflicts of interest.

Data availability:

The data sets analyzed during the current study are available from the corresponding author on reasonable request.

Code availability: The code was analyzed for bioinformation are available from the corresponding author on reasonable request.

Author contribution:

Xia Fang, Shaoyong Gao, Xianghua Yi and Ling Zheng contributed to the conception of the study and manuscription preparation. Gang Wu, Jing Hua, Nana Wang, Pei Zhao contributed to analyze the clinical data. Xuyou Zhu, Mengtian Chan, Hailong Zhu, Tingting Ding and Yu Zeng contributed to collect and perform the bioinformation. Long Zhang and Yuting Liu contributed to the preparation of pathological sections.

Ethics approval: This study was approved by the ethic committee of Tongji Hospital, Tongji University School of Medicine.

Consent for publication: All subjects were informed consent.

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Figures

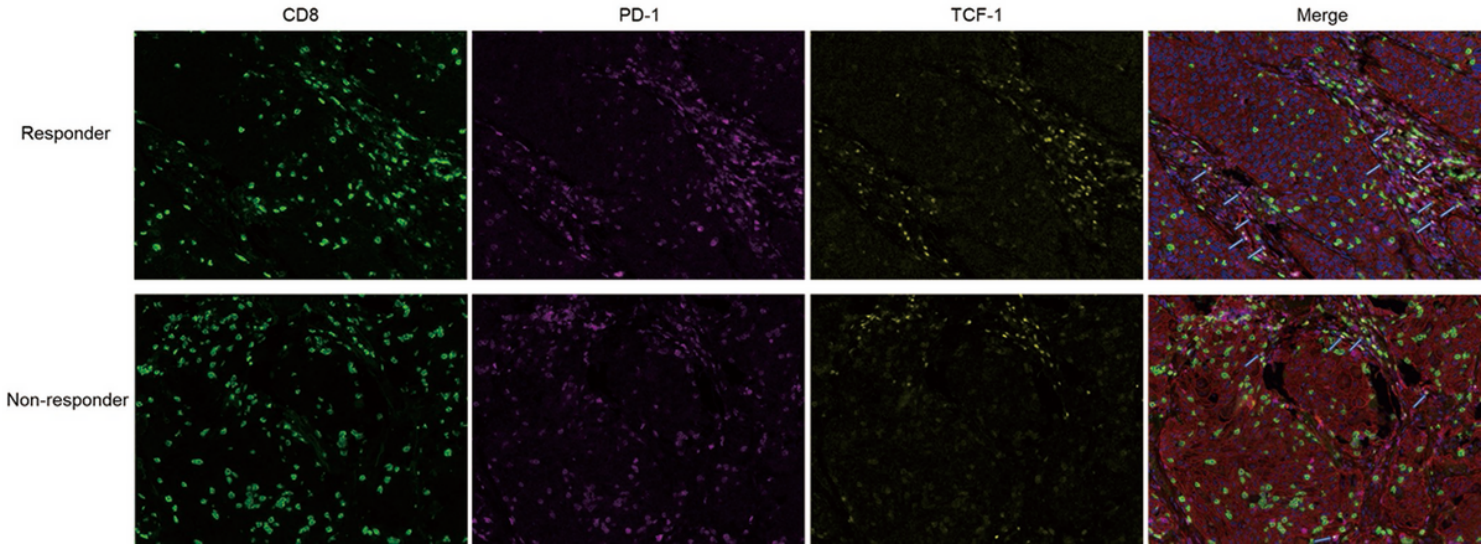

Figure 1

Identification of TCF-1+PD-1+CD8+ T cells in NSCLC. Upper: The expression of TCF-1+PD-1+CD8+ T cells in responder to PD-1 blockade (blue arrow). Lower: The expression of this subpopulation in non-responder to anti-PD-1 therapy (blue arrow).

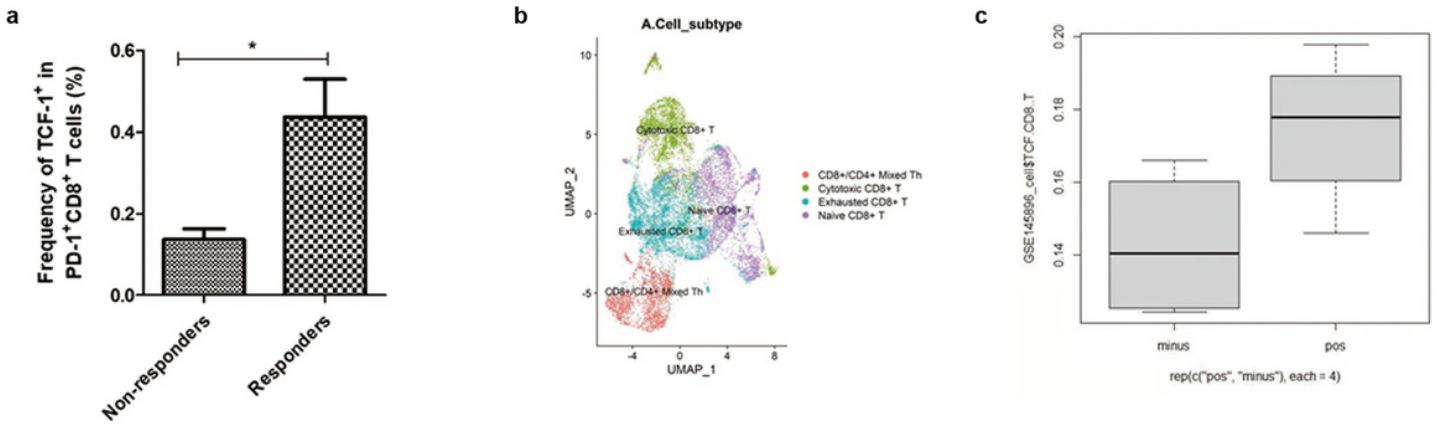

Figure 2

Comparation of TCF-1+PD-1+CD8+ T cells in non-responders and responders. (a): The frequency of TCF-1+ cells in PD-1+CD8+ T cells in non-responders and responders, respectively. (b): CD8+ T cells clustering based on UMAP. (c): Comparation the expression of TCF-1+PD-1+CD8+ T cells in non-responders and responders by analyzing the transcriptomes from human NSCLC with different response to PD-1 blockade (GSE145896).

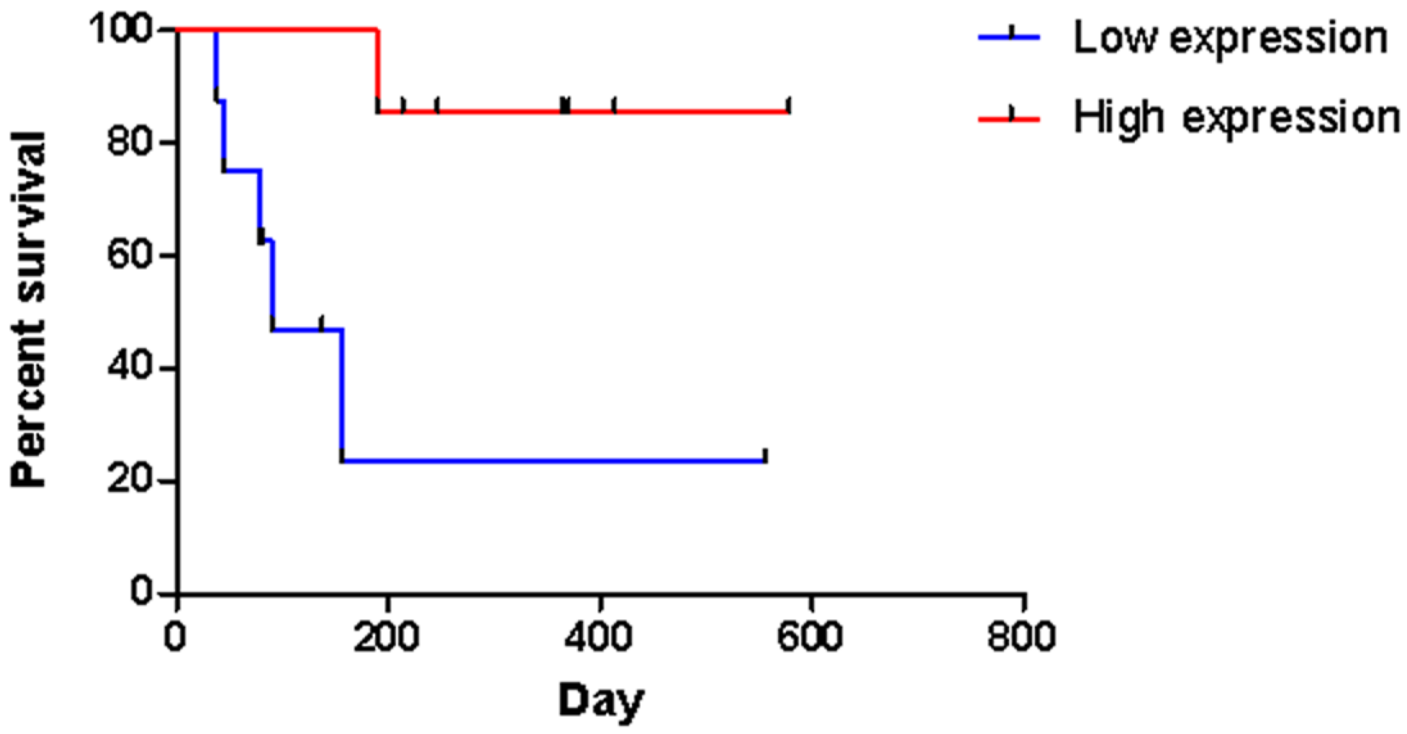

Figure 3

The survival time of patients with PD-1 blockade. Compared with low expression of TCF-1+PD-1+CD8+ T cells group, the patients possessed high expression of TCF-1+PD-1+CD8+ T cells had longer survival time (p=0.0128).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table.S1.xlsx