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Review

Treating accelerated and blast phase myeloproliferative neoplasms: progress and challenges

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***Abstract*:** Myeloproliferative neoplasms (MPNs) are a group of clonal hematologic malignancies that include polycythemia vera (PV), essential thrombocythemia (ET), and myelofibrosis (MF). MPNs are characterized by activating mutations in the JAK/STAT pathway and an increased risk of transformation to an aggressive form of acute leukemia, termed MPN-blast phase (MPN-BP). MPN-BP is characterized by the presence of ⩾20% blasts in the blood or bone marrow and is almost always preceded by an accelerated phase (MPN-AP) defined as ⩾10–19% blasts in the blood or bone marrow. These advanced forms of disease are associated with poor prognosis with a median overall survival (mOS) of 3–5 months in MPN-BP and 13 months in MPN-AP. MPN-AP/BP has a unique molecular landscape characterized by increased intratumoral complexity. Standard therapies used in *de novo* acute myeloid leukemia (AML) have not demonstrated improvement in OS. Allogeneic hematopoietic stem cell transplant (HSCT) remains the only curative therapy but is associated with significant morbidity and mortality and infrequently utilized in clinical practice. Therefore, an urgent unmet need persists for effective therapies in this advanced phase patient population. Here, we review the current management and future directions of therapy in MPN-AP/BP.

Keywords: accelerated phase, blast phase, myeloproliferative neoplasms, novel therapeutics

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Background

Philadelphia chromosone negative (Ph-) myeloproliferative neoplasms (MPNs) are a heterogeneous group of clonal hematologic malignancies that include polycythemia vera (PV), essential thrombocythemia (ET), and myelofibrosis (MF).[1] Activating mutations in the JAK/STAT pathway are the biologic hallmark of the MPNs.[2] MPNs are associated with a risk of transformation to acute leukemia, termed MPN-blast phase (BP), which is characterized by the presence of ⩾20% blasts in the peripheral blood or bone marrow.[3] Transformation to MPN-BP occurs within 10 years from diagnosis in 1%, 4%, and 20% of patients with ET, PV, and MF, respectively.[4,5] Clinically, MPN-BP resembles secondary acute myeloid leukemia (sAML) and is typically preceded by an accelerated phase (MPN-AP) that is defined as presence of ⩾10–19% blasts in the peripheral blood or bone marrow.[1] Rarely, MPNs can transform into acute lymphoblastic leukemia (ALL). In a literature review and report of 18 cases of lymphoid blast transformation of Ph-MPNs, the majority of cases had a B-cell phenotype, a median time to progression to ALL of approximately 10 years and poor prognosis, with a mortality of 80% in the published cases.[6] The mechanisms surrounding the progression to MPN-AP/BP are not entirely understood but are thought to involve the acquisition of somatic mutations and epigenetic alterations in hematopoietic progenitor and stem cells (HPSCs) leading to clonal expansion as well as a tumor supportive proinflammatory microenvironment.[3,7,8] Both MPN-AP and MPN-BP have a poor prognosis with a median overall survival

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(mOS) of approximately 13 months in MPN-AP and 3–5 months in MPN-BP.[3,9] Newer targeted therapies have improved progression-free survival (PFS) but have not significantly impacted overall survival (OS). The only curative therapy is allogeneic hematopoietic stem cell transplant (HSCT) that is utilized in a minority of patients due to the high rate of associated morbidity and mortality in unfit populations. The molecular landscape of MPN-AP/BP differs significantly from that of *de novo* acute myeloid leukemia (AML). MPNs are characterized by driver mutations in Janus Kinase 2 (*JAK2*), myeloproliferative leukemia virus oncogene (*MPL*), and Calreticulin (*CALR*). Over 50% of patients with MPN-CP have additional somatic mutations in epigenetic regulators, splicing modulators, RAS, and TP53. In addition to molecular mutations, cytogenetic abnormalities associated with MPNs often portend increased intratumoral heterogeneity and complexity.[10] Primary myelofibrosis (PMF) is associated with more molecular complexity and an increased frequency of transformation to MPN-AP/BP than both ET and PV.[10] Understanding the molecular landscape driving the progression to MPN-AP/BP is paramount in stratifying patients who are at higher risk of progression and appropriate for risk-adapted therapeutic interventions including HSCT while still in the chronic phase (CP). Here, we provide an overview of the current management of patients with MPN-AP/BP as well as the future direction of therapeutic interventions.

Risk of progression

For the majority of patients who progress from CP disease to MPN-BP, the development of MPN-AP is an obligatory step in disease evolution.[3] Several clinical characteristics associated with progression from MPN-CP to MPN-AP/BP include age $\geq$60 years, red blood cell (RBC) transfusion dependence, leukocytosis, monosomal karyotype, platelet count of $<100\times10^9$/l, and circulating blasts $\geq$3%.[1,11–13] Three widely implemented prognostic scoring systems incorporate these clinical risk factors to risk stratify patients with MF. The Dynamic International Prognostic Scoring System (DIPSS) includes age, presence of systemic symptoms, as well as complete blood count abnormalities including leukocytosis, anemia, and presence of circulating blasts.[14] The DIPSS-plus includes cytogenetic abnormalities, thrombocytopenia, and RBC transfusion dependence.[15] The Mutation Enhanced International Prognostic Score System MIPSS70 incorporates the presence of known high-risk acquired somatic mutations.[16]

Current understanding of molecular basis of advanced forms of disease

Activating driver mutations involving the JAK/STAT pathway are the hallmark of CP-MPNs with *JAK2V617F* occurring in approximately 98% of patients with PV and in 50–60% of patients with ET or MF.[11] In patients with MF, mutations involving *CALR* and *MPL* also contribute to hyperactivity of this signaling pathway and together with *JAK2V617F* account for 90% of driver mutations.[17] The presence of other genomic alterations in *TET2, EZH2*, and *TP53* frequently further alter the biology of the disease and have prognostic implications.[11] The most common somatic mutations in MPN-BP outside of JAK2 occur in *TET2*, *SRSF2*, *IDH1/2* and *TP53*.[11] Whereas, the most common somatic mutations in *de novo* AML occur in *FLT3*, *NPM1*, and *DNMT3A*.[11] At least two distinct routes to leukemic transformation have been proposed: one route includes the acquisition of mutations in the clone bearing a JAK/STAT driver mutation, most commonly *TP53* that is seen in approximately 15% of patients with *JAK2V617F*, 25% of patients with *CALR* mutations, and 0% of patients with *MPL* mutations.[18] Co-operativity between *JAK2* mutations and *TP53* mutations/alterations has been credentialed in murine models, thus establishing the biologic basis of these clinical observations.[19] Alternatively, *de novo* emergence of a leukemic clone distinct from the clone bearing an activating driver mutation can emerge, ultimately leading to a decrease in variant allele frequency (VAF) or loss of pre-existing driver mutation, due to clonal expansion of the leukemic clones over the underlying MPN-clone (Figure 1).[10,11,20] Even with a significant proportion of MPN-BP arising from a potential non-driver mutation MPN-CP clone, the molecular landscape and overall prognosis remains distinct from that in *de novo* AML.

Current therapies

MPN-CP

The goal of therapy for patients with MPN-CP is to prevent thrombotic and hemorrhagic events, reduce the systemic symptom burden and

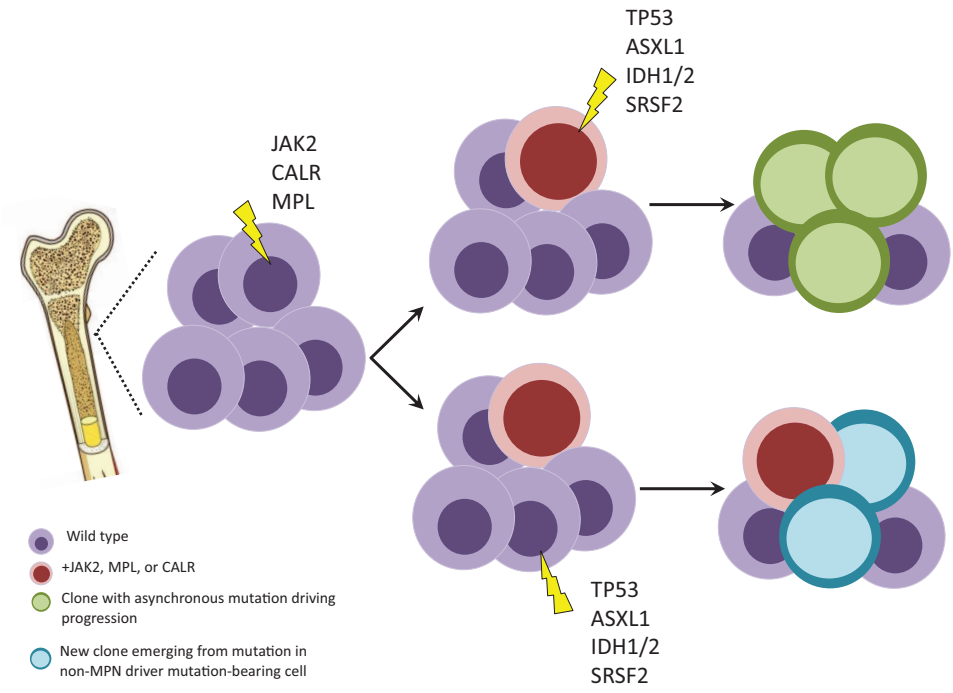

Figure 1. Pathophysiology of progression to MPN-AP/AP.

splenomegaly, and minimize the risk of disease progression to MPN-AP/BP. The only curative therapy remains allogeneic HSCT, which is generally limited to patients with DIPSS intermediate-2 and high-risk MF due to associated morbidity and mortality. Current medical therapeutic agents for CP-MPNs include JAK inhibitors, pegylated interferon-alfa, and nonspecific cytoreductive agents. Ruxolitinib, fedratinib, and pacritinib are the three Food and Drug Administration (FDA) approved JAK inhibitors for select patients with MPN-CP. Fedratinib is typically reserved for patients who have failed ruxolitinib; however, it is approved for use in the front-line setting as well.[21,22] Pacritinib is reserved for patients with MF with severe thrombocytopenia.[23] Momelotinib is another novel JAK inhibitor with positive phase III data seeking approval for MF patients with transfusion-dependent anemia.[24] While these therapeutic agents have demonstrated efficacy in terms of the ability to improve splenomegaly, and alleviate symptom burden, none has demonstrated the ability to effectively deplete the underlying malignant clone or reduce the risk of disease progression.[22,23,25–30] Despite the availability of these targeted agents, the majority of patients treated with selective JAK inhibitors discontinue treatment within 3 years.[31] Interferon derivatives have demonstrated the ability to deplete the underlying malignant clone in a minority of patients with PV and ET; however, whether or not this translates to a reduced risk of disease progression remains controversial and not yet confirmed in prospective studies.[32–34] Nonspecific cytoreductive agents include hydroxyurea and anagrelide, which are effective in preventing thrombotic and hemorrhagic events, but do not target the underlying malignant clone, and have not been shown to improve or reduce progression of disease to AP/BP.[35,36] Rather than evaluating developmental agents based solely on thrombotic risk reduction, spleen size reduction, and symptom burden, there has been shift to develop novel anticlonal agents that can effectively target the underlying malignant clone and prevent progression of disease to AP/BP.

Potentially disease-modifying agents in development for patients with MF include inhibitors of MDM2, BET, BCL-2, PI3K, and telomerase, as well as recombinant PTX2 and an SMAC mimetic.[37] Trials have also evaluated histone deacetylase (HDAC) inhibitors in this patient population; however, toxicity profiles precluded further

development.[38,39] We are hopeful that as our understanding of disease biology continues to grow and more targeted treatment options become available, implementing these approaches will reduce the risk of disease progression and improve OS for CP-MPN. Even with optimal use of our current MPN-CP treatment armamentarium, however, a significant portion of patients with MPNs continue to progress to AP/BP. There remains no standardized approach for the management of MPNs that have progressed to AP or BP.

MPN-AP/BP

Cytotoxic chemotherapy

Cytotoxic chemotherapy with AML-inspired intensive induction regimens such as cytarabine and daunorubicin (7 + 3), fludarabine, cytarabine, and granulocyte colony-stimulating factor (G-CSF), idarubicin (FLAG-IDA), and mitoxantrone, etoposide, and cytarabine (MEC) without consolidative HSCT has been associated with poor outcomes.[4,40,41] Complete response (CR) rates range from 0% to 30% often with incomplete count recovery (CRi), high treatment–related mortality, and short duration of leukemic-free interval.[4,40,41] In a retrospective study of 91 patients with MF-BP, patients were split into 3 treatment groups; 19 received low-intensity therapy, 24 received an AML-like high-intensity induction regimen, and 48 received best supportive care. Low-intensity regimens included monotherapy with vincristine, etoposide, oral-alkylating agents, or low-dose cytarabine (LDAC). High-intensity regimens included 7 + 3 or monotherapy with high-dose cytarabine. No patients in the group receiving high-intensity therapy achieved a CR, and the mOS was 3.9 months. In a subset of patients who reverted to CP disease after high-intensity induction therapy, the mOS was 6.2 months compared with 2.7 months in those with persistently elevated blasts after induction therapy. The incidence of treatment-related mortality, however, was 33%. The mOS in the supportive care and low-intensity therapy arms was 2.1 months and 2.9 months, respectively.[41] Poor response to chemotherapy is likely due to the complex genetic profiles of MPN AP/BP that includes a high frequency of *TP53* mutations.[1,11] Owing to advanced age at diagnosis and often presence of comorbid conditions, patients with MPN BP/AP are frequently not candidates for intensive induction chemotherapy or HSCT.[1]

Hematopoietic stem cell therapy

Consolidative HSCT is the only curative therapy in MPN-AP/BP. The significant morbidity and mortality associated with HSCT often precludes its use in older patients and those with significant comorbidities. In those who are transplant candidates, there are many transplant-related considerations including donor selection, optimal conditioning regimen, and post-transplant complications. In several retrospective studies of patients with MPN-BP who received HSCT, the only factor associated with prolonged OS was achievement of CR prior to transplant.[40,42,43] In a retrospective review of 46 patients with MPN-BP, 42 of the 46 received induction therapy, followed by HSCT. Thirty eight of the 46 patients were assessable of which 24% achieved CR. At 3 years, the PFS and OS rates were 26% and 33%, respectively, and univariate analysis prior to HSCT demonstrated that only pretransplant CR impacted survival. Transplant-related factors such as donor source and conditioning regimen did not impact survival.[43] More recent data, however, suggest that CR is not required for improved outcomes in MPN-BP. A retrospective review of patients 46 patients with MPN-BP who received HSCT demonstrated that pre-HSCT blast count of $<5\%$ *versus* $>5\%$ or $<10\%$ *versus* $>10\%$ did not predict post-HSCT survival.[44] While there are no standard response criteria for patients with MPN AP/BP, the European LeukemiaNet (ELN) criteria are frequently employed to assess response.[45] Elevated circulating blasts and in the bone marrow $>5\%$, however, can be seen in CP-MPNs due to aberrant MPN stem cell trafficking and resultant extramedullary hematopoiesis. A more comprehensive response assessment for MPN-BP has been proposed which incorporates five relevant components of MPN-CP and the leukemic clone: hematologic, clinical, pathologic, cytogenetic, and molecular changes.[46]

Hypomethylating agents

Hypomethylating agents (HMAs) have demonstrated activity in MPN-AP/BP with less toxicity compared with intensive chemotherapy. In a

retrospective analysis of 54 patients with Ph-negative MPNs who progressed to MPN-AP or BP and were treated with azacitidine, 24% achieved a CR.[47] The median duration of response was 9 months, and mOS was 11 months.[47] Another retrospective study that evaluated patients with high-risk MF and MPN-AP/BP, treated with single agent decitabine, demonstrated an overall response rate (ORR) of 29%, with an mOS of 10.5 months in those who responded and 4 months in nonresponders.[48] A retrospective review of a cohort of 410 patients with MPN-BP from the Mayo clinic and AIRC-Gruppo Italiano Malattie Mieloproliferative (AGIMM) treated with HMA monotherapy, the CR rate was only 4%.[12] While there is clearly a benefit for a certain subpopulation of patients with MPN-BP, when used as monotherapy, HMA therapy does not appear to provide a durable response in the majority of treated patients.

Ruxolitinib

Ruxolitinib, a selective JAK 1/2 inhibitor, has also been evaluated in MPN-BP in multiple clinical trials. A single-center phase II study evaluating high-dose ruxolitinib monotherapy was conducted in patients with R/R leukemia. Two of the 18 patients (11%) with MPN-BP treated with ruxolitinib at a dose of 25 mg twice daily attained a CR and one patient (5.5%) attained CRi. Notably, all three responding patients harbored *JAK2V617F*.[49] Overall, the regimen was well-tolerated, but led to limited and transient responses.[49] In a single-center phase I/II study in 27 patients testing three dose levels of ruxolitinib 50 mg twice daily ($n = 4$), 100 mg twice daily ($n = 5$), and 200 mg twice daily ($n = 18$) in patients with R/R AML and ALL, no CR was observed and the study was stopped due to lack of clinical benefit.[50] Similarly to HMA monotherapy, ruxolitinib alone has not demonstrated significant benefit in MPN-AP/BP. Several studies have evaluated ruxolitinib in combination with decitabine in this advanced phase setting. In a multicenter phase II study of 25 patients with MPN AP/BP treated with ruxolitinib at an induction dose of 25 mg twice daily and then 10 mg twice daily in subsequent 28-day cycles plus decitabine at 20 mg/m^2 days 1–5 of a cycle, the ORR was 44% with an mOS of 9.5 months, and the combination was well-tolerated.[51] A single institution phase I study of ruxolitinib including 18 patients with R/R AML and phase II study including 29 patients with MPN-BP of ruxolitinib at a recommended phase II dose of 25 mg twice daily continuously and standard decitabine demonstrated an mOS of 6.9 months.[52] Because of the cross-trial mOS benefit of combination JAK2 inhibitor and HMA when compared with monotherapy data, and an improved toxicity profile when compared with intensive chemotherapy regimens, HMA and ruxolitinib are often administered in combination in this patient population when HSCT is not a therapeutic option for consolidation or as a bridge to HSCT in select patients (Table 1).

Venetoclax

Venetoclax, a BCL-2 inhibitor, in combination with HMA is a well-described low-intensity regimen approved for the treatment of patients with AML.[53] These data have been extrapolated and employed in the anecdotal care of MPN-BP. This regimen has been evaluated in several retrospective studies in this patient population. A retrospective multicenter study of 32 patients with MPN-BP treated with a combination of venetoclax and azacitidine 75 mg/m^2 (days 1–7) or decitabine 20 mg/m^2 (days 1–5) demonstrated a 44% ORR (CR/CRi) and mOS of 8 months.[54] In a single-center study of 31 patients with MPN-BP treated with a combination venetoclax and HMA, 6 of 14 patients (43%) treated in the front-line setting achieved a CR/CRi and 1 patient (7%) achieved a partial response (PR). The mOS was 7 months.[55] In the R/R setting, no patients responded, and mOS was 3 months.[55] There was significant treatment-related toxicity with 83% of patients developing grade 3 or higher infections during the first cycle.[55] Another retrospective multicenter analysis that evaluated venetoclax and HMA or LDAC in 27 patients with MPN-AP or BP similarly demonstrated clinical activity with an ORR of 53% in MPN-BP and 50% in MPN-AP. There was limited survival benefit with an mOS of 6 months for both MPN-BP and MPN-AP.[56] While venetoclax plus HMA has significantly improved outcomes in patients with *de novo* AML, it has not demonstrated convincing beneficial impact in patients with MPN-BP. It is associated with significant toxicity, without survival benefit to merit its use in this patient population outside of a clinical trial.

IDH inhibitors

Mutations in isocitrate dehydrogenase 1/2 (*IDH1/2*) result in aberrant *IDH1/2* activity and impaired myeloid differentiation. The use of small molecule inhibitors targeting these mutations, ivosidenib and enasidenib, which target *IDH1* and *IDH2*, respectively, has led to improved outcomes in *IDH*-mutated AML in both the front-line and R/R setting.[53,57] Importantly, *IDH1* or *IDH2* mutations occur in approximately 22% of MPN-AP/BP cases and thus represent a therapeutic opportunity in a significant portion of this patient population.[58,59] In a retrospective analysis of MPN-BP patients with *IDH1/2* mutations treated with an IDH inhibitor as monotherapy or in combination with ruxolitinib, venetoclax, HMA or intensive chemotherapy, 3 out of 12 (25%) patients achieved a CR with undetectable *IDH1/2* mutations by next generation sequencing (NGS).[59] The mOS was 10 months for all patients and 19 months for responders.[59] Another group conducted a retrospective analysis of patients with *IDH1 or IDH2*-mutated MPN-AP/BP treated with IDH inhibitors. Of 8 patients treated, 2 (25%) achieved a CR, 4 (50%) achieved a PR, and 1 (12.5%) had stable disease. The median OS had not been reached at the median follow-up time of 9 months.[60] There is an ongoing multicenter Myeloproliferative Neoplasms Research Consortium (MPN-RC) phase II study assessing the safety and efficacy of the combination of ruxolitinib and enasidenib in patients with MPN AP/BP or CP MF and at least 5% circulating blasts with an *IDH2* mutation (NCT04281498). Despite persistently low-response rates overall in published data, patients who responded have demonstrated favorable survival outcomes. Therefore, IDH inhibitors are typically incorporated into the treatment regimen for those MPN-AP/BPs with mutations in *IDH1* or *IDH2*.

Future directions

Tumor microenvironment

MPNs are characterized by dysregulated JAK/STAT signaling resulting in an increase in proinflammatory cytokines such as transforming growth factor-beta (TGF-β), tumor necrosis factor alpha (TNFα) and interleukins (ILs) 2, 6, and 8 that have been implicated in supporting malignant clonal hematopoiesis and promoting bone marrow fibrosis.[61] Specifically, IL-2 and IL-8 have demonstrated particular importance in the

Table 1. Therapeutic regimens and associated response rates and median OS.

Regimen	Population	Response rate	Median OS	References
Cytotoxic chemotherapy	MPN-BP	CR 0%	3.9 months	Mesa *et al.*[41]
Ruxolitinib	MPN-BP	CR 11%	–	Eghtedar *et al.*[49]
		CR 0%	–	Pemmaraju *et al.*[50]
Ruxolitinib and decitabine	MPN-AP/BP	ORR (CR/CRi) 44%	9.5 months	Mascarenhas *et al.*[51]
IDH inhibitor	MPN-AP/BP	CR 25%	Not reached at 9 months	Patel *et al.*[60]
IDH inhibitor monotherapy or in combination with ruxolitinib, venetoclax, HMA, or intensive chemotherapy	MPN-BP	CR 25%	10 months	Chifotides *et al.*[59]
HMA	MPN-BP	CR 24%	11 months	Thepot *et al.*[47]
	MPN-AP/BP	ORR 29%	10.5 months	Badar *et al.*[48]
	MPN-BP	CR 4%	–	Tefferi *et al.*[12]
Venetoclax and HMA	MPN-BP	ORR (CR/CRi) 44%	8 months	Gangat *et al.*[54]
	MPN-BP	CR/CRi 43%	7 months	Masarova *et al.*[55]

CR, complete response; Cri, incomplete count recovery; HMA, hypomethylating agent; IDH, isocitrate dehydrogenase; MPN-AP, myeloproliferative neoplasms–accelerated phase; MPN-BP, myeloproliferative neoplasms–blast phase; ORR, overall response rate; OS, overall survival.

prognosis and phenotype of MF. Increased levels of both cytokines have been associated with constitutional symptoms and increased RBC transfusion requirements.[62] In treatment naïve patients, increased levels of circulating IL-8 were found to be associated with inferior survival, increased circulating blasts, and shorter leukemia-free survival.[62] A murine model of MF demonstrated high levels of TGF-β, mCXCL1 (the murine equivalent of h*CXCL8*, the gene coding IL-8) and its receptors CXCR1 and CXCR2.[63] After treatment with reparixin, a CXCR1/2 inhibitor, megakaryocytes demonstrated lower levels of TGF-β compared with control suggesting that IL-8 may be a viable therapeutic target.[63] There is evidence that constitutive JAK/STAT signaling in MF leads to an immunosuppressive tumor microenvironment by upregulation of *PD-L1* expression, checkpoint receptor.[64] Oncogenic JAK2 activity *via* increased STAT3 and STAT5 activation has been shown to enhance *PD-L1* promoter activity and, therefore, PD-L1 expression in murine *JAK2V617F* knock-in model. PD-L1 expression was also shown to be upregulated in human T cells of MF patients when compared with healthy controls.[64] In addition to PD-L1 overexpression, peripheral blood T cells have also demonstrated overexpression of other checkpoint receptors including CTLA4 and TIM-3.[65] Given these data, it was hypothesized that PD-1 inhibition with pembrolizumab may potentiate the immune system to target MPN HPSCs.[64] In a multicenter single-arm phase II study that included 10 patients with advanced MF and single patient with MPN-BP who received pembrolizumab monotherapy, there was no significant clinical or pathologic response. There, however, was evidence of T-cell activation and changes in T-cell clonality with shared epitope demonstrating changes in the immune phenotype suggesting an MPN cell–directed immune repsonse.[66] Despite a lack of clinical or pathologic response to anti-PD1 monotherapy, combination therapy targeting LAG3, CTLA4, or TGF-β may provide additional benefit. Further investigation into the role of targeted combination immunotherapy in MPN AP/BP is warranted.

TP53 pathway dysregulation

Dysregulation of the *TP53* pathway *via* deletion, mutation, or inactivation of p53 plays an important role in the pathogenesis across a diverse array of both solid and hematologic malignancies.[67] TP53 loss is among the most common genomic alterations observed at the time of leukemic transformation in MPNs occurring in 30% of patients.[11] Paired patient samples in MPN-CP and post-MPN AML demonstrated that mutations in *JAK2* and *TP53* were almost always present at high VAFs (>50%) in patients with MPN-BP at the time of transformation. This suggests their presence in the dominant AML clone and consistent with loss of wild-type *TP53*.[11] Inactivation of p53 occurs commonly as a result of overexpression of mouse double-minute homolog 2 (MDM2) and mouse double-minute homolog 4 (MDM4) – two important negative regulators of TP53 transcription and activation.[68] MDM2 is an E3 ubiquitin ligase that regulates the stability of p53 by mediating its degradation thus acting as a negative regulator of the pathway.[69] MDM4 can increase or decrease the E3 ubiquitin ligase activity.[70] Several chromosomal analyses of patients with MPNs have demonstrated an association between abnormalities in chromosomes 1q, which encodes MDM4, with increased risk of disease progression to myelofibrosis and MPN-AP/BP.[67,71,72] In addition, increased expression of MDM2 has been demonstrated in $CD34^+$ HSPCs in MPNs.[73] *JAK2V617F* has been demonstrated to be a negative regulator of TP53 *via* increased MDM2 translation.[74] Several therapeutic candidates are being developed that target reactivation of the p53 pathway.

Expression of MDM2 is upregulated in the HPSCs of patients with *JAK2V617F* MPNs due to upregulation of La antigen, an enhancer of MDM2 translation.[74] A class of drugs called Nutlins, named after the town in which they were discovered (Nutley, NJ), was the first identified selective MDM2 inhibitors.[75] RG7112 is a small molecule inhibitor of p53-MDM2 binding and in preclinical leukemia models demonstrated sensitivity to RG7112 through induction of p53-dependent apoptosis.[76] In a phase I study of patients with R/R leukemias including AML and ALL, RG7112 demonstrated stabilization in p53 levels and transcriptional activation of p53 target genes.[77] The high doses required for efficacy, however, led to significant GI intolerance.[77] A phase II trial evaluated idasanutlin (RG7388, Roche Pharmaceuticals, Basel, ↱ Switzerland), an oral MDM2 inhibitor, in patients with *JAK2V617F positive* PV resistant/intolerant to

hydroxyurea. Subjects enrolled demonstrated a 39% median reduction in the *JAKV617F* VAF at the end of three treatment cycles and a median reduction of 76% at 32 weeks suggesting robust anticlonal activity.[38] Therapy, however, was associated with significant gastrointestinal toxicity leading to treatment discontinuation in greater than 50% of patients.[38] Navtemadlin (KRT232, Kartos Therapeutics, Redwood City, CA, USA), a more potent MDM2 inhibitor demonstrated dose-dependent activity in the reduction of leukemic burden and OS in a patient-derived xenograft (PDX) MPN-BP murine model.[78] Navtemadlin demonstrated clinical activity in a phase Ib dose escalation study in *TP53* WT patients with MPN BP[79] and in a phase II study of patients with R/R MF and DIPSS intermediate 2-risk R/R patients with MF.[80] It is currently being investigated in a multicenter phase Ib/II study in patients with R/R AML (including those with MPN-BP) as monotherapy and in combination with LDAC or decitabine.[81] Interferon-alfa (IFNα) has also demonstrated the ability to decrease the *JAK2V617F* VAF in patients with ET and PV and induce molecular remissions in 17–18% of patients.[33,82,83] In preclinical studies RG7112 and Peg-IFNα 2a, each demonstrated the ability to significantly decrease *JAK2V617F* VAF in MF- and PV-HPSCs. When combined in low doses, RG7112 and Peg-IFNα 2a were able to eliminate *JAK2V617F* HPSCs, suggesting that they work synergistically to enhance p53 expression.[84] Early generation MDM2 inhibitors have demonstrated efficacy in restoring p53 activity. The higher doses, however, needed to be effective as monotherapy have been precluded by GI toxicity and bone marrow suppression, as MDM2 is needed for normal hematopoiesis. ALRN-6924 (Aileron Therapeutics, Cambridge, MA, USA) is a dual MDM4/MDM2 that mimics the inhibitor binding region of TP53.[85] In preclinical studies involving AML cell, ALRN-6924 induced apoptosis and cell cycle arrest as well as improved survival in AML xenograft models.[85] A 71 patient phase I study of ALRN-6924 in advanced solid tumors and lymphomas irrespective of TP53 status was found to be safe with limited myelosuppression, a dose-limiting toxicity well-described in other MDM2 inhibitors.[86] In the solid tumor cohort, the disease-control rate was 59% with a median duration of clinical benefit of 7.5 months.[86] Preliminary results of a phase I/Ib study of ALRN-6924 alone and in combination with cytarabine in 32 patients with AML and myelodysplastic syndrome (MDS) also demonstrated safety with no drug-limiting toxicities and no maximum tolerated dose (MTD) identified. Out of 27 patients, there were two CRs.[87,88] WIP1 is another negative regulator of TP53. Inhibition of WIP1 with GSK2830371 in combination with the MDM2 inhibitor Nutlin-3A synergistically decreased viability and apoptosis in a TP53 WT AML cell line.[89] More potent and selective MDM2 inhibitors like navtemadlin in combination with JAK2 inhibitors, immunomodulatory agents, or other negative regulators of TP53 may mitigate myelosuppression seen in MDM2 inhibitors and lead to significant advances in treatment of MPN-AP/BP.

Wee1 inhibition

Genomic instability due to *JAK2V617F* and *TP53* loss leads to increased dependence on DNA repair mechanisms.[19] Wee1 is an important regulator of G2/M checkpoint of the cell cycle.[19] In a study of a *JAK2V617F* and *Trp53* mutant leukemic mouse model, the combination of adavosertib, a selective Wee1 inhibitor in combination with a PARP inhibitor olaparib, demonstrated significant reduction in the leukocyte count and leukemic burden in both the peripheral blood and bone marrow. In addition, treated mice demonstrated a decrease in spleen size and increase in OS compared with controls. Inhibition of Wee1 is thought to induce replicative stress while PARP inhibition inhibits DNA damage repair. Additional preclinical models evaluating adavosertib demonstrated DNA repair suppression, increase in caspase-mediated apoptosis in ALL cell lines, and a synergistic effect when combined with adriamycin.[90] This cell cycle pathway regulator is a promising target for evaluation in MPN-AP/BP in a clinical setting.

Ruxolitinib and BCL-2 inhibitors

Constitutively activated JAK/STAT signaling is associated with a proinflammatory cytokine profile and overexpression of antiapoptotic B-cell lymphoma proteins BCL-BCL-X_L, BCL-2, and MCL-1 (a STAT target gene).[91] Expression of MCL-1 has represented a barrier to selective BCL-2 inhibition.[91] JAK inhibition leads to a decrease in MCL-1 expression, shifting the burden to BCL-X_L and BCL-2 to maintain survival

and sensitizing the leukemic cells to BCL-2/BCL-X_L inhibitors.[91–93] Navitoclax is a novel small molecule BCL-2/BCL-X_L antagonist. In a phase II multicenter trial of MF patients with prior ruxolitinib exposure and suboptimal response, the combination of ruxolitinib and navitoclax demonstrated an improvement in spleen volume and symptoms in 58% of patients harboring high-risk mutations (HMRs).[94] Zn-d5 is another viable BCL-2 inhibitor under investigation that is a more selective inhibitor of BCL-2 over BCL-X_L and may mitigate some of the on-target myelosuppression seen in combination therapy with venetoclax and HMA.[95] Another novel therapeutic targeting antiapoptotic proteins is the BCL-X_L/BCL-2 proteolysis-targeting chimera (PROTAC). PROTACs are small molecule compounds that cause degradation of target proteins through a ubiquitin proteasome system.[96] 753B is a BCL-X_L/BCL-2 PROTAC that causes BCL-X_L/BCL-2 ubiquitination in cells expressing von-Hippel Lindau (VHL) that spares platelets as VHL is minimally expressed in platelets. It demonstrated efficacy when evaluated in a diverse group of leukemia cell lines and was able to reduce cell viability and increase apoptosis *via* degradation of BCL-X_L in all 17 cell lines as well as BCL-2 in 16 of the 17 leukemia cell lines. In addition, 753B was able to eliminate chemotherapy induced senescent leukemia cells.[97] Inhibition of both JAK/STAT and BCL-2-related pathways may provide synergy and significantly add to the benefit noted in data discussed above that have evaluated venetoclax in this context.

Novel therapies in MDS and AML

Novel therapeutic agents used to that have demonstrated efficacy in MDS and AML and could be explored in MPN-AP/BP as part of a combination regimen include Magrolimab and Eprenetapopt (APR-246). Magrolimab is an anti-CD47 antibody and macrophage checkpoint inhibitor which in combination with both venetoclax and HMA has demonstrated safety and efficacy in MDS and AML. A phase I/II study evaluated venetoclax, azacitidine, and magrolimab in newly diagnosed older, unfit, or high-risk patients with AML. The CR/CRi rate was 94%, and 8-week mortality was 0. In seven patients with TP53 mutation, the CR/CRi was 100%, CR 86%, measurable residual disease (MRD) negative, and CR 57%.[98] Eprenetapopt (APR-246) is a pro-drug that binds to cysteine residues in mutant p53 leading to stabilization of the p53 protein and restoration of the wild-type conformation in addition to increasing oxidative stress and the promotion of tumor cell death.[99] A phase IB/II study of APR-246 in combination with azacitidine in TP53 patients with TP53-mutated MDS or AML demonstrated an ORR of 71% and CR 44%. In the MDS patients, the ORR was 73% and CR was 50%. In the AML patients, the ORR was 64% and CR 36%.[100]

When used in combination with azacitidine, it has demonstrated efficacy in both TP53 mutant and TP53 wild-type AML patients and was been well-tolerated. The phase III trial, however, failed to meet the primary endpoint, and this agent is no longer being developed (Table 2).

Conclusion

Despite the development of a number of novel-targeted therapeutic agents and approaches, MPN-AP/BP carries a dismal prognosis. The cytogenetic and mutational landscape in MPN-AP/BP is associated with increased rates of *TP53* alterations compared with *de novo* AML, rendering patients less responsive to cytotoxic chemotherapy. Current practice for transplant eligible patients remains intensive induction chemotherapy followed by consolidative HSCT. For those ineligible for transplant, current therapies include HMA, BCL-2 inhibitors, and JAK inhibitors, which have demonstrated modest but often transient clinical responses. Novel targeted agents including IDH1/2 inhibitors have demonstrated modest improvement in patients with IDH mutations in retrospective series, and prospective evaluation in this molecularly defined subgroup is still ongoing. The combination of HMA and a BCL-2 inhibitor has demonstrated synergy in poor risk CP-MF but has not led to a significant clinical benefit in the absence of consolidative HSCT. There remains an urgent need for improved therapies in this patient population. Further investigation into combination therapies involving targeted agents that are able to deplete malignant HPSCs and alter the microenvironment that supports these cells may provide improved and more durable responses. Novel agents targeting CXCR1/2 and Wee1 appear promising in the preclinical setting but do not yet have clinical data. Immunotherapeutic agents

Table 2. Mutation frequency in post-MPN AML and *de novo* AML.[18,101]

De novo AML		Post-MPN-AML	
Mutation	**Frequency (%)**	**Mutation**	**Frequency (%)**
ASXL1	30	JAK2V617F	55
FLT3	28	ASXL1	30
NPM1	27	TET2	25
DNMT3A	26	SRSF2	22
IDH1/IDH2	20	RUNX1	20
NRAS/KRAS	12	TP53	17
RUNX1	10	DNMT3A	16
TET 2	8	IDH1	13
TP53	8	IDH2	13
CEBPA	6	CALR	13
KIT	4	MPL	6

AML, acute myeloid leukemia; MPN, myeloproliferative neoplasm.

such as PD-1 pathway inhibitors have not shown promising clinical activity as monotherapy to date. Given the pathophysiology of these diseases, however, it is plausible that combination regimens or novel agents may potentiate the immune system to more effectively target the underlying malignant cell population. Despite slow incremental progress, we are hopeful for better outcomes for patients with MPN AP/BP as new mechanism–based therapeutic agents continue to be developed and combined in innovative approaches.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors have read and agreed to publication of this manuscript.

Author contributions
Helen O. Ajufo: Writing – original draft; Writing – review & editing.

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