ABSTRACT

Myeloproliferative neoplasms (MPNs) are clonal hematopoietic disorders that consist classically of polycythemia vera (PV), essential thrombocythemia (ET), and myelofibrosis (MF). Janus kinase (JAK) inhibitors have become the standard of therapy in treating patients with intermediate- to higher-risk MF. However, JAK inhibitor (JAKi) treatment can be associated with development of resistance, suboptimal response, relapse, or treatment-related adverse effects. With no approved therapies beyond the JAKi class, the estimated median survival, post JAKi failure, is approximately two years or less; therefore, novel therapies are urgently needed in the MF field. In this review, we discuss ruxolitinib use in MPNs as well as causes of ruxolitinib failure or discontinuation. In addition, we review novel therapies being investigated alone or in combination with JAKi administration. We summarize concepts and mechanisms behind emerging novel therapies being studied for MPNs. This review of emerging novel therapies outlines several novel mechanisms of agents, including via promotion of apoptosis, alteration of the microenvironment, activation or inactivation of various pathways, targeting fibrosis, and telomerase inhibition.

Keywords: myeloproliferative neoplasms, MPN, myelofibrosis, JAK inhibitor, ruxolitinib, novel therapy

INTRODUCTION

Myeloproliferative neoplasms (MPNs) are clonal hematopoietic disorders that include polycythemia vera (PV), essential thrombocythemia (ET), and myelofibrosis (MF). The prognosis of patients with MPNs varies, with survival duration from months to decades. Many patients with PV and ET have good long-term prognoses, with 15-year survival of 65% for PV and 73% for ET.[1] Patients with primary myelofibrosis (PMF) have median survival ranging from 2.5–12.3 years, based on the associated existing molecular mutations with prognostic value.[2,3] On the other hand, studies consistently indicate poor prognosis in patients with leukemia transformation with median overall survival ranging from 2.6–6.2 months.[4,5] The most commonly acquired somatic mutation in MF is Janus kinase 2 (JAK2) V617F.[6,7] The next most commonly found mutations include alterations in CALR and MPL, with those negative for any of the three most common driver mutations termed triple negative.[8] Despite improvements in survival over the years, patients still encounter refractory disease, loss of response over time, or adverse events leading to contraindication to the use of JAK inhibitors (JAKis). Unfortunately, there are no approved therapies at this time beyond JAKis for patients with MF. Patients who do not achieve response to JAKis and subsequently develop clonal evolution have overall poor outcomes, with a median survival of approximately 2 years or less.[9,10]

The only potential curative therapy for MF remains allogeneic stem cell transplant (ASCT), which has shown significantly prolonged survival in selected patients.[4,5] However, transplant-related mortality remains high (18–
beyond use of JAKis.

JAK INHIBITORS (JAKis)

JAKi Approval for MPNs

Verstovsek et al.[14] initially demonstrated that patients with MF showed a rapid objective response to the novel agent ruxolitinib, including a significant and clinically meaningful reduction in splenomegaly; patients had significantly improved quality of life, and in longer-term follow-up, improved overall survival. Grade 3 or 4 adverse events occurred in less than 10% of patients; the most common such event was reversible myelosuppression.[15] In the subsequent randomized phase III trials with intermediate or high-risk MF patients (COMFORT I and COMFORT II), patients treated with ruxolitinib demonstrated a greater reduction in spleen volume and disease-related symptom burden than with placebo or best available therapy.[14,16,17] Likewise, a randomized study using ruxolitinib in 222 patients with PV who had an inadequate response to or severe adverse effects from hydroxyurea showed significant efficacy of ruxolitinib in hematocrit control and spleen volume reduction when compared to best available therapy.[16,18] Ruxolitinib became the first drug to be US Food and Drug Administration (FDA) approved for treatment for intermediate- or high-risk MF (PMF and secondary MF) and PV with inadequate response to or intolerant of hydroxyurea.[16]

Fedratinib, a highly selective JAK2 inhibitor, is the second-in-class JAKi that the FDA recently approved for adults with intermediate- or high-risk MF. In a double-blinded, randomized, and placebo-controlled trial enrolling 289 patients with MF (JAKARTA, NCT01437778)[19], 37% of patients achieved the primary outcome of 35% or greater reduction in spleen volume. The median duration of splenic response was 18.2 months, with 40% of patients also experiencing 50% or greater reduction in MF-related symptoms. Currently, the recommended dose of fedratinib is 400 mg orally once daily. Importantly, fedratinib use comes with a “black box” warning for encephalopathy, including Wernicke encephalopathy. Monitoring thiamine level before and during treatment while replacing thiamine as indicated is also recommended.[20,21]

Other JAKis, including momelotinib and pacritinib, are currently being actively investigated in later-stage clinical trials. Each of these JAKis carries unique activity. Momelotinib directly inhibits hepcidin production, which results in increase in iron availability and subsequent improvement in erythropoiesis and benefit in anemia. The clinical efficacy of momelotinib in patients with MF is currently undergoing phase III trial investigation in a study of momelotinib versus danazol for patients with MF, anemia, a defined MPN symptom burden, and splenomegaly (MOMENTUM, NCT04173494).[22] Pacritinib specifically targets both JAK2 and IRAK1, which are thought to be a key driver of MF. Additionally, this drug does not inhibit JAK1, which has been associated with immune dysfunction and worsening cytopenias. An ongoing phase III trial of pacritinib in patients with MF will follow spleen volume reduction as a primary outcome and overall survival as a secondary outcome, focusing specifically on patients with MF and thrombocytopenia (PACIFICA, NCT03165734).[23]

Ruxolitinib Failure

Remarkably, there are still no uniform criteria for the definition of ruxolitinib “treatment failure.” According to a retrospective data analysis with Intercontinental Medical Statistics database, the clinical practice discontinuation rate was 60.9 and 73% at 3 and 6 months, respectively. A large retrospective database analysis also showed discontinuation rates of 41.1 and 48.4% at 3 and 6 months follow-up, respectively.[24] A centralized database supported by 20 European hematology centers also reported a high discontinuation rate of 51.1% (268 of 524 patients) with a median drug exposure of 17.5 months.[19] These studies agree with the most recent retrospective study by Pemmaraju and colleagues[25] in 2020, which showed treatment interruption in 50% of the patients.

Ruxolitinib failure arises from various causes, including primary resistance, suboptimal response, secondary resistance, progression to post-MPN acute myeloid leukemia (AML), and treatment-related toxicities. Kuykendall and colleagues[26] highlight observed reasons for discontinuation, including cytopenias (24 of 64 patients; 38%) with anemia most implicated (21 patients; 33%), non–hematology-related treatment intolerance (6 patients), AML (8 patients), disease progression in symptoms or spleen size (7 patients), suboptimal response (9 patients), and allogeneic hematopoietic stem cell transplant (10 patients). Herein, we explore each cause of treatment failure and present several working definitions. Of note, these definitions are not uniformly agreed upon; however, we believe that exploring the causes of treatment failure will help us to better understand development areas for therapeutic interventions beyond JAKi therapy.

Primary resistance—refractoriness

IWG (International Working Group-Myeloproliferative Neoplasms Research and Treatment) and ELN (European LeukemiaNet) provide practical guidelines on how to assess clinical response to JAKi, such as meaningful improvement in symptoms, spleen size, and transfusion needs, as well as criteria for cytogenetic and molecular remissions.[27] Primary resistance or refractoriness would mean a failure to achieve any clinical benefit.

Suboptimal response

Although definitions may vary from study to study, a general understanding of suboptimal response occurs
when patients do not achieve minimum clinical improvement within approximately 12 weeks or have mixed response with dose reduction or treatment interruptions due to adverse events. While suboptimal response encompasses primary resistance, this group also captures patients with mixed responses and those with clinical responses that do not meet IWG or ELN criteria. Additionally, a study to understand ruxolitinib failure, such as the PAC203 study, describes “failure to benefit” as ruxolitinib use for at least 3 months with less than 10% spleen volume reduction, less than 30% decrease in spleen length, or regrowth to these parameters.

Secondary resistance—relapse
Disease relapse is a loss of previously confirmed clinical response, which can occur for spleen, symptom, or anemia response or improvement. For example, the COMFORT trial II with ruxolitinib treatment aimed to define this as the loss of splenic response as an increase in spleen volume greater than 25% above the on-study nadir.

Leukemic transformation
Leukemic transformation is defined as progression from the MPN state to AML. Leukemia transformation is often referred to as “blast phase,” and the development of more than 10% blasts would also be a concern for disease progression to “accelerated phase.” Disease progression to post-MPN AML was seen in two patients in the COMFORT I study and five patients in the COMFORT II study. Further, five of 40 patients (13%) had progression to AML in a single institution analysis. However, patients require continued monitoring in both short- and longer-term settings to assess over time for leukemic transformation.

Treatment-related toxicities
Treatment-related adverse events include anemia, thrombocytopenia, leukopenia, infections, bleeding, and thromboembolic events, with the highest rates occurring within the first 6 months of the treatment. PAC203 study further defines intolerance as ruxolitinib for 28 days or longer, complicated by development of red cell transfusion requirement or at least grade 3 anemia or thrombocytopenia, and/or hemorrhage while taking less than 20 mg twice a day. Although most experienced grade 1 to 2 adverse events, often reversible, the COMFORT II study identified discontinuation of treatment in two patients due to anemia and in seven patients due to thrombocytopenia.

On the other hand, Palandri and colleagues reported ruxolitinib-related adverse events of 27.5% and unrelated adverse events of 9.2%. Related events included anemia, thrombocytopenia, infection, and neurologic side effects. Unrelated events included solid neoplasm, thrombosis, heart failure, and pleural effusion. Three top clinical reasons for ruxolitinib discontinuation in patients with MF were suboptimal response in spleen size (34.8%), anemia and thrombocytopenia (17.4%), and infectious events (9.2%). Although their study found no significant difference in overall survival between the patients who discontinued ruxolitinib due to suboptimal response versus drug-related toxicity (median survival of 30 vs 13.2 months), they emphasize that this finding still may be clinically relevant. Patients who discontinued ruxolitinib due to toxicity will certainly be most vulnerable with limited therapeutic possibilities after ruxolitinib.

NOVEL THERAPIES, MOVING BEYOND JAK2 INHIBITORS
There is no standard therapeutic approach for patients with intermediate- or high-risk MF who experienced treatment failure, lost response, or developed intolerance to JAKis. Treatments considered for post-JAKi patients unable to qualify for transplant would aim to treat each patient’s specific problem such as anemia, splenomegaly, or symptoms. Moreover, ASCT remains the only potentially curative therapy for the subset of selected, fit patients with MF. Here, we review novel therapies currently undergoing investigation in active clinical trials and their proposed mechanisms. Studies of combination therapies with JAKi plus novel therapies (often referred to as “add-on” or “add-back” studies) and novel agent monotherapies are listed in Tables 1 and 2, respectively.

Combinations With JAKis
JAKis, in combination with various drugs, may lead to clinical benefit in patients with MPNs. Current studies aim to identify optimal drug combinations with JAKi. These studies will be especially valuable in patients who cannot tolerate or become resistant to current JAKi monotherapy. Current drug combination trials with JAKi include azacitidine, HSP90 inhibitor (PU-H71), BCL-xL inhibitor (navitoclax), thalidomide, HDACi (pracinostat), and more (Table 1).

Promotion of Apoptosis
Heaton et al. reported that the level of tumor necrosis factor-alpha (TNF-α) is increased in MF, promoting the clonal dominance of malignant cells. This study found reduced expression of X-linked inhibitor of apoptosis (XIAP) and mitogen-activated protein kinase 8 (MAPK8) in both JAK2V617F-positive murine cells with MPN and human MF cells. Conversely, MF cells had increased cellular inhibitor of apoptosis protein (cIAP) expression in comparison to normal cells. XIAP expression induces apoptosis and regulates cIAPs, which has a role in nuclear factor kappa B (NF-kB) activation involved in TNF-dependent signaling. Overall, this study suggests that MF tumor cells promote their survival by upregulating TNF-α–dependent pathway via decreasing...
XIAP and MAPK8 with a subsequent increase in cIAPs.\footnote{33,34} Targeting this pathway by inhibiting XIAP with AEG35156 (XIAP inhibitor) combined with idarubicin and cytarabine in relapsed/refractory AML cells resulted in all phase II patients achieving response with apoptosis induction.\footnote{35}

**SMAC (second mitochondrial activator of caspase) mimetics**

Du and colleagues\footnote{36} discovered a new protein called second mitochondrial activator of caspase (SMAC) that promotes apoptosis by binding to cIAPs. A SMAC mimetic is designed to bind to cIAP and overcome cIAP-mediated apoptosis-resistant cells, preferentially inhibiting TNF-induced activation. This results in caspase activation and subsequent apoptosis.\footnote{37} Overexpression of SMAC also increases sensitivity to apoptotic stimuli, making this target very important in resistant patients.\footnote{36} A phase Ib study with birinapant (intravenous SMAC mimetic) combined with 5-azacitadine in patients with MDS, including those with refractory disease or with relapse to 5-azacitadine, confirmed clinical efficacy with an acceptable safety profile.\footnote{38} Another SMAC mimetic, oral agent LCL161, showed activity against MPN cells in vitro and in vivo and induced a reduction in splenomegaly in a murine model.

| Combination Study | NCT No. | Malignancy | Phase | No. of Patients Enrolled | Outcome Measures |
|-------------------|---------|------------|-------|--------------------------|-----------------|
| Ruxolitinib + 9-ING-41 vs 9-ING-41 | NCT04218071\footnote{112} | MF | II | 58 | RR |
| Ruxolitinib + ABBV-744 vs ABBV-744 | NCT04454658\footnote{113} | MF | I | 130 | Adverse events, spleen volume reduction, pharmacokinetic profile |
| Ruxolitinib + APG-1252 vs APG-1252 | NCT04354727\footnote{114} | MF | I/II | 60 | DLT, spleen volume reduction |
| Ruxolitinib + Azacitidine\footnote{31} | NCT01787487\footnote{960} | MPN, PMF, SMF | II | 125 | CR, PR, clinical improvement, incidence of adverse events |
| Ruxolitinib + CPI-0610 vs CPI-0610 | NCT02158588\footnote{922} | MPN, MDS, AML, MF | I/II | 271 | Spleen response, RBC transfusion independence rate |
| Ruxolitinib + Daunorubicin | NCT03878193\footnote{115} | Secondary AML, MPN | I/II | 47 | DLT, CCR, CR, Pri, OS, EFS, RFS, remission duration |
| Ruxolitinib + Decitabine\footnote{32} | NCT02257139\footnote{116} | AML, MPN, PMF, SMF | I/II | 34 | MTD, overall response |
| Ruxolitinib + Enasidenib | NCT04281498\footnote{117} | Accelerated/blast-phase MPN, MF | II | 32 | RR |
| Ruxolitinib + Itacitinib | NCT03144687\footnote{118} | MPN | II | 23 | Safety and tolerability, spleen volume reduction |
| Mivebresib +/- Ruxolitinib or Navitoclax | NCT04480086\footnote{119} | MF | I | 130 | Adverse events, change in spleen volume, ORR, pharmacokinetic profile |
| Ruxolitinib + Navitoclax | NCT04472598\footnote{47} | MF | III | 230 | Spleen volume reduction, reduction in TSS*, OS, ORR, leukemia-free survival |
| Ruxolitinib + Navitoclax | NCT04468984\footnote{120} | MF | III | 330 | Spleen volume reduction, reduction in TSS*, OS, ORR, leukemia-free survival |
| Ruxolitinib + Navitoclax vs Navitoclax | NCT03222609\footnote{46} | MF | II | 164 | Spleen volume reduction, change in TSS*, ORR |
| Ruxolitinib + Parsaclisib | NCT02718300\footnote{121} | MPN | II | 90 | DLT, change in spleen volume, adverse events |
| Ruxolitinib + Pevonedistat | NCT03386214\footnote{122} | MF | II | 18 | Safety and tolerability, spleen volume reduction |
| Ruxolitinib + PU-H71 | NCT03373877\footnote{588} | PME, SMF | I | 30 | Adverse events, MTD, RP2D, pharmacokinetic profile |
| Ruxolitinib + TGR-1202 | NCT02495350\footnote{127} | PV, MF | I | 60 | Safety, overall response |
| Ruxolitinib + Thalidomide | NCT03069326\footnote{123} | MF | II | 30 | ORR |
| Ruxolitinib or Fedratinib + Decitabine | NCT04282187\footnote{124} | AML, MPN, PMF, SMF | II | 25 | Remission rate, OS, RFS, mutational data, response rate, OS |
| Fedratinib + Luspatercept | NCT03755518\footnote{125} | PME, post-PV MF | III | 110 | Spleen volume reduction, adverse events, SRR, anemia response |

AML: acute myeloid leukemia; CCR: composite complete remission; CML: chronic myeloid leukemia; CR: complete remission; Cri: complete remission with incomplete marrow recovery; CRR: complete response rate; DLT: dose-limiting toxicity; EFS: event-free survival; JAK: Janus kinase; MDS: myelodysplastic syndrome; MF: myelofibrosis; MPN: myeloproliferative neoplasm; MTD: maximum tolerated dose; ORR: overall response rate; OS: overall survival; PFS: progression-free survival; PME: primary myelofibrosis; PR: partial remission; PV: polycythemia vera; RBC: red blood cell; RFS: relapse-free survival; RP2D: recommended phase 2 dose; RR: response rate; SMF: secondary myelofibrosis; SRR: symptom response rate; TFR: treatment-free events; TSS: total score system.

*Based on the Myelofibrosis Symptom Assessment Form.

Table 1. Selection of current clinical trials of combination therapies with JAK inhibitors

Lee et al: Novel therapies in MPN: Beyond JAK inhibitor monotherapy

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of JAK2V617F-driven MPN. A phase II clinical trial study in patients with PMF, post-PV MF, and post-ET MF further demonstrated an objective response in 38% (6/16) of the patients. The median time to response was about 1.4 months (range, 0.9–9.1 months) with treatment duration of 31.5 months (range, 3.6–55.2 months). Median overall survival has not yet been reached. The most common adverse event leading to dose reduction or study discontinuation was grade 2 fatigue. Overall data demonstrated LCL161’s successful inhibition of tumor activity via antagonizing XIAP and inhibiting cIAPs in high TNF-α–expressing models.

**Inhibition of Bcl-xL**

The Bcl-2 family is a key regulator of apoptosis, promoting the antiapoptotic survival of the cell lines. Its members, such as Bcl-xL, are linked to resistant tumor cells particularly in MF model systems. In previous studies, Bcl-xL inhibitor showed enhancement of apoptotic signals and synergistic cytotoxicity with combined chemotherapy. Navitoclax (Bcl-xL inhibitor) is currently in active clinical investigation in the setting of its hypothesized activity in MF (NCT04041050). A phase II single-arm, multi-center, open-label study using a combination of navitoclax and ruxolitinib demonstrated clinical significance in MF patients: spleen volume reduction of 35% or greater from baseline in 29% (7 of 24) of patients, median total symptom score of 20% improvement from baseline, reduction in driver mutation allelic burden of more than 5% in 42% (10) of patients, and bone marrow fibrosis improvement of at least 1 grade in six patients (NCT03222609). This novel approach is being planned for an upcoming phase III trial (TRANSFORM-1, NCT04472598) that will feature the combination of ruxolitinib with navitoclax versus ruxolitinib plus placebo in the upfront setting for patients with MF.

**Targeting of Hematopoietic Stem Cell/Microenvironment**

CD123 (interleukin-3 receptor alpha subunit) is highly expressed in a variety of hematologic malignancies. Notably, CD123 is expressed in subsets of MPNs, marking a potential therapeutic target. Tagraxofusp (SL-401) is a drug that consists of a chimeric junction between a truncated diphtheria toxin and IL-3 (interleukin-3). The toxin is fused to IL-3 and successfully targets CD123. Once bound to the receptor, the drug is internalized by endocytosis and ultimately leads to cell death. Due to its potent activity against CD123-

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**Table 2. Selection of current clinical trials of novel therapies beyond JAK inhibitors**

| Intervention | NCT No. | Malignancy | Phase | No. of Patients Enrolled | Research Question |
|--------------|---------|------------|-------|--------------------------|------------------|
| AVID200      | NCT03895112 [78] | PMF, SMF | I     | 24                        | MTD, CR, PR, cryptogenic remission, molecular remission, clinical response, bone marrow fibrosis |
| IMG-7289     | NCT03136185 [102] | PMF, SMF | II    | 50                        | Emergent adverse events, safety and tolerability, drug concentration, spleen volume reduction |
| IMG-7289     | NCT04262141 [101] | ET, PV   | II    | 24                        | Hematologic response rates, toxicity, TSS, mutational allele burden, spleen volume reduction, fibrosis score |
| KRT-232      | NCT03669965 [66] | PV        | II    | 20                        | Spleen volume reduction, phlebotomy independence |
| KRT-232      | NCT03662126 [65] | PMF, SMF  | II    | 203                       | Spleen volume reduction, phlebotomy independence |
| LCL161 [40]  | NCT02098161 [126] | PV, PMF, SMF | II | 50                          | ORR, grade 3–4 toxicity, duration of response, time to response |
| Luspatercept [25] | NCT04064060 [77] | MDS, MPN associated MF, beta-thalassemia | III | 665                         | Safety |
| Luspatercept [45] | NCT03194542 [76] | PMF, anemia | II | 103                        | Anemia response |
| Navitoclax   | NCT04041050 [44] | MPN       | II    | 12                        | DLT, pharmacokinetic profile |
| PU-H71       | NCT03935555 [57] | PMF, SMF  | II    | 24                        | Safety and tolerability, pharmacokinetics, treatment response |
| PU-H71       | NCT01393509 [56] | MPN, lymphoma, metastatic solid tumor | I     | 47                        | Safety and tolerability, pharmacokinetics |
| Sotatercept  | NCT01712308 [73] | MDS, MPN, MF, anemia | II | 60                          | Incidence of toxicities, anemia response |
| Tagraxofusp  | NCT02268253 [53] | MF, CMML  | II    | 130                       | Rate response, rate and severity of treatment emergent adverse events |

AML: acute myeloid leukemia; CMML: chronic myelomonocytic leukemia; CR: complete remission; DLT: dose-limiting toxicity; ET: essential thrombocythemia; JAK: Janus kinase; MDS: myelodysplastic syndrome; MF: myelofibrosis; MLFS: morphologic leukemia-free state; MPN: myeloproliferative neoplasm; MTD: maximum tolerated dose; ORR: overall response rate; PMF: primary myelofibrosis; PR: partial remission; PV: polycythemia vera; RP2D: recommended phase 2 dose; SMF: secondary myelofibrosis; TSS: total score system.

*Based on the Myelofibrosis Symptom Assessment Form.

†Based on the Myeloproliferative Neoplasm Symptom Assessment Form.
expressing malignant cells, tagraxofusp was recently approved by the FDA for blastic plasmacytoid dendritic cell neoplasm (BPDCN), a historically aggressive hematologic malignancy that is known to overexpress CD123 in 100% of cases.[61] In a study with MF, 45% (9 of 20) of patients treated with tagraxofusp demonstrated symptom reduction, and all patients (6 of 6) had a reduction in spleen size.[52] A phase I/II clinical trial with tagraxofusp in patients with intermediate- or high-risk and relapsed/refractory MF is currently ongoing (NCT02268253).[53] The most notable toxicity of tagraxofusp is capillary leak syndrome, which is part of the “black box” package label warning for BPDCN approval. This toxicity is actively being monitored in the ongoing phase II MF clinical trial with tagraxofusp.

Another early program in clinical development in the MF field is that of heat shock protein inhibition. Heat shock protein-90 (HSP90) is a chaperone protein that aids in the stability of oncogenes (ie, JAK2) against environmental stress, an essential function for oncogenic transformation.[54,55] A growing interest in HSP90 inhibitors as anticancer therapy is driving current active research. Investigation in PU-H71 (HSP90 inhibitor) as a monotherapy or combined with ruxolitinib for safety and tolerability and pharmacokinetic profile in patients with MPN is ongoing (NCT01393509, NCT03935555, NCT03373877).[56,57,58]

**Activation of the TP53 Pathway**

The TP53 tumor suppressor protein plays an essential role in cellular stability against environmental stressors such as DNA damage and hypoxia. Activation of this protein leads to cell arrest or apoptotic cell death, whereas the loss of this protein promotes oncogenic proliferation and tumorigenesis. Regulation of TP53 is mediated by a TP53-interacting protein called murine double minute clone 2 (MDM2). In normal cells under nonstressed conditions, MDM2 increases ubiquitination and proteasome-dependent degradation of TP53. Conversely, overexpression of MDM2 suppresses the tumor suppressor gene TP53, resulting in oncogenicity.[59,60] The working hypothesis has been that various types of cancer have a higher expression of MDM2.[61] MDM2 is the first long-acting MDM2 antagonist approved by the FDA for blastic plasmacytoid dendritic cell neoplasm (BPDCN), a historically aggressive hematologic malignancy that is known to overexpress CD123 in 100% of cases.[51] In a study with MF, 45% (9 of 20) of patients treated with tagraxofusp demonstrated symptom reduction, and all patients (6 of 6) had a reduction in spleen size.[52] A phase I/II clinical trial with tagraxofusp in patients with intermediate- or high-risk and relapsed/refractory MF is currently ongoing (NCT02268253).[53] The most notable toxicity of tagraxofusp is capillary leak syndrome, which is part of the “black box” package label warning for BPDCN approval. This toxicity is actively being monitored in the ongoing phase II MF clinical trial with tagraxofusp.

**Inactivation of TGF-β (Transforming Growth Factor-Beta) Pathway**

Members of the TGF-β superfamily, which includes growth differentiation factor 11 (GDF11) and activin, are secreted by bone marrow stromal cells and share a role in proliferation and differentiation of the erythroid precursor cells. Sotatercept (ACE011) is a recombinant fusion protein of the extracellular domain of activin receptor type II (ActRII). It binds to activin and GDF11 to inhibit activation of subsequent pathway.[67] Inactivation of GDF11 relieves suppression of terminal erythropoiesis and corrects the abnormal ratio of immature erythroblasts by inducing apoptosis of immature cells.[68,69] Sotatercept improved erythrocytosis in preclinical models of beta-thalassemia, Diamond-Blackfan anemia, and hepcidin transgenic mice.[68,70–72] As anemia is a common complication of PMF, post-PV MF, post-ET MF, and ruxolitinib therapy, sotatercept’s success in improving anemia would greatly aid in mitigating the disease and treatment complication; sotatercept use may also potentially prolong ruxolitinib therapy. A current phase II clinical trial with sotatercept to treat patients with MDS, MPN, and chronic myelomonocytic leukemia (CMML) is ongoing (NCT01712308, NCT01736683).[73,74] Additionally, luspatercept is an investigational erythropoietin maturation agent that neutralizes select TGF-β superfamily ligands to inhibit SMAD protein pathway signaling. In a phase II study with lower-risk MDS patients, luspatercept at high-dose concentration (0.75–1.75 mg/kg) yielded hematologic improvement with a reduction in the number of red blood cell transfusions (52%; 14 of 27 patients).[75] Luspatercept also demonstrated clinical efficacy in enhancing late-stage erythropoiesis in MDS models.[67] Current ongoing trials include luspatercept in patients with PMF and anemia to assess anemia response (NCT03194542).[76] In patients with MDS, MPN-associated MF, and beta-thalassemia to assess the safety of the drug (NCT04064060).[77] Furthermore, AVID200 is an engineered drug that selectively targets TGF-β1 and TGF-β3. A current phase I
study is based on the hypothesis that inhibiting the TGF-β signaling pathway will decrease fibrogenic stimuli, which leads to MF (NCT03895112).[78]

**Targeting Fibrosis**

In terms of the advanced abnormal bone marrow fibrosis state in MF, there is no current standard therapy that specifically aims to reverse marrow fibrosis.[79] Owing to the hypothesis that reduction in marrow fibrosis will restore hematopoiesis and improve cytopenias, more studies aim to identify the fibrosis-driving cells and inhibit their activity. Although progressive bone marrow fibrosis in MF was initially thought to be secondary to mesenchymal stromal cells, tissue fibrosis in other diseases has been linked to fibrocytes. For instance, serum amyloid P (SAP; pentraxin-2), known as fibrocyte inhibitor, has previously shown success in inhibiting progressive fibrotic disease of the kidney.[80] Importantly, it has been demonstrated that patients with MF have low pentraxin-2 (PTX-2) levels, allowing the fibrocyte inhibitor, has previously shown success in inhibiting progressive fibrotic disease of the kidney.[80] Importantly, it has been demonstrated that patients with MF have low pentraxin-2 (PTX-2) levels, allowing the hypothesis that an increase in its level may lead to inhibition of progressive fibrosis in the bone marrow. Based on this rationale, PTX-2 was tested for its efficacy in MF. In xenograft mouse models, PTX-2 significantly prolonged survival and slowed bone marrow fibrosis.[81] PRM151 is a recombinant human PTX-2. It acts as an endogenous regulator of tissue repair by binding to the damaged tissue to prevent and reverse fibrosis. A current phase II trial with PRM151 is targeting patients with PMF, PV, and post-ET MF (NCT01981850).[82] The primary outcome measure of this study is the bone marrow response rate, defined as the percentage of subjects with a reduction in bone marrow fibrosis by at least 1 grade according to World Health Organization criteria.

**Aurora Kinase Inhibition**

Several studies suggest that an increased number of megakaryocytes contributes to bone marrow fibrosis.[83–85] In consideration of these data, the aurora kinase alpha inhibitor (alisertib) has emerged a novel therapeutic target in MF. It successfully induced apoptosis of malignant megakaryocytes and reduced subsequent antifibrotic and antitumor activity in MPN cells.[85] A phase I trial successfully demonstrated the efficacy of aurora kinase alpha inhibition, normalizing megakaryocytes, and reducing bone marrow fibrosis in five of seven patients with MF. Its use also reduced splenomegaly and symptom burden in 29% and 32% of patients, respectively.[86]

**Epigenetic-Directed Therapies**

*Inhibition of BET (bromodomain and extraterminal) family of proteins*

BET family of proteins has been recently put forward as a potential therapeutic target in the MPN field. Bromodomain proteins are chromatin readers that recruit chromatin-regulating enzymes to attempt to modify histone to stimulate gene expression.[87] Their proposed role includes transcribing pro-oncogenes such as c-MYC, BCL2, and CDK6.[88] Successful inhibition of BET-induced apoptosis in murine and human AML cells led to subsequent clinical trials.[89,90] The efficacy of BET-induced apoptosis inhibitors has been demonstrated in vitro in patients who developed post-MPN AML cells. Combination treatment with an HSP90 inhibitor also synergistically worked against previously ruxolitinib-failed malignant cells.[91] A phase I/II study of BET inhibitor (oral drug, CPI-0610) combined with ruxolitinib or alone in patients with MDS, MF, PMN, and AML is currently underway (NCT02158858).[92] The study measures efficacy through spleen volume reduction and red blood cell transfusion independence rate. Preliminary data show clinical efficacy and a generally well-tolerated safety profile in patients with MF and inadequate response or disease refractory to ruxolitinib.[93] This promising program is being planned for upcoming phase III investigation as frontline combination versus JAKi plus placebo. Phase III, randomized study comparing CPI-610 and ruxolitinib with placebo and ruxolitinib in patients with MF has been initiated (MANIFEST-2, NCT04603495).[94] This study will measure splenic and symptom response.

**DNA hypomethylation**

DNA methylation can silence genes involved in tumor suppression and DNA repair. Hypomethylating agents such as azacitidine and decitabine reverse dysregulated DNA methylation. This treatment mechanism likely most benefits patients with aberrant hypermethylation. Masarova and colleagues,[95] in a 2018 phase II study of ruxolitinib combined with azacitidine (RUX + AZA), demonstrated a favorable response in historical comparison with ruxolitinib monotherapy (NCT01787487).[96] Among 46 patients enrolled after a median follow-up of 28 months (range, 4–50+ months), the median time to response was 1.8 months (range, 0.7–19 months), and median overall survival was 28 months (range, 4–39+ months). RUX + AZA had an overall response rate of 72%, including greater than 50% spleen volume reduction in 79% of the patients. Treatment-related toxicities that halted the therapy occurred in four patients (9%) due to significant cytopenias. Of note, spleen volume reduction in this study was measured as spleen length reduction by palpation during physical examination.[95]

**Inhibition of Lysine-Specific Demethylase 1A (LSD1)**

LSD1 is a histone demethylase that removes methyl groups specifically at histone 3 and lysine 4, leading to active transcription. This action ultimately leads to cell proliferation.[97] LSD1 has been linked to the progression of several cancers, including breast and prostate cancer.[98,99] In recent years, LSD1 overexpression has also been found in hematologic malignancies, including MPNs and CMML.[100] IMG-7289 is an irreversible LSD1 inhibitor studied in phase II clinical trial for MPNs.
Telomeres represent specialized tandem repeats of DNA sequence at the end of a eukaryotic chromosome. Telomeres are absent in telomerase expression. Telomerase is a ribonuclear protein complex that synthesizes telomeric DNA onto the chromosome to further protect the DNA and, thereby, enable continued replication. Ex vivo studies in human tissues have identified telomerase expression in about 90% of all malignant tumors. Unlike normal somatic cells, cancer cells escape senescence by acquiring telomerase expression that can aid in maintaining and lengthening telomeres, creating telomere stability and cells’ continued division.

Imetelstat is a competitive inhibitor of telomerase. An in vitro study by Wang and colleagues demonstrated that its use resulted in selective induction of apoptosis in MF stem cells. Imetelstat induced hematologic responses in all patients with ET (18 of 18 patients) and complete hematologic response in 16 patients (89%) with a median follow-up of 17 months (range, 7–32+ months). A pilot study of imetelstat in patients with MF also demonstrated complete remission, although caution was advised against clinically significant myelosuppression. Additionally, a phase II study evaluated its efficacy in patients with intermediate-2 or higher-risk MF and previously treated with a JAKi (NCT02426086). Its preliminary results show dose-dependent median overall survival of 19.9 months at 4.7-mg/kg dose and 29.9+ months at 9.4-mg/kg dose. This finding is comparable to the reported median survival of 13–14 months in MF patients after ruxolitinib failure or discontinuation. A phase III trial of imetelstat in patients with refractory MF is ongoing with a notably novel primary endpoint of median overall survival.

CONCLUSIONS

Ruxolitinib is now FDA approved for three separate indications: intermediate/high-risk MF, PV that is intolerable to hydroxyurea, and steroid-refractory acute graft-versus-host disease for patients aged 12 and older. Despite advancements in treating MPNs with ruxolitinib, multiple reports indicate a high discontinuation rate of ruxolitinib due to resistance, relapse, and treatment-related toxicities. With no approved therapy beyond JAKis, many patients with MF and ruxolitinib failure face a poor prognosis. Fedratinib, recently approved with a broad indication in MF, is the second JAKi now widely available, an agent which may provide an alternative to ruxolitinib or used in the postruxolitinib setting. Studies supporting the use of this drug encourage the routine practice of monitoring for encephalopathy and thiamine deficiency.

With a growing understanding of the pathobiology of MPNs, the field is now seeing an active development of many novel classes of drug targets. We expect current investigations of drugs combined with JAKi treatment (“add-back, or “add-on”) and investigation of novel targeted therapies alone without JAKi treatment to be an emerging area of active clinical development in the coming years. For clinicians, providing details of each therapeutic option and understanding the patient’s goals will be essential for finding an individualized, optimal therapy. Attention must be paid to short- and longer-term novel toxicities in the development of each class of novel therapies, particularly those that may overlap with administration of JAKis.

References

1. Passamonti F, Rumni E, Pungolino E, Malabarba L. Life expectancy and prognostic factors for survival in patients with polycythemia vera and essential thrombocythemia. Am J Med. 2004;117:755–761.
2. Guglielmelli P, Lasho TL, Rotunno G, et al. The number of prognostically detrimental mutations and prognosis in primary myelofibrosis: an international study of 797 patients. Leukemia. 2014;28:1804–1810.
3. Cervantes F, Dupriez B, Passamonti F, et al. Improving survival trends in primary myelofibrosis: an international study. J Clin Oncol. 2012;30:2981–2987.
4. Tam CS, Nussenzveig RM, Popat U, et al. The natural history and treatment outcome of blast phase BCR-ABL—myeloproliferative neoplasms. Blood. 2008;112:1628–1637.
5. Mesa RA, Li C-Y, Ketterling RP, et al. Leukemic transformation in myelofibrosis with myeloid metaplasia: a single-institution experience with 91 cases. Blood. 2005;105:973–977.
6. Levine RL, Wadleigh M, Cools J, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell. 2005;7:387–397.
7. Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med. 2005;352:1779–1790.
8. Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med. 2013;369:2379–2390.
9. Newberry KJ, Patel K, Masarova L, et al. Clonal evolution and outcomes in myelofibrosis after ruxolitinib discontinuation. Blood. 2017;130:1125–1131.
10. Palandri F, Breccia M, Bonifacio M, et al. Life after ruxolitinib: reasons for discontinuation, impact of disease phase, and outcomes in 218 patients with myelofibrosis. Cancer. 2020;126:1243–1252.
11. Ballen KK, Shrestha S, Sobocinski KA, et al. Outcome of transplantation for myelofibrosis. *Biol Blood Marrow Transplant*. 2010;16:358–367.

12. McLornan DP, Szydlo R, Robin M, et al. Outcome of patients with myelofibrosis relapsing after allogeneic stem cell transplant: a retrospective study by the Chronic Malignancies Working Party of EBMT. *Br J Haematol*. 2018;182:418–422.

13. Kröger N, Holler E, Kobbe G, et al. Allogeneic stem cell transplantation after reduced-intensity conditioning in patients with myelofibrosis: a prospective, multicenter study of the Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. *Blood*. 2009;114:5264–5270.

14. Verstovsek S, Gotlib J, Mesa RA, et al. Long-term survival in patients treated with ruxolitinib for myelofibrosis: COMFORT-I and -II pooled analyses. *J Hematol Oncol*. 2017;10.

15. Verstovsek S, Kantarjian H, Mesa RA, et al. Safety and efficacy of INCB018424, a JAK1 and JAK2 inhibitor, in myelofibrosis. *N Engl J Med*. 2010;363:1117–1127.

16. FDA. Highlights of prescribing information: Jakafi (ruxolitinib). Published May 2019. Accessed Mar 28, 2020. www.accessdata.fda.gov/drugsatfda_docs/label/2019/20192s017lbl.pdf

17. Pardanani A, Harrison C, Cortes JE, et al. Safety and tolerability of ruxolitinib in patients with post-essential thrombocytemia myelofibrosis (post-ET MF). *Exp Hematol Oncol*. 2012;366:799–807.

18. Vannucchi AM, Kiladjian JJ, Griesshammer M, et al. Ruxolitinib versus standard therapy for the treatment of polycythemia vera. *N Engl J Med*. 2015;372:426–435.

19. Phase III Study of SAR302503 in intermediate-2 and high risk patients with myelofibrosis (JAKARTA). ClinicalTrial.gov identifier: NCT01437787. Updated Dec 8, 2015. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT01437787

20. Center for Drug Evaluation and Research. FDA approves fedratinib for myelofibrosis. 2019. Accessed Mar 28, 2020. www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-fedratinib-myelofibrosis.

21. Pardanani A, Harrison C, Cortes JE, et al. Safety and efficacy of fedratinib in patients with primary or secondary myelofibrosis: a randomized clinical trial. *JAMA Oncol*. 2015;1:643–651.

22. A study of Momelotinib versus Danazol in symptomatic and anemic myelofibrosis patients (MOMENTUM). ClinicalTrial.gov identifier: NCT04173494. Updated Apr 3, 2021. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT04173494

23. A phase 3 study of Pacritinib in patients with primary myelofibrosis, post polycythemia vera myelofibrosis, or post-essential thrombocythemia myelofibrosis (PACIFICA). ClinicalTrial.gov identifier: NCT03165734. Updated Apr 27, 2021. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT03165734

24. Fonseca E, Silver RT, Kazis LE, et al. Ruxolitinib discontinuation in patients with myelofibrosis: an analysis from clinical practice. *Blood*. 2013;122:2833.

25. Pemmaraju N, Yu J, Parasaruman S, et al. Ruxolitinib (RUX) retreatment in patients (Pts) with myelofibrosis (MF): real-world evidence on pt characteristics and outcomes. *J Clin Oncol*. 2020;38(suppl):abstract e19535.

26. Kuykendall AT, Shah S, Talati C, et al. Between a rux and a hard place: evaluating salvage treatment and outcomes in myelofibrosis after ruxolitinib discontinuation. *Ann Hematol*. 2018;97:435–441.

27. Tesser A, Cervantes F, Mesa R, et al. Revised response criteria for myelofibrosis: International Working Group-Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) and European LeukemiaNet (ELN) consensus report. *Blood*. 2013;122:1395–1398.

28. Gerds AT, Savona MR, Scott BL, et al. Results of PAC203: a randomized phase 2 dose-finding study and determination of the recommended dose of pacritinib. *Blood*. 2019;134(suppl 1):667.

29. Cervantes F, Vannucchi AM, Kiladjian JJ, et al. Three-year efficacy, safety, and survival findings from COMFORT-II, a phase 3 study comparing ruxolitinib with best available therapy for myelofibrosis. *Blood*. 2013;122:4047–4053.

30. Kremyanskaya M, Mascalzinha J, Rampal R, Hoffman R. Development of extramedullary sites of leukemia during ruxolitinib therapy for myelofibrosis. *Br J Haematol*. 2014;167:144–146.

31. Masarova L, Verstovsek S, Bose P, et al. Phase 2 study of ruxolitinib (RUX) in combination with 5-azacitidine (AZA) in patients (pts) with myelofibrosis. *Blood*. 2019;134(suppl 1):1656.

32. Bose P, Verstovsek S, Cortes JE, et al. A phase 1/2 study of ruxolitinib and decitabine in patients with post-myeloproliferative neoplasm acute myeloid leukemia. *Leukemia*. 2020;34:2489–2492.

33. Heaton WL, Senina AV, Pomicter AD, et al. Autocrine Tnf signaling favors malignant cells in myelofibrosis in a Tnfr2-dependent fashion. *Leukemia*. 2018;32:2399–2411.

34. Fleischman AG, Aichberger KJ, Luty SB, et al. TNFα facilitates clonal expansion of JAK2V617F positive cells in myeloproliferative neoplasms. *Blood*. 2011;118:6392–6398.

35. Carter BZ, Mak DH, Morris SJ, et al. XIAP antisense oligonucleotide (AEG35156) achieves target knockdown and induces apoptosis preferentially in CD34+38– cells in a phase 1/2 study of patients with relapsed/refractory AML. *Apoptosis*. 2011;16:67–74.

36. Du C, Fang M, Li Y, et al. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. *Cell*. 2000;102:33–42.

37. Benetatos CA, Mitsuchi Y, Burns JM, et al. Birinapant (TL32711), a bivalent SMAC mimetic, targets TRAF2-associated cIAPs, abrogates TNF-induced NF-κB activation, and is active in patient-derived xenograft models. *Mol Cancer Ther*. 2014;13:867–879.

38. Borthakur G, Foran JM, Wang ES, et al. A phase 1b study of birinapant in combination with 5-azacitidine in patients with myelodysplastic syndrome who are naive, refractory or have relapsed to 5-azacitidine. *Blood*. 2015;126:93.

39. Craver BM, Nguyen TK, Nguyen J, et al. The SMAC mimetic LCL-161 selectively targets JAK2V617F mutant cells. *Exp Hematol Oncol*. 2020;9. DOI: 10.1186/s40164-019-0157-6.

40. Pemmaraju N, Carter BZ, Kantarjian HM, et al. Results for phase II clinical trial of LCL161, a SMAC mimetic, in patients with primary myelofibrosis (PMF), post-polycythemia vera myelofibrosis (post-PV MF) or post-essential thrombocythemia myelofibrosis (post-ET MF). *Blood*. 2016;128:3105.

41. Broecker-Preuss M, Becher-Boveleth N, Müller S, Mann K. The BH3 mimetic drug ABT-737 induces apoptosis and
acts synergistically with chemotherapeutic drugs in thyroid carcinoma cells. *Cancer Cell Int.* 2016;16:27.

42. Parrondo R, de las Pozas A, Reiner T, Perez-Stable C. ABT-737, a small molecule Bcl-2/Bcl-xL antagonist, increases antimitotic-mediated apoptosis in human prostate cancer cells. *PeerJ.* 2013;1:e144.

43. Oltersdorf T, Elmore SW, Shoemaker AR, et al. An evaluation of tolerability and efficacy of Navitoclax in combination with ruxolitinib in patients with myelofibrosis. *Blood.* 2019;134(suppl 1):671.

44. A study evaluating safety and tolerability, and pharmacokinetics of Navitoclax monotherapy and in combination with Ruxolitinib in participants with myeloproliferative neoplasm. ClinicalTrials.gov identifier: NCT04041050. Updated Dec 14, 2020. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT04041050

45. Harrison CN, Garcia JS, Mesa RA, et al. Results from a phase 2 study of Navitoclax in combination with ruxolitinib in patients with primary or secondary myelofibrosis. *Blood.* 2019;134(suppl 1):1.

46. A study evaluating tolerability and efficacy of Navitoclax alone or in combination with Ruxolitinib in participants with myelofibrosis (REFINE). ClinicalTrials.gov identifier: NCT03222609. Updated Apr 22, 2021. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT03222609

47. Study of oral Navitoclax tablet in combination with oral Ruxolitinib tablet when compared with oral Ruxolitinib tablet to assess change in spleen volume in adult participants with myelofibrosis (TRANSFORM-1). ClinicalTrials.gov identifier: NCT04472598. Updated May 4, 2020. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT04472598

48. Lasho T, Finke C, Kimlinger TK, et al. Expression of CD123 (IL-3R-alpha), a therapeutic target of SL-401, on myeloproliferative neoplasms. *Blood.* 2014;124:5577.

49. Yao Y, Hu W, Guo Y, Yang W. Phenotypic characterization of malignant progenitor cells in patients with idiopathic myelofibrosis. *Hematol Oncol Stem Cell Ther.* 2019;12:146–154.

50. Alkharabsheh O, Frankel AE. Clinical activity and tolerability of SL-401 (Tagraxofusp): recombinant diphtheria toxin and interleukin-3 in hematologic malignancies. *Biomedicines.* 2019;7. DOI: 10.3390/biomedicines7010006.

51. Jen EY, Gao X, Li L, et al. FDA approval summary: tagraxofusp-erzs for treatment of blastic plasmacytoid dendritic cell neoplasm. *Clin Cancer Res.* 2020;26:532–536.

52. Premmaraju N, Gupta V, Ali H, et al. Results from a phase 1/2 clinical trial of tagraxofusp (SL-401) in patients with intermediate, or high risk, relapsed/refractory myelofibrosis. *Blood.* 2019;134(suppl 1):558.

53. Tagraxofusp (SL-401) in patients with CMMML or MF. ClinicalTrials.gov identifier: NCT02268253. Updated Jun 4, 2020. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT02268253

54. Neckers L, Workman P. Hsp90 molecular chaperone inhibitors: are we there yet? *Clin Cancer Res.* 2012;18:64–76.

55. Sevin M, Girodon F, Garrido C, de Thonel A. HSP90 and HSP70: implication in inflammation processes and therapeutic approaches for myeloproliferative neoplasm. *Mediators Inflamm.* 2015;2015:1–8.

56. The first-in-human phase 1 trial of PU-H71 in patients with advanced malignancies. ClinicalTrials.gov identifier: NCT01393509. Updated Aug 10, 2020. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT01393509

57. Assess the safety, tolerability oral PU-H71 in subjects taking Ruxolitinib. ClinicalTrials.gov identifier: NCT03935555. Updated Oct 20, 2020. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT03935555

58. Evaluation of Ruxolitinib in combination with PU-H71 for treatment of myelofibrosis. ClinicalTrials.gov identifier: NCT03373877. Updated Oct 22, 2020. Accessed May 20, 2021. www.clinicaltrials.gov/ct2/show/NCT03373877

59. Ashcroft M, Kubbhatu MHG, Vousden KH. Regulation of p53 function and stability by phosphorylation. *Mol Cell Biol.* 1999;19:1751–1758.

60. Kubbhatu MHG, Ludwig RL, Levine AJ, Vousden KH. Analysis of the degradation function of Mdm2. *Cell Growth Differ.* 1999;10:87–92.

61. Levav-Cohen Y, Haupt S, Haupt Y. Mdm2 in growth signaling and cancer: mini review. *Growth Factors.* 2005;23:183–192.

62. Yee K, Martinelli G, Vey N, et al. Phase 1/1b study of RG7388, a potent MDM2 antagonist, in acute myelogenous leukemia (AML) patients (pts). *Blood.* 2014;124:116.

63. A study to evaluate the efficacy, safety, pharmacokinetics and pharmacodynamics of Idasanutlin monotherapy in participants with hydroxyurea-resistant/intolerant polycythemia vera. ClinicalTrials.gov identifier: NCT03287245. Updated Feb 9, 2021. Accessed May 20, 2021. www.clinicaltrials.gov/ct2/show/NCT03287245

64. An open-label, multicenter, phase 1b/2 study of the safety and efficacy of KRT-232 combined with low-dose cytarabine (LDAC) or Decitabine in patients with acute myeloid leukemia (AML). ClinicalTrials.gov identifier: NCT04113616. Updated Dec 7, 2020. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT04113616

65. KRT-232 versus best available therapy for the treatment of subjects with myelofibrosis who are relapsed or refractory to JAK inhibitor treatment (BOREAS). ClinicalTrials.gov identifier: NCT03662126. Updated Feb 2, 2021. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT03662126

66. KRT-232 compared to Ruxolitinib in patients with phlebotomy-dependent polycythemia vera. ClinicalTrials.gov identifier: NCT03669965. Updated Jul 31, 2020. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT03669965

67. Suragani RNVS, Cadena SM, Cawley SM, et al. Transforming growth factor-β superfamily ligand trap ACE-536 corrects anemia by promoting late-stage erythropoiesis. *Nat Med.* 2014;20:408–414.

68. Dussiot M, Maciel TT, Fricot A, et al. An activin receptor IIA ligand trap corrects ineffective erythropoiesis in β-thalassemia. *Nat Med.* 2014;20:398–407.

69. Carrancio S, Markovics J, Wong P, et al. An activin receptor IIA ligand trap promotes erythropoiesis resulting in a rapid induction of red blood cells and haemoglobin. *Br J Haematol.* 2014;165:870–882.

70. Iancu-Rubin C, Mosoyan G, Wang J, et al. Stromal cell-mediated inhibition of erythropoiesis can be attenuated by Sotatercept (ACE-011), an activin receptor type II ligand trap. *Exp Hematol.* 2013;41:155–166.e17.

71. Ear J, Huang H, Wilson T, et al. RAP-011 improves erythropoiesis in zebrafish model of Diamond-Blackfan anemia through antagonizing lefty1. *Blood.* 2015;126:880–890.
72. Langdon JM, Barkatsaki S, Berger AE, et al. RAP-011, an activin receptor ligand trap, increases hemoglobin concentration in hepatic transgenic mice. *Am J Hematol*. 2015;90:8–14.

73. Sotatercept in treating patients with myeloproliferative neoplasm-associated myelofibrosis or anemia. ClinicalTrial.gov identifier: NCT01712308. Updated Aug 5, 2020. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT01712308

74. Study of Sotatercept for the treatment of anemia in low-, or intermediate-1 risk myelodysplastic syndromes (MDS) or non-proliferative chronic myelomonocytic leukemia (CMML). ClinicalTrial.gov identifier: NCT01736683. Updated Jun 25, 2019. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT01736683

75. Platzbecker U, Germing U, Götze KS, et al. Luspatercept for the treatment of anemia in patients with lower-risk myelodysplastic syndromes (PACE-MDS): a multicentre, open-label phase 2 dose-finding study with long-term extension study. *Lancet Oncol*. 2017;18:1338–1347.

76. A safety and efficacy study to evaluate Luspatercept in subjects with myeloproliferative neoplasm-associated myelofibrosis who have anemia with and without red blood cell-transfusion dependence. ClinicalTrial.gov identifier: NCT03194542. Updated Apr 27, 2021. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT03194542

77. A study to evaluate long-term safety in subjects who have participated in other Luspatercept (ACE-536) clinical trials. ClinicalTrial.gov identifier: NCT04064060. Updated Dec 1, 2020. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT04064060

78. MPN-RC 118 AVID200 in myelofibrosis. ClinicalTrial.gov identifier: NCT03895112. Updated May 21, 2021. Accessed May 21, 2021. clinicaltrials.gov/ct2/show/NCT03895112

79. Thiele J, Kvasnicka HM. Grade of bone marrow fibrosis is associated with relevant hematological findings—a clinicopathological study on 865 patients with chronic idiopathic myelofibrosis. *Ann Hematol*. 2006;85:226–232.

80. Nakagawa N, Barron L, Gomez IG, et al. Pentraxin-2 suppresses c-Jun/AP-1 signaling to inhibit progressive fibrotic disease. *J Exp Med*. 2016;213:1723–1740.

81. Verstovsek S, Manshouri T, Pilling D, et al. Role of neoplastic monocyte-derived fibrocytes in primary myelofibrosis. *Blood*. 2014;124:151–152.

82. Mascarenhas J, Kremyanskaya M, Hoffman R, et al. MANIFEST, a phase 2 study of CPI-0610, a bromodomain and extraterminal domain inhibitor (BETi), as mono- or combination therapy in patients with myelofibrosis. ClinicalTrial.gov identifier: NCT02158858. Updated Jan 29, 2021. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT02158858

83. Shi Y, Lan F, Matson C, et al. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell*. 2004;119:941–953.

84. Papadantonakis N, Matsuura S, Ravid K. Megakaryocyte pathology and bone marrow fibrosis: the lysyl oxidase connection. *Blood*. 2012;120:1774–1781.

85. Wang Q, Yang Q, Goldenson B, et al. Targeting megakaryocytic induced fibrosis by AURKA inhibition in the myeloproliferative neoplasms. *Nat Med*. 2015;21:1473–1480.

86. Gangat N, Marinaccio C, Swords R, et al. Aurora kinase A inhibition provides clinical benefit, normalizes megakaryocytes, and reduces bone marrow fibrosis in patients with myelofibrosis: a phase I trial. *Clin Cancer Res*. 2019;25:4898-4906.

87. Belkina AC, Denis GV. BET domain co-regulators in obesity, inflammation and cancer. *Nat Rev Cancer*. 2012;12:465–477.

88. Sehrawat A, Gao L, Wang Y, et al. LSD1 activates a lethal prostate cancer gene network independently of its demethylase function. *Proc Natl Acad Sci USA*. 2018;115:E4179–E4188.

89. Yang Y, Huang W, Qiu R, et al. LSD1 coordinates with the SIN3A/HDAC complex and maintains sensitivity to chemotherapy in breast cancer. *J Mol Cell Biol*. 2018;10:285–301.

90. Sehrawat A, Gao L, Wang Y, et al. LSD1 activates a lethal prostate cancer gene network independently of its demethylase function. *Proc Natl Acad Sci USA*. 2018;115:E4179–E4188.

91. Yang Y, Huang W, Qiu R, et al. LSD1 coordinates with the SIN3A/HDAC complex and maintains sensitivity to chemotherapy in breast cancer. *J Mol Cell Biol*. 2018;10:285–301.

92. Sehrawat A, Gao L, Wang Y, et al. LSD1 activates a lethal prostate cancer gene network independently of its demethylase function. *Proc Natl Acad Sci USA*. 2018;115:E4179–E4188.
102. IMG-7289 in patients with myelofibrosis. ClinicalTrial.gov identifier: NCT03136185. Feb 17, 2021. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT03136185

103. Pettit K, Gerds AT, Yacoub A, et al. A phase 2a study of the LSD1 inhibitor Img-7289 (bomedemstat) for the treatment of myelofibrosis. Blood. 2019;134(suppl 1):556.

104. Kim NW, Piatyszek MA, Prowse KR, et al. Specific association of human telomerase activity with immortal cells and cancer. Science. 1994;266:2011–2015.

105. Bodnar AG, Ouellette M, Frolkis M, et al. Extension of life-span by introduction of telomerase into normal human cells. Science. 1998;279:349–352.

106. Mocellin S, Pooley KA, Nitti D. Telomerase and the association of human telomerase activity with immortal progenitor cells. Blood Adv. 2018;2:2378–2388.

107. Wang X, Hu CS, Petersen B, et al. Imetelstat, a telomerase inhibitor, is capable of depleting myelofibrosis stem and progenitor cells. Blood Adv. 2015;373:920–928.

108. Baerlocher GM, Oppliger Leibundgut E, Ottmann OG, et al. Telomerase inhibitor Imetelstat in patients with essential thrombocythemia. N Engl J Med. 2015;373:902–909.

109. Tefferi A, Lasho TL, Begna KH, et al. A pilot study of the telomerase inhibitor Imetelstat for myelofibrosis. N Engl J Med. 2015;373:908–919.

110. Study to evaluate activity of 2 dose levels of Imetelstat in participants with intermediate-2 or high-risk myelofibrosis (MF) previously treated with janus kinase (JAK) inhibitor. ClinicalTrial.gov identifier: NCT02426086. Updated Mar 3, 2020. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT02426086

111. Geron Corporation. Geron announces plans for imetelstat phase 3 clinical trial in myelofibrosis and other updates. GlobeNewswire. Published May 21, 2020. Accessed Aug 2, 2020. www.globenewswire.com/news-release/2020/05/21/2037258/0/en/Geron-Announces-Plans-for-Imetelstat-Phase-3-Clinical-Trial-in-Myelofibrosis-and-Other-Updates.html Aug

112. Actuate 1901: 9-ING-41 in myelofibrosis. ClinicalTrial.gov identifier: NCT04218071. Updated Mar 9, 2021. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT04218071

113. Safety and tolerability study of oral ABBV-744 tablet alone or in combination with oral Ruxolitinib tablet or oral Navitoclax tablet in adult participants with myelofibrosis. ClinicalTrial.gov identifier: NCT04454658. Updated Mar 21, 2021. Accessed May 21, 2021. clinicaltrials.gov/ct2/show/NCT04454658

114. A study of APG-1252 in patients with myelofibrosis who progressed after initial therapy. ClinicalTrial.gov identifier: NCT04354727. Updated Dec 10, 2020. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT04354727

115. Testing the effect of taking Ruxolitinib and CPX-351 in combination for the treatment of advanced phase myeloproliferative neoplasms. ClinicalTrial.gov identifier: NCT03878199. Updated Nov 12, 2020. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT03878199

116. Ruxolitinib phosphate and decitabine in treating patients with relapsed or refractory or post myeloproliferative acute myeloid leukemia. ClinicalTrial.gov identifier: NCT02257138. Updated Mar 23, 2021. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT02257138

117. Combined Ruxolitinib and Enasidenib in patients with accelerated/blast-phase myeloproliferative neoplasm or chronic-phase myelofibrosis with an IDH2 mutation. ClinicalTrial.gov identifier: NCT04281498. Updated May 21, 2021. Accessed May 21, 2021. clinicaltrials.gov/ct2/show/NCT04281498

118. A study of Itacitinib in combination with low-dose Ruxolitinib or Itacitinib alone following Ruxolitinib in subjects with myelofibrosis. ClinicalTrial.gov identifier: NCT03144687. Updated Jan 26, 2021. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT03144687

119. Safety and tolerability study of Mivebresib tablet alone or in combination with Ruxolitinib tablet or Navitoclax tablet in adult participants with myelofibrosis. ClinicalTrial.gov identifier: NCT04480086. Updated May 19, 2021. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT04480086

120. Study of oral Navitoclax tablet in combination with oral Ruxolitinib tablet to assess change in spleen volume in adult participants With Relapsed/Refractory Myelofibrosis (TRANSFORM-2). ClinicalTrial.gov identifier: NCT04468984. Updated May 17, 2021. Accessed May 20, 2021. www.clinicaltrials.gov/ct2/show/NCT04468984

121. A study of INCB050465 in combination with Ruxolitinib in subjects with myelofibrosis. ClinicalTrial.gov identifier: NCT02718300. Updated Mar 24, 2021. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT02718300

122. Pevonedistat in combination with Ruxolitinib for treatment of patients with myelofibrosis. ClinicalTrial.gov identifier: NCT03386214. Updated Jun 16, 2020. Accessed May 20, 2021. www.clinicaltrials.gov/ct2/show/NCT03386214

123. A clinical study to test the effects of Ruxolitinib and Thalidomide combination for patients with myelofibrosis. ClinicalTrial.gov identifier: NCT03069326. Updated Feb 1, 2021. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT03069326

124. Decitabine with Ruxolitinib or Fedratinib for the treatment of accelerated/blast phase myeloproliferative neoplasms. ClinicalTrial.gov identifier: NCT04282187. Updated Feb 18, 2021. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT04282187

125. A safety trial of Fedratinib in subjects with DIPSS, intermediate or high-risk primary myelofibrosis, post-polycythemia vera myelofibrosis, or post-essential thrombocytocytoma myelofibrosis and previously treated with Ruxolitinib with concomitant luspatercept for subjects with anemia (FREEDOM). ClinicalTrial.gov identifier: NCT04282187. Updated Feb 18, 2021. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT04282187

126. LCL161 in treating patients with primary myelofibrosis, post-polycythemia vera myelofibrosis, or post-essential thrombocytocytoma myelofibrosis. ClinicalTrial.gov identifier: NCT02098161. Updated Apr 1, 2020. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT02098161

127. TGR-1202 + Ruxolitinib PMF PPV-MF PET-MF MDS/MPN Polycythemia vera resistant to hydroxyurea. ClinicalTrial.gov identifier: NCT02493530. Updated Mar 12, 2021. Accessed May 20, 2021. clinicaltrials.gov/ct2/show/NCT02493530