Challenges and Perspectives for Therapeutic Targeting of Myeloproliferative Neoplasms

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Abstract
Myeloproliferative neoplasms (MPNs) are hematopoietic stem cell disorders with dysregulated myeloid blood cell production and propensity for transformation to acute myeloid leukemia, thrombosis, and bleeding. Acquired mutations in JAK2, MPL, and CALR converge on hyperactivation of Janus kinase 2 (JAK2) signaling as a central feature of MPN. Accordingly, JAK2 inhibitors have held promise for therapeutic targeting. After the JAK1/2 inhibitor ruxolitinib, similar JAK2 inhibitors as fedratinib are entering clinical use. Resistance mutations, as seen with other tyrosine kinase inhibitors, have not been described in MPN patients suggesting that functional processes reactivate JAK2 signaling. Compensatory signaling, which bypasses JAK2 inhibition, and other processes contribute to intrinsic resistance of MPN cells restricting efficacy of JAK2 inhibition overall. Combinations of JAK2 inhibition with pegylated interferon-α, a well-established therapy of MPN, B-cell lymphoma 2 inhibition, and others are in clinical development with the potential to enhance therapeutic efficacy. Novel single-agent approaches targeting other molecules than JAK2 are being investigated clinically. Special focus should be placed on myelofibrosis patients with anemia and thrombocytopenia, a delicate patient population at high need for options. The extending range of new treatment approaches will increase the therapeutic options for MPN patients. This calls for concomitant improvement of our insight into MPN biology to inform tailored therapeutic strategies for individual MPN patients.

Pathogenesis of myeloproliferative neoplasms
Myeloproliferative neoplasms (MPNs) are clonal hematopoietic stem cell disorders characterized by excessive generation of mature myeloid blood cells. Three subtypes are recognized, including essential thrombocytosis (ET) presenting with isolated thrombocytosis, polycythemia vera (PV) primarily with polyglobulia, as well as primary myelofibrosis (PMF) with progressive bone marrow fibrosis-inducing cytopenias. ET and PV can progress to myelofibrosis (MF) and all 3 forms have a propensity to transform to acute myeloid leukemia (AML). There is an increased risk for thrombotic and hemorrhagic events relevantly contributing to morbidity and mortality.

On the molecular level, hyperactivation of the Janus kinase 2 (JAK2) signaling pathway is a central feature of MPN. JAK2, a nonreceptor tyrosine kinase, is essential for hematopoietic cytokine signaling by propagating activation of erythropoietin, thrombopoietin (myeloproliferative leukemia virus [MPL]), and granulocyte-macrophage colony-stimulating factor receptors. The JAK2 V617F mutation is present in 95% of PV and 50%-60% of ET and PMF patients and induces activation of the JAK2 kinase domain by relieving inhibitory effects of the pseudokinase domain. JAK2 exon 12 mutations induce JAK2 activation in the majority of JAK2 V617F unmutated PV. In ET and MF, the chaperone calreticulin (CALR) and the thrombopoietin receptor MPL are mutated in 30% and 10% of patients, respectively. Activating mutations of MPL such as W515L converge on JAK2 signaling enhancing analogous pathways as JAK2 V617F. In CALR multiple mutations in exon 9 were identified, which fall in 2 broad categories of type 1 with a 52 base pair deletion or type 2 with a 5 base pair insertion. Altered charge of the CALR C-terminus promotes retention of the mutant CALR in the endoplasmic reticulum, where it can stabilize MPL, thus enhancing MPL-JAK2 signaling. As all known driver mutations converge on activated JAK2, the JAK2 signaling pathway represents a key target for therapy. Genetic deletion of Jak2 in a MPL.W515L-driven MPN murine model consistently ablated the MPN clone, suggesting an essential role of activated JAK2 signaling. There remains a small group of patients with “triple-negative” (ie, JAK2 V617F, CALR, and MPL unmutated) ET or PMF. While triple-negative PMF associates with adverse prognosis, this is less clear in ET where low burden canonical driver mutations as well as atypical JAK2 or MPL mutations or polyclonal hematopoiesis have been described.

Additional mutations prevalently observed across myeloid malignancies, frequently occur also in MPN, particularly in MF, and impact on disease dynamics and prognosis. Mutations in AXL, EZH2, SRSF2, IDH1, and IDH2 have an adverse prognostic impact, therefore, termed “high molecular risk” mutations. Negative prognosis has also been attributed to higher
numbers of concomitant mutations.\textsuperscript{20} The order of acquisition of specific targets seems to play a significant role influencing progenitor cell proliferation, clinical presentation, and risk of thrombosis.\textsuperscript{21,22} Several mutations such as TET2 and DNMT3A may occur before or after JAK2 V617F during MPN development. While “JAK2-first patients” seem to predominantly develop PV-phenotype,\textsuperscript{23} patients with DNMT3A or TET2 mutations acquired before JAK2V617F may mostly present with an ET-phenotype.\textsuperscript{22,23} At the time of transformation to secondary AML, TPS3, and IDH mutations are frequently detected.

### JAK2 inhibitors to target MPN pathogenesis

The central role of activated JAK2 signaling in MPN has fueled the development of JAK2 inhibitors. Ruxolitinib, a JAK1/JAK2 inhibitor approved for the treatment of MF\textsuperscript{24} and hydroxyurea resistant or intolerant PV,\textsuperscript{25} has initiated a new era of molecularly targeted therapy for MPN. JAK2/1 inhibition by ruxolitinib excels by effectively reducing splenomegaly and constitutional symptoms which impact on MPN patients’ quality of life. The need for phlebotomies is lowered in PV.\textsuperscript{25} Based on the COMFORT studies in MF and the RESPONSE studies in PV, ruxolitinib represents a current standard in these entities, whereas ruxolitinib was not found beneficial so far for high-risk PV, ruxolitinib represents a current standard in these entities, whereas ruxolitinib was not found beneficial so far for high-risk ET (MAJIC-ET).\textsuperscript{26} Upon longer follow-up, overall survival of MF patients is extended by ruxolitinib therapy.\textsuperscript{27} The basis for this benefit is not conclusively understood, as ruxolitinib decreases the malignant MPN clone only in a limited number of patients.\textsuperscript{24,29} Clonal evolution is not halted suggesting a rather limited disease-modifying potential.\textsuperscript{0,31}

Ruxolitinib represents a type I JAK2 inhibitor targeting JAK2 in its active, phosphorylated form.\textsuperscript{32} Cell-based investigations suggest that the new type I JAK2 inhibitors, which are currently entering clinical use, may share limitations of ruxolitinib and may not be able to decrease the size or the mutational evolution of the MPN clone.\textsuperscript{33,34} Other principles of JAK2 inhibition like type II inhibitors, target the kinase in the inactive conformation, or mutant selective JAK2 inhibition need to be evaluated for their potential to suppress oncogenic JAK2 signaling more profoundly. Genetic ablation of mutant JAK2 in MPLW515L-driven MPN mouse model abrogates the MPN clone which implies JAK2 dependence of the MPN clone, confirming JAK2 as a central therapeutic target, at least in the mouse models.\textsuperscript{15}

Given current JAK2 inhibitors are not mutant selective, ruxolitinib represents a treatment option both for JAK2 mutated and unmutated MPN patients but relates to anemia and thrombocytopenia. These cytopenias are most prominent at treatment initiation and may stabilize in the further course. Another limitation increasingly evident in clinical practice is loss of responses to ruxolitinib which may occur over time. As shown by long-term follow-up of COMFORT-I, 50% of MF patients lose response over 5 years\textsuperscript{27} suggesting the MPN clone acquires resistance. It is an active area of research which underlying mechanisms drive emergence of resistance to ruxolitinib, as discussed in detail below. As indicated by in vitro studies, cross-resistance extending to new type I JAK2 inhibitors may also occur. Attention has been raised to an increased incidence of herpes zoster as well as nonmelanoma skin cancer in MPN patients treated with ruxolitinib.\textsuperscript{35,36} It is hypothesized that these rare side effects relate to reduced immune surveillance upon JAK1 inhibition with ruxolitinib. In contrast, ruxolitinib-mediated JAK1 inhibition is increasingly utilized for treatment of graft versus host disease to mitigate alloimmune effects.

Despite its limitations, ruxolitinib brings relevant clinical benefit to MPN patients, and similar type I JAK2 inhibitors are currently being developed (Table 1). Fedratinib, a JAK2/FMS-like tyrosine kinase 3 (FLT3) inhibitor, has recently been approved for the treatment of intermediate or high-risk MF. As demonstrated in the JAKARTA trials, its main benefit is in reducing splenomegaly, while the most prevalent adverse events are anemia and thrombocytopenia.\textsuperscript{39} Gastrointestinal side effects relate to its FLT3 inhibitory activity. The approval of fedratinib gives us a second JAK2 inhibitor at hand and broadens our options for targeted therapy of MPN. Of note, the risk of Wernicke’s encephalopathy needs to be considered as highlighted in a black box warning, as clinical development was intermittently halted due to cases of this serious complication. Interference of fedratinib with intestinal thiamine uptake has been reported, and although not conclusive, thiamine levels require monitoring upon fedratinib treatment. The upcoming clinical use of fedratinib in ruxolitinib pretreated patients will inform us on its benefit in patients who lost response to ruxolitinib, even though cell-based studies suggest cross-resistance among these type I JAK2 inhibitors.\textsuperscript{33,34}

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**Table 1**

Selected Clinical Studies With JAK Inhibitors in MPN.

| JAK Inhibitors | Clinical Trial | Design | Clinical Impact | Main Side Effects |
|----------------|---------------|--------|----------------|------------------|
| Ruxolitinib    | MF: COMFORT-I phase 3\textsuperscript{24,27} vs Placebo | Spleen volume reduction | Anemia, thrombocytopenia |
|                | MF: COMFORT-II phase 3\textsuperscript{25,27} vs BAT | Reduction of constitutional symptoms | |
|                | PV: RESPONSE phase 3\textsuperscript{25,36} vs BAT | Survival benefit | |
|                | PV: RESPONSE-2 phase 3\textsuperscript{28} vs BAT | Spleen volume reduction | |
|                | ET: MAJIC-ET phase 2\textsuperscript{29} vs BAT | Hematocrit control | |
|                | MF: JAKARTA phase 3\textsuperscript{30} vs Placebo | Reduction of constitutional symptoms | |
|                | MF: JAKARTA-2 phase 2\textsuperscript{31} vs Placebo | Nonsignificant benefit (complete hematologic response) | |
|                | MF: SIMPLIFY-1 phase 3\textsuperscript{32} vs Rux naive | Spleen volume reduction | |
|                | MF: SIMPLIFY-2 phase 2\textsuperscript{33} vs Rux refractory | Spleen volume reduction (n.s.) | |
|                | MF: PERSIST-1 phase 3\textsuperscript{34} vs BAT | Reduction of constitutional symptoms | |
|                | MF: PERSIST-2 phase 3\textsuperscript{35} vs Rux, in thrombocytopenia | Decreased transfusion dependence | Anemia, thrombocytopenia |

Key studies on JAK2 inhibitors in clinical development are indicated including design, main clinical impact, and most important side effects.

BAT = best available therapy; ET = essential thrombocythemia; JAK = Janus kinase; MF = myelofibrosis; MPNs = myeloproliferative neoplasms; n.s. = non significant; PV = polycythemia vera; Rux = ruxolitinib.
Momelotinib and pacritinib are JAK2 inhibitors with promising profiles for treatment of MF patients with anemia and thrombocytopenia. As they represent a delicate population at high need for options, JAK2 inhibitors tolerable in this setting are desirable. Momelotinib is a JAK1/JAK2 inhibitor similar to ruxolitinib. Additionally, it seems to mitigate anemia via inhibition of type I activin A receptor and decreased hepcidin production.\(^{45}\) Momelotinib has shown benefits in promoting transfusion independence in MF in the SIMPLIFY trials. However, it has not met the endpoint of \(\geq 35\%\) splenomegaly reduction.\(^{41,42}\) Low-grade peripheral neuropathy appeared in almost half of the patients and was mostly irreversible highlighting that benefits and risks need to be well weighed. Pacritinib is a JAK2/FLT3 inhibitor with a particularly nonmyelosuppressive profile.\(^{46}\) In patients with thrombocytopenia \(\leq 100 \times 10^9/L\), pacritinib was tolerable and effective in reducing spleen volume and symptom burden.\(^{44}\) After a clinical hold on the PERSIST studies has been lifted, a renewed phase 3 study in MF with thrombocytopenia is ongoing (PACIFICA). Other type I JAK2 inhibitors such as NS-018 targeting JAK2 and SRC kinases are also in clinical development in line with the central role of JAK2 activation in MPN.

### Resistance to JAK inhibitors

Resistance to tyrosine kinase inhibitors represents a key challenge for the therapy of hematological malignancies and solid tumors\(^ {47}\) and also affects JAK2 inhibitors. It has been shown that response to ruxolitinib is lost in 50\% of patients who initially benefit in a 5-year period.\(^ {27,35}\) Several mechanisms for occurrence of acquired resistance have been proposed.\(^ {48}\) Furthermore, JAK2 inhibition is also hampered by intrinsic resistance of MPN cells interfering with therapeutic efficacy of ruxolitinib overall (Figure 1 and Table 2). The molecular basis of intrinsic resistance to JAK2 inhibition is increasingly understood and proposes targets for combination therapy approaches to enhance therapeutic efficacy.

### Acquired resistance to JAK2 inhibition

#### Genetic resistance

Acquisition of second-site mutations interfering with inhibitor binding have been described in several tyrosine kinases targeted by inhibitors such as BCR-ABL. Of note, second-site mutations in JAK2, which mediate resistance to JAK2 inhibition have not been detected in MPN patients so far. In vitro mutagenesis screens detected resistance mutations in JAK2 including Y931C, G935R, and E864K, which induced partial cross-resistance with other type I JAK2 inhibitors as fedratinib and momelotinib (Figure 1). Interestingly, the JAK2 “gate-keeper” mutation M929I conferred resistance to ruxolitinib, but not to other JAK2 inhibitors.\(^ {53,54}\) In rare patients with hereditary thrombocytosis, germline JAK2 kinase domain mutations mediate insensitivity to JAK2 inhibitors.\(^{49}\) However, these mutations are not acquired under treatment but are preexistent. It is not completely understood why JAK2 resistance mutations are not observed in patients on ruxolitinib although occurring in vitro. Insufficient selective pressure of JAK2 inhibition may play a role.

#### Adaptive resistance by JAK heterodimer formation

MF patients losing response to ruxolitinib were reported to regain sensitivity after pausing JAK2 inhibition with renewed benefit upon reexposure.\(^ {56}\) This observation suggests that acquired resistance relates to adaptive, reversible processes in MPN cells. Long-term exposure to JAK2 inhibitors, including ruxolitinib, fedratinib, and momelotinib, induces resistance of MPN cells via formation of heterodimers of JAK2 with other...
JAK family members such as JAK2-JAK1 and JAK2-tyrosine kinase 2 (TYK2) able to reactivate JAK2 signaling in presence of JAK2 inhibitors33,34 (Figure 1). This adaptation at the signaling level has been confirmed in primary samples from MPN patients on ruxolitinib as well as in murine models. Resistant MPN cells remain dependent on JAK2 and are still affected by JAK2 level has been confirmed in primary samples from MPN patients on ruxolitinib as well as in murine models. Resistant MPN cells remain dependent on JAK2 and are still affected by JAK2 inhibition.34 Adaptive resistance via JAK heterodimer formation was shown to be reversible, which fits the clinical observation of renewed sensitivity to ruxolitinib after a drug holiday.56 Intermittent treatment with JAK2 inhibition may represent an appealing approach. However, cytokine rebound upon cessation of ruxolitinib poses a challenge to this strategy and therapeutic effects may be impeded by reduced target inhibition.57

It has recently been shown that JAK2V617F, unlike wildtype JAK2, has the ability to induce ligand-independent dimerization of the homodimer type I cytokine receptors, which cannot be prevented by ruxolitinib. JAK2 pseudokinase domain seems to be indispensable for the ligand-independent dimerization.58 Besides further elucidating the mechanism of pathogenesis in MPN, this phenomenon may also contribute to the occurrence of resistance to JAK2 inhibitors.

### Intrinsic resistance to JAK2 inhibition

Resistance mechanisms intrinsic to MPN cells which moderate the impact of JAK2 inhibitors, are increasingly understood and propose additional factors, which also need to be targeted to increase therapeutic efficacy of JAK2 inhibitor therapy. 

**Compensatory mitogen-activated protein kinase pathway activation**

Bypass signaling via cell surface tyrosine kinase receptors is involved in resistance to kinase inhibitors in several cancers. Platelet-derived growth factor receptor alpha (PDGFRα) remains activated in MPN in vivo settings upon treatment with ruxolitinib. While JAK2 inhibitors effectively suppress the mitogen-activated protein kinase (MAPK) signaling pathway in vitro, platelet-derived growth factor-PDGFRα signaling is able to bypass JAK2 inhibition in MPN mouse models and mediates compensatory activation of the MAPK pathway (Figure 1). Combined targeting of JAK2 and MAPK/ERK kinase 1/2 (MEK1/2), intermediate kinases in the MAPK pathway, improves therapeutic efficacy of ruxolitinib, suggesting that compensatory MAPK pathway activation is limiting the effects of JAK2 inhibitor therapy.59 These findings propose the MAPK pathway as a mediator of resistance to JAK2 inhibition, which needs to be targeted to improve efficacy of JAK inhibitor therapy.

**Resistance mediated by the bone marrow microenvironment**

Mechanisms of resistance to JAK2 inhibition may also relate to the bone marrow (BM) microenvironment constituted by multiple components including hematopoietic cells as well as stroma with mesenchymal stem cells, endothelium, osteoblasts, neurons, and Schwann cells. Increased levels of inflammatory cytokines are characteristic of the BM microenvironment in MPN patients and murine models.59,60 Paracrine effects from the inflammatory milieu of the BM microenvironment on the MPN clone have been implicated in JAK2 inhibitor resistance. Primary JAK2V617F mononuclear cells from a PMF patient co-cultured with stromal cells were spared from inhibitory effects of a JAK2/JAK3 inhibitor, while sensitivity was restored upon neutralization of inflammatory cytokines suggesting protective effects of the inflammatory niche on the MPN clone.51 Cellular components could also interfere with JAK2 inhibitor efficacy. JAK2V617F positive fibrocytes in bone marrow are not decreased by ruxolitinib suggesting they could contribute to JAK2 inhibitor resistance.52 Of note, an impact of the sympathetic nervous system on protective nestin+ mesenchymal stem cells,62 which support expansion of human HSCs,63 has also been reported. The loss can be restored by treatment with β3-adrenergic agonist, but not with ruxolitinib,64 suggesting this aspect of MPN pathogenesis could also contribute to the ruxolitinib resistance. Further studies are warranted to understand the specific roles of BM microenvironment components in resistance to JAK2 inhibition.

**Ruxolitinib and clonal evolution in MPN**

The order by which mutations are acquired by the MPN clone impact on proliferation of hematopoietic progenitors, clinical presentation, and risk of thrombotic complications, and in addition, could also be important for sensitivity to JAK2 inhibitor therapy. It has been observed that cells from JAK2/TET2 double mutant MPN patients responded better to ruxolitinib when JAK2V617F was acquired first.21 Overall, current JAK2 inhibitors only modestly impact on MPN clone size, although the MPN clone remains JAK2-dependent in the resistance setting.25 In concordance with the notion of limited disease-modifying potential, ruxolitinib does not prevent clonal evolution of MPN with acquisition of additional mutations.30,31 In PMF, 35% of patients develop at least one additional mutation during ruxolitinib treatment, mostly in ASXL1, TET2, EZH2, and TP53. Notably, of these, ASXL1, EZH2, and TP53 mutations associate with adverse prognosis. Clonal evolution with mutations in IDH1, IDH2, and DNMT3A has also been described.20 It becomes evident that clonal evolution in MPN, also when occurring on JAK2 inhibitor treatment, directly impacts on prognosis with shortened survival. This highlights the limited disease-modifying potential of JAK2 inhibitor single-agent therapy calling for enhanced treatment strategies.

### Table 2

**Mechanisms of Resistance to JAK Inhibitors.**

| Type of Resistance | Resistance Mechanism | In Vitro/In Vivo | Reversible | References |
|--------------------|----------------------|-----------------|------------|------------|
| Primary (intrinsic) resistance | Preexisting resistance mutations | Reported in hereditary thrombocytosis | No | Marty et al49 |
| | Bypass signaling via RTK (e.g., PDGFR) | ++/+ | No | Sriviva et al50 |
| | Protective effects of cytokines | ++/+ | Not known | Manshouri et al51 |
| | Bone marrow-fibrocyte-resistance in myelofibrosis | ++/+ | Not known | Verstovsek et al52 |
| Secondary (acquired) resistance | Acquired resistance mutations | ++/- | No | Weigert et al53 |
| | Functional resistance by formation of JAK-heterodimers | ++/+ | Yes | Koppikar et al54 |
| | | | | Meyer et al55 |

Resistance to JAK inhibitors in myeloproliferative neoplasm may occur via intrinsic (primary) mechanisms or may be acquired (secondary) upon prolonged exposure to JAK inhibitors. JAK = Janus kinase; PDGFR = platelet-derived growth factor receptor; RTK = receptor tyrosine kinase.
Novel therapeutic approaches in MPN treatment

To improve therapeutic efficacy, combination therapy approaches addressing additional targets besides JAK2 as well as innovative single-agent therapies need to be explored. Insight into MPN biology has deepened in recent years, and mechanisms of resistance are increasingly understood. Many new targets for therapeutic intervention have been proposed and clinical and preclinical investigations are under way (Tables 3 and 4 and Figure 2).

Combination therapies with JAK2 inhibitors

Genetic studies in MPLW515L-driven MPN mouse model show that the MPN clone remains dependent on activated JAK2 signaling suggesting JAK2 should be targeted. Thus, JAK2 inhibitors represent a rational constituent of MPN therapy. Simultaneous targeting of additional factors involved in MPN development or JAK2 inhibitor resistance has the potential to synergize and enhance specific therapeutic aspects (Table 3).

Targeting parallel signaling pathways: phosphoinositide 3-kinase/AKT and mitogen-activated protein kinase

Activated JAK2 signaling in MPN promotes proliferation, differentiation, and cell survival via activation of phosphoinositide 3-kinase (PI3K)/AKT, signal transducer and activator of transcription 3/5, and MAPK signaling pathways. Both PI3K/AKT and MAPK signaling pathways have been implicated in limiting response to JAK2 inhibitors. Synergism of JAK2 and PI3K/AKT inhibition has been reported in MPN cells, and both AKT and PI3K inhibitors are active in MPN murine models. Preclinical studies demonstrated improved efficacy of combined PI3K inhibition by BEZ235 and ruxolitinib, leading to phase I/II clinical studies evaluating PI3K inhibitors as idealisib, paracelsib, and umbralisib in combination with ruxolitinib in MF (Table 3).

MAPK signaling has been implicated in JAK2 inhibitor resistance in vitro. For in vivo settings, we have shown that PDGFRα signaling bypasses JAK2 inhibition mediating compensatory activation of MEK/extracellular signal-regulated kinases (ERKs). Thus, the MAPK pathway represents an additional therapeutic target in MPN which should be addressed to improve therapeutic efficacy of JAK2 inhibition. Combined JAK2 and MEK inhibition by ruxolitinib and binimetinib enhances treatment effects both in JAK2 and MPL mutant settings evident in murine models and clinical isolates from MPN patients. Combined JAK2/MEK inhibition was particularly effective in reducing bone marrow fibrosis.

Targeting epigenetic regulation

MPN associate with global perturbations of DNA methylation and mutations in epigenetic regulators as DNMT3A, ASXL1, EZH2, IDH1, IDH2, and TET2, which are frequent in other myeloid malignancies, are also prevalent in MPN. The hypomethylating agents (HMAs) azacitidine and decitabine are effective in reducing bone marrow fibrosis. Pan-histone deacetylase inhibition is also explored in MPN to interfere at epigenetic levels in combination with JAK2 inhibiting MEK. Thus, combined HMA/ruxolitinib could provide an intensified therapeutic target for patients with advanced MF and leukemic transformation, a population at a high need for options.

Pan-histone deacetylase inhibition is also explored in MPN to interfere at epigenetic levels in combination with JAK2 inhibition. Histone acetylation determines accessibility of DNA and transcriptional activity and histone deacetylase inhibitors such as panobinostat promote acetylation of histones H3 and H4.
Interfering with JAK2 signaling via increased acetylation of the chaperone heat shock protein 90 (HSP90). JAK2 represents a HSP90 client protein and preclinical studies showed superior effects of combined panobinostat/ruxolitinib in JAK2 mutant MPN. Subsequent phase I/II studies reported responses of splenomegaly and anemia in MF patients. A phase 2 study on long-term tolerability and efficacy of givinostat in MPN is currently active (NCT01761968).

Epigenetic mechanisms are specifically at play to promote the inflammatory milieu in MPN characterized by increased levels of inflammatory cytokines. Inflammation in MPN contributes to constitutional symptoms of patients and relates to activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling. Chromatin changes involving the histone lysine reader bromodomain-containing protein 4 (BRD4), a member of the bromodomain and extraterminal domain (BET) family of proteins, enhances NF-κB signaling, while BRD4/BET inhibition was able to reduce NF-κB pathway activity in MPN mouse models. Combined BRD4/JAK2 inhibition showed promising results in reducing fibrosis and mutant allele burden. Currently, the combination of the BRD4/BET inhibitor CPI-0610 and ruxolitinib is investigated in ruxolitinib pretreated and naïve MF patients in a phase 1/2 study (Table 3). Positive effects, particularly in regard to reduced constitutional symptoms, have been reported. Furthermore, combined BRD4/BET and JAK2 inhibition effectively induced apoptosis in blasts of patients with secondary AML progressed from MPN.

Pevonedistat, which interferes with NF-κB signaling via inhibition of neuronal precursor cell-expressed developmentally down-regulated protein 8 activating enzyme, is also being tested in combination with ruxolitinib in a phase 1 study in MF (NCT03386214).

Table 4
Novel Single-Agent Therapies for MPN in Clinical Development.

| Target | Category | Target Drug | Eligible Diagnosis (MPN) | Study Phase | Clinical Trial Identifier |
|--------|----------|-------------|--------------------------|-------------|--------------------------|
| JAK kinases | JAK1/2 | Momelotinib vs ruxolitinib | MF | 3 | NCT01969838 |
| | | Momelotinib vs danazol | MF | 3 | NCT04173494 |
| | | NS-018 | MF | 1/2 | NCT01423851 |
| JAK2 | Fedratinib | MF | 3 | NCT03755518 |
| | Pacritinib | MF | 2,3 | NCT03165734 |
| | LY2784544 | PV, ET, MF | 2 | NCT01594723 |
| | Itacitinib | MF | 2 | NCT01633372 |
| Interferon-α | Pegylated interferon-α | Peg-IFN-α-2b vs IFN-α | ET | 4 | NCT04226950 |
| | Peg-IFN-α-2a vs IFN-α | ET | 4 | NCT04226950 |
| | Peg-IFN-α-2b vs HU | PV, ET, MF | 3 | NCT01387763 |
| | Peg-IFN-α-2a | PV, ET, MF | 2 | NCT00452023 |
| | Pegylated-proline-interferon-α-2b | Roquep-IFN-α-2b (AOP2014) vs BAT | PV | 2,3 | NCT02218047, NCT03003325 |
| Telomerase | Telomerase | Imetelstat | PV, ET | 2 | NCT01243703 |
| | | | MF | 2 | NCT02426486 |
| Cell cycle | MDM2 | KRT-232 | PV, MF | 2 | NCT03662126 |
| | PIM kinase | TP-3654 | MF | 1 | NCT04176198 |
| | Aurora kinase | Alisertib | MF | 1 | NCT02530619 |
| | Exportin 1 | Selinexor | MF | 2 | NCT03627403 |
| Epigenetics | HDAC | Givinostat | PV, ET, MF | 2 | NCT01761968 |
| | LSD1 | IMG-7289 | PV, ET | 2 | NCT04262141 |
| | | | ET | 2 | NCT04254978 |
| | | | MF | 2 | NCT03136185 |
| | BET | INCB057643 | MF | 1 | NCT04271847 |
| Fibrosis | TGF-β signaling | Solatrecept | MF | 2 | NCT01712308 |
| | | | LSUratecept | MF | 2 | NCT03194542 |
| | | | TGF-β trap (AVID200) | MF | 1/2b | NCT03693112 |
| | SAP/pentraxin 2 | PIM-151 | MF | 2 | NCT01981850 |
| | Other targets | Pemolizumab | MF | 2 | NCT03085400 |
| | | | CD123 | MG8453 + NS593 + spartalizumab/decitabine | MF | 2 | NCT04283526 |
| | | | SMAC mimic | LCL161 | MF | 2 | NCT02286825 |
| | | | HSP90 | PU-H71 | MF, MPN | 1 | NCT03935555, NCT01930509 |

Overview of ongoing clinical studies investigating a therapeutic potential of single-agent therapies in MPNs. BAT = best available therapy; BET = bromodomain and extraterminal domain; ET = essential thrombocythemia; HDAC = histone deacetylase; HSP90 = heat shock protein 90; HU = hydroxyurea; IFN-α = interferon-alpha; JAK = Janus kinase; LSD1 = lysine-specific histone demethylase 1; MDM2 = mouse double minute 2 homolog; MYH = myelofibrosis; MPNs = myeloproliferative neoplasms; PIM = proviral integration site for Moloney murine leukemia virus; PD-1 = programmed cell death protein 1; PV = polycythemia vera; SAP = serum amyloid P component; SMAC = second mitochondria-derived activator; TGF-β = transforming growth factor beta; TIM-3 = T cell immunoglobulin and mucin domain-containing protein 3.
the treatment of AML and BCL-2 inhibition is increasingly explored in MPN. Preclinical studies in JAK2 mutant acute leukemia showed increased efficacy by combined BCL-2/JAK2 inhibition, and BCL-2 inhibition could overcome resistance to JAK2 inhibition in MPN cells. Clinical studies of navitoclax in combination with ruxolitinib or as a single agent are evaluating the potential in MF (Table 3) and encouraging results have been reported in regard to leukocytosis control in ruxolitinib pretreated MF patients.

**Targeting chaperone proteins**

HSP90 is a chaperon protein responsible for correct folding of many signaling proteins including BCR-ABL, FLT3, AKT, and rapidly accelerated fibrosarcoma. PU-H71, a HSP90 inhibitor, induces degradation of JAK2 suggesting also JAK2 as a HSP90 client. Treatment with PU-H71 showed promising results in preclinical MPN models normalizing cytoses and improving survival without myelosuppression. HSP90 inhibition was also able to overcome acquired resistance to JAK2 inhibition.

Several early clinical studies on combined PU-H71/ruxolitinib or PU-H71 as single agent in MF are currently active (Tables 3 and 4). Studies of the HSP90 inhibitor luminespib (AUY922) were halted due to occurrence of gastrointestinal bleeding.

**Targeting cell cycle regulators**

Proviral integration site for Moloney murine leukemia virus (PIM) kinases are implicated as oncogenes in several cancers and PIM activation enhances activation of cyclin-dependent kinase 4/6 (CDK4/6) and JAK2 inhibition. Triple inhibition of PIM, CDK4/6, and JAK2 is under evaluation in MF.

**Immune modulation**

Interferon α (INFα) has long been used to treat MPN given its anti-proliferative and immunomodulatory effect. Furthermore, INFα is able to induce molecular remissions suggesting it as a modality with disease-modifying potential. INFα appears to activate dormant HSCs in mice inducing exhaustion and elimination of JAK2V617F MPN-propagating stem cells. Despite these benefits, INFα therapy is hampered by flu-like side effects often leading to treatment discontinuation. Pegylated forms of INFα have mitigated side effects and increased tolerability and adherence. A combination therapy approach of pegylated INFα with ruxolitinib represents a promising approach and is explored in several studies. While INFα has disease-modifying
activity with reduced MPN clone size, JAK1/JAK2 inhibition with ruxolitinib effectively reduces proliferation and inflammatory symptoms including IFNα-induced side effects, which could lead to improved efficacy, disease modification, and tolerability. Clinical evaluation of pegylated IFNα-2a and ruxolitinib in a phase II study of PV and MF showed substantial reductions of JAK2V617F allele burden with 41% of patients showing a molecular response along with improved cytosis and fibrosis as well as acceptable toxicity (COMBI Study, Table 3).86 The ongoing RUXOPEG study so far showed efficacy in terms of splenomegaly and cytoses along with a favorable tolerability profile and JAK2V617F allele burden reductions at 6 months of treatment.87 These findings demonstrate that pegylated IFNα adds substantial clone suppression to ruxolitinib therapy and that the combination with ruxolitinib improves tolerability of IFNα therapy.

New approaches in MF patients with anemia and thrombocytopenia

Anemia and thrombocytopenia are predictors of shortened survival in MF90 and remain the most common adverse events in patients on ruxolitinib.24 MF patients with these conditions remain the most challenging group to treat. Sotatercept and luspatercept are chimeric trap molecules that prevent ligand binding to activin receptor IIa and IIb, respectively, promoting terminal erythroid differentiation.88,89 Thus, they represent promising anti-anemia agents and are currently being clinically evaluated in MF patients with anemia, both as a monotherapy and in combination with ruxolitinib (Table 4). Six out of 17 (35%) MF patients with anemia on sotatercept showed an anemia response.90 In addition, immunomodulatory imide drugs are investigated for anemia management in MF as single agents and in combination with JAK2 inhibition. Thalidomide and ruxolitinib improved platelet counts in 75% of the MF patients with thrombocytopenia and studies on pomalidomide combinations are ongoing.91 Novel type I JAK2 inhibitors, specifically momelotinib and pacritinib, appear less myelosuppressive and could provide valuable options for MF patients with anemia or thrombocytopenia.41-44

Single-agent therapies

Increasing insight into MPN biology is proposing a growing number of innovative targets for therapy. Several approaches are being tested as an alternative to JAK2 inhibition, rather than a combination with JAK2 inhibitors (Figure 2 and Table 4).

Pegylated interferon-α

Interferon-α has been utilized for MPN therapy with promising results decades before the advent of JAK2 inhibitors, although the specific mechanistic effects relating to interferon-α-mediated immunomodulation are still not entirely dissected. However, flu-like side effects have limited a more extensive use of interferon-α. Modified forms as pegylated IFNα-2a (Peg-IFNα-2a) have substantially improved tolerability and adherence to interferon-α in patients with ET and PV. Once weekly, instead of daily application enabled by a longer half-life and reduced side effects has lowered treatment-related burden and improved the net benefit of interferon-α therapy. Peg-IFNα-2a as a single agent is able to induce molecular responses along with hematologic remissions and limited toxicity in a majority of PV and ET patients.92-94 There is increasing evidence that Peg-IFNα-2a also represents a valid treatment approach in MF mediating decreased mutant allele burden and increased overall survival.95 Interestingly, JAK2V617F mutant MPN cells may preferentially respond to Peg-IFNα-2a as compared to a CALR mutant setting. Higher molecular responses in JAK2V617F than CALR mutant patients have been described.96 Further studies are needed to elucidate the basis for disease modification by interferon-α in MPN.

Pegylated-proline-interferon-α-2b (Ropeg-IFNα-2b) represents another pegylated form of interferon-α with biweekly application and favorable tolerability profile. Direct comparison of Ropeg-IFNα-2b versus best available therapy, including hydroxyurea in PV, showed that Ropeg-IFNα-2b induces meaningful molecular remissions after 3 years of treatment correlating with hematologic response, while tolerability was similar to best available therapy (PROUD-PV, CONTINUATION-PV).97 Ropeg-IFNα-2b has been approved in Europe as monotherapy in PV and is investigated in a phase 2 study for MF. Direct comparison of Ropeg-IFNα-2b to standard therapy in ET is ongoing (Table 4).

Telomerase inhibition

Innovative approaches have focused on interfering with telomere function by inhibition of telomerase. Imetelstat is a 13-mer oligonucleotide telomerase inhibitor covalently modified with lipid extensions. Imetelstat has been successfully tested in ET and MF patients, although patient numbers were small (n = 18 and 33, respectively). In ET, imetelstat induced complete hematologic response in 89%,98 while in MF, 21% of patients achieved hematologic remissions.99 Larger studies are awaited to consolidate these findings. The association of telomerase inhibition with significant myelosuppression poses a caveat for its broad use in ET patients, who are often oligo- or asymptomatic, as well as in MF patients given their prevalent preexistent cytopenias. A potential benefit of imetelstat in MF patients refractory to JAK2 inhibition is of interest and under evaluation (Table 4).

Anti-fibrotic agents

Bone marrow fibrosis represents a key feature of progressed MPN, particularly MF, mediating cytopenia. Cellular components, as well as soluble factors, have been implicated in fibrogenesis in MPN. Atypical megakaryocytes abundant in MF are thought to promote bone marrow fibrosis via secreted cytokines. Heterozygous deletion as well as pharmacologic inhibition of Aurora kinase A reduced fibrosis in MPN in vivo models.100 Alisertib, an Aurora kinase inhibitor evaluated in a phase 1 study in MF, normalized the megakaryocyte lineage and reduced bone marrow fibrosis validating Aurora kinase A as a therapeutic target in MF.101 In addition, clonal monocytedervived fibrocytes have been implicated in bone marrow fibrosis in MF. Inhibition of fibrocyte differentiation by recombinant serum amyloid P component/pentraxin-2, reduced fibrosis in preclinical models.102 Clinical investigations of PRM-151, a recombinant human pentraxin-2 molecule, have shown good tolerability and improved bone marrow fibrosis so far (Table 4). Additional approaches to interfere with bone marrow fibrosis include direct targeting of the fibrogenic cytokine transforming growth factor beta (TGF-β). AVID200 is a recombinant TGF-β receptor-Fc fusion protein acting as a TGF-β1/β3 trap currently investigated in MF. Inhibition of glycogen synthase kinase-3β by a selective inhibitor 9-ING-41 has also shown potential to decrease TGF-β-induced fibrosis102 in MF.

Additional approaches for therapeutic targeting

Therapeutic approaches with evident benefit in AML or other hematologic malignancies are evaluated for efficacy in MPN. They may enhance apoptosis as the mouse double minute 2 homolog (MDM2) inhibitor idasanutlin, the nuclear export inhibitor selinexor, or second mitochondria-derived activator (SMAC) mimetics. MDM2 represents a negative regulator of the tumor suppressor P53 and shows increased expression in MPN.103 The MDM2 inhibitor KRT-232 is under investigation in MF (Table 4). Selective inhibitors of nuclear export processes have shown proapoptotic effects in leukemia settings.104 A clinical
study in MF with the nuclear export inhibitor selinexor inhibitor is ongoing. Second mitochondria-derived activator mimetics interfere with regulators of caspases and beyond AML and myelodysplastic syndrome they hold promise in MF.103 Targeting epigenetic modification processes as with inhibitors of lysine-specific histone demethylase 1 as well as interference with cytokine signaling via interleukin 3 receptor CD123 by tagraxofusp106 has shown promising effects in hematologic malignancies as AML and is under investigation in MPN (Table 4). Immune checkpoint inhibition, which is a promising strategy in solid tumors and hematologic malignancies is explored also for MPN.107 Programmed death-ligand 1/programmed cell death protein 1 signaling seems to play an important role in immune escape in jak2V617F-driven MPN.108 while pembrolizumab, an anti-PD1 monoclonal antibody, is evaluated as monotherapy in MF (Table 4).

**Alternative JAK2 inhibition**

While novel targets involved in apoptosis, cell cycle, epigenetic regulation, and fibrosis are explored, JAK2 remains an important target for the therapy of MPN. Alternative modes to inhibit JAK2, which could provide enhanced efficacy selectively addressing the MPN clone while sparing wild-type hematopoiesis, are of ultimate need. JAK2 inhibitors in current clinical development represent type I JAK2 inhibitors binding to the adenosine triphosphate pocket in the active form of the kinase. Type II JAK2 inhibitors (CHZ868, BBTS594) have recently been explored. They target the inactive conformation of JAK2 and are able to overcome resistance to ruxolitinib. CHZ868 has shown enhanced efficacy in jak2V617F-driven malignancies including MPN109 and B-cell acute lymphoblastic leukemia.109 Of note, type II JAK2 inhibition with CHZ868 preferentially inhibited mutant hematopoietic cells and decreased MPN clone size in preclinical models.110 However, due to their potency, type II JAK inhibitors could be associated with more pronounced cytopenias.110 Much attention is placed on the development of JAK2V617F mutation-specific inhibitors which could spare normal hematopoiesis and selectively target the MPN clone. Strategies to achieve mutation-specificity may include the development of allosteric inhibitors targeting the pseudokinase JH2 (JAK homology 2) domain rather than kinase JH1 domain.111 The successful development of tyrosine kinase 2 inhibitors targeting the pseudokinase domain is encouraging and will hopefully instruct development of JAK2V617F-specific inhibitors.112

**Perspective**

The finding of activated JAK2 signaling as a hallmark of MPN has set the stage for the development and broad use of JAK2 inhibitors. JAK2 inhibition has advanced MPN therapy to a new, mechanism-based level of molecularly targeted therapy. Ruxolitinib, as the first in class JAK2 inhibitor, stands for important advantages as convincing symptom control and effective correction of splenomegaly. We expect additional type I JAK2 inhibitors to consolidate these successes and to extend the benefits of JAK2 inhibitor therapy also to subsets of patients with specific vulnerabilities such as anemia or thrombocytopenia. The approval of additional type I JAK2 inhibitors will enable the choice of specific compounds for specific patients according to their profiles. However, the use of ruxolitinib has revealed important shortcomings of JAK2 inhibitors and has made clear that they are not comparable to tyrosine kinase inhibitors of, for example, BCR-ABL in chronic myeloid leukemia. Reductions in mutant allele burden are modest and clonal evolution progresses. Also, resistance mechanisms differ from BCR-ABL inhibitors with adaptive changes in oncogenic signaling being most prominent while second-site resistance mutations upon JAK2 inhibitor therapy have not been reported in MPN patients so far.

With the development of JAK2 inhibitors, several key questions remain open: First, how to handle loss of response; second, how to address bone marrow fibrosis; third, how to reduce the MPN clone; and forth, how to control progressed MPN. Type II JAK2 inhibition represents a novel mode to target JAK2, which holds the potential to overcome resistance and to reduce the MPN clone. However, type II JAK2 inhibition has not entered clinical development yet. HMAIs and BCL-2 inhibitors, which have shown benefits in acute myeloid leukemia, seem to enhance efficacy of JAK2 inhibition and could represent a valid option to control advanced MPN. Aurora kinase inhibition has shown potent fibrosis reducing effects via acting on MPN megakaryocytes, and the further development is urgently awaited. So far, we still rely on modified forms of interferon-α and allogeneic hematopoietic stem cell transplantation for MPN disease modification. However, given the intense efforts for novel combination and single-agent therapies in early clinical stages, the armamentarium for MPN therapy is expected to expand substantially in the next years. This will bring the challenge and the opportunity to learn how to tailor targeted single agents as well as combination therapies with JAK2 inhibitors to specific subsets and to individual patients in order to maximize therapeutic benefit. This prospect urges further efforts for an improved understanding of MPN biology in regard to resistance, fibrogenesis, clonal evolution, and leukemic transformation. Deepened biological insights should support and inform our clinical choices of therapeutics to address as specifically as possible an individual MPN patient’s most pressing needs.

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