

RESEARCH

Open Access



Clinical implications of aberrant PD-1 expression for acute leukemia prognosis

Yanjie Ruan^{1,2†}, Jiyu Wang^{1†}, Qiuye Zhang³, Huiping Wang¹, Cong Li¹, Xuanxuan Xu⁴ and Zhimin Zhai^{1*}

Abstract

Background Acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) are the most common types of leukemia in adults with an overall poor prognosis. PD-1 alone or combined with other immune checkpoint blockade is a promising research direction for the treatment of acute leukemia (AL) patients. However, clinical implications of aberrant PD-1 expression in peripheral CD4+ and CD8+ T lymphocytes of AML and ALL patients in assessing the prognosis of diseases, remains inconclusive.

Methods In the present study, we used flow cytometry to evaluate PD-1 expression on the surface of CD4+ and CD8+ T lymphocytes in the peripheral circulation of AML and ALL patients and its clinical significance. A total of 53 AML patients, 44 ALL patients and 28 healthy controls were enrolled in this study and peripheral blood specimens were detected by flow cytometry.

Results Our results indicated that percentages of CD4+ PD1+ and CD8+ PD1+ T lymphocytes in newly diagnosed and non-remission groups were significantly higher than healthy control both in AML and ALL patients. The high level of CD4+ PD1+ and CD8+ PD1+ T lymphocytes were respectively poor prognostic indicators of AML patients and ALL patients but had no significant correlation with most common clinical risks.

Conclusions Our findings show that aberrant PD-1 expression correlates with the prognosis of AL patient and may thus serve as poor prognostic indicators. Immunotherapy using PD-1 inhibitors may be a promising strategy for AML and ALL patients with peripheral circulating CD4+ PD1+ and CD8+ PD1+ T lymphocytes positively expressed, respectively.

Keywords PD-1, Acute lymphoma leukemia, Acute myeloid leukemia, CD4+ T lymphocytes, CD8+ T lymphocytes, Prognosis

[†]Yanjie Ruan and Jiyu Wang co-first authors and contributed equally to this work.

*Correspondence:
Zhimin Zhai
zzzm889@163.com

¹ Department of Hematology, Hematology Research Center, The Second Hospital of Anhui Medical University, Hefei 230601, Anhui, China

² Department of Pathology, The Fourth Affiliated Hospital of Anhui Medical University, Hefei 230013, Anhui, China

³ People's Hospital of Taizhou, Fifth Affiliated Hospital of Nantong University, Taizhou 225300, Jiangsu, China

⁴ Jingzhou Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430000, Hubei, China

Introduction

Acute leukemia (AL) is a malignant clonal disease originating from hematopoietic stem cells. Abnormal blast cells and immature cells (leukemia cells) in the bone marrow proliferate in large numbers and inhibit normal hematopoiesis. It is mainly divided into acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) [1]. Chemotherapy is currently the main treatment option for patients with AML and ALL but is limited due to the severe side effects and drug resistance [2]. Cancer immunotherapy has recently been developed to improve the specificity and strength of the immune system against cancer. In recent years we have also



witnessed a breakthrough in the field of oncology that is represented by the development of novel agents: the immune checkpoint inhibitors, which "release the brakes" of the immune system. Immune checkpoint inhibitors, which is currently a hot area of research and may have important therapeutic value [3]. Consistently, PD-1 (Programmed Cell Death Protein 1) and its ligand (PD-L1) inhibitors have been approved by FDA and have been shown to be quite effective in several neoplasms, including leukemia [4].

Almost 30 years ago, Jenkins et al. had shown that effective activation of naive T cells requires the participation of TCR (T cell receptor) and B7/CD28 signals [5]. PD-1 (Programmed death protein 1, CD279) is an inhibitor receptor that belongs to the B7/CD28 family. As early as 1992, PD-1 was identified on T cells undergoing apoptosis by Ishida Y et al. [6]. PD-1 expressed on a broad variety of cells including activated T cells, B cells, monocytes, dendritic cells, and NK cells, except for naive lymphocytes prior to activation [7, 8]. Of note, PD-1 is highly expressed on tumor-specific T cells [9]. PD-1 plays an important role in inhibiting immune responses and promoting self-tolerance through modulating the activity of T-cells, activating apoptosis of antigen-specific T cells and inhibiting apoptosis of regulatory T cells [10–12].

Recent research reported that PD-1 expression is related with poor prognosis of cancers. PD-1/PD-L1-targeted inhibitors play an important role in cancers such as breast cancer, lung cancer, colorectal cancer, gastric cancer, bladder cancer, pancreatic cancer, prostate cancer, DLBCL and so on [10, 13–15]. In this study, we evaluated the expression of PD-1 on CD4+ and CD8+ T lymphocytes in Peripheral Blood and summarized the role of PD-1 which is a crucial factor affecting the prognosis of AML and ALL patients.

Materials and methods

Patients

102 AL patients including 57 AML patients, 45 ALL patients and 28 healthy controls enrolled in our study were recruited from September 2016 to August 2019 in the Second Hospital of Anhui Medical University. All diagnosed patients with AML and ALL were divided into newly diagnosed (ND) group, complete remission (CR) group, and Non-remission (NR) group according to the 2016 National Comprehensive Cancer Network (NCCN) guidelines 2nd Edition. All healthy volunteers enrolled in this study have no abnormal liver and kidney function, no autoimmune diseases, no history of immunosuppressive drugs. This study was approved by the Institutional Review Board (IRB) Institutional of the Second Hospital of Anhui Medical University. All patients enrolled in the study have signed informed consent.

PD-1 analysis

Peripheral blood mononuclear cells (PBMCs) were separated by density gradient centrifugation (Ficoll-Hypaque, Amersham Bio-sciences, Sweden), and washed with phosphate-buffered saline (PBS). After washing, 100 μ L PBMCs was incubated with monoclonal antibodies and analyzed by flow cytometer (CytoFLEX, Beckman Coulter, USA), and EXPO 32 Multicomp software was used for data acquisition and analysis. The lymphocyte population was gated as H1 by FSC, SSC and CD45. The T lymphocyte population was defined with CD3+ cell population in H1 gate. PD-1 subsets were stained and identified by the phenotype of CD279+ on CD4+ and CD8+ T lymphocytes. We analyzed percentages of CD4+ T lymphocytes and CD8+ T lymphocytes, and the expression of PD-1 on the membrane surface of these two cell populations. The following monoclonal antibodies were purchased from Beckman Coulter Immunology (Miami): FITC-labeled CD3 (clone No.UCHT1), PE-labeled CD4 (clone No.13B8.2), ECD-labeled CD8 (clone No.SFC121Thy2D3), PC7-labeled CD45 (clone No.J33), APC-labeled CD279 (clone No. PD1.3).

Statistical analysis

All statistical analysis was performed by using SPSS19.0 software (IBM, Chicago, IL, USA) and GraphPad Prism 8.0.2 (GraphPad Software Inc., La Jolla, CA). For quantitative data with a normal distribution, the t-test is used for comparison. For multiple independent samples, the One-way ANOVA test was used for comparison. Quantitative data with non-normal distribution from two independent samples was compared by a non-parametric Mann–Whitney test. For multiple samples were compared using the Kruskal & Wallis Test (non-parametric ANOVA). To evaluate correlations, Spearman's correlation coefficient was applied. Overall survival (OS) was used and defined as the time from date of diagnosis until the date of death. The prognostic value was evaluated by Kaplan–Meier survival curves. Generate high and low PD1 expression groups based on the median survival time. Log-rank test was applied for evaluating the differences between the comparison of groups. $p < 0.05$ was considered statistically significant.

Results

Patient characteristics

A total of 102 patients with acute leukemia (AL) were included in this analysis, comprising 57 with Acute Myeloid Leukemia (AML) and 45 with Acute Lymphoblastic Leukemia (ALL). Additionally, 28 healthy individuals served as controls for comparison. The average age of AML patients was 52 years (range: 5–85), with 27

(47.4%) being male and 30 (52.6%) female. Among ALL patients, the average age was 26 years (range: 5–72), with 21 (46.7%) males and 24 (53.3%) females. Healthy controls had an average age of 42 years (range: 24–60), with 12 (42.9%) males and 16 (57.1%) females. Table 1 presents the clinical characteristics of the study participants. There were no significant differences in gender and age among the groups.

Percentage of CD4+ and CD8+ T lymphocytes in Peripheral Blood from AL Patients

We observed that the proportion of CD4+ T lymphocytes in each AML group were increased in various degrees compared to the healthy control (Fig. 1A). The highest percentage of CD4+ T lymphocytes was detected in AML-CR group, and it was significantly higher ($p=0.0019$) than healthy control (Fig. 1A). In contrast, the percentage of CD8+ T lymphocytes in AML-ND was significantly reduced ($p=0.0066$) compared to control (Fig. 1B). We also evaluated the ratio of CD4/CD8 in peripheral blood from AL patients and healthy control, and it was significantly higher in AML-ND ($p=0.0124$) and AML-CR ($p=0.0124$) groups than control (Fig. 1C). We also evaluated the percentage of CD4+ T lymphocytes in ALL patients and healthy control, but the results were not statistically significant (Fig. 1D). The percentage of CD8+ T lymphocytes in AML-NR was significantly higher ($p=0.0390$) than AML-ND (Fig. 1E). The ratio of CD4/CD8 was significantly reduced in AML-NR group compared to control ($p=0.0345$) and AML-ND group ($p=0.0347$) (Fig. 1F). The proportion of CD4+ and CD8+ T lymphocytes and ratio of CD4/CD8 are important indicators for evaluating immune function. Generally, the normal range of CD4/CD8 ratio is about 1.4–2.0. As shown in Fig. 1, abnormal CD4/CD8 ratio was associated with immune dysfunction in patients.

Expression of PD-1 on CD4+ and CD8+ T lymphocytes in peripheral blood of AL patients

Our results showed that PD1 expression on CD4+ T lymphocytes was significantly increased both in AML patients and ALL patients compared to healthy controls (Fig. 2). The percentage of CD4+ PD1+ T lymphocytes in the peripheral blood of AML-CR group was significantly increased compared to controls ($p=0.0463$) (Fig. 2A, B and C). Especially, PD-1 expression on CD4+ T lymphocytes in AML-ND ($p<0.0001$) and AML-NR ($p<0.0001$) groups was also significantly elevated than the control group (Fig. 2A, B and C). Similarly, we observed significant elevated percentages of CD4+ PD1+ lymphocytes in ALL-ND ($p=0.0023$), ALL-CR ($p=0.0003$) and ALL-NR ($p<0.0001$) groups compared to those in the healthy control (Fig. 2A, D and E).

Moreover, we observed that PD1 expression on CD8+ T lymphocytes was significantly increased both in AML patients and ALL patients compared to healthy controls (Fig. 3). Levels of CD8+ PD1+ T lymphocytes in AML-ND ($p<0.0001$), AML-CR ($p=0.0016$) and AML-NR ($p<0.0001$) were obviously elevated than those in controls (Fig. 3A, B and C). Percentage of CD8+ PD1+ lymphocytes in ALL-ND ($p<0.0001$), ALL-CR group ($p=0.0055$) and ALL-NR group ($p<0.0001$) were significantly higher than those in controls (Fig. 3A, D and E).

Changes in PD-1 expression on CD4+ and CD8+ T lymphocytes of newly diagnosed AL patients after effective treatment

To further elucidate the changes in PD-1 levels on T cells in newly diagnosed AL patients after effective treatment, we assessed PD-1 expression on CD4+ and CD8+ T lymphocytes in 8 AML patients and 4 ALL patients who achieved complete remission after therapy. Our

Table 1 Characteristics of healthy controls and AL patients

Group	Number	Median age (range)	Gender (M/F)	Low risk	Medium risk	High risk	No data
AL	102	43 (5–85)	48/54	5	9	36	52
AML	57	52 (5–85)	27/30	5	9	18	25
AML-ND	17	60 (16–85)	8/9	1	4	3	9
AML-CR	13	50 (5–71)	3/10	3	2	3	5
AML-NR	27	52 (17–78)	16/11	1	3	12	11
ALL	45	26 (5–72)	21/24	0	0	18	27
ALL-ND	12	33 (12–70)	6/6	0	0	6	6
ALL-CR	12	25 (7–72)	4/8	0	0	2	10
ALL-NR	21	26 (5–62)	11/10	0	0	10	11
Healthy controls	28	42 (24–60)	12/16				

AML-ND Newly diagnosed patients with AML, AML-CR Complete remission patients with AML, AML-NR Non-remission patients with AML, ALL-ND Newly diagnosed patients with ALL, ALL-CR Complete remission patients with ALL, ALL-NR Non-remission patients with ALL

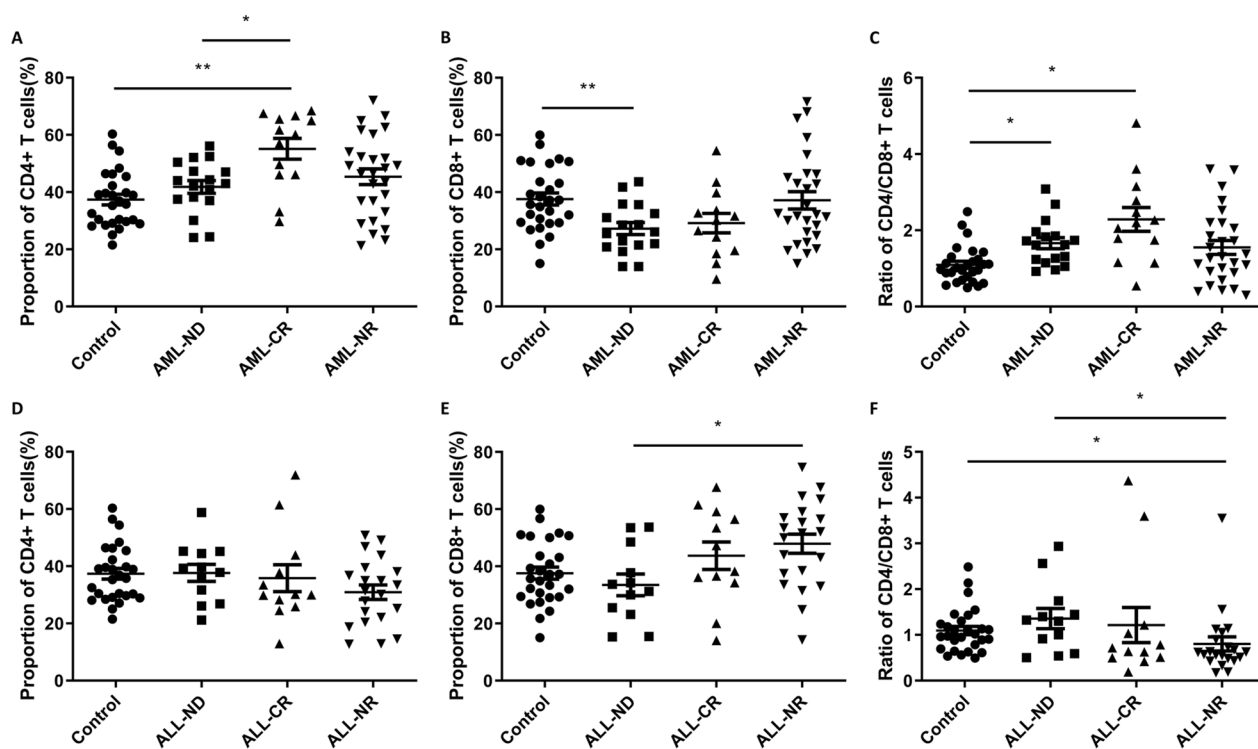


Fig. 1 The proportion of CD4+, CD8+ T lymphocytes to lymphocytes and the ratio of CD4/CD8 in the peripheral blood of healthy control and AL patients. **A** The proportion of CD4+ T lymphocytes to lymphocytes in the peripheral blood of healthy control and AML patients. **B** The proportion of CD8+ T lymphocytes to lymphocytes in the peripheral blood of healthy control and AML patients. **C** The ratio of CD4/CD8 in the peripheral blood of healthy control and AML patients. **D** The proportion of CD4+ T lymphocytes to lymphocytes in the peripheral blood of healthy control and ALL patients. **E** The proportion of CD8+ T lymphocytes to lymphocytes in the peripheral blood of healthy control and ALL patients. **F** The ratio of CD4/CD8 in the peripheral blood of healthy control and ALL patients. * $p < 0.05$, ** $p < 0.01$

results revealed a significant decrease in PD-1 expression on both CD4+ and CD8+ T lymphocytes in AML patients after effective treatment ($p = 0.0040$ and $p = 0.0173$, Fig. 4A, B). In ALL patients, the proportion of CD4+ PD1+ T lymphocytes significantly decreased ($p = 0.0165$, Fig. 4C), while CD8+ PD1+ showed a declining trend after treatment, albeit without statistical significance ($p = 0.0806$, Fig. 4D).

Relationship between clinical characteristics of AL patients and PD-1 expression on CD4+ and CD8+ T lymphocytes

In this study, we grouped all patients with AL according to different types of clinical factors, including gender, minimal residual disease (MRD), disease classification, and risk stratification. The results suggested that there was no significant difference in the level of CD4+ PD1+ in the peripheral blood of male and female AML patients (Fig. 5A), but the level of CD8+ PD1+ in male patients was significantly higher than that in female patients (Fig. 5B). In addition, the correlation between PD1 and MRD was also analyzed. We found that the percentage of CD4+ PD1+ T lymphocytes in AML patients

was not significantly correlated with MRD, and there was no significant correlation between CD8+ PD1+ and MRD (Fig. 5C, D, E and F). Although there was no significant difference in CD4+ PD1+ levels among AML patients of different disease types (Fig. 5G), the CD8+ PD1+ levels of M2 AML patients were significantly higher than those of M4 ($p < 0.01$) (Fig. 5H). We also analyzed the relationship between PD-1 expression and risk stratification in AML patients, which indicated that there were no significant differences in PD-1 expression on CD4+ and CD8+ T lymphocytes among low-risk, intermediate-risk, and high-risk groups (Fig. 5I and J). Additionally, we have included the comparative results of baseline characteristics, including risk stratification, for the high PD-1 and low PD-1 groups of patients, which showed that there were no significant differences in baseline characteristics, including risk stratification, between patients in the high PD-1 group and those in the low PD-1 group (Table 2 and Table 3).

The results showed that there was no significant difference in the levels of CD4+ PD1+ and CD8+ PD1+ T lymphocytes between male and female ALL patients

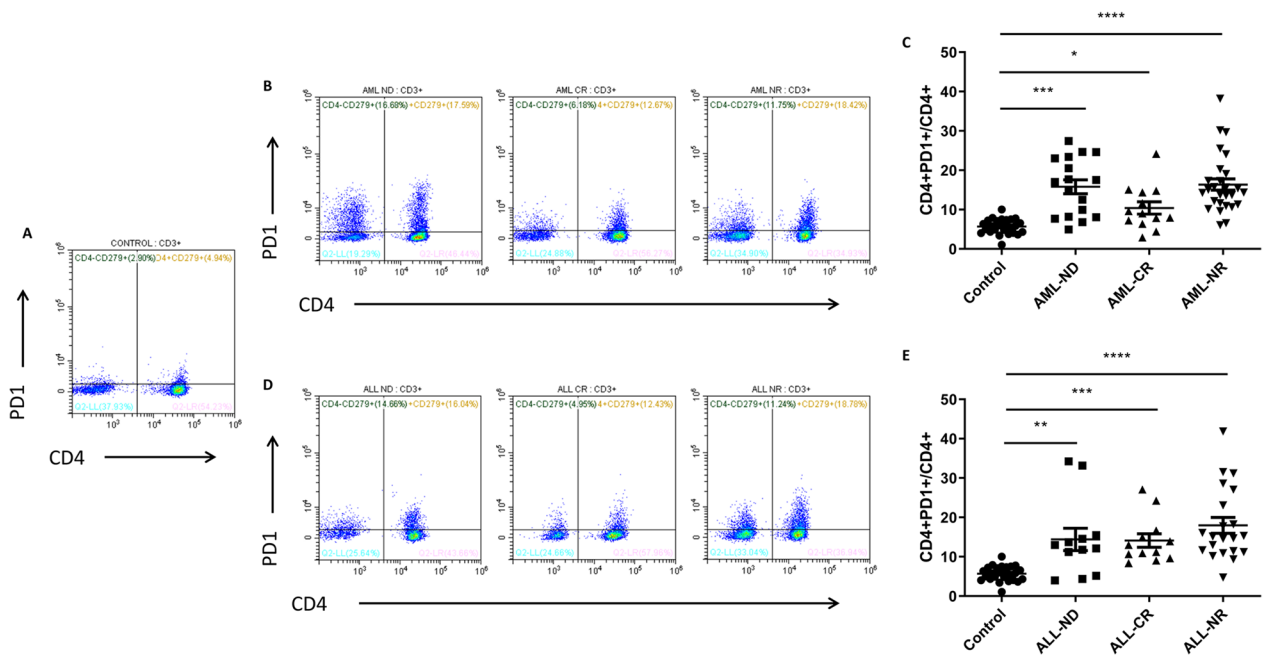


Fig. 2 The expression of PD-1 in CD4+T lymphocytes from AL patients and controls. **A** Representative flow cytometry plots of CD3+CD4+CD279+T lymphocytes in healthy controls. **B** Representative flow cytometry plots of CD3+CD4+CD279+T lymphocytes in AML patients. **C** The expression of PD-1 in CD4+T lymphocytes from AML patients. **D** Representative flow cytometry plots of CD3+CD4+CD279+T lymphocytes in ALL patients. **E** The expression of PD-1 in CD4+T lymphocytes from ALL patients. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

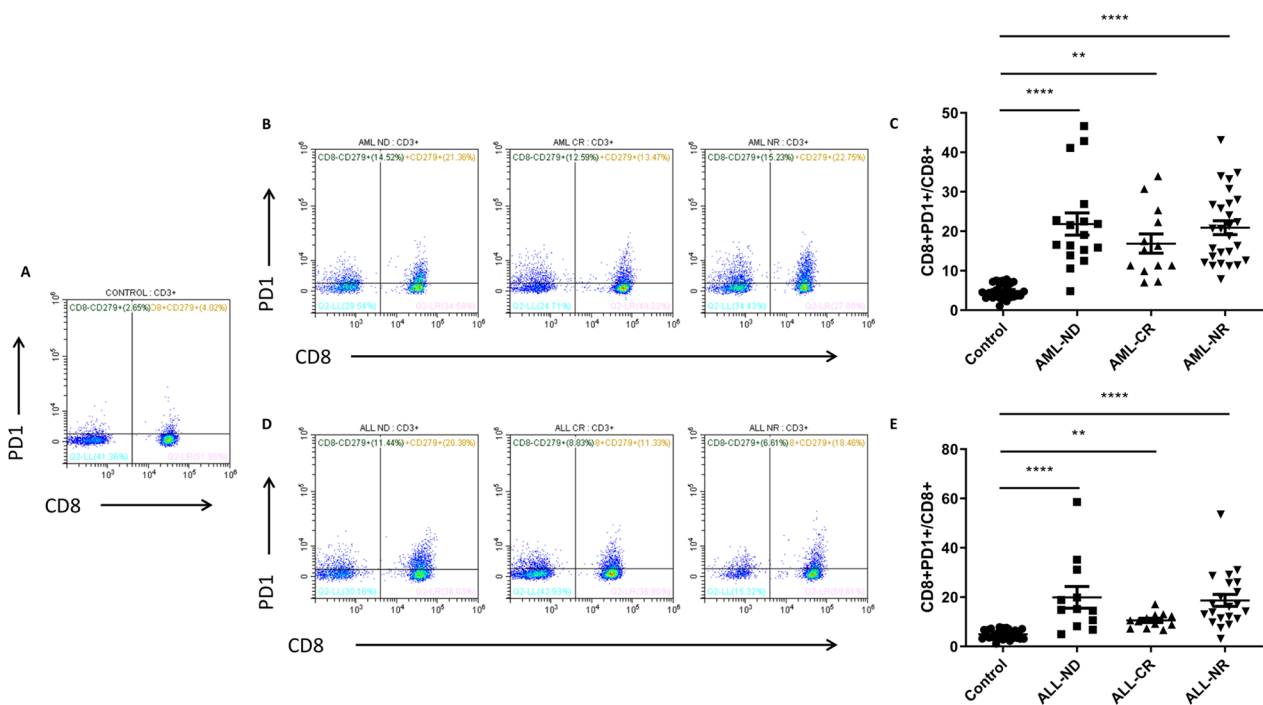


Fig. 3 The expression of PD-1 in CD8+T lymphocytes from AL patients and controls. **A** Representative flow cytometry plots of CD3+CD8+CD279+T lymphocytes in healthy controls. **B** Representative flow cytometry plots of CD3+CD8+CD279+T lymphocytes in AML patients. **C** The expression of PD-1 in CD8+T lymphocytes from AML patients. **D** Representative flow cytometry plots of CD3+CD8+CD279+T lymphocytes in ALL patients. **E** The expression of PD-1 in CD8+T lymphocytes from ALL patients. **** $p < 0.0001$, ** $p < 0.01$

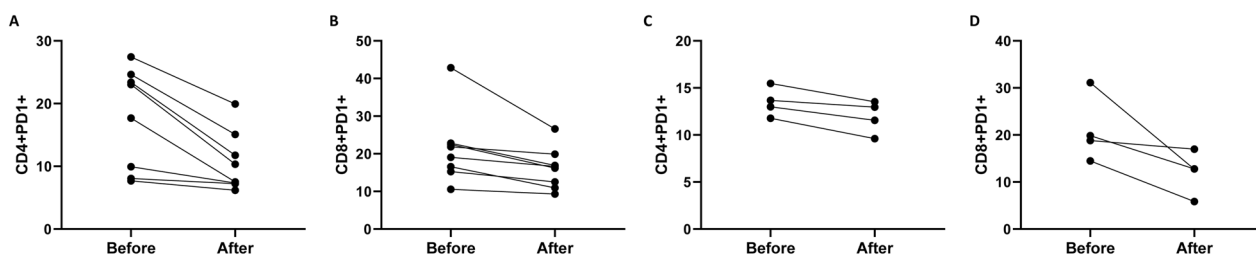


Fig. 4 Comparison of PD-1 expression in AL patients at the time of initial diagnosis and after achieving complete remission through effective therapy. **A** Changes in CD4+ PD1+ T lymphocytes in AML patients, n=8, p=0.0040. **B** Changes in CD8+ PD1+ T lymphocytes in AML patients, n=8, p=0.0173. **C** Changes in CD4+ PD1+ T lymphocytes in ALL patients, n=4, p=0.0165. **D** Changes in CD8+ PD1+ T lymphocytes in ALL patients, n=4, p=0.0806

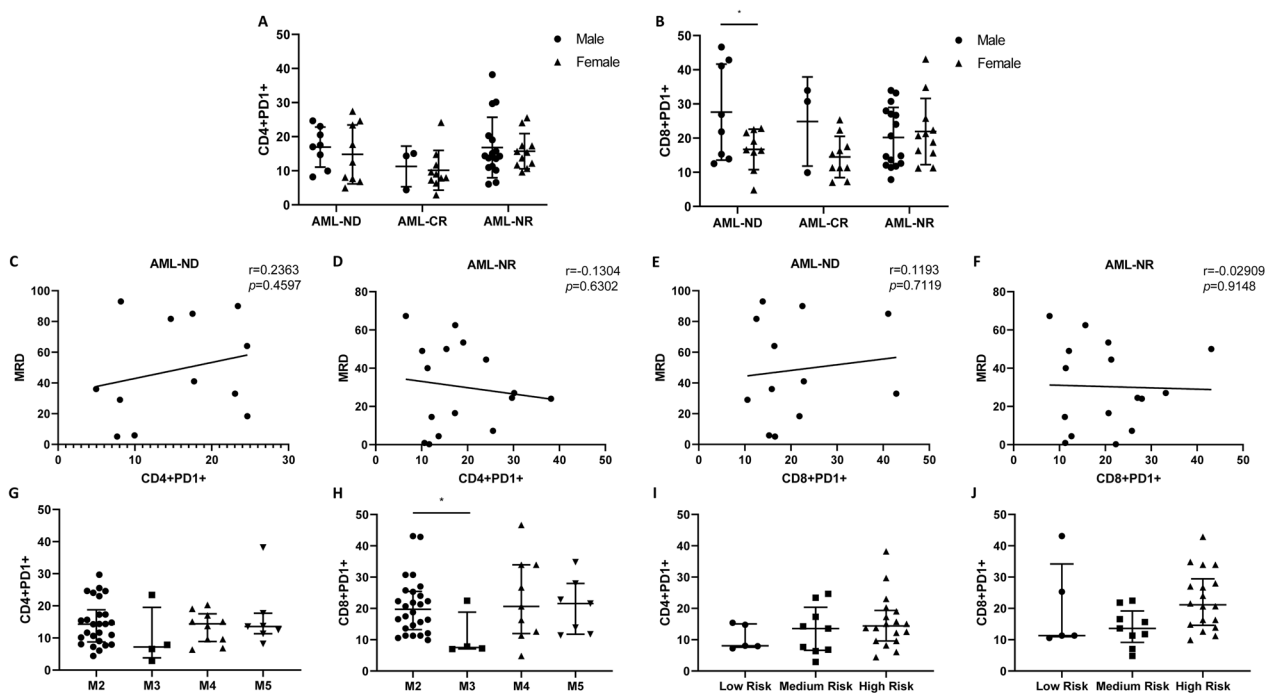


Fig. 5 Clinical correlation of PD-1 in AML patients. **A** The level of CD4+PD1+ T lymphocytes in male and female AML patients. **B** The level of CD8+ PD1+ T lymphocytes in male and female AML patients. **C** Correlations between MRD and CD4+ PD1+ T lymphocytes in newly diagnosed patients with AML. **D** Correlations between MRD and CD4+ PD1+ T lymphocytes in non-remission patients with AML. **E** Correlations between MRD and CD8+ PD1+ T lymphocytes in newly diagnosed patients with AML. **F** Correlations between MRD and CD8+ PD1+ T lymphocytes in non-remission patients with AML. **G** The level of CD4+ PD1+ T lymphocytes in different types of AML patients. **H** The level of CD8+ PD1+ T lymphocytes in different types of AML patients. **I** The level of CD4+ PD1+ T lymphocytes in AML patients with different risk stratifications. **J** The level of CD8+ PD1+ T lymphocytes in AML patients with different risk stratifications. Each point represents an individual. The horizontal bar represents the average. *p < 0.05

(Fig. 6A, B). In addition, there was no significant difference in PD1 expression on CD4+ and CD8+ T lymphocytes neither ALL patients younger than 14 years old nor ALL patients older than 14 years old (Fig. 6C, D). Next, correlations between MRD and PD1 expression in ALL patients was analyzed. There was no relationship between the percentage of CD4+ PD-1+ T

lymphocytes and MRD in peripheral blood of ALL patients (Fig. 6E, F). There was no relationship between the percentages of CD8+ PD-1+ T lymphocytes and MRD (Fig. 6G, H). Finally, we analyzed the expression of PD1 in BCR-ABL positive ALL patients. It is worth noting that we found that the levels of CD4+ PD1+ and CD8+ PD1+ in newly diagnosed ALL patients with BCR-ABL positive were higher than those in the

Table 2 Comparison of baseline characteristics in low and high CD4+PD1+ AML patients

Variable	Total number (n=57)	Low CD4+PD-1+ (n=28)	High CD4+PD-1+ (n=29)	Statistic	p
Gender, n (%)				$\chi^2=2.998$	0.088
Male	27 (47.37)	11 (36.67)	16 (59.26)		
Female	30 (52.63)	19 (63.33)	11 (40.74)		
Risk, n (%)				-	1.000
Low risk	5 (15.62)	3 (17.65)	2 (13.33)		
Medium risk	9 (28.12)	5 (29.41)	4 (26.67)		
High risk	18 (56.25)	9 (52.94)	9 (60.00)		

Table 3 Comparison of baseline characteristics in low and high CD8+PD1+ AML patients

Variable	Total number (n=57)	Low CD8+PD-1+ (n=28)	High CD8+PD-1+ (n=29)	Statistic	p
Gender, n (%)				$\chi^2=0.449$	0.503
Male	27 (47.37)	12 (42.86)	15 (51.72)		
Female	30 (52.63)	16 (57.14)	14 (48.28)		
Risk, n (%)				-	0.138
Low risk	5 (15.62)	3 (17.65)	2 (13.33)		
Medium risk	9 (28.12)	7 (41.18)	2 (13.33)		
High risk	18 (56.25)	7 (41.18)	11 (73.33)		

negative group, but there was no significant difference. The opposite is true in relapsed ALL patients (Fig. 6I–L).

Relationship between PD1 expression in peripheral circulation and survival in AL patients

We evaluated the relationship between PD1 expression in peripheral circulation and survival in AL patients. We found that the AML patients with the higher percentage of CD4+PD1+ in peripheral blood had shorter OS than those with the lower percentage (median 90 vs 525 days, $p=0.0005$) (Fig. 7A). Additionally, there is no significant difference in OS of AML patients with high levels of CD8+PD1+ compared with low levels (median 150 vs 360 days, $p=0.4234$) (Fig. 7B). On the other hand, the median OS of patients with ALL with a high level of CD4+PD1+ T lymphocytes in the peripheral blood was 1440 days and the low level was 330 days, but the difference between the two was not statistically significant ($p=0.1336$) (Fig. 7C). ALL patients with low levels of CD8+PD1+ in the peripheral blood have a significant survival advantage over patients with high levels of

CD8+PD1+ in the peripheral circulation (median 720 vs 1620 days, $p=0.0284$) (Fig. 7D). To determine whether aberrant PD-1 expression is an independent factor affecting prognosis in AL patients. Univariate analysis was used, and the results were shown in Table 4. Higher CD4+PD1+ ($p=0.042$) in AML patients and higher CD8+PD1+ ($p=0.019$) in ALL patients were found to predict OS (Table 4). Together, these results indicate that the high level of CD4+PD1+ T lymphocytes and CD8+PD1+ were respectively poor prognostic indicators of AML patients and ALL patients.

Discussion

Traditional therapies combined with immune checkpoint inhibitors including anti-PD-1 antibody have shown better therapeutic efficacy in a variety of cancer types, including acute leukemia [16–18]. It is known that the occurrence and development of acute leukemia is closely related to the reduction of the body’s immune level, especially the abnormality of cellular immunity. T lymphocytes perform cellular immune function, and patients with leukemia are often accompanied by changes in the number of T cell subsets and functional impairment. CD3+ cells are usually used to define the total number of T lymphocytes. CD4+ cells are the main response cells in the immune response. CD8+ cells can produce cell-mediated cytotoxicity on target cells, and at the same time have a regulatory immunosuppressive effect on CD4+ cells [19–21]. In the present study, we used flow cytometry to evaluate PD-1 expression on the surface of CD4+ and CD8+ T lymphocytes in the peripheral circulation of AML and ALL patients and its clinical significance. Our results showed that the CD4/CD8 ratio in the peripheral circulation of AML patients and newly diagnosed ALL patients was increased to varying degrees compared to healthy controls. The proportion of CD4+ and CD8+ T lymphocytes and ratio of CD4/CD8 are important indicators for evaluating immune function. Generally, the normal range of CD4/CD8 ratio is about 1.4–2.0. Abnormal CD4/CD8 ratio was associated with immune dysfunction in patients, the unstable CD4+ / CD8+ ratio was not conducive to the balance of cellular immune responses.

Recent studies have found that the PD-1 expression is up-regulated in lung cancer, gastric cancer, hepatocellular carcinoma, multiple myeloma, breast cancer, renal cell carcinoma and melanoma [22–28]. The high expression of PD-1 continuously activates the PD-1/PD-L1 signaling pathway, thereby inhibiting various signaling pathways. In addition, some studies have shown that high expression of PD-1 may be a poor prognostic factor in some malignant tumors such as lymphoma [29, 30], lung cancer [31] and breast cancer [32]. At present, PD-1/PD-L1

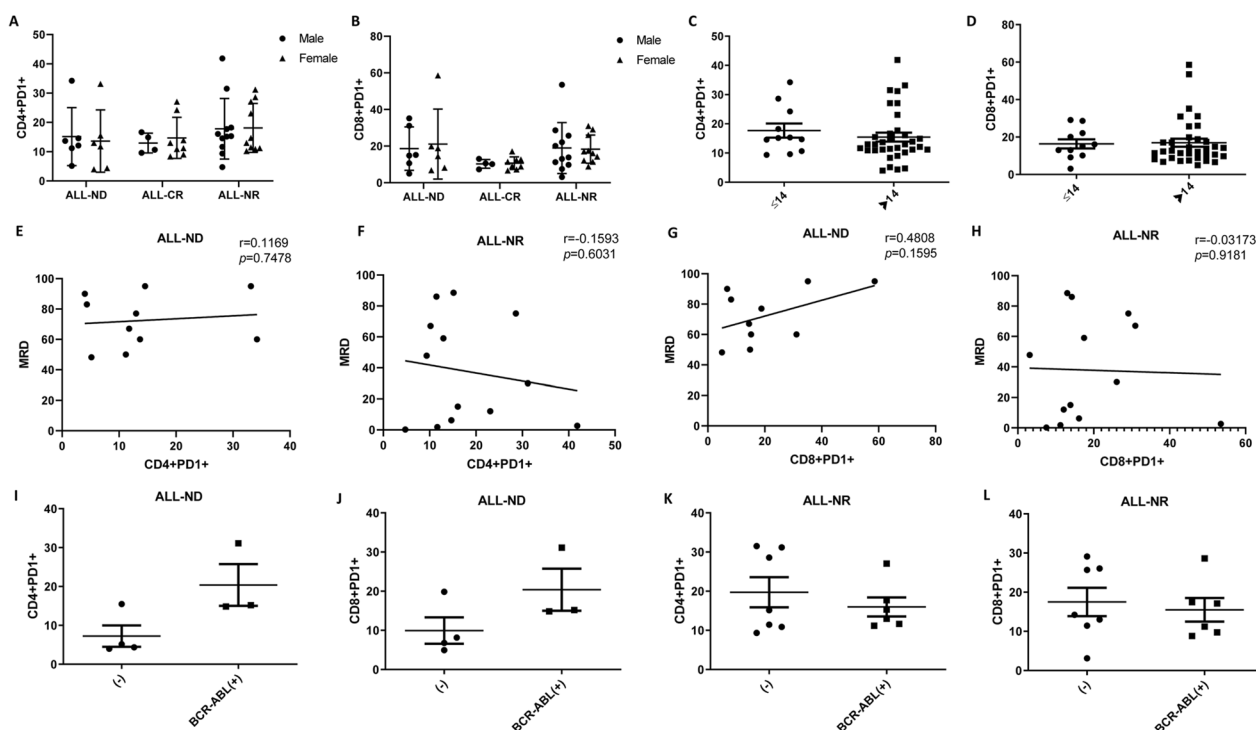


Fig. 6 Clinical correlation of PD1 in ALL patients. **A** The level of CD4+PD1+T lymphocytes in male and female ALL patients. **B** The level of CD8+PD1+T lymphocytes in male and female ALL patients. **C** The level of CD4+PD1+T lymphocytes in different age groups of ALL patients. **D** The level of CD8+PD1+T lymphocytes in different age groups of ALL patients. **E** Correlations between MRD and CD4+PD1+T lymphocytes in newly diagnosed patients with ALL. **F** Correlations between MRD and CD4+PD1+T lymphocytes in non-remission patients with ALL. **G** Correlations between MRD and CD8+PD1+T lymphocytes in newly diagnosed patients with ALL. **H** Correlations between MRD and CD8+PD1+T lymphocytes in non-remission patients with ALL. **I** The level of CD4+PD1+T lymphocytes in BCR-ABL+ group of ALL-ND patients. **J** The level of CD8+PD1+T lymphocytes in BCR-ABL+ group of ALL-ND patients. **K** The level of CD4+PD1+T lymphocytes in BCR-ABL+ group of ALL-NR patients. **L** The level of CD8+PD1+T lymphocytes in BCR-ABL+ group of ALL-NR patients. The horizontal bar represents the average

monoclonal antibodies have made breakthroughs in clinical trials for the treatment of non-small cell lung cancer, which further indicates that PD-1 and its ligands play an important role in anti-cancer therapy. It also provides a new targeted therapy idea for the first and second-line treatment of malignant tumors.

In hematological malignancies, PD-1 expression has been reported increased in patients with Hodgkin's lymphoma, diffuse large B-cell lymphoma and chronic lymphocytic leukemia [33–35]. Furthermore, PD-1+T cells have also been proven in follicular lymphoma as an independent prognostic factor of overall survival [36, 37]. As far as the field of leukemia is concerned, current studies have reported that PD-1 is highly expressed in chronic lymphocytic leukemia and AML patients, and the expression level correlates with prognosis. However, clinical Implications of aberrant PD-1 expression in peripheral CD4+ and CD8+T lymphocytes of AML and ALL patients in assessing the prognosis of diseases, remains inconclusive. The differential expression of PD-1 on different types of T cells in the peripheral circulation

of AL patients and its relationship with the clinical characteristics and prognosis are still worthy of further investigation.

Our results indicated that expression levels of PD-1 on CD4+ and CD8+T lymphocytes were significantly increased in newly diagnosed and non-remission patients compared to healthy controls both in AML and ALL patients. According to our analysis, the relationship between high PD1 expression on different T cell types and prognosis is different. To further investigate the clinical significance of PD-1 in AL, this study first analyzed the relationship between the expression of PD-1 and its clinical characteristics. Except for the increased CD8+PD1+ levels in M2 AML patients, there was no significant association between the most common clinical indications and PD-1 expression in ALL and AML patients, indicating that PD-1 expression is not affected by general clinical manifestations and common hematologic indicators. The high level of CD4+PD1+ and CD8+PD1+ T lymphocytes were respectively poor prognostic indicators of AML patients and ALL patients

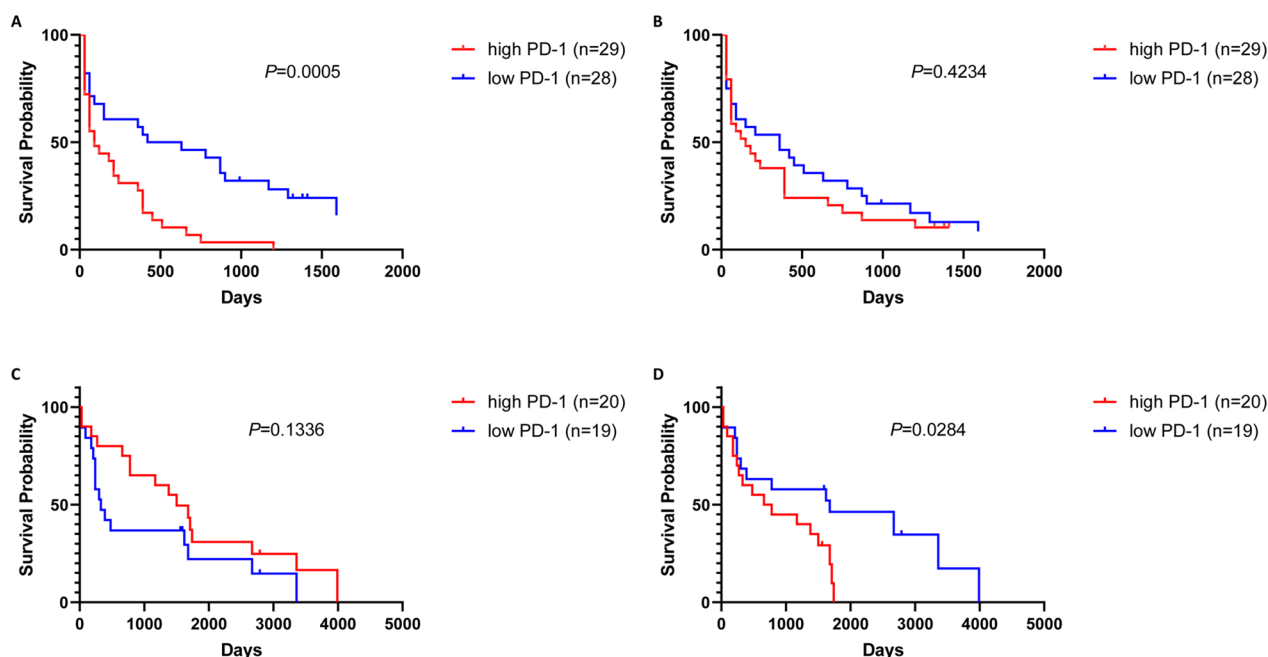


Fig. 7 Kaplan–Meier survival curve of overall survival (OS) according to immune cell specific PD-1 expression. **A** Kaplan–Meier analysis of OS according to PD-1 expression in CD4+T lymphocytes of AML patients. **B** Kaplan–Meier analysis of OS according to PD-1 expression in CD8+T lymphocytes of AML patients. **C** Kaplan–Meier analysis of OS according to PD-1 expression in CD4+T lymphocytes of ALL patients. **D** Kaplan–Meier analysis of OS according to PD-1 expression in CD8+T lymphocytes of ALL patients

Table 4 Cox regression analysis for OS

Groups	Variable	HR	95% CI	p value
AML	CD4+PD1+	1.791	1.021–3.142	0.042
	CD8+PD1+	1.742	0.987–3.075	0.055
ALL	CD4+PD1+	0.775	0.381–1.577	0.483
	CD8+PD1+	2.675	1.174–6.095	0.019

but had no significant correlation with most common clinical risks. We found that the AML patients with the higher percentage of CD4+ PD1+ in peripheral blood had shorter OS than those with the lower percentage. ALL patients with low levels of CD8+PD1+ in the peripheral blood have a significant survival advantage over patients with high levels of CD8+PD1+ in the peripheral circulation. Together, these results were indicated that the high level of CD4+PD1+T lymphocytes and CD8+ PD1+ were respectively poor prognostic indicators of AML patients and ALL patients. High expression of PD-1 in the peripheral blood of AML and ALL patients was related to their poor prognosis.

To conclude, our evidence may be limited though, but preliminarily reveals the expression level of PD-1 in peripheral circulation of newly diagnosed or non-remission AML and ALL patients was significantly higher than healthy persons. The high level of CD4+ PD1+ and

CD8+ PD1+ T lymphocytes were respectively poor prognostic indicators of AML patients and ALL patients but had no significant correlation with most common clinical risks. Aberrant PD-1 expression correlates with the prognosis of AL patient and may thus serve as poor prognostic indicators. Immunotherapy using PD-1 inhibitors may be a promising strategy for AML and ALL patients with peripheral circulating CD4+PD1+ and CD8+ PD1+ T lymphocytes positively expressed, respectively.

Author contributions

ZZ designed the experiment. RY and ZQ performed all the experiments. WJ wrote the manuscript. WJ and RY analyzed the data. WH, LC and XX collected the clinical specimens.

Funding

This work was partly supported by The Key Research and Development Plan of Anhui Province, China (No. 201904a07020058), Natural Science Research Project of Provincial Universities (No. KJ2018ZD019), the National Natural Science Foundation of China (GrantNo.81670179) and the Research Fund Project of Anhui Medical University (No. 2019xkj053).

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Declarations

Ethics approval consent to participate

This study was approved by the Institutional Review Board Institutional of the Second Hospital of Anhui Medical University.

Competing interests

The authors declare no competing interest.

Received: 6 October 2022 Accepted: 9 September 2023

Published online: 27 September 2023

References

- de Haas V, Ismaila N, Advani A, Arber DA, Dabney RS, Patel-Donnelly D, et al. Initial diagnostic work-up of acute leukemia: ASCO clinical practice guideline endorsement of the college of American pathologists and American society of hematology guideline. *J Clin Oncol*. 2019;37:239–53.
- Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. *Nat Rev Immunol*. 2008;8:59–73.
- Ljunggren HG, Jonsson R, Hoglund P. Seminal immunologic discoveries with direct clinical implications: the 2018 nobel prize in physiology or medicine honours discoveries in cancer immunotherapy. *Scand J Immunol*. 2018;88:e12731.
- Salmaninejad A, Valilou SF, Shabgah AG, Aslani S, Alimardani M, Pasdar A, et al. PD-1/PD-L1 pathway: Basic biology and role in cancer immunotherapy. *J Cell Physiol*. 2019;234:16824–37.
- Jenkins MK, Schwartz RH. Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness in vitro and in vivo. *J Exp Med*. 1987;165:302–19.
- Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J*. 1992;11:3887–95.
- Hu B, Sun M, Wang Z, Zheng Y, Cai W, Shi HH, et al. Prognostic value of programmed cell death-ligand 1 expression in tumor-infiltrating lymphocytes and viral load in peripheral blood mononuclear cells for Epstein-Barr virus-positive nasopharyngeal carcinoma. *Clin Chem*. 2020;66:1219–27.
- Gordon SR, Maute RL, Dulken BW, Hutter G, George BM, McCracken MN, et al. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. *Nature*. 2017;545:495–9.
- Ahmadzadeh M, Johnson LA, Heemskerck B, Wunderlich JR, Dudley ME, White DE, et al. Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. *Blood*. 2009;114:1537–44.
- Han Y, Liu D, Li L. PD-1/PD-L1 pathway: current researches in cancer. *Am J Cancer Res*. 2020;10:727–42.
- Messenheimer DJ, Jensen SM, Afentoulis ME, Wegmann KW, Feng Z, Friedman DJ, et al. Timing of PD-1 blockade is critical to effective combination immunotherapy with anti-OX40. *Clin Cancer Res*. 2017;23:6165–77.
- Iwai Y, Hamanishi J, Chamoto K, Honjo T. Cancer immunotherapies targeting the PD-1 signaling pathway. *J Biomed Sci*. 2017;24:26.
- Xu-Monette ZY, Zhou J, Young KH. PD-1 expression and clinical PD-1 blockade in B-cell lymphomas. *Blood*. 2018;131:68–83.
- Wang X, Wu WKK, Gao J, Li Z, Dong B, Lin X, et al. Autophagy inhibition enhances PD-L1 expression in gastric cancer. *J Exp Clin Cancer Res*. 2019;38:140.
- Ajona D, Ortiz-Espinosa S, Moreno H, Lozano T, Pajares MJ, Agorreta J, et al. A combined PD-1/CTLA4 blockade synergistically protects against lung cancer growth and metastasis. *Cancer Discov*. 2017;7:694–703.
- Larkin J, Hodi FS, Wolchok JD. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N Engl J Med*. 2015;373:1270–1.
- Forde PM, Chaft JE, Smith KN, Anagnostou V, Cottrell TR, Hellmann MD, et al. Neoadjuvant PD-1 blockade in resectable lung cancer. *N Engl J Med*. 2018;378:1976–86.
- Wunderlich M, Manning N, Sexton C, O'Brien E, Byerly L, Stillwell C, et al. PD-1 inhibition enhances blinatumomab response in a UCB/PDX model of relapsed pediatric B-cell acute lymphoblastic leukemia. *Front Oncol*. 2021;11:642466.
- Siska PJ, Rathmell JC. T cell metabolic fitness in antitumor immunity. *Trends Immunol*. 2015;36:257–64.
- Groni M, Brevi A, Cattaneo E, Rovida A, Bordini J, Bertilaccio MTS, et al. CD4+ T cells sustain aggressive chronic lymphocytic leukemia in Emu-TCL1 mice through a CD40L-independent mechanism. *Blood Adv*. 2021;5:2817–28.
- Bozorgmehr N, Okoye I, Oyegbami O, Xu L, Fontaine A, Cox-Kennett N, et al. Expanded antigen-experienced CD160(+)CD8(+)effector T cells exhibit impaired effector functions in chronic lymphocytic leukemia. *J Immunother Cancer*. 2021;9(4):e002189.
- Sheng QJ, Tian WY, Dou XG, Zhang C, Li YW, Han C, et al. Programmed death 1, ligand 1 and 2 correlated genes and their association with mutation, immune infiltration and clinical outcomes of hepatocellular carcinoma. *World J Gastrointest Oncol*. 2020;12:1255–71.
- Shrestha R, Prithviraj P, Anaka M, Bridle KR, Crawford DHG, Dhungel B, et al. Monitoring immune checkpoint regulators as predictive biomarkers in hepatocellular carcinoma. *Front Oncol*. 2018;8:269.
- Saar M, Narits J, Magi L, Aaspollu H, Vapper A, Kase M, et al. Expression of immune checkpoint PD-1 in non-small cell lung cancer is associated with tumor cell DNA-dependent protein kinase. *Mol Clin Oncol*. 2021;15:211.
- Yu K, Gu Y, Zhang P, Fang H, Cao Y, Wang J, et al. Intratumoral PD-1(+) CD8(+) T cells associate poor clinical outcomes and adjuvant chemotherapeutic benefit in gastric cancer. *Br J Cancer*. 2022;127:1709–17.
- Costa F, Vescovini R, Marchica V, Storti P, Notarfranchi L, Dalla Palma B, et al. PD-L1/PD-1 pattern of expression within the bone marrow immune microenvironment in smoldering myeloma and active multiple myeloma patients. *Front Immunol*. 2020;11:613007.
- Yuan K, Wu J, Zhao Y, Lyu S, Zhou Q, Shi F, et al. Consistent expression of PD-L1 in tumor microenvironment with peripheral PD-1/PD-L1 in circulating T lymphocytes of operable breast cancer: a diagnostic test. *Diagn Pathol*. 2022;17:68.
- Kim MH, Ko GH, Lee JH, Lee JS, Kim DC, Yang JW, et al. PD-1 expression and its correlation with prognosis in clear cell renal cell carcinoma. *In Vivo*. 2021;35:1549–53.
- Kim S, Nam SJ, Park C, Kwon D, Yim J, Song SG, et al. High tumoral PD-L1 expression and low PD-1(+) or CD8(+) tumor-infiltrating lymphocytes are predictive of a poor prognosis in primary diffuse large B-cell lymphoma of the central nervous system. *Oncoimmunology*. 2019;8:e1626653.
- Dong L, Lv H, Li W, Song Z, Li L, Zhou S, et al. Co-expression of PD-L1 and p-AKT is associated with poor prognosis in diffuse large B-cell lymphoma via PD-1/PD-L1 axis activating intracellular AKT/mTOR pathway in tumor cells. *Oncotarget*. 2016;7:33350–62.
- Sasaki H, Tatematsu T, Okuda K, Moriyama S, Yano M, Fujii Y. PD-1 gene promoter polymorphisms correlate with a poor prognosis in non-small cell lung cancer. *Mol Clin Oncol*. 2014;2:1035–42.
- Muenst S, Soysal SD, Gao F, Obermann EC, Oertli D, Gillanders WE. The presence of programmed death 1 (PD-1)-positive tumor-infiltrating lymphocytes is associated with poor prognosis in human breast cancer. *Breast Cancer Res Treat*. 2013;139:667–76.
- Gamaleldin MA, Ghallab OM, Nadwan EA, Abo Elwafa RA. PD-1 and PD-L1 gene expressions and their association with Epstein-Barr virus infection in chronic lymphocytic leukemia. *Clin Transl Oncol*. 2021;23:2309–22.
- Xia B, Wu DW, Wang TT, Guo SQ, Wang Y, Yang HL, et al. Expressions and prognostic significance of PTEN and PD-1 protein in patients with classical Hodgkin's lymphoma. *Zhonghua xue ye xue za zhi = Zhonghua xueyexue zazhi*. 2018;39:839–44.
- Karakatsanis S, Papadatos SS, Syrigos N, Marinou L, Poulou E, Papanikolaou A. Clinical significance of PD-1 and PD-L1 molecules in patients with diffuse large B-cell lymphoma, not otherwise specified: correlation with clinical and pathological findings. *J BUON*. 2021;26:569–79.
- Yang ZZ, Kim HJ, Villasboas JC, Chen YP, Price-Troska T, Jalali S, et al. Expression of LAG-3 defines exhaustion of intratumoral PD-1(+) T cells and correlates with poor outcome in follicular lymphoma. *Oncotarget*. 2017;8:61425–39.
- Beck Enemark M, Monrad I, Madsen C, Lystlund Lauridsen K, Honore B, Plesner TL, et al. PD-1 expression in pre-treatment follicular lymphoma predicts the risk of subsequent high-grade transformation. *Onco Targets Ther*. 2021;14:481–9.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.