Disease modifying agents of myeloproliferative neoplasms: a review

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Abstract
The identification of driver mutations in Janus kinase (JAK) 2, calreticulin (CALR), and myeloproliferative leukemia (MPL) has contributed to a better understanding of disease pathogenesis by highlighting the importance of JAK signal transducer and activator of transcription (STAT) signaling in classical myeloproliferative neoplasms (MPNs). This has led to the therapeutic use of novel targeted treatments, such as JAK2 inhibitors. More recently, with the development of next-generation sequencing, additional somatic mutations, which are not restricted to MPNs, have been elucidated. Treatment decisions for MPN patients are influenced by the MPN subtype, symptom burden, and risk classification. Although prevention of vascular events is the main objective of therapy for essential thrombocythemia (ET) and polycythemia vera (PV) patients, disease-modifying drugs are needed to eradicate clonal hematopoiesis and prevent progression to more aggressive myeloid neoplasms. JAK inhibitors are a valuable therapeutic strategy for patients with myelofibrosis (MF) who have splenomegaly and/or disease-related symptoms, but intolerance, refractory, resistance, and disease progression still present challenges. Currently, allogeneic stem cell transplantation remains the only curative treatment for MF, but it is typically limited by age-related comorbidities and high treatment-related mortality. Therefore, a better understanding of the molecular pathogenesis and potential new therapies with the aim of modifying the natural history of the disease is important. In this article, I review the current understanding of the molecular basis of MPNs and clinical studies on potential disease-modifying agents.

Key Words Myeloproliferative neoplasms, Polycythemia vera, Essential thrombocythemia, Myelofibrosis

INTRODUCTION

Classical myeloproliferative neoplasms (MPNs), also called Philadelphia-negative MPNs, are clonal hematopoietic disorders characterized by the excessive production of terminally differentiated blood cells [1]. Classical MPNs include three main diseases: PV, ET, and MF, which have frequent disease-related complications such as venous and arterial thrombosis, hemorrhages, and transformation to acute myeloid leukemia [2]. The identification of driver mutations in JAK2, CALR, and MPL has contributed to a better understanding of disease pathogenesis, implicating near-universal upregulation of JAK-STAT signaling [2, 3], and has led to the development and therapeutic use of novel targeted treatments, such as JAK2 inhibitors [4-6]. More recently, according to the development of next-generation sequencing, additional somatic mutations have been identified that are not restricted to MPNs and occur in other myeloid malignancies, including acute myeloid leukemia (AML) [7, 8], myelodysplastic syndrome (MDS) [9, 10], or in some elderly patients without overt myeloid malignancies [11-13]. Moreover, aging, the bone marrow microenvironment, and other genetic factors such as germline predisposition, order of mutation acquisition, and variant allele frequency have been demonstrated as key factors influencing clonal outgrowth [14].

Treatment decisions for MPN patients are influenced by the MPN subtype, symptom burden, and risk classification. Even though the main objective of ET and PV therapy is to prevent vascular events, there is a need for disease-modifying drugs that can eradicate clonal hematopoiesis and prevent progression to more aggressive myeloid neoplasms [15-17]. JAK inhibitors are a valuable therapeutic strategy for patients with MF who have splenomegaly and/or disease-related symptoms [4, 5, 18]. However, allogeneic stem cell transplantation remains the only curative treatment for MF, but...
it is typically limited by age-related comorbidities and high treatment-related mortality [19, 20]. Therefore, a better understanding of the molecular pathogenesis and potential new therapies to modify the natural course of the disease is important.

Here, the present paper reviews the current understanding of the molecular basis of MPNs and potential disease-modifying therapies.

MOLECULAR LANDSCAPE OF MPNS

Activation of JAK-STAT signaling

Constitutive activation of the JAK-STAT pathway is a hallmark of classical MPNs [2, 3]. Documented drivers in classical MPNs include JAK2V617F and exon 12 mutations [21-24], mutations in MPL [25], and mutations in exon 9 of CALR [26, 27]. The JAK2V617F mutation is the result of a guanine to thymine somatic mutation at nucleotide 1849 in exon 14 of the JAK2 gene, which leads to a single amino acid substitution from valine to phenylalanine at codon 617 [2]. This mutation is found in 95% of PV and 50-60% of ET and primary myelofibrosis (PMF) [21-24]. JAK2 exon 12 mutations have also been found in 1% to 2% of PV patients, most of whom have JAK2V617F negative PV [28].

MPL is a cell surface receptor for thrombopoietin (TPO) [29]. MPL mutations at tryptophan W515 located at the boundary of the transmembrane and cytosolic domains of MPL are present in 3% of ET and 5% of PMF, with the most frequent mutations being W515L and W515K [30]. Several other substitutions, such as W515R, W515A, and W515G, have also been reported [31].

CALR is a protein that resides in the lumen of the endoplasmic reticulum (ER), where it functions as a molecular chaperone for many glycoproteins, assisting in their folding and contributing to calcium homeostasis. The two most frequent mutations are type 1 and type 2 mutations [32, 33]. The type 1 mutation is a 52 bp deletion (c.1092-1143del, p.1367fs*46), whereas the type 2 mutation is a 5 bp insertion (c.1154_1155insTTGTC, p.K385fs*47). The distribution of these CALR mutation types varies depending on the MPN subtype; in PMF, the type 1 mutation is more prevalent than type 2 (70% vs. 13%, respectively), but in ET, type 1 and type 2 mutations are more evenly distributed (51% vs. 39%, respectively) [34].

Other somatic mutations

The recent development of next-generation sequencing has allowed the identification of several mutations in patients with a variety of myeloid neoplasms, including MPNs [2, 35-45]. The most commonly affected genes are those important in epigenetic regulation, messenger RNA splicing, transcriptional mechanisms, and signal transduction. Although reported somatic mutations lack specificity because they can be found in a broad range of myeloid neoplasms, there is evidence to suggest that the identification of certain non-driver mutations in MPN patients is associated with a greater risk of disease progression or shortened survival [19, 46-48].

Mutations in epigenetic regulators: Mutations targeting DNA methylation regulators (TET2, DNMT3A, and IDH1/2) were identified across MPN subtypes. All TET2 mutations in MPN are loss-of-function point mutations or deletions and are present in approximately 10% to 20% of MPN subtypes [49]. DNMT3A mutations are less frequent than TET2 mutations in MPNs (5-10%) [42]. These two types of mutations increase the self-renewal of JAK2V617F hematopoietic stem cells (HSCs) and play an important role in disease initiation [50]. They also induce disease progression when they occur as secondary mutations, but their role in myelofibrosis and leukemic transformation remains elusive [50]. Interestingly, IDH1/2 mutations were mutually exclusive with mutations in TET2, and TET2 loss-of-function mutations were associated with similar epigenetic defects as IDH1/2 mutants [51].

Mutations in EZH2 occur in 13% of patients with MF [37]. EZH2 encodes a histone H3 lysine 27 (H3K27) methyltransferase and is the catalytic subunit of the polycomb repressive complex (PRC2), which is required for maintenance of HSCs [52]. Mutations in ASXL1 occur in approximately 25% of PMF [2], the second most common epigenetic regulator mutation in MPN after TET2 [3, 38]. Similar to EZH2, the loss of ASXL1 impairs PRC2-mediated suppression of leukemia oncogenes in hematopoietic progenitors [53]. As discussed above, the EZH2 and ASXL1 abnormalities result in inhibition of the PRC2 complex, which reduces H3K27 methylation and, in turn, promotes H3K27 acetylation, ultimately exerting a gain-of-function effect through the recruitment of bromodomains and extra-terminal (BET) family proteins [54]. In a JAK2(V617F)/Ezh2-null mouse model, a bromodomain inhibitor significantly attenuated H3K27 acetylation levels at the promoter regions of PRC2 targets and downregulated their expression, leading to the abrogation of MF-initiating cells [55]. Moreover, Kleppe et al. [56] showed that BET inhibitors reduce NF-κB-induced inflammation and bone marrow fibrosis in MPN models, and combination treatment with BET and JAK inhibitors showed improved efficacy.

Mutations in splicing machinery: Mutations in components of the RNA spliceosome machinery, including SF3BI, SRSF2, U2AF1, and ZRSR2, are involved in MPN patients, especially ET and MF, as well as other myeloid malignancies [43, 57]. SRSF2 encodes a protein that is a member of the serine/arginine-rich splicing factor family; it binds to exonic splicing enhancer (ESE) sequences in pre-messenger RNA (pre-mRNA). SRSF2 mutations alter SRSF2’s normal sequence-specific RNA binding activity, thereby altering the recognition of specific exonic splicing enhancer motifs to drive recurrent mis-splicing of key hematopoietic regulators [58]. In particular, mutations in the spliceosome components U2AF1 and SRSF2 are known to lead to mis-splicing and nonsense-mediated decay of EZH2 [58, 59] and are associated with MPNs with poor prognoses [19].
malignancies in samples from patients with post-MPN AML versus chronic MPN phase revealed somatic mutations targeting TP53 tumor-suppressing function in 20% to 30% of patients with leukemic transformation, but are uncommon in the chronic phase of MPN [44, 60]. RUNX1 encodes a transcription factor that binds to enhancers and promoters to regulate normal hematopoiesis [61]. Various RUNX1 mutations and chromosomal aberrations are found in 10% of MPNs to secondary leukemia transitions [45, 62].

Signal transduction gene mutations, such as NRAS, SH2B3, CBL, NFI, and FLT3, are associated with an increased risk of leukemic transformation and typically occur more frequently in the blast phase than in the chronic phase of MPN [2, 60, 62].

**Molecular-based therapy in ET and PV**

Although the efficacy of interferon-α (IFN-α) has shown efficacy in the treatment of ET and PV for more than 20 years and has provided an alternative option to chemotherapeutic agents, toxicity and the need for frequent parenteral application of conventional formulations led to a high proportion of patients discontinuing treatment [63, 64]. Because pegylated (peg) forms have been shown to have increased tolerance and efficacy in IFNα-treated hepatitis patients, there is renewed interest in using pegylated IFN, and clinical trials have been performed to elucidate its role in the upfront and salvage treatment of patients with ET and PV. The objectives included not only high rates of hematologic responses, but also molecular responses in JAK2-mutated ET and PV patients. In a phase 2 trial, with a median follow-up of 42 months, a complete hematologic response was achieved in 76% of patients with PV and 77% of those with ET. This was accompanied by a complete molecular response (CMR) (i.e., undetectable JAK2V617F) in 18% and 17% of PV and ET patients, respectively [65]. In patients with PV, there was a sustained decrease in the allelic burden, from a median of 64% to 8%, in those treated for 60 months. In contrast, the majority of ET patients did not experience a significant decrease in the JAK2V617F allele burden. In patients treated with hydroxyurea or aspirin, the CALR burden remained stable [67]. Ropeginterferon α-2b is a monopegylated IFN-α developed for treating PV, which consists of a single positional isomer resulting in an extended elimination half-life, enabling less frequent dosing and improved tolerability, and supporting long-term patient compliance [68]. As reported in data from an open-label, randomized phase study (PROUD-PV and CONTINUATION-PV) that evaluated the efficacy and safety of ropeginterferon α-2b in comparison to hydroxyurea treatment, ropeginterferon α-2b was more effective in achieving durable hematological and molecular remissions than hydroxyurea and was well tolerated during long-term application [69]. In particular, in the CONTINUATION-PV study, patients treated with ropeginterferon α-2b showed a steady decrease in the mean absolute JAK2V617F allele burden to less than half of the baseline level by month 36. In contrast, in the hydroxyurea group, the reduction was transient and was lost by month 36, suggesting the possibility of the disease-modifying potential of IFN-α-based therapy. Moreover, in a post-hoc analysis, a lower JAK2V617F allele burden correlated with a complete hematologic response at 12, 24, and 36 months in the ropeginterferon α-2b group. However, further study on the clinical relevance of the molecular response during ropeginterferon α-2b is needed.

**Table 1. ‘Add-on’ approaches to ruxolitinib being studied in clinical trials.**

| Agent (class) | Drug class | Phase (NCT number) | Reference |
|--------------|------------|--------------------|-----------|
| CPI-0610     | BET inhibitor | 2 (NCT02158858)   | Mascaréñas et al. [70] |
| Navitoclax   | BCL-2/BCL-xL antagonist | 2 (NCT03222609) | Harrison et al. [71] |
| Umbralisib   | PI3K inhibitor | 1 (NCT02493530)   | Moyo et al. [76] |
| Parsaclisib  | PI3K inhibitor | 2 (NCT02718300)   | Daver et al. [77] |
| Idelalisib   | PI3K inhibitor | 1 (NCT02436135)   | -         |
BET inhibitor, together with a JAK inhibitor, can result in the reduction of serum levels of inflammatory cytokines, bone marrow fibrosis (BMF), and mutant cell burden [56]. A phase 2 study of CPI-0610 alone or as an “add-on” to ruxolitinib (CPI-0610+ruxolitinib) (NCT02158858) provided clinical benefits in MF patients with inadequate responses or those refractory to ruxolitinib. Through improvement in BMF and anemia responses, its potential for disease modification has been suggested [70].

**Targeting anti-apoptotic protein Bcl-xl:** The anti-apoptotic protein Bcl-xl is regulated by JAKs and the combined targeting of JAK2. Furthermore, it has been demonstrated that Bcl2/-xl is synergistic in preclinical JAK2V617F MPN models and helps overcome acquired resistance to ruxolitinib [71]. A phase 2 single-arm, multicenter study (NCT03222609) enrolled adults diagnosed with MF. Eligible patients received ≥12 weeks of continuous ruxolitinib therapy and had persistent splenomegaly that required a new therapy while continuing their current stable dose of ruxolitinib. Navitoclax was initiated at 50 mg QD and was escalated to 300 mg QD based on tolerability. The primary endpoint was the percentage reduction in the spleen volume (SVR) from baseline. Secondary endpoints included the effect on total symptom score (TSS), BMF, anemia response, and safety. Plasma cytokine levels were measured at baseline and at weeks 12, 24, and 48. The median percentage change from baseline was calculated for the 140 panel analytes. The combination of navitoclax with ruxolitinib was well tolerated and led to clinically meaningful SVR, improvements in TSS, encouraging reductions in BMF, and cytokine modulation. This study demonstrated that apoptotic induction with navitoclax might be an important therapeutic option for patients with MF to prevent or reverse JAK2 resistance and to modify MF biology [72].

**Targeting phosphatidylinositol-3-kinase delta:** The phosphatidylinositol-3-kinase/Akt/mammalian target of the rapamycin (PI3K/Akt/mTOR) cascade integrates cellular growth and proliferation signals downstream of JAK-STAT, and constitutive activation of this pathway is central to MPN pathogenesis [73]. Preclinical studies have shown that inhibitors of this pathway, both alone and synergistically in combination with ruxolitinib or fedratinib, reduce proliferation and induce apoptosis of JAK2V617F/MPLW515L MPN cell lines and primary cells [73-75].

Updated results from a trial in which umbralisib, a selective inhibitor of the delta isoform of PI3K with a superior tolerability profile, was “added on” to ruxolitinib (stable dose for ≥8 wk) in patients with an insufficient response to the latter, were recently presented (NCT02493530) [76]. Two out of 23 patients achieved complete response (CR). An additional 11 patients showed clinical improvement based on anemia, spleen, and/or symptom responses. To note, determination of sub-optimal response to ruxolitinib for patient eligibility for this trial was left up to the physician’s discretion. Parsaclisib is another PI3K delta isoform-specific inhibitor that has been studied in combination with ruxolitinib in an ongoing “add-on” trial (NCT02718300); however, a sub-optimal response to ruxolitinib is clearly defined in this trial (palpable spleen >10 cm or 5–10 cm, with active symptoms of myelofibrosis after at least 6 months of ruxolitinib and with a stable dose over the preceding 8 weeks or longer) [77]. Parsaclisib exhibited a good tolerability profile in this trial, but a switch from daily to weekly dosing after 8 weeks of combination therapy (to mitigate toxicities) appeared to correlate with some loss of response. A similar phase 1 trial of idelalisib added to ruxolitinib (stable dose for ≥4 wk) has been completed (NCT02436135).

**Novel agents under study as monotherapies**
Several investigational agents are being studied as mono-therapies in ruxolitinib-resistant or ineligible patients.

**Telomerase inhibitor:** The telomerase inhibitor imetelstat generated considerable excitement when seven durable complete and partial responses from 33 patients with MF, with reversal of BMF in all four patients who had a complete response, were reported in a pilot study [78]. The results from the phase 2 imetelstat trial in patients with DIPSS intermediate-2/high-risk myelofibrosis who had failed therapy with a JAK inhibitor (NCT02426086) were presented at the 2018 American Society of Hematology (ASH) annual meeting [79]. In this study, two doses of imetelstat (9.4 mg/kg or 4.7 mg/kg IV, every 3 wk) were administered to 107 patients with intermediate-2 or high-risk MF that was relapsed/refractory to prior JAK inhibitor therapy (i.e., either no reduction in splenomegaly after 12 weeks or worsening splenomegaly at any time after the start of the JAK inhibitor therapy). The lower dose (4.7 mg/kg) arm (N=48) was closed to new patient entry due to insufficient activity after an interim analysis, and the patients were allowed a dose escalation. At the time of the clinical cutoff (ASH Annual Meeting 2018), 9.4 mg/kg administered every 3 weeks resulted in a 10.2% spleen response and 32% symptom response. Importantly, after a median follow-up of 22.6 months, the median survival in the 9.4 mg/kg arm was not reached, while the median OS was 19.9 months in the 4.7 mg/kg arm [79]. Although no formal study has reported survival for patients who are truly relapsed/refractory to JAK inhibitors, observed OS after imetelstat therapy was in marked contrast to the 13-14 months reported by several groups studying patients who discontinued ruxolitinib [80-82].

**Murine double minute 2 (MDM2) inhibitor:** Preclinical studies have shown that JAK2V617F/MMP leads to overexpression of murine double minute 2 (MDM2) in MPN [83], and upregulation of MDM2 protects the clonal HSCs driving the disease from apoptosis. An open-label phase 2 trial with the MDM2 inhibitor KRT-232 is currently enrolling patients who failed JAK inhibitor therapy (NCT03662126).

**Microenvironment and fibrosis**
Megakaryocytes in PMF exhibit impaired maturation associated with downregulation of the transcription factor GATA1 [84]. These atypical megakaryocytes may contribute to bone marrow fibrosis by releasing cytokines such as trans-
forming growth factor (TGF-β). The aurora kinase A (AURKA) inhibitor alisertib has been shown to promote the differentiation of PMF megakaryocytes and ameliorate bone marrow fibrosis in vivo in mouse models of PMF [85]. In a phase 1 clinical trial of alisertib in 24 patients with myelofibrosis, 63% of whom had prior exposure to JAK inhibitor therapies, alisertib reduced splenomegaly and symptom burden in 29% and 32% of patients, respectively, despite not consistently reducing the degree of inflammatory cytokines. Moreover, correlative studies showed normalization of megakaryocyte morphology, restoration of GATA1 staining, and reduction of BMF in five of seven patients for whom sequential marrows were available. Further study of AURKA inhibition as a therapeutic option for myelofibrosis is planned (NCT02530619) [86]. Fibrosis-driving cells in PMF bone marrow are reported to be clonal, neoplastic, and derived from monocytes [87]. The anti-fibrotic agent, PRM-151, is intravenously administered (every 4 wk) and recombinant pentraxin-2 molecule, also known as serum amyloid protein. The results from 18 patients, 9 of whom received PRM-151 alone and 9 that received a combination with ruxolitinib in an open-label extension study, are presented. The median time of study was 30.9 months, and the drug was well tolerated. The mean best percent change (by palpation) in spleen size from baseline was -37%, with a median percent reduction of -26.1%. The mean best percent improvement in the MPN-SAF TSS was -54%, with a median percent reduction of TSS of -64%. Interestingly, even the patients on PRM-151 monotherapy showed similar benefits in terms of spleen size and symptom burden as those receiving PRM-151+ruxolitinib. In addition, an overall improvement in the BM reticulin and collagen fibrosis grade was observed [88].

There are some potential strategies aimed at reversing bone marrow fibrosis. Galunisertib, a small-molecule inhibitor of the TGF-β receptor 1 kinase ALK5, blocks excessive collagen production in JAK2V617F and MPLW515L mouse models [89]. Sotatercept and luspatercept are activin receptor type IIa ligand traps designed to sequester natural ligands to the TGF-β receptor and inhibit signaling. These agents are currently in clinical trials for patients with MF and anemia [90, 91].

**Immunotherapy**

The effect of recombinant interferon to prevent the development of marked splenomegaly, anemia, and florid myelofibrosis in early myelofibrosis was tested [92]. Early data on the combination of ruxolitinib and pegylated IFN-α from the ongoing RUXOPEG study showed that this combination was generally well tolerated, and the preliminary efficacy results were encouraging [93].

Mutant calreticulin binds to the thrombopoietin receptor, MPL (requirement for the lectin-dependent function of mutant calreticulin to bind to the extracellular domain of MPL) to serve as a potential tumor antigen in MPN [94]. This led to the development of novel, vaccine-based approaches to target this immunogenic mutant protein [95, 96], but these have not yet entered clinical practice.

**CONCLUSIONS**

Although the identification of driver mutations in JAK2, CALR, and MPL of classical MPN have contributed to a better understanding of classical MPN pathogenesis, no drug therapy has clearly been proven to be disease-modifying. Over the past decade, it has been shown that treatment with ruxolitinib improves splenomegaly and associated symptoms regardless of driver mutation status and survival advantage in patients with intermediate-2/high-risk MF. However, drug-related cytopenias, refractory, resistance to ruxolitinib after 2-3 years of therapy, and disease progression remain a challenge [80, 97]. In particular, patients have shown poor survival after ruxolitinib discontinuation, particularly in the presence of clonal evolution and/or declining platelet counts, while on ruxolitinib [80-82, 98]. Therefore, clinical studies to identify novel agents with disease-modifying properties are underway as either solo or ‘add-on’ therapies to ruxolitinib. In addition, strategies targeting bone marrow fibrosis and immunotherapeutic approaches are also being studied.

**Authors’ Disclosures of Potential Conflicts of Interest**

No potential conflicts of interest relevant to this article were reported.

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