Remodeling the Bone Marrow Microenvironment – A Proposal for Targeting Pro-inflammatory Contributors in MPN

Jonas Samuel Jutzi and Ann Mullally

1 Division of Hematology, Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, United States, 2 Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, United States, 3 Cancer Program, Broad Institute, Cambridge, MA, United States

Philadelphia-negative myeloproliferative neoplasms (MPN) are malignant bone marrow (BM) disorders, typically arising from a single somatically mutated hematopoietic stem cell. The most commonly mutated genes, JAK2, CALR, and MPL, lead to constitutively active JAK-STAT signaling. Common clinical features include myeloproliferation, splenomegaly and constitutional symptoms. This review covers the contributions of cellular components of MPN pathology (e.g., monocytes, megakaryocytes, and mesenchymal stromal cells) as well as cytokines and soluble mediators to the development of myelofibrosis (MF) and highlights recent therapeutic advances. These findings outline the importance of malignant and non-malignant BM constituents to the pathogenesis and treatment of MF.

Keywords: MPN, JAK2, CALR, MPL, inflammation, megakaryocytes, monocytes, mesenchymal stromal cells

INTRODUCTION

Myeloproliferative neoplasms (MPN) are a group of clonal malignant bone marrow (BM) diseases, originating from a hematopoietic stem cell (HSC) which acquired a MPN phenotypic driver mutation (i.e., in JAK2, CALR, or MPL), leading to constitutively active JAK-STAT signaling (1, 2). Although the pathogenesis of MPN is cell-intrinsic to hematopoietic cells, MPN cells also exert cell-extrinsic effects resulting in chronic inflammation that perturbs the BM niche, and which in turn contributes to the MPN phenotype and renders the niche less supportive of normal hematopoiesis (i.e., the malignant self-perpetuating niche) (3).

The three main MPN clinical entities are polycythemia vera (PV), displaying an increase in red blood cells, essential thrombocythemia (ET), presenting with increased platelets and primary myelofibrosis (PMF), showing fibrosis of the BM. Common features of MPN, most pronounced in myelofibrosis (MF) patients, are increased levels of pro-inflammatory cytokines, leading to chronically increased inflammation in the BM and resulting in constitutional symptoms (e.g., fatigue, weight loss).

Eradicating malignant MPN cells in patients has so far failed in settings other than allogeneic HSC transplantation and in a minority of patients with PV and ET treated with interferon (4, 5). Another, complimentary approach to break the vicious cycle of aberrant “cross talk” between malignant hematopoiesis and the BM microenvironment is to inhibit the secretion of...
pro-inflammatory cytokines in both malignant and non-malignant cell populations. This has the potential to limit the expansion of the malignant hematopoietic clone and slow down or even prevent MPN disease progression.

In this review, we focus on secreted pro-inflammatory factors of MPN, cell-autonomous and cell non-autonomous contributors to MPN as well as novel approaches targeting these factors.

**CYTOKINES AND SOLUBLE MEDIATORS**

A wide variety of immune-modulatory cytokines are elevated in MPN patients, including IL-1, IL-6, IL-8, IL-10, IL-11, IL-17, TNFα, and TGFβ (6–10). Most of the listed cytokines are either pro-inflammatory like IL1 or directly pro-fibrotic factors as in the case of transforming growth factor beta (TGFβ), with the exception of IL-10 which has an anti-inflammatory role. While MPN is caused by genetic mutations in HSC, its progression is often driven, at least in part, by inflammation. Cytokines like IL-1, IL-6, and TGFβ have been identified to contribute to the pathogenesis of fibrosis and osteosclerosis of the BM (11). NFκB signaling is frequently increased in MPN patients and required for downstream expression of pro-inflammatory cytokines like IL-8 (12). IL-8 itself has been implicated in leukemic transformation in MF patients (10, 13). In patients with PV, IL-12 levels correlate with hematocrit levels, IL-1 with leukocytosis, and IFNγ with the risk of thrombosis. Lastly, MIP1β has been shown to be associated with shorter overall survival (14). In patients with ET a recent longitudinal study on more than 400 patients described an ET-specific inflammatory cytokine signature compromising CCL11 (eotaxin), CXCL1 (GROα), and epidermal growth factor (EGF) (15). Finally, chemical mediators such as reactive oxygen species (ROS) have also been associated with inflammation-induced genomic instability and DNA damage in JAK2V617F-positive MPN patients, and this topic has been reviewed elsewhere (16–18). In summary, it is now apparent that circulating cytokines are perturbed in MPN, not just in established MF, but also in PV and ET. Furthermore, these studies provide indirect evidence that inflammation is not just an “innocent bystander” in MPN, but also contributes to clinically relevant outcomes.

**CELLULAR CONTRIBUTORS TO INFLAMMATION**

Inflammation is increasingly thought to play an important role in the development of chronic myeloid malignancies like MPN as well in progression to acute leukemia (19–22). Several different cell types are involved in initiating and/or perpetuating inflammation. In this review, we address four major cellular contributors of inflammation in the context of MPN.

**Hematopoietic Stem and Progenitor Cells**

Recent advances in single-cell approaches have uncovered MPN-specific lineage-trajectories and transcriptional programs.
CD14+/CD34+ monocytes, obtained from MF patients were able to induce an MF-like phenotype in immunocompromised mice (33). Mice developed splenomegaly, reticulin fibrosis and megakaryocyte clustering (33). Moreover, under TGFβ stimulation, fibrocytes lose their CD34+ and CD45-positivity and express smooth-muscle actin (a-SMA) (34), making them myofibroblast-like. Myofibroblasts are contractile, fibrosis-causing and collagen-secreting cells (35).

Taken together, these studies support the idea that monocytes and their derivates contribute to MF and are therefore potential candidates for future targeted therapies.

**Megakaryocytes**

Megakaryocytes are increased in the BM of MF patients, resulting in the overproduction of pro-fibrotic cytokines and are therefore considered to be a major cellular driver of BM fibrosis (36–39). Woods and colleagues found activation of Jak/Stat signaling and expansion of megakaryocytes in Jak2V617F-Pf4iCre mice, which was developed to restrict Cre recombinase-mediated excision to megakaryocytes and its progeny (40). Using Jak2V617F-Pf4iCre mice, Zahn et al. showed that Jak2V617F-mutant megakaryocytes promote the expansion of hematopoietic stem and progenitor cells (HSPCs) in mice (41). A recent study verified that the expansion of HSPCs was due to constitutively active thrombopoietin/MPL signaling, resulting in increased megakaryocytes, and causing HSPC expansion through cell non-autonomous mechanisms (42). Moreover, expression of mutant Jak2 in megakaryocytes was sufficient to induce fibrosis and erythropoiesis, the latter due to increased levels of IL6 (42). This finding supports other studies showing a cell non-autonomous effect of the Jak2-mutant clone on wildtype cells (40). While there have been earlier reports suggesting that Pf4iCre does not restrict recombination solely to megakaryocytic-lineage cells (i.e., "leaky" recombination in other lineages) (43, 44), a recent study by Mansier et al. investigated this specifically in the context of Jak2V617F. Using Pf4iCre, the authors detected Jak2V617F expression in a fraction of HSCs (45), suggesting that recombination in HSC cannot be excluded as a contributing factor to some of the findings in the earlier studies focused on the cell non-autonomous effects of megakaryocytes in Jak2V617F-driven MPN (40–42).

Comprehensive single-cell sequencing is revolutionizing the field of hematology by providing high-resolution profiling of hematopoietic cell populations and by re-defining the hematopoietic hierarchy in normal and malignant hematopoiesis (46–48). Gene set enrichment analysis of megakaryocyte precursors (MKPs) revealed enrichment of inflammatory pathways in MF MKPs as compared to MKPs from healthy donors (HD) (23). A subset of these MKPs (displaying similar expression profiles between HD and MF MKPs), showed high expression of known mediators of MF (PDGA, CCL5, and CXCL5) (23). Most MF MKPs however, had a distinct transcriptional profile from HD MKPs, indicating the expansion of an aberrant megakaryocyte population in MF (23). Some MKP populations display selective expression of AURKA, a kinase that has previously been proposed as a therapeutic target in MF (39). In addition, there have been several studies focused on the contributions of platelets to inflammation in MF, a topic that was recently reviewed by Oyarzún and Heller (49).

In summary, megakaryocytes have been shown to contribute to MPN pathology, by fueling the proliferation of malignant and wildtype cells through cell non-autonomous effects, while also promoting inflammation and MF.

**Mesenchymal Stromal Cells**

It has been appreciated that BM mesenchymal stromal cells (MSCs) contribute to inflammation (3) and to the pathogenesis of MF (50–52). Importantly, it has been shown that MSC do not harbor JAK2V617F (30, 53–55).

In experimental mouse models, perturbation of MSCs has been shown to induce BM fibrosis by indirectly influencing HSCs, as in the case of deletion of the retinoblastoma gene (Rb), a cell-cycle regulator in hematopoiesis. A study by Walkley et al. showed that genetic knockout of Rb in the entire hematopoietic system using the inducible MxCre system leads to a myeloproliferative phenotype and extramedullary hematopoiesis (56). However, this was not the result of an HSC cell-intrinsic phenotype but due to cell-extrinsic Rb-dependent crosstalk between HSCs and the BM niche (56). Another example where perturbation of MSC in experimental mouse models induced MF is in mice deficient in the expression of the retinoic acid receptor gamma (RARγ−/−), specifically in the BM niche. Wildtype BM transplanted into RARγ−/− mice showed an MPN phenotype mirroring several features of human MF (57), again highlighting the role of MSCs in driving MF phenotypes in vivo.

Specific subgroups of MSCs have been identified to be cellular drivers of BM fibrosis, including the Leptin receptor (Lepr) and Gli1+ MSCs (58, 59). Lepr+ MSCs differentiate into myofibroblasts in the context of thrombopoietin (TPO) overexpression-induced MF, accompanied by upregulation and secretion of proteins linked to MF (e.g., collagen) (58). Gli1+ and Lepr+ MSCs do not express the common hematopoietic surface marker CD45, highlighting a different process of myofibroblast differentiation as compared to monocyte-derived fibrocytes which are CD45+ (59). Blockade of the platelet-derived growth factor receptor a (Pdgfra), a driver of BM fibrosis in Lepr+ MSCs cells, strongly suppressed MSC growth. Conversely, Pdgfra overexpression increased MSCs and extramedullary hematopoiesis. These findings highlight PDGFRA signaling as a potential therapeutic target in MF patients (58). Martinau and colleagues performed whole transcriptome profiling of MSCs from patients with MF and from HD and found a clear pro-fibrotic and inflammatory signature in MSCs from patients with MF (60). MSCs from patients with MF overexpressed pro-inflammatory factors (e.g., TGFβ1, BMP2) and ECM components (e.g., glycosaminoglycans, chondroitin sulfate, and heparan sulfate) (50).

In summary, as the field has developed a better understanding of the cellular components of the BM microenvironment, this has led to a shift away from focusing solely on cell-intrinsic contributions to myeloid malignancies to a more holistic view of HSPCs in their BM niche.
THERAPEUTIC TARGETING OF SOLUBLE MEDIATORS, THE MALIGNANT BONE MARROW AND CELLULAR CONTRIBUTORS OF MPN-DRIVEN INFLAMMATION

Simplified, there are two main approaches to treating MF. Firstly, the eradication of the malignant hematopoietic clone and secondly, the modulation of cellular components and soluble mediators including through inhibiting signaling pathways in MF.

Targeting Soluble Inflammatory Mediators

Inflammation plays a role in all MPN subgroups, most pronounced in MF patients. It has been shown that inhibiting specific cytokines like IL-1β or the NFκB pathway can either decrease hematopoietic cell growth ex vivo (61) or even diminish fibrosis in vivo (62). Targeting soluble mediators in MF patients serves predominantly to ameliorate constitutional symptoms and reduce frequent comorbidities like MF-associated anemia. In patients with MF, reduction of pro-inflammatory cytokines induced by treatment with the JAK1/2 inhibitor, ruxolitinib correlated with symptomatic improvement (63). More recently, Fisher et al., using mass cytometry, found a limited effect on the levels of pro-inflammatory cytokines in MF patients treated with ruxolitinib (28) with plasma cytokine levels remaining markedly abnormal despite JAK2 inhibition (28). Some of the elevated cytokines were responsive to ex vivo pharmacological inhibition of the NFκB and/or the MAP kinase signaling pathway (28), highlighting the importance of these pathways for future cytokine-directed therapies in MF.

Momelotinib, a JAK1/2 inhibitor, which also inhibits the activin A receptor type 1 (ACVR1) has shown significant improvement in anemia in treated MF patients (64, 65). It is thought that the anemia response may be mediated via an indirect mechanism resulting in suppression of hepcidin and releasing storage iron to promote erythropoiesis (66, 67). Another agent, currently in a phase II study for MF patients (NCT03194542), is luspatercept, a TGFβ super family ligand-binding fusion protein which reduces downstream SMAD signaling, and acts as an erythroid maturation agent (68, 69). Notably, luspatercept recently gained FDA-approval for the treatment of anemia associated with beta-thalassemia and for myelodysplastic syndrome (MDS)-related anemia (NCT02631070, NCT03682536) (70, 71). INCBO39110, a JAK1 inhibitor was tested in a phase II clinical trial for MF patients and aimed to reduce elevated cytokine levels to improve constitutional symptoms (72). Plasma pro-inflammatory cytokine levels (e.g., CRP, IL-6, VEGF) were significantly decreased in most patients. JAK2V617F allele burden, however, was non-significantly changed (72). In about half of the patients, red blood cell transfusions could be reduced by 50% or more during the duration of the study, spleen volume was slightly decreased and effects on myelopoiesis were mild (72).

Targeting Malignant Hematopoietic Cells

The first targeted therapy for MPN patients was introduced in 2011 when the JAK1/2 inhibitor ruxolitinib (INCB-018424) gained FDA-approval for the treatment of patients with intermediate and high-risk MF (13, 73). This approach led to a decrease in spleen size and reduction in constitutional symptoms and a better 5-year overall survival, however, ruxolitinib does not substantially reduce the JAK2V617F variant allele fraction (74–77). Limitations in targeting JAK2 are caused by the dependency of normal hematopoiesis on JAK2, resulting in on-target toxicity in the form of anemia and thrombocytopenia in patients with MF treated with JAK2 inhibitors (77, 78). Fedratinib is a selective JAK2-kinase inhibitor which also showed significant reduction in spleen size and improvement in constitutional symptoms in patients with MF and was recently was FDA-approved as both a first line and second-line therapy (following ruxolitinib failure) in MF (79–82). Several other JAK inhibitors are currently in late phase clinical trials (e.g., momelotinib and pacritinib) and will likely gain FDA-approval also.

Targeting megakaryocytes selectively has shown efficacy in several preclinical and early phase clinical studies (38, 39). Three approaches regulating megakaryocyte maturation have shown benefits. First, anagrelide, a megakaryocyte maturation inhibitor (83), was shown to be effective in ET patients (84). Second, targeting AURKA which was recently shown to be differentially expressed in JAK2-mutant MkPs in MF (23), with alisertib (MLN8237) promoted megakaryocyte polyploidization and to reduced MF in preclinical studies (39) and has shown some benefits in MF patients (85). Third, bodememstat (IMG-7289), an inhibitor of LSD1, an enzyme essential for platelet formation (86), was recently granted FDA fast-track designation for the treatment of ET patients (NCT04254978). In murine models of MPN, IMG-7289 has shown efficacy in reducing inflammation, splenomegaly and fibrosis, in addition to prolonged survival (87). IMG-7289 killed Jak2V617F-mutant cells selectively and synergized with Jak inhibition in pre-clinical MPN mouse models (87). Bodememstat is currently in phase IIb clinical trials for MF patients (NCT03136185).

Recently, Psaila et al. showed differential increased expression of G6B in JAK2-mutant HSPCs in MF (as compared to wildtype HSPCs from the same patient) (23). G6B is an immunoreceptor tyrosine-based inhibition motif (ITIM)-containing inhibitory receptor, normally expressed exclusively on mature megakaryocytes in normal hematopoiesis (23, 88, 89). The authors identified JAK2-mutant HSPCs using G6B expression and validated this cell surface marker as a candidate for specifically targeting JAK2-mutant HSPCs in MF, using a bi-specific antibody (against CD34 and G6B), as a potential future novel therapeutic strategy (23).

As PMF is characterized by the progressive deposition of ECM proteins (90), another therapeutic approach is to normalize the composition of the ECM. Lysyl oxidases (LOXs) have been demonstrated to be important in this process by cross-linking collagens and elastins through deamination of lysins and hydroxylysins, resulting in a stiffer ECM consistency (91). Lysyl oxidases are expressed in immature megakaryocytes and
downregulated in mature megakaryocytes but upregulated in MF patient megakaryocytes and in murine models of MF (38, 92, 93). Lysyl oxidase inhibition has shown efficacy in Gata1-low (38) and JAK2V617F mouse models of MF (94–96). However a recent phase 2 study of simtuzumab, a monoclonal inhibitor of LOX2 did not reduce bone fibrosis in patients with MF (97).

In conclusion, more effectively targeting cellular components of malignant hematopoiesis in MPN remains an ongoing goal within the field.

Targeting the Bone Marrow Niche
Therapeutically targeting the BM stroma has gained more attention in the treatment of MF (59, 98). As highlighted before, GLI1+ MSC were shown to be an important driver of MF in mouse models highlighting them as a potential therapeutic target. GI1 as well as Pthch1 are known hedgehog (Hh) target genes, previously shown to be increased in MPN patients (99). Treatment with the GI1 inhibitor, GANT61 in a JAK2V617F MF mouse model reduced the expression of mediators of inflammation and fibrosis significantly (e.g., MMP9, CXCR4, endothelin 1) (59). Moreover, treatment also reduced Stat5 expression in JAK2V617F-mutant cells, thereby decreasing pro-inflammatory signaling in the BM and interrupting the self-reinforcing cycle of inflammation, myofibroblast differentiation and ECM deposition. Ex vivo treatment of primary human MPN MSCs with GANT61 reduced the expression of both a-SMA and GLI1 and increased apoptosis (as compared to vehicle treatment) (59). These findings suggest selective targeting of GI1-positive myofibroblasts by the inhibitor, making it an attractive candidate for potential clinical use in MPN patients (59).

The NfkB pathway has been shown to be activated in JAK2 mutated MPN. Recently, a potential combinatorial therapeutic approach for MPN patients has been proposed, by targeting inflammation through reduction of NfkB activity using BET inhibition in combination with JAK inhibition (62). Using MPN mouse models, Kleppe et al. showed that increased NfkB activity in MPN is partly cell-extrinsic, highlighting the importance of targeting the BM microenvironment. The BET inhibitor, JQ1 showed potent anti-fibrotic effects and cooperated with Jak inhibition to ameliorate inflammation (62). Moreover, the NFKB pathway has also been shown to be upregulated in CALR mutated MPN HSPCs (24), suggesting that BET inhibition might also be effective in CALR-mutant MPN patients. Preliminary data using the BET inhibitor, CPI-0610 in MF patients either alone or in combination with ruxolitinib (MANIFEST study), showed beneficial effects. CPI-0610 alone or as ab add-on to ruxolitinib was well-tolerated and showed a reduction in BM fibrosis, spleen size and amelioration of anemia in MF patients (100, 101).

Taken together, these studies underscore the importance of treatment strategies for MPN that target the BM niche and highlight the potential for combinatorial targeting of both the malignant hematopoietic clone and the BM microenvironment to have enhanced efficacy.

CONCLUSION
MPN comprise a group of clonal malignant hematopoietic disorders with common features such as myeloproliferation and systemic inflammation. While genetic driver mutation-specific targeted therapy is at the center of MPN research, recent evidence highlights the importance of regulating inflammation in MPN. Malignant and non-malignant cellular contributors such as megakaryocytes and monocytes, as well as the BM niche, promote disease progression and cause considerable morbidity. This emphasizes the importance of a broader approach to simultaneously inhibit several pathogenic contributors in MPN, with the goal of improving treatment outcomes. Ongoing studies will shed light on the efficacy (and potential toxicity) of combining targeted therapies with anti-inflammatory approaches for the treatment of MPN.

AUTHOR CONTRIBUTIONS
JJ drafted the manuscript. Both authors designed the outline for the manuscript and edited and approved the manuscript.

FUNDING
This work was supported by the NIH (R01HL131835 to AM), the MPN Research Foundation (AM), the Gabrielle’s Angel Foundation for Cancer Research (AM), and the German Research Foundation (DFG, JU3104/2-1 to JJ). AM is a Scholar of The Leukemia & Lymphoma Society.

REFERENCES
1. Marneth AE, Mullally A. The molecular genetics of myeloproliferative neoplasms. Colds Perspect Med. (2019) 10:e034876. doi: 10.1101/coldsperpect. a034876
2. Mead AJ, Mullally A. Myeloproliferative neoplasm stem cells. Blood. (2017) 129:1607–16. doi: 10.1182/blood-2016-10-696005
3. Schepers K, Pietras EM, Reynaud D, Flach J, Binnewies M, Garg T, et al. Myeloproliferative neoplasia remodells the endosteal bone marrow niche into a self-reinforcing leukemic niche. Cell Stem Cell. (2013) 13:285–99. doi: 10. 1016/j.stem.2013.06.009
4. Kildjian J-J, Cassinat B, Chevet S, Turlure P, Cambier N, Roussel M, et al. Pegylated interferon-alpha-2a induces complete hematologic and molecular responses with low toxicity in polycythemia vera. Blood. (2008) 112:3065–72. doi: 10.1182/blood-2008-03-143537
5. Gisslinger H, Klade C, Georgiev P, Krochmalczyk D, Gercheva-Kyuchukova L, Egyed M, et al. Ropoginterferon alfa-2b versus standard therapy for polycythaemia vera (PROUD-PV and CONTINUATION-PV): a randomised, non-inferiority, phase 3 trial and its extension study. Lancet Haematol. (2020) 7:e196–208. doi: 10.1016/s2352-3026(19)30236-4
6. Panteli KE, Hatzimichael EC, Bouranta PK, Katsaraki A, Seferiadik S, Stebbing J, et al. Serum interleukin (IL)−1, IL−2, sIL−2Ra, IL−6 and thrombopoietin levels in patients with chronic myeloproliferative disorders. Br J Haematol. (2005) 130:709–15. doi: 10.1111/j.1365-2457.2005. 05674.x
7. Boissinot M, Cleyrat C, Vilaine M, Jacques Y, Corre I, Hermouet S. Anti-inflammatory cytokines hepatocyte growth factor and interleukin-11 are over-expressed in Polycythemia vera and contribute to the growth of clonal erythroblasts independently of JAK2V617F. Oncogene. (2011) 30:990–1001. doi: 10.1038/onc.2010.479
10. Tefferi A, Vaidya R, Caramazza D, Finke C, Lasho T, Pardanani A. Circulating interleukin-17 serum levels in patients with chronic myeloproliferative diseases. *Tumor J.* (2008) 95:404–5. doi: 10.1177/030098160909500326

11. Tefferi A. Pathogenesis of myelofibrosis with myeloid metaplasia. *J Clin Oncol.* (2005) 23:8520–30. doi: 10.1200/JCO.2004.00.9316

12. Buss H, Handschick K, Jurmann N, Pekkonen P, Beurerlein K, Müller H, et al. Cyclin-dependent kinase 6 phosphorylates NF-κB P65 at Serine 536 and contributes to the regulation of inflammatory gene expression. *PLoS One.* (2012) 7:e51847. doi: 10.1371/journal.pone.0051847

13. Barbui T, Tefferi A, Vannucchi AM, Passamonti F, Silver RT, Hoffman R, et al. Philadelphia chromosome-negative classical myeloproliferative neoplasms: revised management recommendations from European LeukaemiaNet. *Leukemia.* (2018) 32:1057–69. doi: 10.1038/s41375-018-0077-1

14. Vaidya R, Gangat N, Jimma T, Finke CM, Lasho TL, Pardanani A, et al. Plasma cytokines in polycythaemia vera: phenotypic correlates, prognostic relevance, and comparison with myelofibrosis. *Am J Hematol.* (2012) 87:1038–5. doi: 10.1002/ajh.22395

15. Nina FØ, Jacob G, Miriam B, Melissa I, Sm S, Nageswara RT, et al. Evaluation of interleukin-17 serum levels in patients with chronic myeloproliferative diseases. *Br J Haematol.* (2010) 150:176–86. doi: 10.1111/j.1365-2141.2009.07876.x

16. Hermouet S, Godard A, Pineau D, Corre I, Raher S, Lippert E, et al. Abnormal production of interleukin (Il)-11 and Il-8 in polycythaemia vera. *Blood.* (2008) 111:4722–9. doi: 10.1182/blood-2008-06-161895

17. Hasselbalch HC. Perspectives on chronic inflammation in essential thrombocythemia. *Blood Rev.* (2011) 25:63–72. doi: 10.1016/j.brev.2010.07.004

18. Koschmieder S, Chatain N. Role of inflammation in the biology of myelofibrosis. *Frontiers in Immunology.* doi: 10.3389/fimmu.2018.02985

19. Carobbio A, Tiele J, Passamonti F, Rumi E, Ruggeri M, Rodeghiero F, et al. Risk factors for arterial and venous thrombosis in WHO-defined essential thrombocythemia: an international study of 891 patients. *Blood.* (2011) 117:5857–9. doi: 10.1182/blood-2011-02-339002

20. Goette NP, Lev PR, Heller PG, Kornblith LI, Korin L, Molinas PC, et al. Monocyte IL-2Ra expression is associated with thrombosis and the JAK2V617F mutation in myeloproliferative neoplasms. *Cytokine.* (2010) 51:67–72. doi: 10.1016/j.cyto.2010.04.011

21. Lai HY, Brooks SA, Craver BM, Morse SI, Nguyen TK, Haghighi N, et al. Defective negative regulation of Toll-like receptor signaling leads to excessive TNF-α in myeloproliferative neoplasm. *Blood Adv.* (2019) 3:122–31. doi: 10.1182/bloodadvances.2018026450

22. Fisher DAC, Miner CA, Engle K, Hu H, Collins TB, Zhou A, et al. Cytokine production in myelofibrosis exhibits differential responsiveness to JAK-STAT, MAP kinase, and NFκB signaling. *Leukemia.* (2019) 33:1978–95. doi: 10.1038/s41375-019-01379-y

23. Psaila B, Wang G, Rodriguez-Meira A, Li R, Heuston EF, Murphy L, et al. Targeting megakaryocytic-induced fibrosis in myeloproliferative neoplasms induces bone marrow fibrosis in PMF. *J Exp Med.* (2016) 213:1723–40. doi: 10.1084/jem.20160283

24. Nam AS, Kim K-T, Chaligne R, Izzo F, Ang C, Taylor J, et al. Somatic mutations and cell identity linked by genotyping of transcriptomes. *Mol Cell.* (2019) 78:477–92.e8. doi: 10.1016/j.molcel.2020.04.008

25. Carobbio A, Tiele J, Passamonti F, Rumi E, Ruggeri M, Rodeghiero F, et al. Risk factors for arterial and venous thrombosis in WHO-defined essential thrombocythemia: an international study of 891 patients. *Blood.* (2011) 117:5857–9. doi: 10.1182/blood-2011-02-339002

26. Calaminus SDJ, Guitart A, Sinclair A, Schachterner H, Watson SP, Holyoke TL, et al. Lineage tracing of Pf4-cre marks hematopoietic stem cells and their progeny. *PLoS One.* (2012) 7:e35136. doi: 10.1371/journal.pone.0035136

27. Nagy Z, Vögtle T, Geer MJ, Mori J, Heising S, Nunzio GD, et al. The Gp1ba-EGF as principle growth factor for megakaryocytes and platelets from patients with idiopathic myelofibrosis. *Blood Adv.* (2019) 3:122–31. doi: 10.1182/bloodadvances.2018026450

28. Goette NP, Lev PR, Heller PG, Kornblith LI, Korin L, Molinas PC, et al. Monocyte IL-2Ra expression is associated with thrombosis and the JAK2V617F mutation in myeloproliferative neoplasms. *Cytokine.* (2010) 51:67–72. doi: 10.1016/j.cyto.2010.04.011

29. Lai HY, Brooks SA, Craver BM, Morse SI, Nguyen TK, Haghighi N, et al. Defective negative regulation of Toll-like receptor signaling leads to excessive TNF-α in myeloproliferative neoplasm. *Blood Adv.* (2019) 3:122–31. doi: 10.1182/bloodadvances.2018026450

30. Fisher DAC, Miner CA, Engle K, Hu H, Collins TB, Zhou A, et al. Cytokine production in myelofibrosis exhibits differential responsiveness to JAK-STAT, MAP kinase, and NFκB signaling. *Leukemia.* (2019) 33:1978–95. doi: 10.1038/s41375-019-01379-y

31. Herzog EL, Bucala R. Fibrocytes in health and disease. *Exp Hematol.* (2010) 38:548–56. doi: 10.1016/j.exphem.2010.03.004

32. Eyden B. The myofibroblast: a study of normal, reactive and neoplastic cells. *CLIN IMMUNOL.* (2005) 120:371–8. doi: 10.1016/j.clim.2004.08.007

33. Manshouri T, Verstovsek S, Harris DM, Veletic I, Zhang X, Post SM, et al. Primary myelofibrosis: morpho-clinical correlations. *Blood.* (2018) 132:1057–69. doi: 10.1016/j.blood.2018.05.030

34. Carobbio A, Tiele J, Passamonti F, Rumi E, Ruggeri M, Rodeghiero F, et al. Risk factors for arterial and venous thrombosis in WHO-defined essential thrombocythemia: an international study of 891 patients. *Blood.* (2011) 117:5857–9. doi: 10.1182/blood-2011-02-339002
46. Notta F, Zandi S, Takayama N, Dobson S, Gan OI, Wilson G, et al. Distinct routes of lineage development reshape the human blood hierarchy across ontology. Sci New York N Y (2015) 351:aaeb2116. doi: 10.1126/science.aab2116

47. Rodriguez-Fraticelli AE, Wolokol SL, Weinreb CS, Panero R, Patel SH, Jankovic M, et al. Clonal analysis of lineage fate in native haematopoiesis. Nature. (2018) 553:212–6. doi: 10.1038/nature25168

48. Giustacchini A, Thongjuea S, Barkas N, Woll PS, Povinelli BJ, Booth CAG, et al. Single-cell transcriptomics uncovers distinct molecular signatures of stem cells in chronic myeloid leukemia. Nat Med. (2017) 23:692–702. doi: 10.1038/nm.4336

49. Oyarzún CPM, Heller PG. Platelets as mediators of thromboinflammation in chronic myeloproliferative neoplasms. Front Immunol. (2019) 10:1373. doi: 10.3389/fimmu.2019.01373

50. Desterke C, Martinaud C, Ruzehbaj N, Bousse-Kerdiles M-C. Inflammation as a keystone of bone marrow stroma alterations in primary myelofibrosis. Mediat Inflamm. (2015) 2015:1–16. doi: 10.1155/2015/415024

51. Kuter DJ, Bain B, Mufti G, Bagg A, Hasserjian RP. Bone marrow fibrosis: pathophysiology and clinical significance of increased bone marrow stromal fibres: review. Br J Haematol. (2007) 139:351–62. doi: 10.1111/j.1365-2141.2007.06807.x

52. Kraman R, Schneider RK, DiRocco DP, Machado F, Fleig S, Bondzí PE, et al. Perivascular Gli1+ progenitors are key contributors to injury-induced organ fibrosis. Cell Stem Cell. (2014) 16:51–66. doi: 10.1016/j.stem.2014.01.004

53. Mercier F, Monczak Y, François M, Prchal J, Galipeau J. Bone marrow mesenchymal stromal cells of patients with myeloproliferative disorders do not carry the JAK2-V617F mutation. Exp Hematol. (2009) 37:416–20. doi: 10.1016/j.exphem.2008.11.008

54. Pieri L, Guglielmelli P, Bogani C, Bosi A, Vannucchi AM. (MPD-RC) MDRC. J Clin Oncol. (2017) 35:212–6. doi: 10.1002/jco.265168

55. Bacher U, Asenova S, Badbaran A, Zander AR, Alchalby H, Fehse B, et al. Pathomechanisms of bone marrow fibrosis and an important cellular therapeutic target. Cancer Cell. (2017) 32:516–7. doi: 10.1016/j.ccell.2017.03.055

56. Decker M, Martinez-Morenín L, Wang G, Lee Y, Liu Q, Leslie J, et al. Leptin-receptor-expressing bone marrow stromal cells are myofibroblasts in primary myelofibrosis. Blood. (2016) 127:2391–405. doi: 10.1182/blood-2016-03-643544

57. Walkley CR, Olsen GH, Dworkin S, Fabb SA, Swann J, McArthur GA, et al. The clinical revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. (2016) 127:2391–405. doi: 10.1182/blood-2016-03-643544

58. Jankovic M, et al. Clonal analysis of lineage fate in native haematopoiesis. J Hematol Oncol. (2017) 10:55. doi: 10.1186/s13045-017-0417-z

59. Kantarjian HM, Silver RT, Komrokji RS, Mesa RA, Tacke R, Hassan CN. Ruxolitinib for myelofibrosis—an update of its clinical effects. Clin Lymphoma Myeloma Leukemia. (2015) 15:638–45. doi: 10.1016/j.cllm.2013.09.006

60. Verstovsek S, Vora A, Gorin A, Lipton J, Thomas DA, et al. Safety and efficacy of TG101348, a selective JAK2 inhibitor, in myelofibrosis. Blood. (2011) 129:2373–89. doi: 10.1182/blood-2011-04-330843

61. Kraman R, DiRocco DP, Humphreys BD. Understanding the origin, activation and regulation of producing myofibroblasts for treatment of fibrotic disease. J Pathol. (2013) 231:273–89. doi: 10.1002/path.4253

62. Mercier F, Monczak Y, François M, Prchal J, Galipeau J. Bone marrow mesenchymal stromal cells of patients with myeloproliferative disorders do not carry the JAK2-V617F mutation. Exp Hematol. (2009) 37:416–20. doi: 10.1016/j.exphem.2008.11.008

63. Walkley CR, SheA JM, Sims NA, Purton LE, Orkin SH. RB regulates interactions between hematopoietic stem cells and their bone marrow microenvironment. Cell. (2007) 129:1081–95. doi: 10.1016/j.cell.2007.03.055

64. Martinaud C, Desterke C, Konopacki J, Pieri L, Torosian F, Golub R, et al. Osteogenic potential of mesenchymal stromal cells contributes to primary myelofibrosis. Cancer Res. (2015) 75:4753–65. doi: 10.1158/0008-5472.can-14-3696

65. Estrov Z, Kurzrock R, Wetzler M, Kantarjian H, Blake M, Harris D, et al. Suppression of chronic myelogenous leukemia colony growth by interleukin-1 (IL-1) receptor antagonist and soluble IL-1 receptor: a novel application for inhibitors of IL-1 activity. Blood. (1991) 78:1476–84.

66. Kleppe M, Kocher R, Zou L, Galen P, Van Hill CE, Dong L, et al. Dual targeting of oncogenic activation and inflammatory signaling increases therapeutic efficacy in myeloproliferative neoplasms. Cancer Cell. (2018) 33:29–43. doi: 10.1016/j.ccell.2017.11.009

67. Verstovsek S, Kantarjian H, Mesa RA, Pardanani AD, Cortes-Francjo J, Thomas DA, et al. Safety and Efficacy of INC0081442, a JAK1 and JAK2 inhibitor, in myelofibrosis. New Engl J Med. (2010) 363:1117–27. doi: 10.1056/nejmoa1002028

68. Pardanani A, Laborde RR, Lasho TL, Finke C, Begna K, Al-Kali A, et al. Safety and efficacy of CYT387, a JAK1 and JAK2 inhibitor, in myelofibrosis. Leukemia. (2013) 27:1322–7. doi: 10.1038/leu.2013.71

69. Pardanani A, Gotlib J, Gupta V, Roberts AW, Wadleigh M, Sirhan S, et al. Update on the long-term efficacy and safety of momelotinib, a JAK1 and JAK2 inhibitor, for the treatment of myelofibrosis. Blood. (2013) 122:108. doi: 10.1182/blood.v122.10.108

70. Asshoff M, Petzer V, Warr MR, Haschka D, Tymoszuk P, Demetz E, et al. Momelotinib inhibits ACVR1/ALK2, decreases hepcidin production and ameliorates anemia of chronic disease in rodents. Blood. (2017) 129:1823–30. doi: 10.1182/blood-2016-09-740092

71. Bose P, Verstovsek S. Developmental therapeutics in myeloproliferative neoplasms. Clin Lymphoma Myeloma Leukemia. (2017) 17:534–52. doi: 10.1016/j.clml.2017.02.014

72. Fenaux P, Kiladjian JJ, Platzebecker U. Luspatercept for the treatment of anemia in myelodysplastic syndromes and primary myelofibrosis. Blood. (2019) 135:790–4. doi: 10.1182/blood-2018-11-876888

73. Platzebecker U, Germing U, Götzke KS, Kiewe P, Mayer K, Chromik J, et al. Luspatercept for the treatment of anemia in patients with lower-risk myelodysplastic syndromes (PANCE-MDS): a multicentre, open-label phase 2 dose-finding study with long-term extension study. Lancet Oncol. (2017) 18:1338–47. doi: 10.1016/s1470-2045(17)30615-0

74. Kantarjian R, DiRocco DP, Humphreys BD. Understanding the origin, activation and regulation of producing myofibroblasts for treatment of fibrotic disease. J Pathol. (2013) 231:273–89. doi: 10.1002/path.4253

75. Estrov Z, Kurzrock R, Wetzler M, Kantarjian H, Blake M, Harris D, et al. Suppression of chronic myelogenous leukemia colony growth by interleukin-1 (IL-1) receptor antagonist and soluble IL-1 receptors: a novel application for inhibitors of IL-1 activity. Blood. (1991) 78:1476–84.
82. Pardanani A, Tefferi A, Jamieson C, Gabrail NY, Lebedinsky C, Gao G, et al. phase 2 randomized dose-ranging study of the JAK2-selective inhibitor fedratinib (SAR302503) in patients with myelofibrosis. *Blood Cancer J.* (2015) 5:e335. doi: 10.1038/bcj.2015.63

83. Espasandin YR, Glombotsky AC, Gozdzielski M, Lev PR, Goette NP, Molinas FC, et al. Anagrelide platelet-lowering effect is due to inhibition of both megakaryocyte maturation and proplatelet formation: insight into potential mechanisms. *J Thromb Haemost.* (2015) 13:631–42. doi: 10.1111/jth.12850

84. Gisslinger H, Gotic M, Holowiecki J, Penka M, Thiele J, Kvasnicka H-M, et al. Anagrelide compared with hydroxyurea in WHO-classified essential thrombocythemia: the ANAHYDRET Study, a randomized controlled trial. *Blood.* (2013) 121:1720–8. doi: 10.1182/blood-2012-07-443770

85. Gangat N, Marinaccio C, Swords R, Watts JM, Gurbuxani S, Rademaker A, et al. Aurora kinase a inhibition provides clinical benefit, normalizes megakaryocytes, and reduces bone marrow fibrosis in patients with myelofibrosis: a phase I trial. *Clin Cancer Res.* (2019) 25:4898–906. doi: 10.1158/1078-0432.ccr-19-1005

86. Spriessl A, Schulte JH, Weber S, Necke M, Händschke K, Thor T, et al. Lysine-specific demethylase 1 restricts hematopoietic progenitor proliferation and is essential for terminal differentiation. *Leukemia.* (2012) 26:2039–51. doi: 10.1038/leu.2012.157

87. Jutzi J, Klepe M, Dias J, Stechel H, Shank K, Teruya-Feldstein J, et al. LSD1 inhibition prolongs survival in mouse models of MPN by selectively targeting the disease clone. *Hemasphere.* (2018) 2:e54. doi: 10.1007/h49.100000000000045

88. Senis YA, Tomlinson MG, García Á, Dumon S, Heath VL, Herbert J, et al. A comprehensive proteomics and genomics analysis reveals novel transmembrane proteins in human platelets and megakaryocytes including G6b-B, a novel immunoreceptor tyrosine-based inhibitory motif protein. *Mol Cell Proteomics.* (2006) 6:548–64. doi: 10.1074/mcp.d000007-mcp200

89. Coxon CH, Geer MJ, Senis YA. ITIM receptors: more than just inhibitors of cell function. *Blood.* (2013) 122:666. doi: 10.1182/blood.v122.21.666.666

90. Leiva O, Ng SK, Matsuura S, Chitalia V, Lucero H, Findlay A, et al. Novel lysyl oxidase inhibitors attenuate hallmarks of primary myelofibrosis in mice. *Int J Hematol.* (2019) 110:699–708. doi: 10.1007/s12185-019-02751-6

91. Bhagwat N, Keller MD, Rampal RK, Shank K, Stanchina E, De Rose K, et al. Improved efficacy of combination Of JAK2 and hedgehog inhibitors in myelofibrosis. *Blood.* (2013) 122:666. doi: 10.1182/blood.v122.21.666.666

92. Harrison CN, Patriarca A, Mascarenhas J, Kremyanskaya M, Hoffman R, Schiller GJ, et al. Preliminary report of MANIFEST, a phase 2 Study of CPI-0610, a bromodomain and extraterminal domain inhibitor (BETi), in combination with ruxolitinib, in JAK inhibitor (JAKi) treatment naïve myelofibrosis patients. *Blood.* (2019) 134:4164. doi: 10.1182/blood-2019-128211

93. Mascarenhas J, Kremyanskaya M, Hoffman R, Bose P, Talpaz M, Harrison CN, et al. MANIFEST, a phase 2 study of CPI-0610, a bromodomain and extraterminal domain inhibitor (BETi), As monotherapy or "add-on" to ruxolitinib, in patients with refractory or intolerant advanced myelofibrosis. *Blood.* (2019) 134:670. doi: 10.1182/blood-2019-127119

**Conflict of Interest:** AM has received honoraria from Blueprint Medicines, Roche, and Incyte and receives research support from Janssen.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Jutzi and Mullally. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.