Programmed Death-Ligand 1 (PD-L1) Expression in Patients with Primary or Secondary Myelofibrosis

Francisco Socola[1], Moayed Ibrahim[2], Catherine Murphree[2], Kirtesh Patel[3], Matthew Mastrodomenico[3], Nakhle S. Saba[2], Hana Safah[2], and Janet Schmid[4]

[1]AdventHealth Orlando
[2]Tulane University John W Deming Department of Medicine
[3]Delta Pathology Group
[4]Pathology Department at Tulane University

October 9, 2023

Abstract

It has been described in mice models that Primary Myelofibrosis (PMF) with JAK2-V617F mutation has an increased expression of programmed death-ligand 1 (PD-L1) in megakaryocytes leading to cancer immune evasion by inhibiting the T-lymphocytes. To prove this hypothesis, we quantified PD-L1 expression on 29 bone marrow (BM) biopsies. We created a scoring system to quantify PD-L1 expression in megakaryocytes. We obtained 14 BM with JAK2 positive PMF, 5 JAK2 negative PMF and 10 patients with normal BM biopsies. PD-L1 expression was higher in the JAK2 positive group compared to the control group with a score of 212.6 vs 121.1 (t-value 2.05,p-value 0.025). In addition, the score was higher in the PMF group regardless of JAK2 mutational status when compared to the control group with score of 205.9 vs 121.1(t-value 2.12,p-value 0.021). There was no difference in the PD-L1 score between the JAK2 negative vs the control group 187.2 vs 121.1 (t-value 1.02,p-value 0.162). These findings suggest that PMF patients with a JAK2 mutation have a higher PD-L1 expression in megakaryocytes compared to the control group. We postulate that the combination of checkpoint and JAK2 inhibitors may be an active treatment option in JAK2 mutated PMF given the higher PD-L1 expression.

Article title

Programmed Death-Ligand 1 (PD-L1) Expression in Patients with Primary or Secondary Myelofibrosis

Short title

PD-L1 Expression in Myelofibrosis

Author affiliations

Moayed Ibrahim, MD[1], Catherine Murphree, MD[1], Kirtesh Patel MD[2], Matthew Mastrodomenico, MD[2], Nakhle S. Saba, MD[1], Hana Safah, MD[1], Janet Schmid[3], Francisco Socola, MD[4]

[1]Section of Hematology and Medical Oncology, Deming Department of Medicine, Tulane University, New Orleans, LA, USA

[2]Delta Pathology Group, Shreveport, LA, USA

[3]Pathology Department at Tulane University, New Orleans, USA

[4]Hematology and Bone Marrow Transplant , AdventHealth Hospital Orlando, Orlando, FL, USA

MI: mibrahim1@tulane.edu, MM: Matthew.Mastrodomenico@lcmchealth.org, KP: Kirtesh.Patel@lcmchealth.org, CM: cmurphree@tulane.edu, NS: Nsaba@tulane.edu, HS: Hsafah@tulane.edu, JS: jschmid@tulane.edu, FS: francisco.socola@adventhealth.com

Corresponding author

Francisco Socola , MD

Attending Physician

Hematology and Bone Marrow Transplantation

AdventHealth Hospital Orlando

2415 N. Orange Ave., Ste. 601 Orlando, FL 32804

tel: 407.303.5932 | fax 407.303.0586 | E: francisco.socola@adventhealth.com

Word count

Text: 2656, Abstract: 200

Figue/table count

Figures: 5, tables: 2

Reference Count: 11

Key points

PMF patients with a JAK2 mutation have a higher PD-L1 expression than patients with JAK2-unmutated PMF or control groups.

A lower PD-L1 expression in PMF resulted in longer overall survival compared to patients with a higher PD-L1 expression score.

Abstract

It has been described in mice models that Primary Myelofibrosis (PMF) with JAK2-V617F mutation has an increased expression of programmed death-ligand 1 (PD-L1) in megakaryocytes leading to cancer immune evasion by inhibiting the T-lymphocytes. To prove this hypothesis, we quantified PD-L1 expression on 29 bone marrow (BM) biopsies. We created a scoring system to quantify PD-L1 expression in megakaryocytes. We obtained 14 BM with JAK2 positive PMF, 5 JAK2 negative PMF and 10 patients with normal BM biopsies. PD-L1 expression was higher in the JAK2 positive group compared to the control group with a score of 212.6 vs 121.1 (t-value 2.05,p-value 0.025). In addition, the score was higher in the PMF group regardless of JAK2 mutational status when compared to the control group with score of 205.9 vs 121.1(t-value 2.12,p-value 0.021). There was no difference in the PD-L1 score between the JAK2 negative vs the control group 187.2 vs 121.1 (t-value 1.02,p-value 0.162). These findings suggest that PMF patients with a JAK2 mutation have a higher PD-L1 expression in megakaryocytes compared to the control group. We postulate that the combination of checkpoint and JAK2 inhibitors may be an active treatment option in JAK2 mutated PMF given the higher PD-L1 expression.

Keywords

Primary myelofibrosis, secondary myelofibrosis, PD-L1, immunotherapy, JAK/STAT pathway

Introduction

Primary myelofibrosis (PMF) is a myeloproliferative neoplasm (MPN) characterized by constitutional activation of the JAK-STAT signaling pathway and bone marrow (BM) fibrosis which leads to decreased peripheral blood counts, pro-inflammatory state, and a potential for transformation to acute myeloid leukemia (AML). The median age at presentation is 65 years [2,3]. JAK2-V617F is the most common mutation in PMF

and is found in 50-60% of patients [4]. The only curative treatment option currently available is allogeneic stem cell transplant. However, most patients are ineligible because of advanced age and comorbidities [5]. Ruxolitinib and fedratinib, JAK2 inhibitors, are the only FDA approved treatment for intermediate/high risk PMF patients [6,7]. However, those drugs have their limitations and only improve symptoms and decrease splenomegaly, without an overall survival benefit [6,7]. Therefore, there is a significant unmet need for treatment options in this patient population.

Prestipino et al. discovered that mice models with JAK2-V617F mutated MPN generally have an increased expression of PD-L1 that leads to cancer immune evasion by inhibiting the antitumor effect of the T lymphocytes against cancer cells [1]. Checkpoint inhibitors are monoclonal antibodies that block the interaction between PD-L1 and its receptor, allowing the immune system to fight cancer cells with an enhanced antitumor response. Pembrolizumab was the first checkpoint inhibitor approved by the FDA for patients with metastatic non-small-cell lung cancer with >50% PD-L1 expression in tumor cells by immunohistochemistry stain. In this subset of patients, pembrolizumab was more effective than systemic chemotherapy [9].

In this paper, we compared the PD-L1 expression among patients with JAK2-mutated PMF versus JAK2-unmutated PMF patients versus normal controls without no PMF or JAK2 mutation.

Patient and methods

Study population

We collected bone marrow biopsies of patients with PMF done at Tulane Medical Center from 1990 to 2019. All these patients had a known JAK2 status and a well-preserved specimen for adequate PD-L1 staining. We only used the initial bone marrow biopsy obtained to diagnose patients with PMF. We collected 10 additional samples of patients that came to clinic with transient cytopenias from benign hematologic conditions. These patients had normal karyotype, FISH panel for myelodysplastic syndrome (MDS), and a negative 64-gene next generation sequencing (NGS) myeloid mutation panel. An institutional review board approval was obtained before collecting these bone marrow samples and all participants gave informed consent. The authors analysed the data to which all authors had access. All relevant participant data was deidentified and shared as appropriate in the text.

PD-L1 immunohistochemistry staining

We used the FDA approved test PD-L1 IHC 28-8 pharmDx to assess the PD-L1 expression on the bone marrow biopsies. This is a qualitative immunohistochemical assay which uses monoclonal mouse anti-PD-L1 Clone 22C3 intended for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded tissues of different cancers such as non-small cell lung cancer and gastric/gastroesophageal junction adenocarcinoma. This test is used to identify patients who may be treated with pembrolizumab.

Bone marrow sections of 4-5 μm were made with tissues mounted on microscope slides then placed in a 58 ± 2 °C oven for 1 hour. The slides were stained with the PD-L1 IHC 22C3 pharmDx reagent and the samples were incubated. Finally, all the slides were numbered and labeled with codes.

Interpretation of PD-L1 expression

The slides were reviewed and scored independently by two clinical pathologists specialized in in interpreting PD-L1 expression in solid tumors. PD-L1 was scored according to the quantity and intensity in the megakaryocytic lineage of cells. The quantity of PD-L1 expression was graded from 0 to 100% while the intensity was graded from 1+ to 3+, according to the level of intensity. The result was a PD-L1 score calculated by multiplying percentage by intensity of PD-L1 in the bone marrow megakaryocytes. This score will be detailed further in the statistical analysis section.

JAK2 mutation and NGS myeloid mutation panel

Patients with PMF diagnosis had a JAK2-V617F mutation status known while the patients diagnosed with

PMF after 2018 also had a myeloid NGS done. The controls had the myeloid NGS done en sure they did not have any clonal diseases. The genes included in our inhouse myeloid NGS mutation panel are: ABL1, ASXL1, ATM, ATRX, BCOR, BCORL1, BRAF, CALR, CBL, CBLB, CDKN2A, CEBPA, CSF3R, DAXX, DNMT3A, EED, EGFR, ETV6, EZH2, FBXW7, FLT3, GATA1, GNAS, HRAS, IDH1, IDH2, IKZF1, JAK1, JAK2, JAK3, KAT6A, KIT, KMT2A, KRAS, MPL, NF1, NOTCH1, NPM1, NRAS, PDGFRA, PHF6, PRPF40B, PTEN, PTPN11, RAD21, RB1, RUNX1, SETBP1, SF1, SF3A1, SF3B1, SH2B3, SMARCB1, SMC1A, SMC3, SRSF2, STAG2, SUZ12, TET2, TP53, U2AF1, U2AF2, WT1 and ZRSR2.

Results

We studied the bone marrow biopsies of 29 patients in total; 14 patients had JAK2-mutated PMF, 5 JAK2-unmutated PMF, and 10 controls with negative bone marrow biopsies. The median age of the whole group was 57 years, with 34% males and 66% females. The main clinical characteristics of the myelofibrosis patients is described in the**table 1.** PD-L1 expression and intensity is described in**table 2.** We have a few examples how the PD-L1 expression and intensity was quantified in the bone marrow biopsies, shown in**figures 1-4** .

The average PD-L1 expression score for the JAK2-mutated PMF group was 212.57, the JAK2-unmutated PMF group 187.2, and the control group 121.1. There was a statistically significant difference between the PD-L1 score between the JAK2-mutated PMF group vs the control group (t-value 2.05 and p-value of 0.025) and when we compared the PMF group regardless of the JAK2 status vs the control group (t-value 2.12 and p-value of 0.021). However, there was not a statistically significant difference when we compared the PD-L1 expression between the JAK2 negative vs the control group (t-value 1.02 and p-value 0.162).

Myelofibrosis patients with a PD-L1 score <250 had a median overall survival of 130 months vs PD-L1 score >250 of 64 months (hazard ratio 2.63: 95% CI, 0.82 to 8.4; P=0.09), it was numerically longer but not statistically significant as shown in**figure 5** .

Discussion

The presented results show that PD-L1 score was higher in the PMF group vs the control group regardless of JAK2 mutation status, which turned out to be statistically significant. This helps build on the data reported by Prestipino et al that showed oncogenic JAK2 activity led to STAT phosphorylation which in turn enhanced PD-L1 promoter activity and PD-L1 protein expression in JAK2 mutant cells [1]. In addition, PD-L1 expression was higher on primary cells isolated from patients with JAK2-mutated MPNs as compared to healthy individuals and declines upon JAK2 inhibition [1]. Moreover, Lee et al were able to demonstrate that PD-L1 expression was significantly associated with overt myelofibrosis and JAK2 mutational status [9]. Moreover, in the previously mentioned study, there were 4 patients who were found to have a particularly high PD-L1 expression that also harbored the JAK2 mutation [9].

This supports further that PD-L1 may play a more important role than previously realized in MPNs and should perhaps be a future target in our current small armamentarium of viable drugs. To the best of our knowledge, there have only been 2 small phase II trials in which the investigators tested the utility of PD-L1 inhibition (pembrolizumab and nivolumab) in patients with PMF. Hobbs, et al conducted a phase 2, single arm study of pembrolizumab in patients with Dynamic International Prognostic Scoring System (DIPSS) intermediate-2 or greater, primary, or secondary, post essential thrombocythemia or post polycythemia vera MF who were ineligible for or previously treated with ruxolitinib [9]. This study had 10 patients, 5 with JAK2 mutation who were treated with pembrolizumab without objective clinical responses. However, an important takeaway from this data showed that flow cytometry, T-Cell receptor (TCR) sequence and proteomics demonstrated changes in the immune makeup of patients, suggesting improved T cell responses [10]. Although this study was terminated early as no objective clinical responses were seen, the latter changes mentioned suggest that perhaps PD-L1 inhibition is not enough to elicit a clinical response and combination therapy may be more effective.

Another study, in which Dalle et al investigated the efficacy and safety of single agent nivolumab in 8 adult patients with myelofibrosis, was also terminated early due to failure to meet predetermined efficacy endpoint

(primary endpoint was objective response rate (ORR) defined as complete response (CR), partial response (PR) and clinical improvement (CI) after 8 doses) [11]. The median duration of enrolled patients on the study was 5.4 months with a median number of cycles of 3. Unfortunately, in this study, none of the patients responded to nivolumab therapy. These patients showed more advanced disease including intermediate 2 and high risk DIPSS score with 5 patients failing ruxolitinib and 7 with clonal evolution, i.e., progressive disease [11].

It is important to note that these two described studies had very small sample sizes, with most patients in the high risk DIPSS category, multiple previous lines of therapy, and complex mutational status or clonal evolution. However, they were able to characterize changes in the patient's immune milieu after administration of PD-L1 blockade [10,11]. It's noteworthy to the authors that both studies employed PD-L1 blocked only after patients had undergone multiple lines of therapy, in a relapsed or refractory setting, raising the question that perhaps this is not the right setting to use this line of therapy. In addition, it would be interesting to expand further on the hypothesis that perhaps PD-L1 blockade is not enough and combination therapy with ruxolitinib may be more effective in patients with PMF.

Conclusions

We found that the PD-L1 expression in the bone marrow megakaryocytes of JAK2-mutated PMF patients was higher than the control group. This may confirm the hypothesis that the oncogenic JAK2 mutation enhances the PD-L1 promoter activity and its expression in JAK2 positive patients. We hypothesize that patients with high PD-L1 expression may benefit from checkpoint inhibitors, in combination with JAK2 inhibitors, in the upfront setting of therapy.

Author contributions

FS, MM, KP, CM,and MI contributed substantially to original draft writing while HS, NS and FS contributed significantly to reviewing and editing.

Acknowledgements

We thank the patients for the permission to conduct this research.

Funding

Carol Lavin Bernick Faculty Grant Program at Tulane University School of Medicine.

Declarations of interest

The authors have no competing interests to declare.

Figue/table Captions

Visual abstract: Myelofibrosis patients with a PD-L1 score <250 had a median overall survival of 130 months vs PD-L1 score >250 of 64 months, P=0.09.

Figure 1.76 y/o woman with JAK2 positive PMF (Expression 100%, intensity +3)

Figure 2. 28 y/o woman with anemia and normal BM BX (Expression 0%, intensity 0)

Figure 3. 73 y/o woman with JAK2 negative PMF (Expression 78%, intensity +2)

Figure 4. 60 y/o man with JAK2 negative PMF (Expression 100%, intensity +3)

Table 1.- Main characteristics of patients with myelofibrosis.

Table 2.- Level of expression and intensity of PD-L1 on the 29 bone marrow biopsies

Figure 5.- Median Overall Survival according to a PD-L1 socre >250 vs <250.

References:

1. Prestipino A, Emhardt AJ, Aumann K, O'Sullivan D, Gorantla SP, Duquesne S, Melchinger W, Braun L, Vuckovic S, Zeiser R. Oncogenic JAK2V617F causes PD-L1 expression, mediating immune escape in myeloproliferative neoplasms. Sci Transl Med. 2018 Feb 21;10(429)
2. Thiele J, Kvasnicka HM, Tefferi A, Barosi G, Orazi A, Vardiman,et al. Myelofibrosis. In: Swerdlow SH, Campo E, Harris NL,Jaffe ES, Pileri SA, Stein H, et al., editors. WHO classification oftumours of haematopoietic and lymphoid tissues. Lyon:WHO; 2008. p. 44–7.
3. Savona MR. Are we altering the natural history of primarymyelofibrosis? Leuk Res. 2014;38(9):1004–12.
4. Ahmed RZ, Rashid M, Ahmed N, Nadeem M, Shamsi TS Coexisting JAK2V617F and CALR Exon 9 Mutations in Myeloproliferative Neoplasms - Do They Designate a New Subtype? Asian Pac J Cancer Prey. 2016;17(3):923-6.
5. Deeg HJ, Bredeson C, Farnia S, Ballen K, Gupta V, Mesa RA, Popat U, Hari P, Saber W, Seftel M, Tamari R, Petersdorf EW. Hematopoietic Cell Transplantation as Curative Therapy for Patients with Myelofibrosis: Long-Term Success in all Age Groups. Biol Blood Marrow Transplant. 2015;21(11):1883. Epub 2015 Sep 11.
6. Harrison C, Kiladjian JJ, Al-Ali HK, et al. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. N Engl J Med 2012; 366:787.
7. Verstovsek S, Mesa RA, Gotlib J, et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. N Engl J Med 2012; 366:799.
8. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, Gottfried M, Peled N, Tafreshi A, Cuffe S, O'Brien M, Rao S, Hotta K, Leiby MA, Lubiniecki GM, Shentu Y, Rangwala R, Brahmer JR; KEYNOTE-024 Investigators. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. N Engl J Med. 2016 Nov 10;375(19):1823-1833. Epub 2016 Oct 8.
9. Lee, Sze-Hwei., Lin, Chien-Chin., Wie, Chao-Hong., Chang, Ko-Ping., Yuan, Chang-Tsu., Tsai, Cheng-Hong., Liu, Jia-Hao. House, Hsin-An., Tang, Jih-Lu., Chou, Wen-Chien., Tien, Hwei-Fang. PD-L1 expression in megakaryocytes and its clinicopathological features in primary myelofibrosis patients. The journal of Pathology: Clinical Research. September 2021.
10. Hobbs, Gabriela. Bozkus, Cansu Cimen, Moshier, Erin, Doughterty Mikaela., Bar-Nata, Michal., Sandy, Lonette., Johnson, Kathryn., Foster, Julia Elise., Som, Tina., Macrae, Molly., Marble, Hetal., Salama, Mohamed., El Jamal, Siraj M., Zubizarreta, Nicole., Wadleigh, Martha., Stone, Richard., Bhardwaj, Nina., Iancu-Rubin, Camelia., Mascarenhas, John. PD-1 inhibiation in advanced myeloproliferative neoplasms. Blood advances. December 2021.
11. Dall, Iman Abou Kantarjian, Jagop., Daver, Naval., Masarova, Lucia., Pemmaraju, Naveen. Bose, prithivaj., Garcia-Manero, Guillermo., Verstovsek, Srdan. Phase II study of single agente nivoulmab in patients with myelofibrosis. Ann Hematol. December 2021.

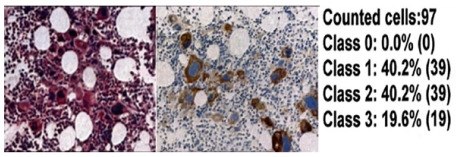

Figure 1: 76 y/o woman with JAK2 positive PMF (Expression 100%, intensity +3)

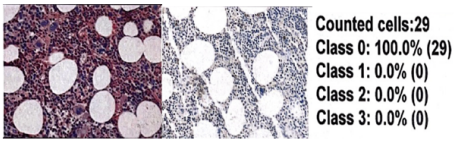

Figure 2: 28 y/o woman with anemia and normal BM BX (Expression 0%, intensity 0)

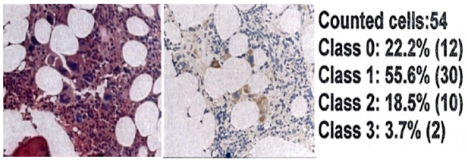

Figure 3: 73 y/o woman with JAK2 negative PMF (Expression 78%, intensity +2)

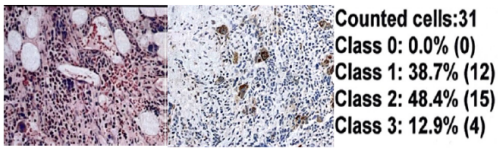

Figure 4: 60 y/o man with JAK2 negative PMF (Expression 100%, intensity +3)

Hosted file

figure 5.pptx available at https://authorea.com/users/672810/articles/671743-programmed-death-ligand-1-pd-l1-expression-in-patients-with-primary-or-secondary-myelofibrosis

Hosted file

table 1.docx available at https://authorea.com/users/672810/articles/671743-programmed-death-ligand-1-pd-l1-expression-in-patients-with-primary-or-secondary-myelofibrosis

Hosted file

Table 2.docx available at https://authorea.com/users/672810/articles/671743-programmed-death-ligand-1-pd-l1-expression-in-patients-with-primary-or-secondary-myelofibrosis