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## Investigational histone deacetylase inhibitors (HDACi) in myeloproliferative neoplasms

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### Abstract

**Introduction**—The Philadelphia chromosome negative myeloproliferative neoplasms (MPN) mainly comprise polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (MF), primary or post-PV/ET). Therapy in PV and ET focuses on minimizing thrombosis and bleeding risk, while in MF, prolongation of survival is an important goal. Different cytoreductive agents are employed in high risk PV and ET, while the JAK inhibitor ruxolitinib is the cornerstone of therapy in MF. Histone deacetylase inhibitors (HDACi) are pleiotropic agents with diverse epigenetic and non-epigenetic actions, selectively in transformed cells. A number of HDACi have been or are being investigated in MPN.

**Areas covered**—The mechanisms of action of HDACi in neoplastic cells are summarized, and the preclinical rationale and data supporting their development in MPN specifically examined, particularly their synergism with JAK inhibitors. Major findings of clinical trials of HDACi, both alone and in combination with ruxolitinib, in MPN are then discussed, with particular attention to their toxicities and disease-modifying effects.

**Expert opinion**—HDACi are clearly active in MPN, and there is good preclinical rationale for this. Their combination with ruxolitinib in MF is promising, but the long-term tolerability of these agents is an important concern. Further development in PV or ET appears unlikely.

### Keywords

histone deacetylase inhibitors; myeloproliferative neoplasms; epigenetic; myelofibrosis; ruxolitinib; JAK inhibitors; rational combinations; polycythemia vera; essential thrombocythemia

### 1. Introduction

The term “myeloproliferative syndromes”, coined by William Dameshek in 1951,(1) has classically been used to refer to chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF), disorders characterized by the overproduction of mostly mature blood cells of one or more myeloid lineages. The

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modern category of “myeloproliferative neoplasms” (MPN) additionally encompasses chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia (CEL), not otherwise specified, MPN, unclassifiable (MPN-U) and mastocytosis, although the most recent iteration of the World Health Organization (WHO) classification of myeloid neoplasms recognizes mastocytosis as a separate disease entity owing to its unique clinical and pathological features.(2) The identification of clonal markers underlying most or all of these conditions, e.g., the Philadelphia chromosome (Ph)(3) and its fusion protein product BCR-ABL(4) in CML, *JAK2*(5–9) *MPL*(10, 11) and *CALR*(12, 13) mutations in PV, ET and PMF, *CSF3R* mutations(14) in CNL, *KIT* mutations in mastocytosis,(15) etc. was key to the change in terminology from “syndromes” or “disorders” to “neoplasms”.

While CML represents a molecularly defined entity, the Ph-negative MPN, generally referred to as MPN, are much more molecularly heterogeneous, and although the activating *JAK2* V617F mutation is encountered frequently (approximately 95% of cases of PV and 50% of cases of ET and PMF) and is a phenotypic “driver” mutation, it is not considered the disease-initiating mutation.(16–18) Median survival in the three classic Ph-negative MPN ranges from being similar to that of the general population in ET(19) and 18.9 years in PV(20) to about 6.5 years in PMF.(21) Accordingly, the goal of therapy in ET and PV centers on prevention of thrombosis and bleeding, whereas in PMF and in post-ET/PV myelofibrosis, prolongation of survival takes precedence.(22) There is evidence of universal activation of JAK-STAT (Janus kinase - signal transducer and activator of transcription) signaling across the spectrum of MPN,(23, 24) and the *JAK1/2* inhibitor ruxolitinib is the first agent to improve survival in myelofibrosis in randomized, controlled trials.(25–27) However, the survival benefit of ruxolitinib in myelofibrosis is modest, and has been attributed to the dramatic reduction in circulating pro-inflammatory cytokine levels with associated improvements in appetite, weight, hypocholesterolemia, performance status and cachexia.(28, 29) Thus, there still remains a major unmet need for additional disease-modifying agents in myelofibrosis. Many classes of agents have been investigated, especially in combination with ruxolitinib,(30) prominent among them being histone deacetylase inhibitors (HDACi).

## 2. Histone deacetylase inhibitors (HDACi)

The acetylation status of histones regulates access of transcription factors to DNA and influences levels of gene expression.(31) In general, while increased histone acetylation is associated with open and active chromatin and increased transcription, deacetylated histones are associated with condensed chromatin and transcriptional repression.(32) Histone deacetylase (HDAC) activity diminishes acetylation of histones, causing compaction of the DNA/histone complex.(31) However, this is an over-simplified view, and acetylation status is often associated with the functionality of a genomic element, beyond simply determining open versus closed chromatin structure. For example, “bivalent” promoters, carrying both trimethylated H3K4 and H3K27, are not closed or condensed, but not acetylated, either.(33) The same applies to regions of DNA occupied by the zinc finger protein CTCF (reviewed in ref. (34)). Bivalent promoters are not active transcriptionally because of the need for recruitment of other factors for transcription, while CTCF-connected domains maintain the spatial organization of DNA, and are not transcriptionally active themselves.(33, 34) There

are at least 18 human HDACs (Table 1), grouped by their homology to yeast proteins into four classes: classes I (HDACs 1, 2, 3 and 8), II (HDACs 4, 5, 6, 7, 9 and 10) and IV (HDAC 11) all contain a zinc ion in their active site and are inhibited by the “pan”-HDACi, while the class III HDACs (sirtuins 1–7) are nicotinamide adenine dinucleotide (NAD<sup>+</sup>)-dependent enzymes that are not inhibited by currently available HDACi.(31, 32) Besides histones, HDACs deacetylate a host of non-histone proteins of fundamental importance in cancer, such as the nuclear transcription factors p53, E2F1, GATA1, FoxO3A, c-Myc and nuclear factor kappa B (NF-κB), hypoxia-inducible factor 1α (HIF-1α), the estrogen and androgen receptor complexes, the DNA repair enzymes Ku70 and WRN, the chaperone protein heat shock protein 90 (HSP90), STAT3, β-catenin, α-tubulin and the nuclear import protein importin-α7; indeed, these enzymes may be better termed “protein deacetylases”, or simply “deacetylases”.(31, 32) Although the most accurate nomenclature is “lysine deacetylases” (KDACs, and “lysine acetyltransferases” (KATs) rather than histone acetyltransferases (HATs) for enzymes that catalyze the opposite reaction),(35) the original terminology has largely persisted in the literature, and these enzymes and their pharmacologic inhibitors continue to be referred to as HDACs and HDACi, respectively. HDACs also have a critical role in modulating the balance between pro- and anti-apoptotic proteins, and activate death receptor pathways.(31) Consequently, HDACi have wide-ranging effects, selectively in transformed cells, such as induction of cell cycle arrest (through induction of the endogenous cyclin-dependent kinase (CDK) inhibitor p21, among other mechanisms), differentiation, and apoptosis.(31, 32) HDAC inhibition may also affect tumor cell survival by blocking tumor angiogenesis, and by inhibiting intracellular stress response pathways. (31) More recent work has revealed many new insights into the mechanisms of action of HDACi (reviewed in ref. (36) and summarized in Figure 1). Importantly, HDACi disrupt cell cycle checkpoints, induce DNA damage and proteotoxic (endoplasmic reticulum) stress, and inhibit both homologous and non-homologous end-joining (NHEJ) DNA repair mechanisms. (36)

Despite their pleiotropic actions and relative selectivity for malignant cells, the clinical utility of HDACi has largely been restricted to T-cell lymphomas. Thus, the pan-HDACi, vorinostat (Zolinza®, Merck)(37) and belinostat (Beleodaq®, Spectrum),(38) and the class I HDACi romidepsin (Istodax®, Celgene)(39) are all approved for the treatment of cutaneous and/or peripheral T-cell lymphoma (CTCL/PTCL). Additionally, the pan-HDACi, panobinostat (Farydak®, Novartis), is approved in combination with bortezomib and dexamethasone for previously treated patients with multiple myeloma. HDACi have also been studied extensively in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML, but responses to monotherapy have been modest (10–20%),(40) and it does not appear that the combination of HDACi with either hypomethylating agents in MDS (41, 42) or intensive chemotherapy in AML adds therapeutic benefit. HDACi continue to be investigated in MPN in both preclinical and clinical settings. In the following sections, we summarize the current status of these agents in MPN and speculate about their future role in their therapeutic arena.

### 3. HDACi in MPN

Therapy in ET and PV is risk-adapted with the goal of minimizing thrombosis and bleeding risk. In both diseases, hydroxyurea is the preferred agent when cytoreductive therapy is indicated, and most patients should receive aspirin.(22) Additionally, all patients should have aggressive management of cardiovascular risk factors.(22) The hematocrit goal in PV is <45%,(43) and this is usually accomplished with the use of phlebotomy in low risk patients. Anagrelide is the preferred second line cytoreductive agent in ET.(22) Recently, ruxolitinib was approved for the treatment of patients with PV who have an inadequate response to or are unable to tolerate hydroxyurea.(44) Therapy of myelofibrosis (PMF or post-ET/PV MF) centers around ruxolitinib, which has salutary effects on constitutional symptoms and splenomegaly, as well as a proven survival benefit.(25–27) Finally, allogeneic hematopoietic stem cell transplantation should be offered to higher risk MF patients with a less than 5-year life expectancy and remains the only potentially curative option.(22, 45)

#### Preclinical data with HDACi in MPN (Table 2)

Apart from the canonical actions of JAKs in transducing cytokine signals from their cognate receptors to activate intracellular pathways,(46) JAK2 has a number of nuclear actions. Both wild type JAK2 and JAK2 V617F translocate to the nucleus and phosphorylate histone H3 (HH3) at Tyr41, thereby preventing the binding of heterochromatin protein 1 alpha (HP1 $\alpha$ ) to chromatin at this residue, leading to derepression of transcription.(47) JAK2 V617F also phosphorylates the arginine methyltransferase PRMT5, greatly impairing its ability to methylate its histone substrates and thus regulates chromatin modification by facilitating transcription, promoting myeloproliferation.(48) JAK2 additionally phosphorylates p27, an endogenous CDK inhibitor involved in cell cycle regulation, thus promoting its degradation in the cytoplasm.(49) Finally, JAK2 V617F up-regulates the CDC25A phosphatase, a key regulator of the G1/S cell cycle checkpoint, through a translational mechanism involving STAT5 and the translational initiation factor eIF2 $\alpha$ , potentially invoking another explanation for *JAK2* V617F-driven expansion of hematopoietic cells.(50) HDACi induce p21(51) and p27,(32) trigger DNA damage which transformed cells are unable to repair,(52, 53) down-regulate checkpoint kinase 1 (Chk1),(54) a key regulator of the G2/M cell cycle checkpoint, interfere with the intra-S-phase(55) and mitotic spindle checkpoints(56–58) and impair multiple processes of DNA repair.(59–61) Finally, HDAC activity is required for STAT5-induced activation of transcription,(62) and panobinostat has been shown to down-regulate phospho-STAT5 (p-STAT5) and phospho-STAT3 (p-STAT3) in CTCL models *in vitro* and *in vivo*.(63)

There is preclinical evidence for increased expression of HDACs in Ph-negative MPN, particularly PMF.(64, 65) The suppressor of cytokine signaling (SOCS) proteins are negative regulators of JAK-STAT signaling, competing with STATs for docking at the cytokine receptor, promoting ubiquitinylation and proteasomal degradation of JAK2, and interfering with its catalytic function.(66) Class I HDACs, primarily HDAC8, down-regulate SOCS1 and SOCS3, and HDACi inhibit JAK-STAT signaling by increasing the expression of SOCS1 and SOCS3 in primary MPN cells.(67, 68) The HDACi givinostat has been shown to inhibit proliferation of MPN cells bearing *JAK2* V617F through a specific down-modulation

of the JAK2 V617F protein and inhibition of its downstream signaling (i.e., phospho-JAK2 V617F, p-STAT5, p-STAT3).(69) Besides these inhibitory effects on JAK2-STAT5-extracellular signal regulated kinase 1/2 (ERK1/2) phosphorylation, givinostat also down-regulates the hematopoietic transcription factors NF-E2 and C-MYB.(70) NF-E2 is often overexpressed in MPN patients, and mice expressing an NF-E2 transgene in hematopoietic cells exhibit many features of MPN, a phenotype transplantable to secondary recipient mice.(71) Givinostat and hydroxyurea at low doses potentiate each other's pro-apoptotic effects in JAK2 V617F<sup>+</sup> cell lines and are additive in freshly isolated PV cells bearing *JAK2* V617F.(72) Vorinostat has also been shown to be highly effective in preclinical studies in PV, both *in vitro* and *in vivo*.(73) Vorinostat markedly inhibited proliferation and induced apoptosis in cells expressing *JAK2* V617F, accompanied by inhibition of phosphorylation of JAK2, STAT5, STAT3, AKT, and ERK1/2, and significantly inhibited *JAK2* V617F-expressing mouse and human PV hematopoietic progenitor cells (HPCs).(73) Furthermore, vorinostat significantly down-regulated NF-E2 and up-regulated SOCS1 and SOCS3.(73) Finally, vorinostat normalized blood counts and markedly reduced splenomegaly in *JAK2* V617F knock-in mice compared with placebo, and also decreased the mutant allele burden in these mice.(73) NF-E2 transgenic mice exhibit hypoacetylation of HH3; vorinostat treatment restores physiological levels of HH3 acetylation, reduces NF-E2 expression and normalizes platelet counts in these mice.(71)

JAK2 is a client of the chaperone protein, HSP90, and treatment of PV and ET cell lines and mouse models with HSP90 inhibitors leads to degradation of JAK2, inhibition of JAK-STAT signaling, normalization of peripheral blood counts and improved survival, without degradation of JAK2 in normal tissues or substantial toxicity.(74) Synergistic induction of apoptosis is observed in cultured and primary MPN cells with combined inhibition of JAK2 and HSP90, with marked reduction in phospho-JAK2, p-STAT5, phospho-AKT (p-AKT; Akt activation is an important downstream consequence of JAK2 V617F signaling in MPN)(75) and the anti-apoptotic protein, Bcl-xL, with concomitant induction of the pro-apoptotic protein, Bim.(76) Acetylation of HSP90 with resultant down-regulation of HSP90 client proteins is an important action of HDAC6 inhibitors.(77) Thus, panobinostat blocks the autophosphorylation and downstream signaling of JAK2 V617F, and disrupts the chaperone association of JAK2 V617F with HSP90, promoting proteasomal degradation of JAK2 V617F.(78) Panobinostat induces apoptosis of JAK2 V617F<sup>+</sup> cells and synergizes with JAK2 inhibitors in doing so, with greater cytotoxicity against primary CD34<sup>+</sup> MPN cells than normal CD34<sup>+</sup> HPCs.(78) The combination of ruxolitinib and panobinostat had a more profound effect on splenomegaly, as well as on bone marrow and spleen histology, in mouse models of *JAK2* V617F-driven MPN, than either agent alone.(79) These findings have fueled interest in clinical trials of ruxolitinib-based combinations with HDACi. However, the bone marrow microenvironment has been shown to protect MPN cells from the combination of ruxolitinib and vorinostat through the secretion of soluble factors from the stroma; this protective effect was completely abrogated by pharmacological inhibition of the c-Jun N-terminal kinase (JNK) and phosphatidylinositol-3-kinase (PI3K) pathways.(80) Sequential treatment of CD34<sup>+</sup> cells from patients with PMF with DNA methyltransferase inhibitors (DNMTi) followed by HDACi has been shown to reduce the number of total cells, CD34<sup>+</sup> cells and HPCs, particularly *JAK2* V617F<sup>+</sup> HPCs.(81) This effect is, in part, due to up-

regulation of the chemokine receptor, CXCR4, which is suppressed by promoter methylation in PMF.(82, 83)

### Clinical trials of HDACi in MPN (Tables 3 and 4)

Givinostat (50 mg twice daily for 24 weeks) was evaluated in a pilot study in *JAK2* V617F<sup>+</sup> patients with PV (n = 12), ET (n = 1) or MF (n = 16).(84) Patients with a baseline platelet count < 100 × 10<sup>9</sup>/L were excluded. The median treatment duration was 20 weeks and reasons for treatment discontinuation included disease progression in six patients, grade 2 thrombocytopenia in one, unrelated psychiatric symptoms in one and withdrawal of consent in two. One MF patient had a prolonged QTc at baseline and was therefore ineligible; this patient experienced a further slight QTc prolongation on study treatment, which was discontinued after day 9. Eleven PV/ET patients and seven MF patients completed 24 weeks of treatment. Dose reduction was necessary in 10 patients, and temporary withholding of the drug in 15. Among the 13 PV/ET patients, 7 (54%) had a clinical response by the 2009 European LeukemiaNet (ELN) criteria,(85) including 1 complete response (CR), and six of eight had a spleen response at the end of the study. Among the 16 patients with myelofibrosis (6 PMF + 5 post-ET MF + 5 post-PV MF), 3 (19%) achieved a major response according to EUMNET criteria,(86) but by the 2006 IWG-MRT criteria,(87) 2 patients had clinical improvement (CI) and 5 stable disease (SD). 38% of MF patients had a spleen response. The mean *JAK2* V617F allele burden decreased from 55% at study entry to 47% and 41% after 12 and 24 weeks, respectively, of givinostat treatment in PV/ET patients, but there was no change among MF patients. Most toxicities were grade 1 or 2, and no grade 4 toxicity occurred. The most frequent drug-related adverse events (AEs) were diarrhea, nausea, abdominal pain, thrombocytopenia, fatigue, QTc prolongation, weight loss, anorexia and rash. Pruritus disappeared in most patients.(84)

Givinostat (50 or 100 mg daily) was also tested in combination with hydroxyurea in 44 patients with PV who were unresponsive to maximum tolerated doses (MTD) of hydroxyurea in a phase II study.(88) Patients had to have *JAK2* V617F<sup>+</sup> disease, and be unresponsive to hydroxyurea at the MTD for at least 3 months, as defined by the ELN.(89) Thrombocytopenia < 100 × 10<sup>9</sup>/L was an exclusion criterion. Treatment lasted up to 24 weeks, and assessment of response occurred at 12 and 24 weeks. Responses, by ELN 2009 criteria,(85) were achieved in 55% and 50% of patients receiving 50 or 100 mg of givinostat, respectively. Pruritus was controlled in 64% and 67% of patients in the 50 and 100 mg groups, respectively. The regimen was well tolerated: eight patients (18%) discontinued, four in each treatment arm; grade 3 AEs were reported in one patient (4.5%) in each treatment arm. The most frequent drug-related grade 2 AEs were thrombocytopenia and diarrhea. The only grade 3 toxicities were nausea and anemia in the 50 mg and 100 mg groups, respectively. No grade 4 toxicity was reported.(88)

A phase II study of vorinostat (400 mg once daily) enrolled 19 patients with ET and 44 with PV.(90) There was a high discontinuation rate (52%), mainly because of non-hematologic AEs, such as fatigue, renal impairment, diarrhea, hair loss, weight loss, nausea, unspecified pain, headache and leg ulcers. 48% of patients completed 24 weeks of therapy. Most AEs reported by patients who completed the study were fatigue and gastrointestinal. The

intention-to-treat response rate (RR) was 35%, and responses were independent of *JAK2* mutational status among ET patients (only one PV patient was *JAK2* V617F-negative). Pruritus, present in 19% of patients at study initiation, resolved in all patients, while the prevalence of splenomegaly decreased from 50% to 27% ( $p = 0.03$ ). 65% of 43 evaluable patients had a decrease in the *JAK2* V617F allele burden ( $p = 0.006$ ), but the median decrease among molecularly responding patients was only 5.6%, and no patient had a complete molecular response.(90)

Mascarenhas *et al.* studied panobinostat in a phase I trial in 18 patients (10 PMF + 5 post-PV MF + 3 post-ET MF) with myelofibrosis.(91) 12 (67%) patients were positive for *JAK2* V617F. Panobinostat was administered three times weekly; three successive cohorts of six patients each received 20 mg, 30 mg and 25 mg, respectively. Reversible thrombocytopenia was the dose limiting toxicity (DLT) in both the 20 mg and 30 mg dose cohorts. No patient developed clinically significant bleeding attributable to the study drug. Grade 3/4 neutropenia occurred in 17%, but there were no cases of febrile neutropenia. There were no grade 3/4 non-hematologic AEs. Diarrhea (89%), musculoskeletal AEs (33%) and fatigue (33%) were common. Only five patients received 6 cycles and were evaluable for response (using the 2006 IWG-MRT criteria)(87); 3 of these patients experienced CI with 100% reduction in palpable splenomegaly, and 2 SD. Two of these five patients also experienced improvement in their anemia on panobinostat. One patient had a sustained, dramatic reversal of bone marrow histopathologic abnormalities after cycles 16 and 30, and another had a significant reduction in marrow fibrosis (from grade 4 to grade 1) after 24 cycles of therapy. Most patients treated for longer than a month had symptomatic benefit, and significant reduction in splenomegaly was observed at the end of six cycles. The authors concluded that a minimum of 12 cycles of treatment with single agent panobinostat was required in order to achieve improvement in blood counts and possibly longer to achieve reversal of bone marrow histopathological abnormalities.(91)

Panobinostat was also investigated in a phase II trial in myelofibrosis ( $n = 35$ , 24 PMF + 5 post-ET MF + 6 post-PV MF) at a dose of 40 mg three times a week.(92) About two-thirds (65.7%) of patients were *JAK2* V617F<sup>+</sup>. 33 patients discontinued study treatment, 19 before completing cycle 3, mostly because of AEs. The median number of days on study was only 57. Twenty patients had a dose modification, and of the remaining 15, 12 had a treatment duration of < 33 days. Thrombocytopenia occurred in 71.4% of patients, and was grade 3/4 in 60%. For neutropenia, the corresponding percentages were 20% and 14.3%. Gastrointestinal AEs were extremely frequent (diarrhea, 80%; nausea, 57.1%; anorexia, 54.3%; vomiting 40%) but mostly grades 1 and 2 in severity. Fatigue occurred in 57.1% of patients, and was grade 3/4 in 31.4%. Only one patient met the 2006 IWG-MRT criteria(87) for CI. Correlative studies demonstrated increased acetylation of intracellular targets, e.g., tubacin, HH3 and HSP90, inhibition of JAK-STAT downstream signaling (p-STAT5, p-STAT3, p-AKT, phospho-mitogen activated protein kinase 3/1 (p-MAPK3/1)), down-regulation of STAT5, STAT3 and AKT, anti-apoptotic PIM1 and Mcl-1 and the pro-inflammatory cytokines interleukin-6 (IL-6), tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and MIP1 $\beta$ , induction of Bim and a reduction in the median *JAK2* V617F allele burden from 36.82% pre-treatment to 14.88% before cycle 2 to 0.86% before cycle 4.(92)

Our group has evaluated the pan-HDACi, pracinostat, at a dose of 60 mg three times weekly for three out of every four weeks in a phase II study in 22 patients with myelofibrosis.(93) 82% of patients were positive for *JAK2* V617F, and the majority of patients were symptomatic at study entry and had significant palpable splenomegaly. 27% of patients experienced a reduction in splenomegaly, but none of these met the 2006 IWG-MRT criteria(87) for CI. Two patients had CI in terms of anemia. 17% of *JAK2* V617F<sup>+</sup> patients had a reduction in allele burden (median 11.4%), although this was not significant when the entire cohort of *JAK2* V617F<sup>+</sup> patients was analyzed as a whole. The major toxicity of pracinostat in this trial was fatigue, occurring in 91% of patients. 13% and 21% of patients, respectively, had grade 3/4 neutropenia and thrombocytopenia. At the time of publication, all but one patient had discontinued pracinostat, mostly due to lack of response or disease progression.(93)

In light of these observations, and the established role of ruxolitinib in myelofibrosis, efforts are ongoing to combine HDACi such as pracinostat (NCT02267278) or panobinostat (NCT01693601, NCT01433445) with ruxolitinib. Preliminary results from a phase Ib trial of panobinostat and ruxolitinib in patients with MF have been published in abstract form.(94) Patients (n = 61, 38 escalation phase + 23 expansion phase) were required to have palpable splenomegaly 5 cm below the left costal margin and intermediate-1, -2 or high risk disease by the International Prognostic Scoring System,(95) and received ruxolitinib 5–15 mg twice daily and panobinostat 10–25 mg three times a week, every other week. Grade 4 thrombocytopenia and grade 3 nausea were identified as DLTs, and the higher doses of both drugs were confirmed to be the recommended phase 2 doses (RP2D). Among 34 patients treated at the RP2D, 21% discontinued due to AEs. The most common grade 3/4 hematologic AEs in these patients, regardless of attribution, were anemia (32%) and thrombocytopenia (29%), while grade 3/4 non-hematologic AEs included diarrhea (18%), asthenia (12%) and fatigue (9%). 57% and 39% of patients achieved a 35% reduction in spleen volume at weeks 24 and 48, respectively. 4 of 12 evaluable patients had improved bone marrow fibrosis at week 48, and 5 of 17 (29%) *JAK2* V617F<sup>+</sup> patients in the expansion phase of the trial had a 20% decrease in allele burden by week 48.(94)

#### 4. Conclusion

HDACi are active in MPN, both preclinically and in the clinic. However, these agents have chronic, predominantly low grade, toxicities that make their long-term tolerability a concern, especially since these drugs need to be administered for long periods of time in order for disease-modifying effects to emerge, at least in MF, where the need to alter the underlying disease biology is greatest. Current development strategies are focusing on combinations with ruxolitinib, employing low doses of HDACi.

#### 5. Expert Opinion

As discussed in this review, there is strong preclinical rationale and modest evidence of clinical efficacy to support the continued development of HDACi in MPN. However, considering that the MPN are chronic disorders with relatively long survival, the long-term tolerability of these drugs becomes an important concern. This is particularly relevant



because the disease-modifying effects of these drugs in MF appear to take time to emerge. (91)

In PV, a disease with a long natural history, while HDACi are clearly active, their continued development is uncertain and appears unlikely. The recent approval of ruxolitinib for the treatment of hydroxyurea-resistant or -intolerant patients with PV represents an important advance in the management of this disease.(44) While HDACi appear to control troublesome symptoms of PV such as pruritus,(84, 88, 90) so does ruxolitinib.(44) Longer term follow-up of the RESPONSE trial has shown a steady decrease in the *JAK2* V617F allele burden among patients originally randomized to ruxolitinib (22% mean reduction at week 80, 40% by week 208).(96, 97) While the significance of *JAK2* V617F allele burden reduction in MPN remains unknown, these findings underscore the fact that this action is not unique to HDACi. Finally, given the success of pegylated interferon alfa-2a in PV,(98, 99) a longer acting interferon, ropeginterferon alfa-2b, which allows administration every 2 weeks, has been developed.(100) In a phase 1/2 study, it produced a cumulative overall response rate of 90% (47% CR plus 43% partial response, PR). A complete molecular response was achieved in 21% and a partial molecular response in 47%. There were no DLTs.(100) Given the above, it is difficult to envision a role for long-term therapy with HDACi with their attendant toxicities (fatigue, gastrointestinal, thrombocytopenia, neutropenia) in PV.

Thrombocytopenia is an on-target effect of HDACi due to hyperacetylation of tubulin, an important constituent of the platelet microtubule cytoskeleton, leading to decreased megakaryocyte maturation and proplatelet formation, ultimately impeding the release of platelets from megakaryocytes.(101, 102) This has led some to propose development of HDACi as a treatment for ET, which is characterized by thrombocytosis.(103) However, there is no evidence that the platelet count *per se* affects the risk of thrombosis or hemorrhage in ET (Falchi L, Bose P, Newberry KJ, Verstovsek S, submitted), which raises the question whether simply lowering the platelet count is a clinically relevant therapeutic goal in ET, the most indolent among the classic MPN. Risk factors for inferior survival in ET include age  $\geq 60$ , leukocytosis  $\geq 11 \times 10^9/L$  and prior thrombosis.(104) Risk factors for thrombosis in ET include age over 60, history of thrombosis, presence of cardiovascular risk factors and the *JAK2* V617F mutation.(105, 106) Patients with ET can have significant symptoms,(107) and ruxolitinib is being developed for this indication.

The need for disease-modifying agents is greatest in MF, where ruxolitinib currently is the cornerstone of treatment. A number of JAK inhibitors have been investigated in the clinic, but many of them have been discontinued due to toxicity.(108) Of the ones still in development, pacritinib,(109) which appears to be nonmyelosuppressive, and momelotinib, (110) which may actually improve the anemia of MF, are in the most advanced stages of clinical testing; however, the “full clinical hold” recently placed on the development of pacritinib by the US Food and Drug Administration is of concern. This underscores the importance of developing rational ruxolitinib-based combinations(30). Many classes of agents are being tested, either alone or in combination with ruxolitinib: these include HDACi, DNMTi, HSP90 inhibitors, PI3K/Akt/mTOR pathway inhibitors, hedgehog inhibitors, anti-fibrotic agents and telomerase inhibitors (reviewed in ref. (111)). Currently available JAK inhibitors are “type I” inhibitors that bind to and stabilize the active

conformation of JAK2.(112) An interesting mechanism of reactivation of JAK-STAT signaling despite the presence of a type I inhibitor is “JAK2 inhibitor persistence”, characterized by heterodimerization between activated JAK2 and JAK1 or TYK2.(113) This may be circumvented by “type II” JAK2 inhibitors, which stabilize the inactive state of the kinase.(112, 114) The anti-fibrotic agent PRM-151 (recombinant human pentraxin-2) has shown encouraging results in MF as a single agent and trials are ongoing.(115) The telomerase inhibitor imetelstat produced responses in 21% of patients in a pilot study in patients with higher risk MF, including reversal of bone marrow fibrosis in those patients who obtained a CR, albeit at the expense of significant myelosuppression, and responses did not correlate with baseline telomere length.(116) Ruxolitinib-based combinations with PI3K inhibitors(117) and hedgehog inhibitors(118) have so far been disappointing. The combination of ruxolitinib with DNMTis may be most efficacious in patients with accelerated or blast phase MPN and those with myelodysplastic syndrome/MPN “overlap” syndromes.(119) As discussed above, the combination of ruxolitinib and panobinostat in MF is promising thus far,(94) and appears to lead to faster spleen responses and *JAK2* V617F allele burden reductions than ruxolitinib alone,(25, 26) although the clinical relevance of the latter phenomenon remains unproven. Evaluation of pracinostat in combination with ruxolitinib is ongoing (NCT02267278). The key to the future role, if any, of HDACi in MF will be their long-term tolerability and disease-modifying benefits over those of ruxolitinib alone,(25, 26) which may be best assessed by regression/reversal of bone marrow fibrosis and, of course, improved overall survival.

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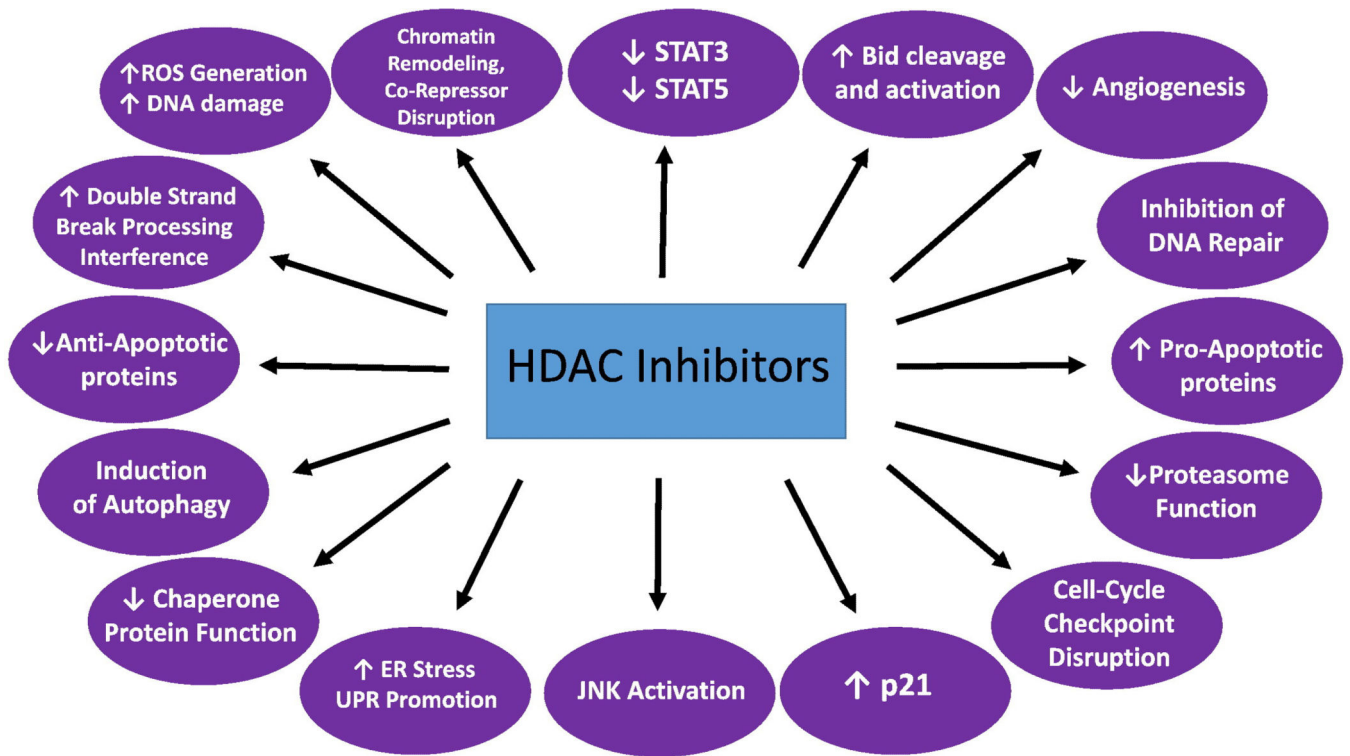


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**Highlights box**

- HDACi are active, both preclinically and clinically, in MPN.
- HDAC6 inhibitors down-regulate JAK2 and JAK2 V617F by disrupting the chaperone function of HSP90 and synergize with JAK2 inhibitors.
- HDACi have significant chronic toxicities, e.g., diarrhea, fatigue, thrombocytopenia.
- Long-term therapy is required for disease-modifying effects of HDACi in MF to emerge.
- Ongoing trials are investigating combinations of HDACi with ruxolitinib in myelofibrosis.



**Figure 1.** Mechanisms of HDACi lethality. Reproduced, with permission, from (36).

**Table 1**

Human histone deacetylase: classification, localization, and inhibitors. Reproduced, with permission, from (120).

Class	Enzymes	Localization	Expression	Drugs
I	HDAC 1, 2, 3, and 8	Nucleus	Ubiquitous	Vorinostat, trichostatin A, belinostat, panobinostat, romidepsin, etinostat, and mocetinostat
IIa	HDAC 4, 5, 7, and 9	Nucleus and cytoplasm	Tissue-specific	Vorinostat, trichostatin A, belinostat, panobinostat, romidepsin, and ricolinostat (HDAC6 inhibitor)
IIb	HDAC 6 and 10	Cytoplasm		
III	Sirtuins 1–7	Variable	Variable	Nicotinamide, dihydrocoumarin, and naphthopyranone
IV	HDAC 11	Nucleus and cytoplasm	Ubiquitous	Vorinostat, trichostatin A, belinostat, and panobinostat

Abbreviations: HDAC, histone deacetylase.

**Table 2**

Mechanisms of action of histone deacetylase inhibitors in myeloproliferative neoplasms

Preclinical observation	Mechanistic explanation	Related preclinical findings, if any	Author and reference
Increased HDAC expression in MPN	HDACi inhibit the function of HDACs		Wang <i>et al.</i> (64) Skov <i>et al.</i> (65)
HDACi up-regulate SOCS1 and SOCS3	Class I HDACs, mainly HDAC8, down-regulate SOCS1/3, negative regulators of JAK-STAT signaling		Chen <i>et al.</i> (67) Gao <i>et al.</i> (68)
HDACi down-regulate JAK2, a client of HSP90	HDAC6 inhibitors disrupt chaperone function of HSP90 through acetylation	HDAC inhibitors and HSP90 inhibitors synergize with JAK2 inhibitors in MPN models	Guerini <i>et al.</i> (69) Akada <i>et al.</i> (73) Wang <i>et al.</i> (78) Fiskus <i>et al.</i> (76)
HDACi down-regulate the transcription factor, NF-E2	NF-E2 is overexpressed in MPN patients and its overexpression confers a transplantable MPN phenotype in mice		Amaru Calzada <i>et al.</i> (70) Kaufmann <i>et al.</i> (71)

Abbreviations: HDAC, histone deacetylase; HDACi, histone deacetylase inhibitor; HSP90 heat shock protein 90; MPN, myeloproliferative neoplasm; SOCS, suppressor of cytokine signaling; JAK, Janus kinase; STAT, signal transducer and activator of transcription.

**Table 3**  
Completed, published clinical trials of HDACi monotherapy in myeloproliferative neoplasms.

HDACi studied	Phase	Population studied	Efficacy	Toxicity	Author and reference
Givinostat (starting dose 50 mg bid × 24 weeks)	IIA	12 PV, 1 ET, 16 MF; all <i>JAK2</i> V617F+	PV/ET: 1 CR, 6 PR; MF: 3 major responses; spleen ↓ in 75% of PV/ET and 38% of MF; pruritus resolved in most patients	Well tolerated; dose ↓ in 10; interrupted in 15; 2 stopped due to AEs	Rambaldi <i>et al.</i> (84)
Givinostat (50 or 100 mg/d plus MTD of hydroxyurea for maximum 24 weeks)	II	PV (N = 44); all unresponsive to MTD of hydroxyurea	ORR 55% at 50 mg/d; 50% at 100 mg/d; 64% and 67% had control of pruritus	Grade 3 AEs in only 1 patient (4.5%) in each arm	Finazzi <i>et al.</i> (88)
Vorinostat (400 mg/d × 24 weeks)	II	44 PV, 19 ET	Pruritus 19% to 0%; splenomegaly 50% to 27%; ↓ in <i>JAK2</i> V617F allele burden in 65%	High rate (44%) of discontinuation due to toxicity	Andersen <i>et al.</i> (90)
Panobinostat (20, 30 or 25 mg tiw in 4-week cycles)	I	MF (N = 18); PMF 56%, post-PV MF 28%; post-ET MF 17%	Only 5 evaluable; 3 CI-spleen, 2 SD. Anemia improved in 2; responses deepened with time	DLT was reversible thrombocytopenia	Mascarenhas <i>et al.</i> (91)
Panobinostat (40 mg tiw in 4-week cycles)	II	MF (N = 35); PMF 68.6%, post-PV MF 17.1%; post-ET MF 14.3%	1 patient achieved an IWG-MRT response (3%)	Poorly tolerated; diarrhea 80%, thrombocytopenia 71.4%; high early rate of discontinuation	DeAngelo <i>et al.</i> (92)
Pracinostat (60 mg qod × 3 weeks per	II	MF (N = 22)	IWG-MRT CI-anemia in 9%; 36% had	Fatigue (91%); grade 3/4 neutropenia in	Quintas-Cardama <i>et al.</i> (93)

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HDACi studied	Phase	Population studied	Efficacy	Toxicity	Author and reference
month)			clinical benefit; ↓ spleen (27%)	13%, thrombocytopenia in 21%	

Abbreviations: PV, polycythemia vera; ET, essential thrombocythemia; MF, myelofibrosis; bid, twice daily; tid, three times a week; qod, every other day; MTD, maximum tolerated dose; DLT, dose-limiting toxicity; AE, adverse event; SD, stable disease; CR, complete response; PR, partial response; CI, clinical improvement; IWG-MRT, International Working Group for Myelofibrosis Research and Treatment; JAK2, Janus kinase; HDACi, histone deacetylase inhibitor; PMF, primary myelofibrosis.



**Table 4**

Ongoing clinical trials of ruxolitinib-HDACi combinations in myelofibrosis.

HDACi studied	Phase	Clinicaltrials.gov identifier	Population studied	Efficacy	Toxicity	Author and reference, if available
Panobinostat (tiw qow or every week)	I/II	NCT01693601 (PRIME)	PMF or post-PV/ET MF, int-2 or high risk, in CP or AP	N/A	N/A	N/A
Panobinostat	I/II	NCT01433445	PMF or post-PV/ET MF, int or high risk, palpable spleen 5 cm below LCM (N = 38 in escalation phase and 23 in expansion phase)	In expansion phase (n = 23), 57% and 39% had SVR at week 24 and 48; 29% had 20% ↓ in JAK2 V617F allele burden	RP2D: RUX 15 mg bid and PAN 25 mg tiw qow; 21% at RP2D discontinued due to AEs; grade (32%), 3/4 AEs: anemia (32%), thrombocytopenia (29%), diarrhea (18%), asthenia (12%), fatigue (9%)	Harrison <i>et al.</i> (94)
Pracinostat (started after RUX alone for first 3 months; starting dose 60 mg qod × 3 days every 3 weeks)	II	NCT02267278	PMF or post-PV/ET MF, int or high risk, palpable spleen 5 cm below LCM	N/A	N/A	N/A

Abbreviations: HDACi, histone deacetylase inhibitor; RUX, ruxolitinib; PMF, primary myelofibrosis; post-PV/ET MF, post-polycythemia vera/essential thrombocythemia myelofibrosis; LCM, left costal margin; N/A, not applicable; CP, chronic phase; AP, accelerated phase; tiw, three times a week; qow, every other week; qod, every other day; int, intermediate; PAN, panobinostat; RP2D, recommended phase 2 dose; bid, twice daily; SVR, spleen volume reduction; JAK2, Janus kinase 2.