Still a burning question: the interplay between inflammation and fibrosis in myeloproliferative neoplasms

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Purpose of review
Bone marrow fibrosis is the progressive replacement of blood-forming cells by reticulin fibres, caused by the acquisition of somatic mutations in hematopoietic stem cells. The molecular and cellular mechanisms that drive the progression of bone marrow fibrosis remain unknown, yet chronic inflammation appears to be a conserved feature in most patients suffering from myeloproliferative neoplasms.

Recent findings
Here, we review recent literature pertaining to the role of inflammation in driving bone marrow fibrosis, and its effect on the various hematopoietic and nonhematopoietic cell populations.

Summary
Recent evidence suggests that the pathogenesis of MPN is primarily driven by the hematopoietic stem and progenitor cells, together with their mutated progeny, which in turn results in chronic inflammation that disrupts the bone marrow niche and perpetuates a disease-permissive environment. Emerging data suggests that specifically targeting stromal inflammation in combination with JAK inhibition may be the way forward to better treat MPNs, and bone marrow fibrosis specifically.

Keywords
bone marrow fibrosis, inflammation, myeloproliferative neoplasms

INTRODUCTION

Bone marrow fibrosis is defined by the accumulation and disorganization of extracellular matrix (ECM) fibres throughout the marrow. This phenomenon has detrimental effects on blood cell replenishment and function. An array of underlying conditions has been associated with bone marrow fibrosis, ranging from chronic and acute blood cancers, lymphoma, multiple myeloma and solid tumours metastasizing to the bone marrow, to metabolic diseases, various immune diseases, infections and aging [1]. Interestingly, inflammation is constitutive of all these conditions, implying that pro-inflammatory signals are paramount during bone marrow fibrosis. The Philadelphia-negative myeloproliferative neoplasms (MPNs) represent a prime example of this intricate relationship, as MPN-induced bone marrow fibrosis is systematically associated with underlying inflammation. Therefore, MPN-centred studies will be the focus of this review.

MPNs are malignancies of the hematopoietic stem cell (HSC) arising as a consequence of clonal proliferation, driven in most patients by somatically acquired driver mutations in the *JAK2*, *CALR*, or *MPL* genes that constitutively activate JAK-STAT signalling [2]. The three primary MPN clinical subtypes are polycythaemia vera, characterized by an increase in red blood cells, essential thrombocythemia, presenting with increased platelets and primary myelofibrosis (PMF), the prototypical example of fibrosis of the bone marrow.

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KEY POINTS

- Malignant hematopoietic cells create a chronically inflamed microenvironment via secretion of pro-inflammatory cytokines that severely disrupts the normal bone marrow niche.
- The bone marrow niche, particularly stromal cells, can also contribute to chronic inflammation in MPN, perpetuating a disease-permissive environment.
- Specifically targeting stromal inflammation, together with JAK inhibition, is a promising approach to reduce bone marrow fibrosis and treat MPN.

Growing evidence suggests that although the pathogenesis of MPNs is primarily cell-intrinsic to hematopoietic stem and progenitor cells (HSPCs), the malignant clone also acts in a cell-extrinsic manner, resulting in chronic inflammation that perturbs the bone marrow niche (Fig. 1a). In turn, this intricately intertwined process significantly contributes to the MPN phenotype and corrupts the niche to become less supportive of healthy haematopoiesis and instead creating a self-perpetuating malignant milieu that is permissive for disease development and progression [3,4].

INFLAMMATION: AN EARLY EVENT IN MYELOID MALIGNANCIES

Inflammation is a critical physiological process that mediates host defence against invading pathogens, injury, and other insults. Inflammation evolves as an acute response that is quickly suppressed upon restoration of tissue homeostasis. Persistence of inflammation or failure to efficiently resolve inflammatory insults can have serious consequences for tissue maintenance and function. This is particularly true for malignancy-induced inflammation as long as malignant cells are present or even expand.

A common feature of myeloid malignancies is the early elevation of pro-inflammatory cytokines, leading to chronic inflammation in the bone marrow and subsequently resulting in constitutional symptoms, such as fatigue and weight loss [5]. Regarding disease initiation, two schools of thought exist: mutant hematopoietic cells may trigger inflammation, or, alternatively, inflammatory cues may be responsible for genotoxic stress and thereby induce malignancy. Although the pro-inflammatory function of mutant clones has been consistently reported [6▪,7], both options are not mutually exclusive. Indeed, there is also growing evidence that chronic inflammation often precedes the development of myeloid malignancies, thereby creating a milieu that is well suited to the development of haematological malignancies.

Age-related inflammation, also known as inflammaging, showcases how inflammatory signals can precede and prime for myeloid malignancy development and is another example of continuous, although often 'low-grade', inflammation. The complex and multifactorial process of aging is the primary risk factor for many conditions, including haematological malignancies [8]. Along these lines, a recent report demonstrated that aged HSCs are epigenetically reprogrammed, leading to a predisposition to leukaemia [9]. Although aged HSCs appear inherently altered, extrinsic inflammatory cues may drive malignancies. Valletta *et al.* [10▪] performed RNA sequencing of different nonhematopoietic populations, including mesenchymal stromal cells (MSCs), endothelial cells and osteoblasts, in young and old mice. Importantly, inflammatory pathways appeared to be consistently upregulated and the authors showed that increased stromal Il-6 was a key regulator of the age-related decrease in erythropoiesis. Helbling *et al.* [11▪▪] described similar findings and noted a decreased stromal production of hematopoiesis-supporting factors, at the expense of pro-inflammatory molecules. This remodelling of the stromal niche is reminiscent of MPN-associated features, suggesting that the aged stroma may favour and/or trigger myeloid malignancies. Future studies conducting single-cell RNA sequencing (RNAseq) of the aged stroma will certainly enable further characterization of the aging bone marrow stroma and its contribution to myeloid malignancies.

Recent work revealed that in MPN patients, malignant clones may emerge decades before the diagnosis, dating back the occurrence of somatic driver mutations to infancy or even in embryos [12▪▪,13▪▪]. Inflammaging, or inflammatory events during the life of the patient, may influence clonal haematopoiesis and determine the evolution of malignant clones. Moreover, a study demonstrated that transition from young age to adulthood was already associated with increased inflammation [11▪▪]. Thus, rather than being an elderly specific phenomenon, the inflammation dating back to adolescence may direct clonal haematopoiesis.

STROMAL INFLAMMATION AS THE SOIL OF FIBROSIS

High-resolution studies in mice and humans based on cell purification methods, RNAseq and single-cell RNAseq demonstrated that myeloid malignancies are accompanied with mesenchymal inflammation [3,10▪,11▪▪,14–16,17▪,18▪] (Fig. 1a).

Already in 2013, Schepers *et al.* [3] demonstrated in a chronic phase CML mouse model that CML-exposed stromal cells display an inflammatory

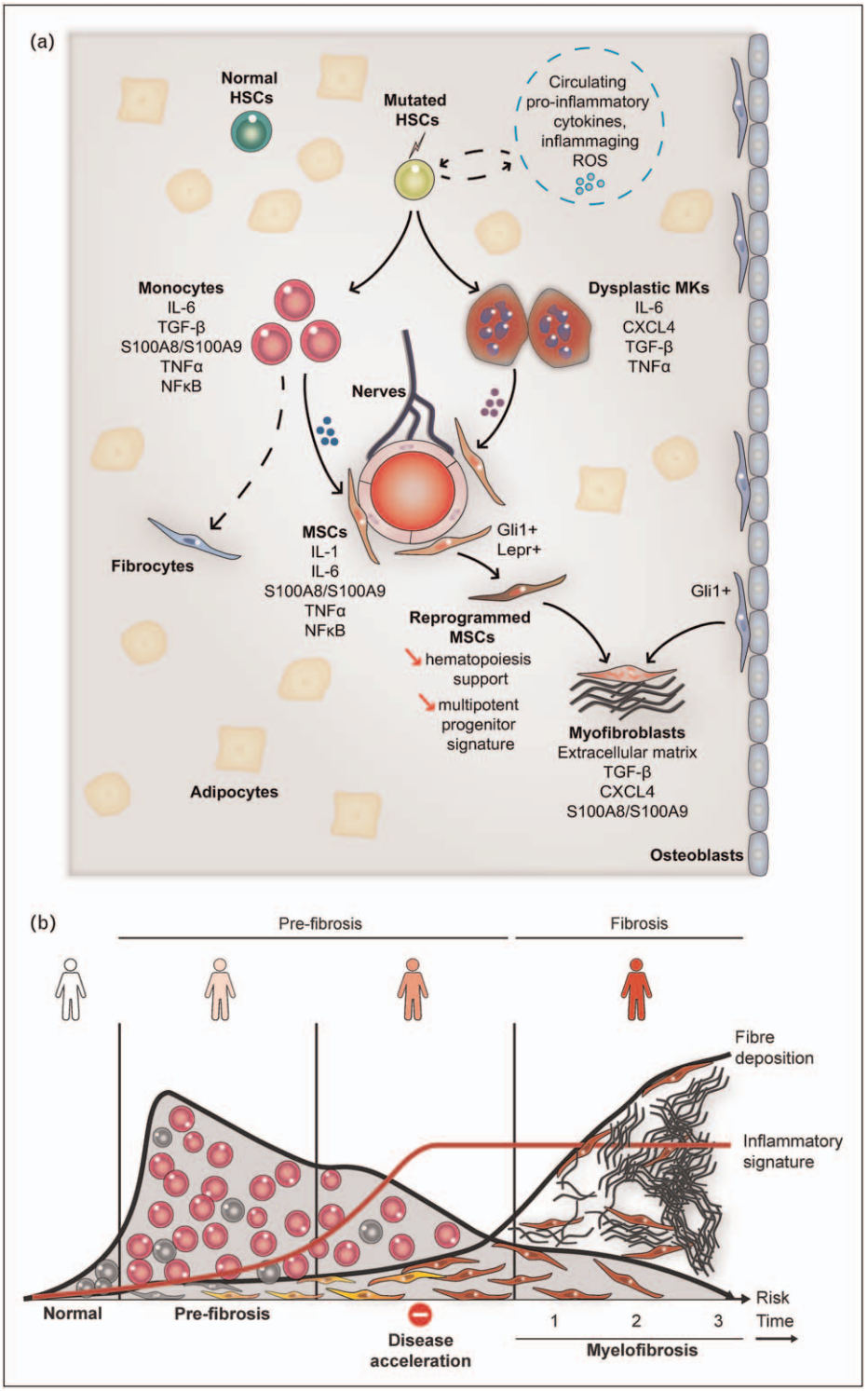

FIGURE 1. Inflammation and fibrosis during myeloproliferative neoplasm progression. (a) Cell interactions in the bone marrow microenvironment. Inflammatory cues precede and/or result from MPN-driving mutations. Monocytes and megakaryocytes fuel stromal inflammation, early during MPN progression. MSCs undergo a transcriptional reprogramming and eventually differentiate into fibrosis-driving myofibroblasts. (b) Scheme of putative kinetics and correlation of inflammation and fibre deposition during MPN. Elevation of inflammation occurs before overt fibrosis. This early inflammatory context accelerates disease progression and provides a window of opportunity for therapeutic intervention. MPN, myeloproliferative neoplasm; MSCs, mesenchymal stromal cells.

signature, with enhanced expression of IL-1, TNFα and Nf-κβ signalling components. They demonstrated that this stromal inflammation is caused by leukemic myeloid cells, which stimulate MSCs to overproduce functionally altered osteoblastic lineage cells, which accumulate in the bone marrow cavity as 'inflammatory myelofibrotic cells'.

The functional heterogeneity of MSCs, and the plausible specialization of MSC subsets towards inflammation or fibrosis, have been only recently addressed. Our lab has recently performed single-cell RNAseq of nonhematopoietic bone marrow cells, and two distinct MSC subpopulations emerged as fibrosis-driving clusters in well established murine models of MPN associated with fibrosis [18▪]. Importantly, we dived into the stepwise activation of fibrosis-driving cells to determine which changes in the stroma occur in the presence of a myeloid-mutated hematopoietic clone before fibrosis is present. This approach helps to better define the initiation phase of fibrosis and priming of the stroma for fibrosis. Interestingly, inflammation in the fibrosis-driving MSC compartment was a key feature in the prefibrotic phase (reticulin fibres not present yet, but MPN phenotype), whereas manifestation of pro-fibrotic features of MSCs (as TGFβ and ECM production) was only noticed weeks later (Fig. 1b). This leads to the hypothesis that stromal inflammation is crucial for priming the stroma and an initiator for disease progression and fibrosis. Disease progression from the early prefibrotic inflammatory to the fibrotic stage remained poorly investigated so far. A better characterization of the prefibrotic mesenchyme may provide a window of opportunity for early therapeutic intervention and reveal novel therapeutic targets. Indeed, although diverse studies reported delayed or halted fibrosis upon therapeutic intervention, no tractable approach seems to achieve reversion of the phenotype (in addition to allogeneic stem cell transplantation). This suggests that fibrosis may not be reversible, advocating for early intervention at the prefibrotic stage.

Inflammation in the bone marrow microenvironment may be confined to a locally restricted specialized niche. A recent study performed single-cell RNAseq combined with laser capture microdissection to characterize the different niches of the mouse bone marrow [19▪]. Interestingly, MSCs were found to be major producers of cytokines and to reside in the vicinity of arteries and sinusoids. Spatial transcriptomics in the context of MPN may provide additional information on the cellular interplay that occurs at inflammatory and fibrotic sites.

Although mesenchymal inflammation precedes the fibrotic transformation, it may also contribute to sustain fibrosis. In recent years, we and others identified $Gli1^+$ and $LepR^+$ stromal cells as fibrosis-driving cells, both differentiating into myofibroblasts that produce ECM proteins and subsequently deposit fibres in the bone marrow [20,21] (Fig. 1a). Interestingly, we observed that the $Gli1^+$-derived pro-fibrotic mesenchymal lineage upregulated inflammatory pathways, showing that although mesenchymal inflammation is an early feature of MPN, it is sustained in the fibrotic stroma and leads to a self-reinforcement of the fibrotic niche (Fig. 1b).

A variety of inflammatory pathways have been associated with mesenchymal inflammation but the critical factors mediating progression to full-blown fibrosis remain elusive. IL-6, a cytokine associated with both inflammation and fibrosis, was shown to be upregulated in MPN [22]. IL-6 stimulation of primary murine bone marrow-MSCs *in vitro* leads to the acquisition of myofibroblastic features, such as upregulation of α-SMA [17▪], suggesting that IL-6 may be instrumental in the initiation of fibrosis. CXCL4 also appears to be an interesting biomarker and inflammatory mediator during bone marrow fibrosis progression. Spatial localization of CXCL4 shifts from hematopoietic cells at early stage (prefibrosis), to stromal cells at late stage (fibrosis). Such spatial specificity may also apply to other inflammatory components and be instrumental in bone marrow fibrosis development. In line with these findings, we also demonstrated disease-specific upregulation of the pro-inflammatory alarmin complex S100A8/S100A9 specifically in fibrosis-driving cells in both murine MPN models and in patient samples. Under normal circumstances, S100A8/S100A9 are not expressed in stromal cells but we validated also a spatial specificity of their expression in the progression of fibrosis starting in the mutated clone and later dominating in the fibrosis-driving cells. Crucially, we demonstrated that these genes show spatial specificity, are associated with inflammation and are potential therapeutic targets [17▪,18▪]. We provided proof-of-concept that S100A8/S100A9 might be an actionable biomarker as treatment with the anticancer drug Tasquinimod, inhibiting the binding of S100A8/S100A9 to TLR4, which ameliorated the MPN phenotype and bone marrow fibrosis in a murine model of JAK2V617F-driven disease [18▪].

THE GENOTYPE–PHENOTYPE QUESTION: WHY DO SOME PATIENTS WITH JAK2-MUTATED DISEASE DEVELOP MYELOPROLIFERATIVE NEOPLASM AND OTHERS NEVER?

It remains unclear how the various MPN subtypes can share the very same driver mutation (i.e. JAK2V617F),

yet result in highly variable phenotypes. More specifically – why do some patients with the same mutation present with fibrosis at the time point of diagnosis whilst others never develop fibrosis at all? Moreover, numerous myeloid malignancies are associated with inflammation, and even stromal inflammation, yet rarely result in fibrosis.

There is evidence that these differences could be a result of different MPN cytokine profiles. In MPN, several studies have highlighted the deregulation of inflammatory cytokines [23,24]. Specifically, interleukin (IL)-8, IL-12 and IL-15 have been identified as predictors of inferior survival in primary MPN myeloid malignancies [25]. A recent longitudinal serum cytokine profiling study of 400 essential thrombocythemia patients identified an essential thrombocythemia subtype-specific cytokine signature [26▪]. Although cytokines are prime candidates to fuel the inflammatory niche and support the malignant clone, dysregulated levels of reactive oxygen species (ROS) appear to also contribute to inflammation-induced genomic instability and DNA damage, further driving MPN progression [27,28]. ROS are also able to activate TGF-β, a known master regulator of fibrosis, which is abundantly secreted in MPNs, particularly by dysplastic megakaryocytes. TGF-β is a pleiotropic cytokine, juggling anti-inflammatory and pro-fibrotic properties, together with an important role in maintaining HSC quiescence and growth suppression. Interestingly, it was recently described that mutated Jak2 HSCs are resistant to this TGF-β growth suppressive effect and gain a selective advantage that contributes to their clonal expansion and myeloproliferation [29].

Together, these studies highlight the important contribution of inflammation in MPNs regardless of disease subtype, and that it is not simply a 'consequence' of MPN but rather an active participant in the development and progression of the disease.

DOES THE CELLULAR INTERPLAY CAUSE THE GENOTYPE–PHENOTYPE DISCREPANCY AND SHAPE A PRO-INFLAMMATORY ENVIRONMENT?

Recent advances in single-cell technologies are starting to provide new insights into the cellular and molecular mechanisms that underpin many diseases and specifically MPNs, and how these different cell types communicate and influence each other. In particular, in the context of stromal inflammation and fibrosis, two cell types have moved into the centre of the disease pathogenesis: megakaryocytes and monocytes that both directly interact with the fibrosis-driving stromal cells as receptor–ligand interactions of single cell data sets indicated (Fig. 1a).

In an elegant work, Psaila *et al.* [6▪] demonstrated that aberrant megakaryopoiesis observed in PMF likely stems from aberrant differentiation of HSPCs, and showed that early multipotent stem cells are biased towards megakaryocyte differentiation, having increased expression of megakaryocyte-associated genes, findings, which were similarly highlighted by Tong *et al.* [30▪]. Importantly, megakaryocytes progenitors were heterogeneous and demonstrated distinct expression of inflammatory mediators in MPN. Rodriguez-Meira *et al.* [7] further elegantly depicted that even more immature hematopoietic stem and progenitor cells from MPN patients are enriched for inflammatory pathways involving TNF-α and interferon signalling. Together, this data suggests that the disease-inducing malignant clone acquires pro-inflammatory programs early in development and highlights the mutual interactions of HSPCs with an inflamed niche, further supporting concepts of spatial specificity of inflammation in different disease stages.

Mature megakaryocytes, whilst rather rare in healthy marrows, are significantly increased and atypical in the bone marrow of PMF patients; they grow in clusters and are found in atypical localizations (e.g. close to the endosteal bone), are hyperlobated and enlarged, and were shown to secrete high levels of pro-inflammatory and pro-fibrotic cytokines. Megakaryocytes are considered to be major cellular drivers of bone marrow fibrosis [31], as they can directly stimulate the malignant clone via dysregulated pro-inflammatory cytokine secretion but can also recruit and activate $Gli1^{+}$ stromal cells that eventually deposit fibrosis in the bone marrow [17▪,20]. Our recent work highlighted the role of CXCL4 (platelet-factor 4), a megakaryocyte-specific cytokine, as an inflammatory mediator in PMF, specifically contributing to fibrosis development and stromal cell reprogramming [17▪]. Various studies show that JAK2V617F mutations restricted to the megakaryocyte lineage activate JAK-STAT signalling, and lead to megakaryocytes and HSPC expansion in mice via the constitutive activation of the thrombopoietin receptor (MPL) [32–34]. Interestingly, some megakaryocyte progenitor populations display selective expression of AURKA, a kinase involved in megakaryocyte polyploidization, which can be targeted with the selective AURKA inhibitor MLN8237 [31]. A recent study by Guo and colleagues identified discrete changes in platelet transcripts in PMF patients, showing that more than 1000 genes were differentially expressed. They could create a three-gene 'fibrotic signature'

that discriminates between patients with and without fibrosis [35▪], which could soon be used as a biomarker.

However, many questions remain. For example, how does megakaryocytes–stroma crosstalk work? Is this intracellular communication via extracellular vesicles, or more directly through cell-to-cell contact? Furthermore, is inflammation 'transmitted' from one cell type to another, or is it simply a stress response?

MONOCYTES AS PRO-INFLAMMATORY CELLS IN STROMAL REPROGRAMMING AND FIBROSIS

Beyond megakaryocytes, leukocytes are increasingly recognized as major contributors to myeloid malignancies. In particular, recent studies highlighted that activated monocytes are an important source of pro-fibrotic cytokines and pro-thrombotic factors. In the context of myelofibrosis, Fisher *et al*. [36] used mass cytometry to analyse cytokine secretion in hematopoietic populations. Interestingly, monocytes were found to be the major source of myelofibrosis-associated cytokines. Another study in polycythaemia vera patients confirmed the critical pro-inflammatory function of monocytes, and described the emergence of a phenotypically distinct pro-inflammatory subset upon disease progression [37]. Monocytes may also be critical players in the cross-talk with the MPN microenvironment and induce stromal inflammation [38]. Thus, myeloid cells can drive the activation of fibrosis-driving cells, and future work will not only focus on the fibrosis-driving cells but also the altered 'niche cross-talk' to better understand disease initiation and progression.

Monocytes have also been proposed to foster fibrosis via the generation of fibrocytes. Fibrocytes are monocyte-derived cells that harbour features of both hematopoietic and stromal cells, and they have been shown to be expanded in fibrotic marrows [39]. Using SAP (PRM-151), an inhibitor of fibrocyte differentiation, a study reported a significant decrease of fibrosis in PMF mouse models, providing further evidence for the contribution of monocytes to fibrosis [39]. PRM-151 is an antifibrotic immunomodulator and already tested in phase II clinical trials in idiopathic lung fibrosis.

TARGETING INFLAMMATION TO HALT THE FIBROTIC TRANSFORMATION

Treating bone marrow fibrosis associated with myeloid malignancies is a major clinical challenge and unmet need. The only curative treatment option is an allogeneic stem cell transplant, which aims to restore bone marrow function but is associated with significant risks of morbidity and mortality in elderly patients [40]. Moreover, recent studies show that deregulation of the bone marrow microenvironment, including MSCs, can worsen MPNs, suggesting that treating both the malignant clone and the dysregulated microenvironment is paramount for complete disease resolution. The JAK1/JAK2 inhibitor Ruxolitinib was the first JAK inhibitor to be approved by the Food and Drug Administration (FDA) for the treatment of PMF and improves constitutional symptoms, yet has a very modest effect on fibrosis [41,42]. At present, there are 16 active and recruiting studies registered on clinicaltrials.gov for the treatment of bone marrow fibrosis in the context of MPN. The majority of registered studies involve JAK inhibition, some with combined inhibition of PI3K-delta or lysine-specific demethylase 1A (LSD1), yet no trial aims to specifically target inflammation.

As highlighted above, targeting inflammation in MPN/PMF, potentially even in combination with JAK inhibition, appears to be a promising approach to tackle fibrosis in MPN or potentially in myeloid malignancies per se. A recent study highlights that the bromodomain and extra-terminal (BET) inhibitor JQ1, which targets the constitutively active NfκB pathway, cooperates with Ruxolitinib to reduce inflammatory cytokines in serum, reduce disease burden, and improve bone marrow fibrosis in the murine model of PMF [43]. As the NfκB pathway is also upregulated in CALR-mutated HSPCs [44], it is also likely that BET inhibition would be beneficial in CALR-mediated MPNs. Early data using the BET inhibitor CPI-0610 in PMF patients, either as a monotherapy or with Ruxolitinib, reduced bone marrow fibrosis, spleen size and ameliorated cytopenias [45,46]. The TNF-α inhibitor Etanercept ameliorated patient symptoms yet failed to reduce BM fibrosis [47].

Together, data from these studies suggest that broadly targeting inflammation may not be sufficient to reduce fibrosis but specifically targeting stromal inflammation may prove more successful in reducing bone marrow fibrosis. Our own studies using Tasquinimod also significantly supported the hypothesis that reducing inflammation in myeloid malignancies might be beneficial in at least halting fibrosis progression [18▪]. Our data showed that Tasquinimod treatment ameliorated the MPN phenotype, alleviated the fibrosis-specific anaemia and reduced fibrosis in the JAK2V617F-induced murine model of PMF, highlighting its potential to treat both the 'intrinsic' myeloproliferation and 'extrinsic' inflammation in MPNs.

Targeting specific cell populations, such as megakaryocytes, monocytes or fibrosis-driving cells that communicate by inflammatory signalling, may be a promising approach for MPNs. Yet, it remains unclear whether targeting one cell type alone is effective to reduce fibrosis. Recently, Psaila *et al.* [6▪] highlighted G6B as a marker in JAK2-mutated HSPCs, which can be specifically targeted using a bi-specific antibody and shows promise as a novel immunotherapy. Therapeutically targeting the bone marrow microenvironment, or MSCs specifically, is another promising approach. We previously showed that treating $Gli1^+$ cells, which are fibrosis-driving cells, with the Gli inhibitor GANT61 reduces inflammation and fibrosis, suggesting that selective targeting of Gli is an attractive therapeutic avenue [20].

CONCLUSION

The pathogenesis of MPN illustrates well how inflammation drives hematological malignancies, and particularly, a fibrotic transformation of the stromal microenvironment. Nevertheless, the inflammatory status of the bone marrow does not systematically predict the emergence of fibrosis, suggesting that complex cellular and molecular interactions grant disease progression. The critical contribution of inflammatory cues derived from MSCs, megakaryocytes and monocytes has recently come to light, although many questions remain regarding the cross-talk engaged between these cell types. Dissecting further cell–cell interactions within the inflamed bone marrow niche may provide tractable approaches for therapy.

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Conflicts of interest

There are no conflicts of interest.

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