Leukemia Research 79 (2019) 52–59

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Research paper

Programmed Cell Death Receptor (PD-1) Ligand (PD-L1) expression in Philadelphia chromosome-negative myeloproliferative neoplasms

Jen-Chin Wang*, Chi Chen, Ajay Kundra, Sreenath Kodali, Anita Pandey, Ching Wong, Tony Cheung, Vladimir Gotlieb, Gardith Joseph, Sophia Tribie

Division of Hematology/Oncology, Brookdale University Hospital Medical Center, Brooklyn, NY 11212, USA

ARTICLE INFO

Keywords:
PD-1
PD-L1
Myeloproliferative neoplasm (MPN)

ABSTRACT

Programmed Cell Death Receptor (PD-1) and its Ligand (PD-L1) pathway inhibitor therapy has been explored in the field of oncology treatment mainly for solid tumors. In hematologic malignancies, there is limited information except for Hodgkin's lymphoma, and there is even less information regarding myeloproliferative neoplasm (MPN). Therefore, we explored this by first measuring PD-1 and PD-L1 levels (percentage of positive cells) in 63 patients with Philadelphia chromosome-negative MPN (*Ph(-)* MPN), including 16 MF (12 PMF, 2 post-PV-MF, 2 post-ET-MF), 29 ET, and 18 PV. We found there was no significant difference in PD-1 or PD-L1 levels between the different MPN groups but that there was a significant difference when PV, ET and MF were grouped as MPN and compared with controls, of all immune cells including $CD4^+$, $CD8^+$, $CD14^+$ and $CD34^+$ progenitor cells. We further found a higher incidence of higher expression levels (more than 50% of cells with positive expression) of PD-1 and PD-L1 (20% and 26%, respectively) in the $CD34^+$ cells; in contrast, we found a low incidence (0.08–1.8%) in the immune cells in MPN patients. PD-1 and PD-L1 levels were also measured by MFI methods, and we obtained similar results except the measurements by percentage appeared to be more sensitive than the MFI methods. We found no correlation between PD-1 and PD-L1 expression levels and clinical features including WBC, platelet counts, hemoglobin levels, presence or absence of the JAK2, MPL, or CALR gene mutation, or splenomegaly. Since MPN represents stem cell disorders, the presence of elevated expression of PD-1 and PD-L1 in these cells suggests that the exploration of PD-1 and PD-L1 pathway inhibitor therapy may be worthwhile in *Ph(-)* MPN.

1. Introduction

The programmed death-1 (PD-1) ligand (PD-L1) signaling system and cytotoxic Tlymphocyte-associated antigen 4 (CTLA-4) system are two important immune check point mechanisms that are crucial for immune modulation to maintain the duration and amplitude of physiological immune responses to minimize collateral tissue damage. It is now clear that tumors co-opt these immune checkpoint pathways as major mechanisms of immune resistance [1–3]. PD-1 is expressed on the surface of activated T cells, B cells, and macrophages; this suggests that compared to CTLA-4, which is only present in T cells, the PD-1 and PD-L1 axis negatively regulates immune responses more broadly [4,5]. PD-L1 is expressed on resting B cells, T cells, macrophages, and dendritic cells (DCs), and the expression of PD-L1 is further upregulated on these cells by various stimuli including inflammatory cytokines such as IFN-γ, IL-12, GM-CSF, and IL-4 [6,7] which are secreted by tumor or stromal cells. PD-1 and PD-L1 are highly expressed in different tumors, such as lymphomas and pancreatic and renal cell carcinomas [8–12]. Expression of PD-L1 on tumor cells further confers a potent escape mechanism from host T cell immunity.

PD-1 and PD-L1 pathway inhibitor therapy has been explored as an oncology treatment recently. The Food and Drug Administration (FDA) has approved these inhibitors for the treatment of melanoma, non-small cell lung cancer (NSCLC), re-current or metastatic head and neck squamous cell carcinoma (HNSCC), renal cell carcinoma (RCC), and recently classical Hodgkin lymphoma (cHL) [13–20]. PD-1 and PDL-1 pathway inhibitor therapy has been less explored for use in hematologic malignancy, especially for myeloproliferative neoplasm. The current study is an extension of our preliminary studies on the PD-1 and PD-L1 pathway in BCR–ABL(-) myeloproliferative neoplasm (MPN) [21].

* Corresponding author.
E-mail address: jcwang0005@gmail.com (J.-C. Wang).

https://doi.org/10.1016/j.leukres.2019.02.010
Received 8 August 2018; Received in revised form 25 February 2019; Accepted 27 February 2019
Available online 28 February 2019
0145-2126/

2. Materials and methods

2.1. Patients

Sixty-three patients with MPN diseases (12 PMF, 2 post-PV-MF, 2 post-ET-MF, 29 ET, and 18 PV) and 25 normal healthy volunteer controls (CTR) were studied. Their diagnoses was made according to the World Health Organization classification [22]. Patients who were treated with ruxolinitinib, other immunomodulatory drugs (IMiD) or interferon were excluded. The median age of the MPN group was 61 (interquartile range 54–73); the CTR group had a median age of 48 (interquartile range 44–59).

2) JAK 2 V617 F, MPL and calreticulin gene mutation analysis was done with qRT-PCR methods by either the Genoptix Bio-Pharma Company (Carlsbad, CA) or GenPath Lab (Elamwood Park, NJ). The assay sensitivity was > 1%. All patients provided informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Brookdale University Hospital Medical Center Institutional Review Board.

2.2. Flow cytometry analysis of PD-1 and PD-L1 expression

Peripheral blood samples were collected from patients and healthy individuals in heparin-anticoagulated tubes. Leukocytes were obtained from whole blood after lysing RBCs using RBC lysis buffer (**Quiagen**). To quantify surface bound PD-1 or PDL-1, leukocytes were incubated with CD279-APC and CD274-PE as well as CD4-FITC, CD8-FITC, CD14-PCP, or CD34-FITC (BD Biosciences) and were subjected to flow cytometric analysis. The flow data were further analyzed using FlowJo (v 7.6.2). PD-1 and PD-L1-positive cells were expressed as a percentage of the gated $CD4^{bright}$, $CD8^+$, $CD14^+$, or $CD34^+$ cells. Representative dot plots (Fig. 1) showed the flow cytometric analysis of peripheral leukocytes after staining for CD4-FITC, CD8-FITC, CD14-PCP, or CD34-FITC in addition to CD279-APC and CD274-PE. A first gate was applied to identify $CD4^{bright}$ (Fig. 1A), $CD8^+$ (Fig. 1D), $CD14^+$ (Fig. 1G), or $CD34^+$ (Fig. 1J) cells. Subsequent dot plots showed the surface expression of PD-1 in $CD4^{bright}$ (Fig. 1B), $CD8^+$ (Fig. 1E), $CD14^+$ (Fig. 1H), and $CD34^+$ (Fig. 1K) cells and the surface expression of PD-L1 in $CD4^+$ (Fig. 1C), $CD8^+$ (Fig. 1F), $CD14^+$ (Fig. 1I), or $CD34^+$ (Fig. 1L) cells. Representative flow cytometric histograms of PD-1 and PDL-1, in $CD4^+$, $CD8^+$, $CD14^+$ and $CD34^+$ cells overlaid with their respective isotype controls in dashed lines are shown in Fig. 2. The same procedures were performed to measure the PD-1 and PD-L1 levels by analysis of MFI (mean fluorescence intensity) with flow cytometry in MPN patients compared with controls (Figs. S1–S4).

2.3. Statistical analyses

Statistical analyses was performed with paired or unpaired Student's t-tests and one-way ANOVA for comparison of groups. The results are given as the mean with standard error and 95% confidence intervals (CIs). Data are expressed as the median and interquartile range if considered to not have a normal distribution.

Mann–Whitney U tests were used for nonparametric data, and data are expressed as the median (interquartile range). $p < 0.05$ was considered significant. The correlation of PD-1 and PD-L1 levels with clinical parameters were made by comparing PD-1 and PD-L1 levels in WBCs ($> 10.0 \times 10^9$ /L vs. $< 10 \times 10^9$), platelet counts ($> 400 \times 10^9$/L vs. $< 400 \times 10^9$), HB (> 13 g/dL vs. < 13), splenomegaly (yes vs. no) (splenomegaly was measured with either CT or sonogram images and a few by physical examination), and JAK2 status (positive vs. negative) (Allele-burden, > 50% vs. < 50%) using Student's t-test. The correlations between the percentage of blast cells to PD-1 and PD-L1 levels in MF patients were calculated using Spearman correlation methods.

3. Results

3.1. PD-1 and PD-L1 levels in MPN

All values were calculated as the percentage of positive cells in different types of immune cells or progenitor cells over the respective total cell number. MF included PMF, post-ET MF and post-PV MF. There is a wide range of distribution of PD-1 and PD-L1 expression in various types of cells; this could be secondary to the heterogenous distribution of PD-1 and PD-L1 in the same types of cells or other unknown factors such as non-apparent infections. All of the samples were assayed for PD-1 and PD-L1 expression within 24 h. We also assayed the PD-1 and PD-L1 levels in relation to the time the samples were collected; this analysis revealed that PD-1 and PD-L1 increased as the time from the sample collection to processing increased (data are shown).

3.2. CD4 + PD-1 and PD-L1 levels

As shown in Fig. 3, both PD-1 and PD-L1 levels significantly increased in MF but not ET or PV ($p < 0.05$). If we grouped ET, PV and MF together, then MPN exhibited elevated levels compared to controls ($p < 0.001$), and there was no significant difference among the different sub-categories of MPN. The results (PD-1 levels) were expressed as the mean ± SE as follows: ET (9.65 ± 1.48) (n = 24), PV (15.68 ± 4.52) (n = 15), MF (17.97 ± 3.2) (n = 16), MPN (13.72 ± 1.72) (n = 55) and control (7.22 ± 0.92) (n = 25). The PD-L1 levels were ET (18.74 ± 4.07) (n = 24), PV (21.47 ± 6.37) (n = 15), MF (28.68 ± 6.02 (n = 16), MPN (22.38 ± 3.01) (n = 55) and control (7.35 ± 1.29) (n = 25).

3.3. $CD8^+$ PD-1 and PD-L1 levels

As shown in Fig. 4, PD-1 levels were significantly increased in ET, PV, and MF samples compared to controls ($P < 0.05$). The differences were more significant when we grouped ET, PV and MF together as MPN and compared that value to the control ($P < 0.001$), while there were no differences among the subcategories of MPN. For the PD-L1 levels, only the MF samples were significantly different than the controls ($p < 0.05$); again when we grouped ET, PV and MF together as MPN, it was significantly different compared to the controls ($P < 0.001$). The PD-1 levels were as follows: ET (16.75 ± 3.25) (n = 25), PV (30.79 ± 3.25) (n = 15), MF (33.79 ± 6.99) (n = 16), MPN (25.38 ± 3.15) (n = 56) and control (9.46 ± 1.82) (n = 26). The PD-L1 levels were as follows: ET (15.63 ± 2.66) (n = 25), PV (12.72 ± 3.52) (n = 15), MF (23.82 ± 6.78) (n = 16), MPN (17.19 ± 2.48) (n = 56) and control (7.01 ± 0.73) (n = 26).

3.4. $CD14^+$ PD-1 and PD-L1 levels

As shown in Fig. 5, the PD-1 levels in PV and ET but not MF were significantly elevated compared to controls ($p < 0.05$); if we grouped ET, PV and MF together as MPN, then this value was significantly different than the control ($P < 0.001$). Similarly, there was no difference between the subcategories of MPN; for PD-L1 levels, MF was significantly different than controls but not ET or PV, but it was significantly different ($p < 0.001$) if we grouped ET, PV and MF together as the MPN value compared to the control, while there was no difference between each subcategory of MPN. The PD-1 levels were as follows: ET (22.35 ± 2.35) (n = 29), PV (24.01 ± 3.88) (n = 18), MF (23.31 ± 5.22) (n = 14), MPN (23.03 ± 1.96) (n = 61) and control (11.06 ± 1.42) (n = 22). The PD-L1 levels were as follows: ET (11.35 ± 2.36) (n = 29), PV (14.20 ± 3.38) (n = 18), MF (26.35 ± 8.16) (n = 14) MPN (15.63 ± 2.47) (n = 61) and control (3.96 ± 0.64) (n = 22).

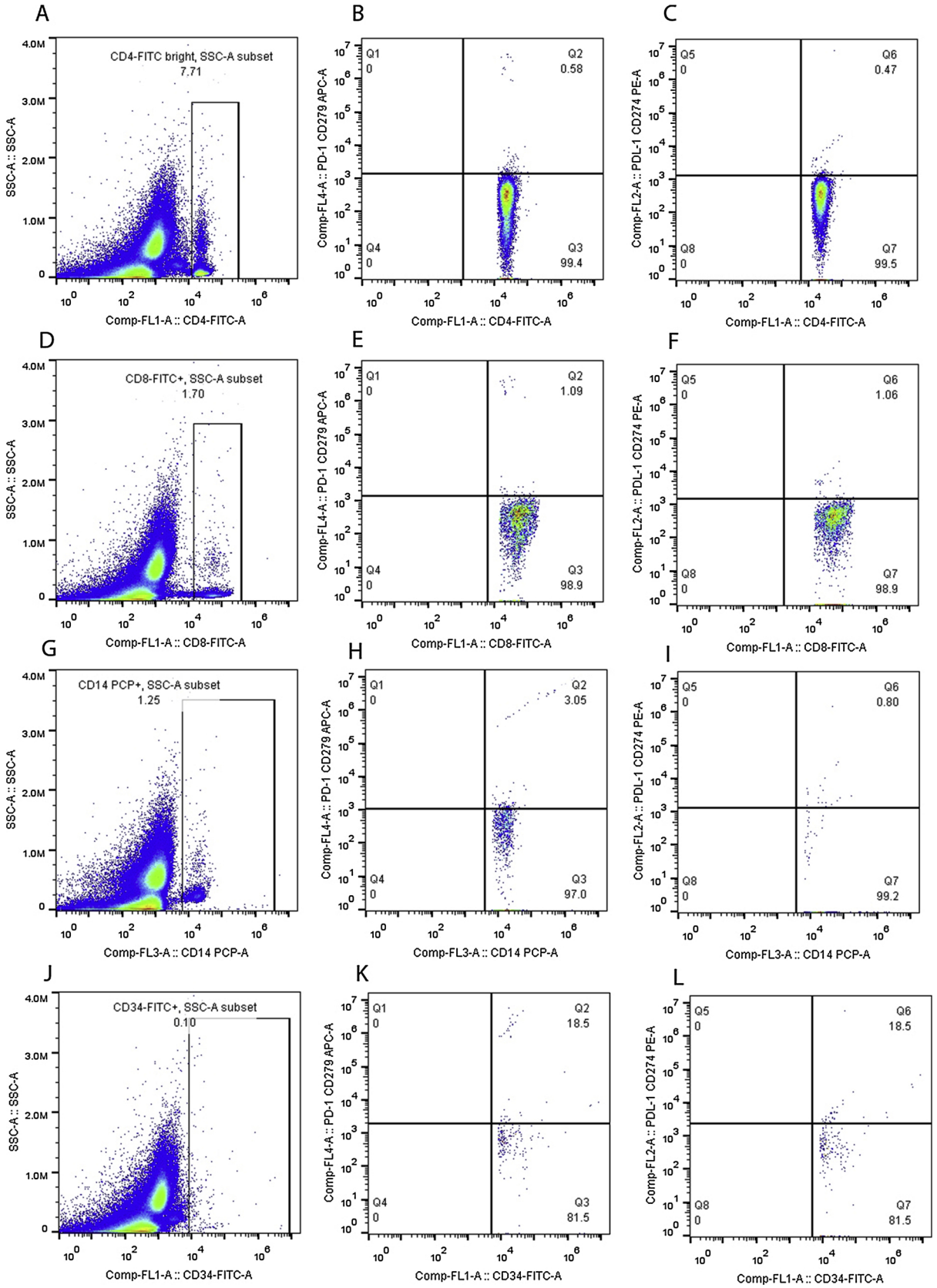

Fig. 1. Flow cytometric analysis of the surface expression of PD-1 and its ligand PD-L1 in distinct lymphocyte subsets, monocytes, and CD34$^+$ progenitor cells in peripheral blood from a healthy donor. Representative dot plots show flow cytometric analysis of peripheral leukocytes after staining for CD4-FITC, CD8-FITC, CD14-PCP, or CD34-FITC, in addition to CD279-APC and CD274-PE. A first gate was applied to identify CD4^{bright} (A), CD8$^+$ (D), CD14$^+$ (G), or CD34$^+$ (J) cells. Subsequent dot plots show the surface expression of PD-1 in CD4^{bright} (B), CD8$^+$ (E), CD14$^+$ (H), and CD34$^+$ (K) cells and the surface expression of PD-L1 in CD4$^+$ (C), CD8$^+$ (F), CD14$^+$ (I), or CD34$^+$ (L) cells.

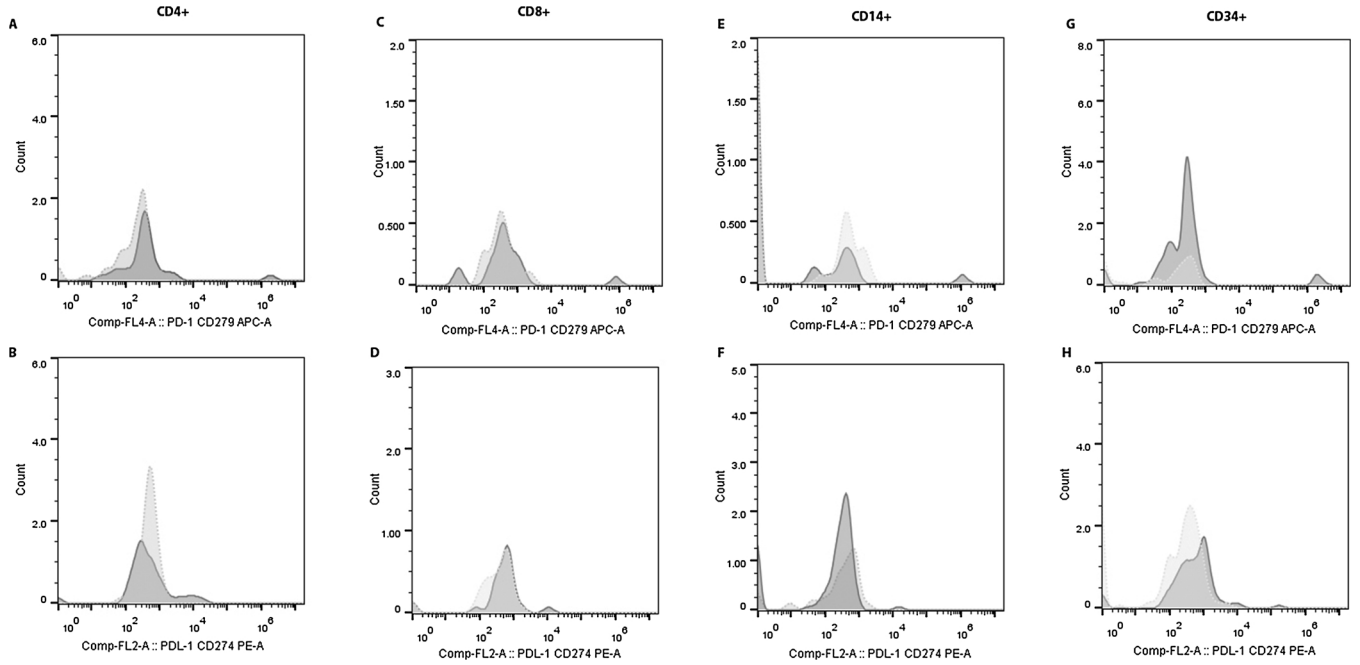

Fig. 2. Representative flow cytometric histograms of PD-1 (CD279; panels A, C, E and G) or PDL-1 (CD274; panels B, D, F, and H) represented with solid lines in gated $CD4^+$ (A and B), $CD8^+$ (C and D), $CD14^+$ (E and F) and $CD34^+$ (G and H) cells. The histograms are overlaid with the respective isotype controls represented with dashed lines.

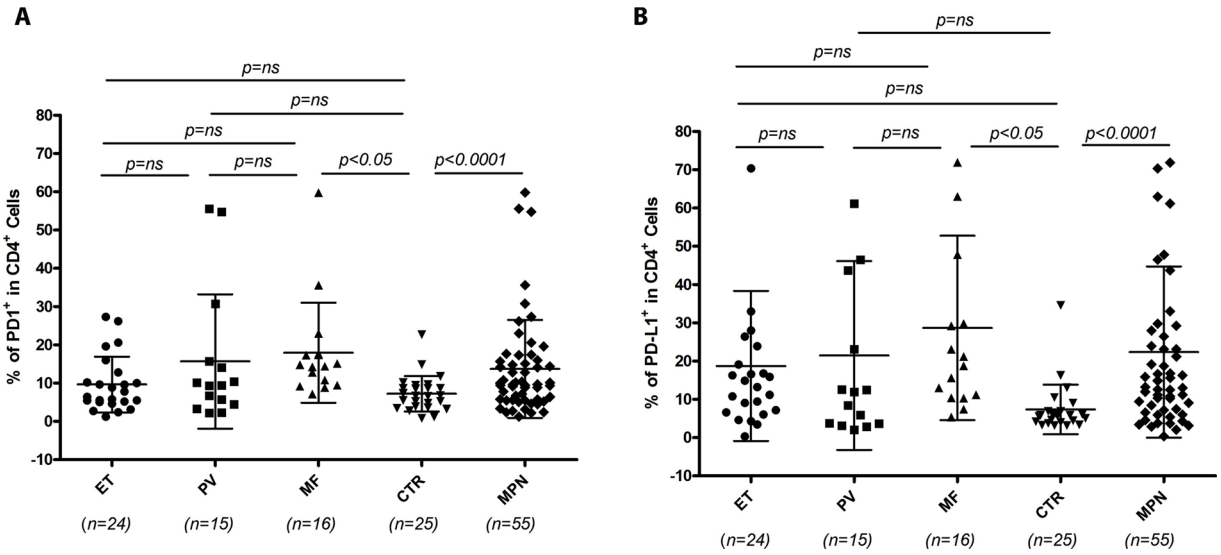

Fig. 3. CD4+ PD-1 and PD-L1 levels. a) PD-1 levels were enumerated as the percentage of $CD4^+$ cells out of the total $CD4^+$ cells. For both the PD-1 and PD-L1 levels, the MF values were significantly increased compared to the controls ($P < 0.05$). If we grouped ET, PV and MF together as the MPN group and compared to the controls ($p < 0.001$), there were no significant differences among the different subcategories of MPN. The PD-1 levels were expressed as the mean ± SE as follows: ET (9.65 ± 1.48) (n = 24), PV (15.68 ± 4.52) (n = 15), MF (17.97 ± 3.2) (n = 16), MPN (13.72 ± 1.72) (n = 55) and control (7.22 ± 0.92) (n = 25). The PD-L1 levels were as follows: ET (18.74 ± 4.07) (n = 24), PV (21.47 ± 6.37) (n = 15), MF (28.68 ± 6.02 (n = 16), MPN (22.38 ± 3.01) (n = 55) and control (7.35 ± 1.29) (n = 25).

3.5. $CD34^+$ PD-1 and PD-L1 levels

As shown in Fig. 6, the PD-1 levels were significantly elevated in PV but not ET or MF compared to controls ($P < 0.05$) and we grouped ET, PV and MF together as MPN, the value was highly significantly elevated compared to controls ($P < 0.001$), and there were no significant differences among the different subcategories of MPN. For PD-L1 levels, PV and ET but not MF were significantly elevated compared to the controls and if we grouped ET, PV, MF together as MPN, the value was highly significantly elevated compared to the control ($P < 0.0001$), and there were no differences among the subcategories of MPN. The PD-1 levels were as follows: ET (24.03 ± 4.51) (n = 21), PV (47.59 ± 7.58) (n = 13), MF (22.18 ± 8.15) (n = 9), MPN (30.76 ± 3.91) (n = 43) and control (11.52 ± 1.94) (n = 14). The PD-L1 levels were as follows: ET (39.31 ± 6.63) (n = 21), PV (53.76 ± 7.93) (n = 11), MF (31.48 ± 7.57) (n = 9), MPN (41.47 ± 4.43) (n = 41) and control (11.94 ± 2.25) (n = 14).

3.6. Higher percentage of PD-1 or PD-L1 expression in $CD34^+$ cells (more than 50%)

A high percentage of PD-1 or PD-L1 (> 50%) expression has been

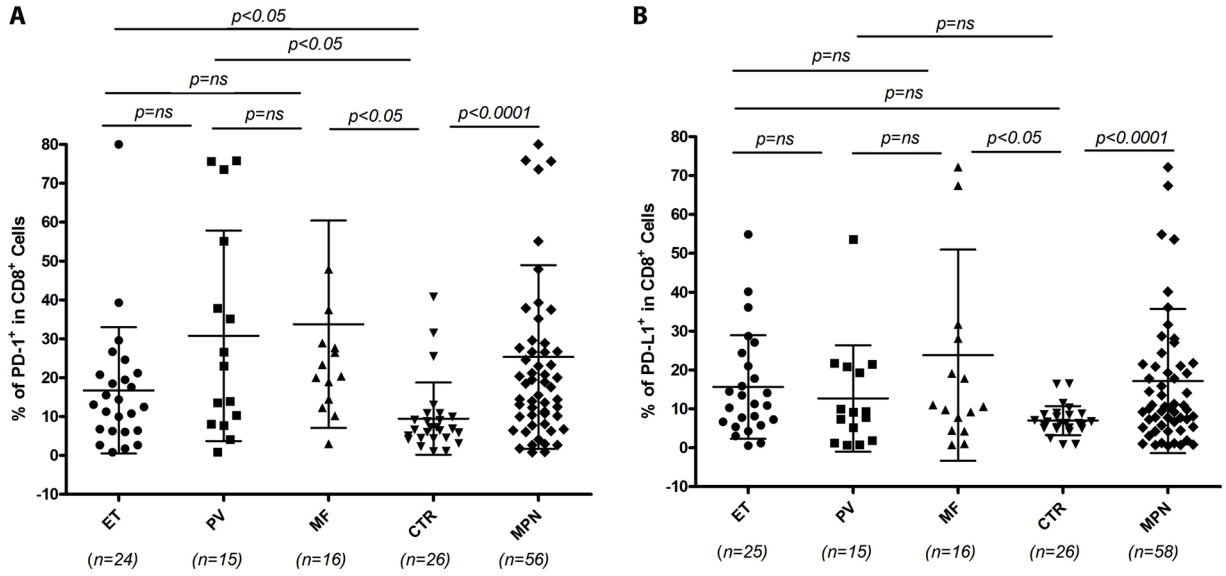

Fig. 4. CD8+ PD-1 and PD-L1 levels. a) CD8+ PD-1 levels. PD-1 levels were significantly increased in ET, PV, and MF compared to the controls ($P < 0.05$). When we grouped ET, PV and MF together as MPN, the value was even more significantly different than the controls ($P < 0.001$), while there was no difference among each subcategory of MPN. The values were as follows: ET (16.75 ± 3.25) (n = 25), PV (30.79 ± 3.25) (n = 15), MF (33.79 ± 6.99) (n = 16) and control (9.46 ± 1.82) (n = 26). b) CD8 + PD-L1 levels. Only MF was significantly different than the control ($p < 0.05$). When we grouped ET, PV and MF together as MPN, the value was also highly significantly different than the control ($P < 0.001$). The PD-L1 levels were as follows: ET (15.63 ± 2.66) (n = 25), PV (12.72 ± 3.52) (n = 15), MF (23.82 ± 6.78) (n = 16), MPN (17.19 ± 2.48) (n = 56) and control (7.01 ± 0.73) (n = 26).

found to be associated with excellent response to PD-1 inhibitor antibody therapy in solid tumors [23]. Therefore, we also calculated the percentage of PD-1 and PD-L1 expression in various cell types. As shown in Figs. 3–6, we found higher levels of expression with more than 50% of PD-1 and PD-L1 in only 0.05% and 1.18% of $CD4^+$ cells, 1.2% and 0.08% of $CD8^+$ cells, 1.8% and 1.5% of $CD14^+$ cells, respectively. However, we found a high percentage of expression (> 50%) in $CD34^+$ cells with PD-1 and PD-L1 levels of 20% and 26%, respectively. We also examined whether higher expression of PD-1 and PD-L1 levels in various cell types occurred in the same patients, but we did not observe this.

3.7. MFI measurements of PD-1 and PD-L1 levels in $CD4^+$, $CD8^+$, $CD14^+$, and $CD34^+$ cells

As shown in Figs. S1–S4, in general similar results were obtained. When we grouped ET, PV, and MF together as MPN, the value was highly significantly elevated for both PD-1 and PD-L1 levels in $CD4^+$ and $CD8^+$ cells ($P < 0.05$) but not in $CD14^+$ cells. In $CD34^+$ cells, PD-1 and not PD-L1 levels were significantly elevated ($P < 0.05$). It appeared that the calculation with the percentage of positive cells was more sensitive than the MFI measurement. This may be due to heterogenous distribution of PD-1 and PD-L1 levels in the same MPN cells.

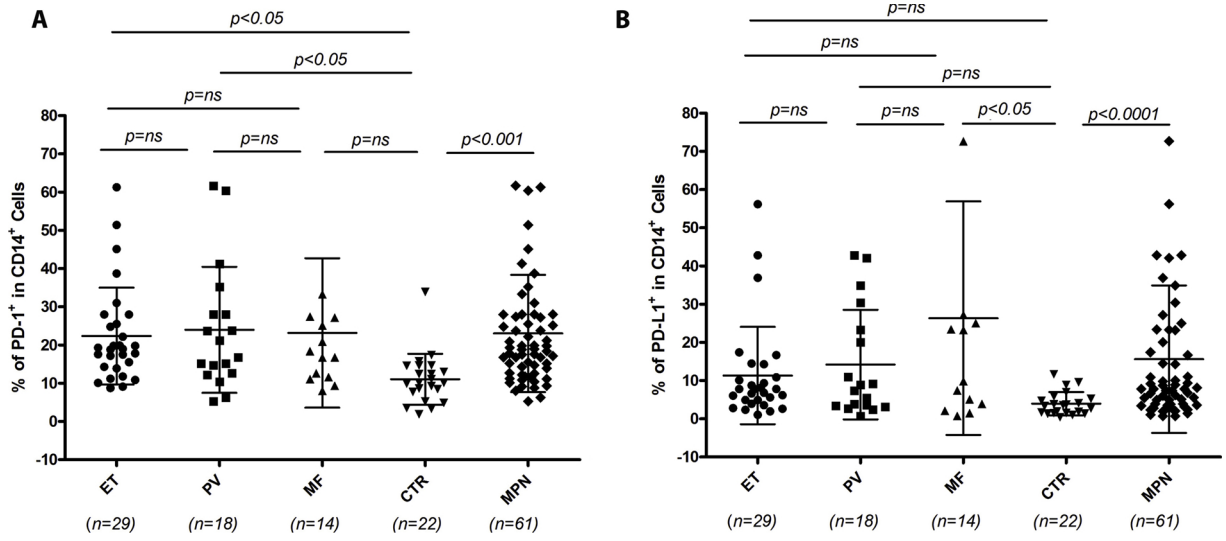

Fig. 5. CD14+ PD-1 and PD-L1 levels. a) CD14+ PD-1 levels. PV and ET but not MF were significantly elevated compared to the control ($p < 0.05$). If we grouped ET, PV and MF together as MPN, then this value was highly significantly different than the control ($P < 0.001$), while there was no difference among the subcategories of MPN. The PD-1 levels were as follows: ET (22.35 ± 2.35) (n = 29), PV (24.01 ± 3.88) (n = 18), MF (23.31 ± 5.22) (n = 14), MPN (23.03 ± 1.96) (n = 61) and control (11.06 ± 1.42) (n = 22). b) CD14 PD-L1 levels. MF was significantly different than the controls, but ET and PV were not significant. If we grouped ET, PV and MF together as MPN, the value was highly significantly different than the control ($p < 0.001$), there was no difference among the subcategories of MPN. The PD-L1 levels were as follows: ET (11.35 ± 2.36) (n = 29), PV (14.20 ± 3.38) (n = 18), MF (26.35 ± 8.16) (n = 14), MPN (15.63 ± 2.47) (n = 61) and control (3.96 ± 0.64) (n = 22).

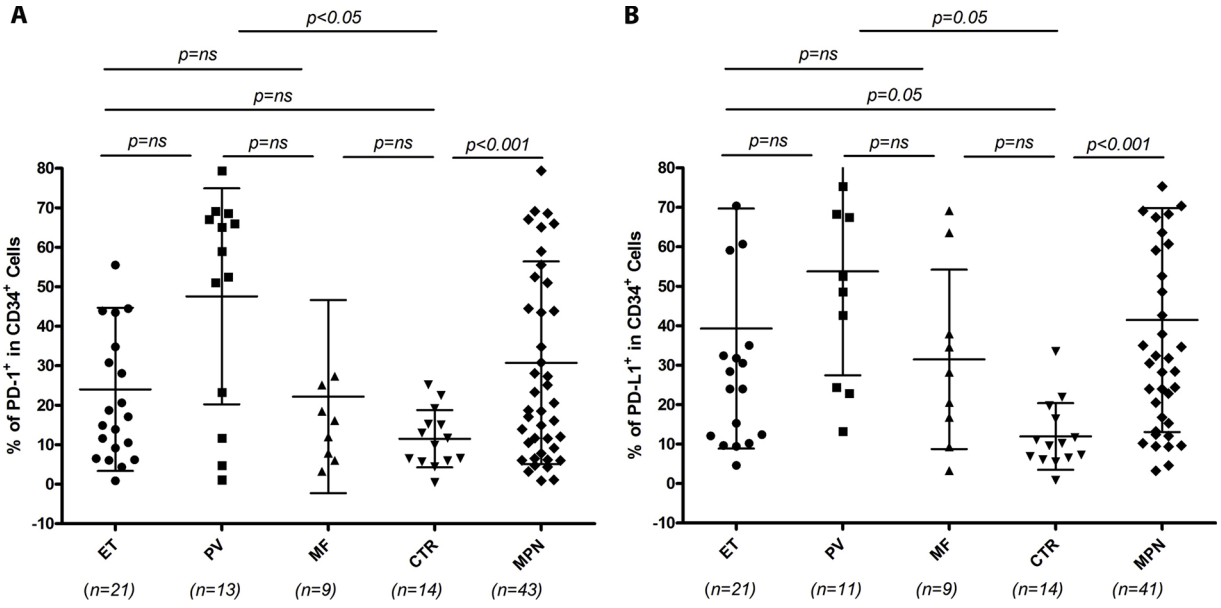

Fig. 6. CD34+ PD-1 and PD-L1 levels. a) PD-1 (Left): PV but not ET or MF was significantly elevated compared to the control ($P < 0.05$). When we grouped ET, PV, and MF together as MPN, the value was highly significantly elevated compared to the control ($P < 0.001$), and there was no significant difference between the different subcategories of MPN. The values were as follows: ET (24.03 ± 4.51) (n = 21), PV (47.59 ± 7.58) (n = 13), MF (22.18 ± 8.15) (n = 9), MPN (30.76 ± 3.91) (n = 43) and control (11.52 ± 1.94) (n = 14). b) PD-L1 levels. PV and ET but not MF were significantly elevated compared to the control. If we grouped ET, PV, and MF together as MPN, the value was highly significantly elevated compared to the control ($P < 0.0001$), while there was no difference between the subcategories of MPN. The PD-1 levels were as follows: ET (39.31 ± 6.63) (n = 21), PV (53.76 ± 7.93) (n = 11), MF (31.48 ± 7.57) (n = 9), MPN (41.47 ± 4.43) (n = 41) and control (11.94 ± 2.25) (n = 14).

3.8. PD-1 and PD-L1 levels related to JAK2, MPL, and CALR mutations and other clinical features

The percentages of positive PD-1 and PD-L1 expression in $CD4^+$, $CD8^+$, $CD14^+$, and $CD34^+$, cells compared to patients with or without hydroxyurea treatment were not significantly different (data not shown). Therefore, patients who were on hydroxyurea treatment were included in the current studies. PD-1 and PD-L1 levels were compared to JAK2, CALR, and MPL mutation status in patients with MPN diseases. Thirty-five subjects were JAK2+, including 2 PV—MF, 1 ET—MF, 2 PMF, 13 PV, and 17 ET; 5 patients were CALR+, including 3 ET and 2 PMF; and one patient was MPL +. We found no difference in the percentage of PD-1 and PD-L1 positive cells between JAK2+ and Jak2– patients. Additionally, there were no differences between CALR + and CALR- patients. There was no significant difference when we grouped the CALR, JAK2 and MPL mutation positive patients together and compared that value with those of the triple negative group. Similarly, we compared the PD-1 and PD-L1 levels with WBC counts, Hb levels, platelet counts, and splenomegaly status. No significant differences were found.

3.9. Percentage of blast cells in MF patients in relation to PD-1 and PD-L1 levels

Because elevated PD-1 and PD-L1 levels had been described in patients with acute leukemia [23,24], the PD-1 and PD-L1 levels were correlated to the percentage of blast cells in MF patients. We found that there was no correlation, as shown in Table S-1.

4. Discussion

Our current studies showed that the PD-1 and PD-L1 levels were significantly increased than controls when ET, PV and MF were grouped together as MPN but no difference noted among individual subgroup. In some parts, subgroups of MPN, may not be significant from controls as an individual group, which likely is due to insufficient statistical power. Our studies agree with the preliminary results from Craig et al., who demonstrated that the levels of PD-L1 protein and mRNA were higher in splenic hematopoietic cells, including megakaryocytes and myeloid cells, from MF patients compared to healthy donors [25]. There was also a recent publication by Prestipino et al. [26] which showed elevated levels of PD-L1 as measured by MFI, among 32 JAK2 mutated patients when compared to controls. The results were similar to our study, although our study was more detailed and included more patients.

We found a high incidence of high percentages of PD-1 and PD-L1 expression (more than 50%) in $CD34^+$ cells (20% and 26% of PD-1 and PD-L1, respectively), while only approximately 0.01%–1.8% was detected in the other immune-reactive $CD4^+$, $CD8^+$ or $CD14^+$ cells in MPN patients. Since high expression of PD-L1 in tumors is indicative of excellent response to immunotherapy [27], our current studies indicate that exploration of PD-1 and PD-L1 inhibitor immunotherapy in MPN will be worthwhile. We are expecting the NCI clinical trial of PD-1 inhibitors in MPN will provide the answers [28].

The transcriptional control mechanisms of PD-1 and PD-L1 expression remain largely unknown. PD-L1 regulation is clearly more understood; the promotor contains an ISRE/IRF1 module of the JAK/STAT-responsive promoter with a stat protein-binding site [29,30], and the enhancer contains dual AP-1–binding sites [31]. Therefore, in acute leukemia, lung cancer, etc., increased PD-L1 in the cancerous cells is caused by T cell-activation by interferon gamma secretion and upregulation of PD-L1 production [32–34]. Increased PD-L1 from EBV-transformed lymphoblastoid cell lines resulted from latent membrane protein 1–mediated, JAK/STAT-dependent promoter and AP-1–associated enhancer activity, which then stimulated PD-L1 expression [31]. In myeloma, enhanced PD-L1 expression in plasma cells is caused by IFN-Ɣ and Toll-like receptor (TLR) ligands through promotor stimulation and the MEK/ERK pathway [35]. Oncoproteins such as ALK/NPM can induce PD-L1 through the STAT protein binding site in the PD-L1 promotor [36]. Transcriptional control of PD-1 remains largely unknown, and its expression is reported to rely on multiple transcription factors such as NFAT [37], T-bet [38], and Blimp-1 [39].

We have found increased MDSC levels in patients with MPN [40] and that TLR-2 increased in patients with ET and PV [41]. MDSC interacts with activated T cells which secrete IL-10 which, in turn, activates STAT3 phosphorylation leading to B7-H1 expression [42]. Increased TLR-2 expression with inflammatory changes in MPN leads to activation of the MERK/ERK pathway and then the STAT pathway, which leads to activation of the PD-L1 promotor and eventually PD-L1 expression. Persistent inflammatory antigenic stimulation caused downregulation of T-bet, which resulted in more severe exhaustion of $CD8^+$ T cells which then caused enhanced PD-1 production [43,44]. This may explain the increased PD-1 and PD-L1 expression found in patients with MPN.

Conflicts of interest

Jen C Wang, MD, received research grants from Celgene Corporation, USA. All other authors declare no conflicts of interest with pharmaceutical companies.

Acknowledgement

This work is support by the Brookdale Research Foundation.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.leukres.2019.02.010.

References

[1] C. Kyi, M.A. Postow, Checkpoint blocking antibodies in cancer immunotherapy, FEBS Lett. (October) (2013) [Epub ahead of print].
[2] D.M. Pardoll, The blockade of immune checkpoints in cancer immunotherapy, Nat. Rev. Cancer 12 (March (4)) (2012) 252–264 Review.
[3] R.J. Greenwald, G.J. Freeman, A.H. Sharpe, The B7 family revisited, Annu. Rev. Immunol. 23 (2005) 515–548.
[4] W. Zou, L. Chen, Inhibitory B7 family molecules in the tumour microenvironment, Nat. Rev. Immunol. 8 (2008) 467–477.
[5] T. Okazaki, T. Honjo, PD-1 and PD-1 ligands: from discovery to clinical application, Int. Immunol. 19 (2007) 813–824.
[6] G.J. Freeman, A.J. Long, Y. Iwai, K. Bourque, T. Chernova, H. Nishimura, L.J. Fitz, N. Malenkovich, T. Okazaki, M.C. Byrne, H.F. Horton, L. Fouser, L. Carter, V. Ling, M.R. Bowman, B.M. Carreno, M. Collins, C.R. Wood, T. Honjo, Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation, J. Exp. Med. 192 (October (7)) (2000) 1027–1034.
[7] W. Zou, L. Chen, Inhibitory B7-family molecules in the tumour microenvironment, Nat. Rev. Immunol. 8 (2008) 467–477.
[8] Y. Iwai, M. Ishida, Y. Tanaka, T. Okazaki, T. Honjo, N. Minato, Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade, Proc. Natl. Acad. Sci. U. S. A. 99 (September (19)) (2002) 12293–12297.
[9] H. Dong, S.E. Strome, D.R. Salomao, et al., Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion, Nat. Med. 8 (793) (2002) [Erratum, Nat Med 2002;8:1039.].
[10] R. Yamamoto, M. Nishikori, T. Kitawaki, T. Sakai, M. Hishizawa, M. Tashima, T. Kondo, K. Ohmori, M. Kurata, T. Hayashi, T. Uchiyama, PD-1-PD-1 ligand interaction contributes to immunosuppressive microenvironment of Hodgkin lymphoma, Blood 111 (2008) 3220–3224.
[11] T. Nomi, M. Sho, T. Akahori, K. Hamada, A. Kubo, H. Kanehiro, S. Nakamura, K. Enomoto, H. Yagita, M. Azuma, Y. Nakajima, Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer, Clin. Cancer Res. 13 (2007) 2151–2157.
[12] R.H. Thompson, H. Dong, C.M. Lohse, B.C. Leibovich, M.L. Blute, J.C. Cheville, Kwon ED (2007) PD-1 is expressed by tumor infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma, Clin. Cancer Res. 13 (2007) 1757–1761.
[13] Y. Iwai, M. Ishida, Y. Tanaka, T. Okazaki, T. Honjo, N. Minato, Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 12293–12297.
[14] Adminstration USFaD, Fda Approves Keytruda for Ad- Vanced Melanoma, (2014).
[15] Administration USFaD, Fda Approves Keytruda for Ad- Vanced Non-Small Cell Lung Cancer, (2015).
[16] Administration USFaD, Fda Approves Opdivo for Advanced Melanoma, (2014).
[17] Administration USFaD, Fda Expands Approved use 0f Opdivo to Treat Lung Cancer, (2015).
[18] Administration USFaD, Nivolumab (Opdivo) for Hodgkin Lymphoma, 2016 (2015).
[19] Administration USFaD, Fda Approves Opdivo to Treat Ad- Vanced Form of Kidney Cancer, (2015).
[20] Administration USFaD, Fda Approves Keytruda (Pembrolizumab) for Head and Neck Cancer, (2016).
[21] A. Kundra, S. Baptiste, C. Chen, H. Sindhu, J.-C. Wang, Programmed Cell Death Receptor (PD-1), PD-1 Ligand (PD-L1) expression and myeloid derived suppressor cells (MDSC) in myeloid neoplasms implicate the mechanism of IMiD treatment of myelofibrosis, Blood. 122 (21) (2013) 2837.
[22] W. Vardiman, J. Thiele, D.A. Arber, et al., The 2008 revision of the world health organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes, Blood 114 (5) (2009) 937–951.
[23] H. Tamura, K. Dan, K. Tamada, K. Nakamura, Y. Shioi, H. Hyodo, et al., Expression of functional B7-H2 and B7.2 costimulatory molecules and their prognostic implications in de novo acute myeloid leukemia, Clin. Cancer Res. 11 (2005) 5708–5717.
[24] X. Chen, S. Liu, L. Wang, W. Zhang, Y. Ji, X. Ma, Clinical significance of B7-H1 (PD-L1) expression in human acute leukemia, Cancer Biol. Ther. 7 (2008) 622–627.
[25] R. Craig, S.R. Tripp, M. Deininger, M.E. Salama, Programmed death ligand (PD-L1) expression is increased in spleens of myelo- fibrosis patients, United States and Canadian Academy of 2016 Annual Meeting (2016) Abstract 1353.
[26] A. Prestipino, A.J. Emhardt, K. Aumann, D. O'Sullivan, S.P. Gorantla, S. Duquesne, W. Melchinger, L. Braun, S. Vuckovic, M. Boerries, H. Busch, S. Halbach, S. Pennisi, T. Poggio, P. Apostolova, P. Veratti, M. Hettich, G. Niedermann, M. Bartholomä, K. Shoumariyeh, J.S. Jutzi, J. Wehrle, C. Dierks, H. Becker, A. Schmitt-Graeff, M. Follo, D. Pfeifer, J. Rohr, S. Fuchs, S. Ehl, F.A. Hartl, S. Minguet, C. Miething, F.H. Heidel, N. Kröger, I. Triviai, T. Brummer, J. Finke, A.L. Illert, E. Ruggiero, C. Bonini, J. Duyster, H.L. Pahl, S.W. Lane, G.R. Hill, B.R. Blazar, N. von Bubnoff, E.L. Pearce, R. Zeiser, Oncogenic JAK2V617F causes PD-L1 expression, mediating immune escape in myeloproliferative neoplasms, Sci. Transl. Med. 10 (February (429)) (2018), https://doi.org/10.1126/scitranslmed.aam7729 pii: eaam7729.
[27] M. Reck, D. Rodríguez-Abreu, A.G. Robinson, R. Hui, T. Csőszi, A. Fülöp, M. Gottfried, N. Peled, A. Tafreshi, S. Cuffe, M. O'Brien, S. Rao, K. Hotta, M.A. Leiby, G.M. Lubiniecki, Y. Shentu, R. Rangwala, J.R. Brahmer, KEYNOTE-024 investigators, Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer, N. Engl. J. Med. 375 (November (19)) (2016) 1823–1833 Epub 2016Oct 8.
[28] PD-1 Inhibition in Advanced Myeloproliferative Neoplasms – (PI, John Mascarenhas, MD), https://clinicaltrials.gov/ct2/show/NCT03065400.
[29] S.J. Lee, B.C. Jang, S.W. Lee, et al., Interferon regu- latory factor-1 is prerequisite to the constitutive expression and IFN-gamma-induced upregula- tion of B7-H1 (CD274), FEBS Lett. 580 (3) (2006) 755–762.
[30] M.R. Green, S. Monti, S.J. Rodig, P. Juszczynski, T. Currie, E. O'Donnell, et al., Integrative analysis reveals selective 9p24.1 amplification, increased PD-1 ligand expression, and further induction via JAK2 in nodular sclerosing hodgkin lymphoma and primary mediastinal large B-cell lymphoma, Blood 116 (2010) 3268–3277.
[31] M.R. Green, S. Rodig, P. Juszczynski, J. Ouyang, P. Sinha, E. O'Donnell, D. Neuberg, M.A. Shipp, Constitutive AP-1 activity and EBV infection induce PD-L1 in hodgkin lymphomas and posttransplant lymphoproliferative disorders: implications for targeted therapy, Clin. Cancer Res. 18 (March (6)) (2012) 1611–1618, https://doi.org/10.1158/1078-0432.CCR-11-1942 Epub 2012 Jan 23.
[32] H. Kronig, L. Kremmler, B. Haller, C. Englert, C. Peschel, R. Andreesen, et al., Interferon-induced programmed death-ligand 1 (PDL1/B7-H1) expression increases on human acute myeloid leukemia blast cells during treatment, Eur. J. Haematol. 92 (3) (2014) 195–203.
[33] C. Berthon, V. Driss, J. Liu, K. Kuranda, X. Leleu, N. Jouy, et al., In acute myeloid leukemia, B7-H1 (PD-L1) protection of blasts from cytotoxic T cells is induced by TLR ligands and interferongamma and can be reversed using MEK inhibitors, Cancer Immunol. Immunother. 59 (12) (2010) 1839–1849.
[34] S.J. Lee, B.C. Jang, S.W. Lee, Y.I. Yang, S.I. Suh, Y.M. Park, S. Oh, J.G. Shin, S. Yao, L. Chen, I.H. Choi, Interferon regulatory factor-1 is prerequisite to the constitutive expression and IFN-gamma-induced upregulation of B7-H1 (CD274), FEBS Lett. 580 (February (3)) (2006) 755–762 Epub 2006 Jan 9.
[35] J. Liu, A. Hamrouni, D. Wolowiec, V. Coiteux, K. Kuliczkowski, D. Hetuin, A. Saudemont, B. Quesnel, Plasma cells from multiple myeloma patients express B7-H1 (PD-L1) and increase expression after stimulation with IFN-{gamma} and TLR ligands via a MyD88-, TRAF6-, and MEK-dependent pathway, Blood 110 (July (1)) (2007) 296–304 Epub 2007 Mar 15.
[36] M. Marzec, Q. Zhang, A. Goradia, P.N. Raghunath, X. Liu, M. Paessler, H.Y. Wang, M. Wysocka, M. Cheng, B.A. Ruggeri, M.A. Wasik, Oncogenic kinase NPM/ALK induces through STAT3 expression of mmunosuppressive protein CD274 (PD-L1, B7-H1), Proc. Natl. Acad. Sci. U. S. A. 105 (December (52)) (2008) 20852–20857, https://doi.org/10.1073/pnas.0810958105 Epub 2008 Dec 16. PubMed PMID: 19088198; PubMed Central PMCID: PMC2634900.
[37] K.J. Oestreich, H. Yoon, R. Ahmed, J.M. Boss, NFATc1 regu- lates PD-1 expression upon T cell activation, J. Immunol. 181 (7) (2008) 4832–4839.
[38] C. Kao, K.J. Oestreich, M.A. Paley, A. Crawford, J.M. Angelosanto, M.A. Ali, et al., Transcription factor T-bet represses expression of the inhibitory receptor PD-1 and sustains virus-specific CD8+ T cell responses during chronic infection, Nat. Immunol. 12 (May (7)) (2011) 663–671, https://doi.org/10.1038/ni.2046.
[39] P. Lu, B.A. Youngblood, J.W. Austin, A.U. Mohammed, R. Butler, R. Ahmed, et al., Blimp-1 represses CD8 T cell expression of PD-1 using a feed-forward transcriptional circuit during acute viral infection, J. Exp. Med. 211 (3) (2014) 515–527.
[40] J.C. Wang, A. Kundra, M. Andrei, S. Baptiste, C. Chen, C. Wong, H. Sindhu,

Myeloid-derived suppressor cells in patients with myeloproliferative neoplasm, Leuk. Res. 43 (2016) 39–43, https://doi.org/10.1016/j.leukres.2016.02.004 Epub 2016 Feb 16. PubMed PMID: 26943702.

[41] Guanfang Shi, Hui Chen, Cherif Abdelmalek, Andrei Bandarchuk, Abdullah Khawer Mahmood, Wong Ching, Madhumati Kalavar, Vladimir Gotlieb, Tony W. Cheung, Jen-Chin Wang, Derangements of Toll-like receptors, inflammatory cytokines, and reactive oxygen species in Philadelphia chromosome-negative myeloproliferative neoplasm: implicate roles of inflammation in the pathogenesis, Blood 128 (2016) 1945.

[42] L. Pinton, S. Solito, V. Damuzzo, S. Francescato, A. Pozzuoli, A. Berizzi, S. Mocellin, C.R. Rossi, V. Bronte, S. Mandruzzato, Activated T cells sustain myeloid-derived suppressor cell-mediated immune suppression, Oncotarget. 7 (January (2)) (2016) 1168–1184, https://doi.org/10.18632/oncotarget.6662 PubMed PMID: 26700461; PubMed Central PMCID: PMC4811451.

[43] C. Berthon, V. Driss, J. Liu, K. Kuranda, X. Leleu, N. Jouy, et al., In acute myeloid leukemia, B7-H1 (PD-L1) protection of blasts from cytotoxic T cells is induced by TLR ligands and interferongamma and can be reversed using MEK inhibitors, Cancer Immunol. Immunother. 59 (12) (2010) 1839–1849.

[44] S.J. Lee, B.C. Jang, S.W. Lee, Y.I. Yang, S.I. Suh, Y.M. Park, S. Oh, J.G. Shin, S. Yao, L. Chen, I.H. Choi, Interferon regulatory factor-1 is prerequisite to the constitutive expression and IFN-gamma-induced upregulation of B7-H1 (CD274), FEBS Lett. 580 (February (3)) (2006) 755–762 Epub. 2006 Jan 9.