Deactylase inhibition in myeloproliferative neoplasms

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Summary Myeloproliferative neoplasms (MPN) are clonal haemopoietic progenitor cell disorders characterized by the proliferation of one or more of the haemopoietic lineages (myeloid, erythroid and/or megakaryocytic). The MPNs include eight haematological disorders: chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), systemic mastocytosis (SM), chronic eosinophilic leukemia, not otherwise specified (CEL, NOS), chronic neutrophilic leukemia (CNL), and unclassifiable MPN (MPN, U). Therapeutic interventions for MPNs include the use of tyrosine kinase inhibitors (TKIs) for BCR-ABL1+ CML and JAK2 inhibitors for PV, ET and PMF. Histone deacetylase inhibitors (HDACi) are a novel class of drugs capable of altering the acetylation status of both histone and non-histone proteins, thereby affecting a repertoire of cellular functions in neoplastic cells including proliferation, differentiation, immune responses, angiogenesis and survival. Preliminary studies indicate that HDACi when used in combination with tyrosine kinase or JAK2 inhibitors may overcome resistance to the latter agents and enhance the pro-apoptotic effects on MPN cells. This review provides a review of pre-clinical and clinical studies that have explored the use of HDACi as potential therapeutics for MPNs.

Keywords Myeloproliferative neoplasms · Deacetylase inhibition · Epigenetic therapy · Chronic myelogenous leukemia

Introduction

Myeloproliferative neoplasms

The World Health Organization (WHO) classification of haemopoietic and lymphoid neoplasms was revised in 2008 [1]. This represented a major improvement over the 2001 WHO classification system, whereby myeloid neoplasms are now classified into five categories: acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN), MDS/MPN, and myeloid and/or lymphoid malignancies associated with eosinophilia and platelet-derived growth factor receptor (PDGFR) or fibroblast growth factor receptor 1 (FGFR1) rearrangements. MPN are sub-classified into eight separate entities: chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), systemic mastocytosis (SM), chronic eosinophilic leukemia, not otherwise specified (CEL, NOS), chronic neutrophilic leukemia (CNL), and unclassifiable MPN (MPN, U).

The MPN are clonal haemopoietic stem cell disorders characterized by proliferation of one or more haemopoietic...
lineages. Occurrence is primarily in the 5th to 7th decade of life, however, CML and ET have been described in children. The incidence of MPN as a group is 6-10/100,000 population annually [2]. Collectively, MPN are characterised by a hypercellular bone marrow with effective haemopoiesis. Over time splenomegaly and hepatomegaly occur as a result of sequestration of blood cells and extra-medullary proliferation of haemopoietic cells. Despite an initially indolent course MPN progress over time and have the potential to transform to acute leukaemia. This manifests clinically as progressive organomegaly, worsening of peripheral cytopenias and an increasing numbers of circulating blast cells.

The diagnosis of CML requires the presence of BCR-ABL1, while its absence is required for all other MPN. CML remains the prototype for the identification and classification of myeloid neoplasms. The enhanced tyrosine kinase activity of BCR-ABL1 results in constitutive activation of a number of signal transduction pathways resulting in the leukemic phenotype observed in CML. BCR-ABL1 provided the first bona fide therapeutic target for tyrosine kinase inhibitors (TKIs). This has revolutionized the management of CML, a disorder previously fatal without allogeneic stem cell transplantation.

PV, ET, and PMF make up the non-leukemic MPN. Clonal proliferation is responsible for the overlapping expansion of erythropoietic, granulopoietic, and megakaryocytic components in the marrow and, in advanced disease, the liver and spleen. A single acquired point mutation (V617F) of the cytoplasmic Janus-associated tyrosine kinase (JAK2) occurs (heterozygous or homozygous) in the marrow and blood of almost all patients with PV and in approximately 50% of patients with ET and PMF, and is responsible for the uncontrolled myeloproliferation associated with these disorders. JAK2 is involved in transducing signals from cytokines and growth factors including erythropoietin (EPO), granulocyte-macrophage colony-stimulating factor (GM-CSF) and thrombopoietin (TPO). The mutation occurs in a highly conserved region of the pseudokinase domain that is believed to negatively regulate JAK2 signaling. In PV, the presence of JAK2 homozygosity increases with time. Additional MPN-associated molecular markers include mutations of MPL, TET2 and KIT but the diagnostic utility of MPL and TET2 mutations is limited by a low mutational frequency.

In SM, presence of the D816V KIT mutation is expected but not essential for diagnosis. CEL, NOS should be distinguished from both PDGFR-rearranged or FGFR1-rearranged neoplasms and hyper eosinophilic syndrome (HES). CNL is a rare myeloproliferative disease with only 150 cases reported [1] and is characterized by sustained neutrophilia, bone marrow hypercellularity and hepatosplenomegaly. The BCR-ABL1 fusion gene is undetectable in CNL and further discrimination is required to distinguish CNL from other MPNs.

Prognosis

Prior to any effective therapy the median survival for CML was 2–3 years [3]. Subsequently, interferon-γ (IFN-γ)-based approaches resulted in 10 year overall survival (OS) rates of approximately 25% [4]. The 10-year OS for allogeneic haemopoietic stem cell transplantation (HSCT) varied widely from 10 to 70% depending on patient age, phase of disease and donor type [5, 6]. In the current era of tyrosine kinase inhibitor (TKI) therapy 5-year OS with imatinib is 80–95% [5, 7], however, acquired resistance to imatinib in patients leads to a clinical impasse. In addition to BCR-ABL1 gene amplification resulting in overexpression of BCR-ABL1 protein, or point mutations that prevent the binding of the inhibitor to the kinase domain [8, 9], several groups have demonstrated other forms of BCR-ABL1-independent imatinib resistance [10–12]. Interestingly, BCR-ABL1-independent imatinib-resistant K652 cells display aberrant protein acetylation and increased sensitivity to histone deacetylase inhibitors (HDACi) [12].

PV and ET are relatively indolent disorders that result in a modest reduction of survival, particularly evident after the first decade from diagnosis. In contrast, PMF has a more aggressive clinical course with a median survival of approximately 5 years, although younger patients with low-risk disease may experience survival in excess of 10 years [13]. With the possible exception of IFN-γ use in PV [14–16], therapy for classical MPN is aimed at ameliorating the signs and symptoms of myeloproliferation, including anaemia and/or thrombocytopenia, and to reduce the risk of thrombosis. Patients with PMF and post-ET/PV MF have progressive cytopenias, extra-medullary haemopoiesis (manifesting as splenomegaly and/or hepatomegaly), significant constitutional symptoms, the potential for blastic transformation and subsequently, premature death [17, 18]. A number of novel therapeutic strategies have been explored in these patients including farnesyl-transferase inhibition [19], proteosome inhibition [20] and immunomodulation with lenalidomide [21] but with limited efficacy. Since current therapies rarely offer more than a palliative benefit, the urgent need for improved therapeutic options for PMF and post-ET/PV MF has resulted in therapeutic targeting of the JAK2V617F mutation, a common molecular link that unifies the pathogenesis of these MPNs. A selective JAK1/2 inhibitor, INCB018424, demonstrated prolonged survival in a preclinical murine model of JAKV617F+MPN [22] and preliminary results from a phase 1/2 study resulted in objective and subjective improvements in patients with PMF and post-ET/PV MF [22]. These targeted therapeutic
Epigenetic therapy in MPN

Despite the general acceptance that genetic changes underlie the pathogenesis of cancer there is an increasing body of evidence demonstrating that in malignant cells epigenetic changes result in the abnormal transcription of structurally intact genes. In general, malignant tissue is characterized by global hypermethylation of DNA [23, 24]. In addition, malignant tissues also demonstrate increased histone deacetylation that results in chromatin condensation and transcriptional repression of tumor suppressor genes [24–26]. Collectively, these post-translational modifications of histones and DNA methylation result in transcriptional silencing of tumour suppressor and cell differentiation genes, thus promoting cell survival by blocking apoptosis and senescence. Importantly, epigenetic but not genomic changes have the potential to be modulated for therapeutic benefit. Novel therapeutic approaches are now being developed to target some of these molecular lesions, including epigenetic alterations, by making use of methyltransferase and histone deacetylase inhibitors. However, the extent and specific role of epigenetic modifications in MPN remain poorly understood. Furthermore, increased knowledge of the linkage between genetic and epigenetic changes that promote tumourogenesis and the identification of appropriate biomarkers represents a prerequisite for successful epigenetics-directed MPN therapy. The production of pro-inflammatory cytokines by stromal elements and constitutive activation of aberrant signal transduction pathways by malignant cells could result in disease progression. Indeed, the constitutive activation of the JAK/STAT pathway in JAKV617F+MPN can result in subsequent epigenetic silencing of tumour suppressor genes [27, 28], suggesting that these changes result in further evolution of the disease.

Epigenetic events in MPN are most prevalent in PMF as evidenced by genome-wide methylation studies demonstrating a high rate of methylation in samples from PMF patients [29, 30]. In addition, a decreased level of acetylation as a result of increased histone deacetylase activity has also been observed [30]. Most of the work on gene methylation in MPN has focused on a limited number of genes, in particular, the status of the suppressors of cytokine signaling family proteins (SOCS) [31–34]. The increased number of CD34-positive cells in the peripheral blood of PMF patients has been attributed to the reduced membrane expression of C-X-C chemokine receptor type 4 (CXCR4) [35, 36] which is secondary to increased methylation of the CpG islands of the CXCR4 promoter [37]. Ex vivo treatment of CD34-positive cells from PMF patients with hypomethylating agents rectified the abnormal homing and proliferation of these cells [38]. Wang et al., (2008) demonstrated increased HDAC levels in the CD34-positive cell fractions of patients with PMF [30]. Given these changes, it would seem reasonable to explore epigenetic therapy as a therapeutic option in MPN, especially PMF, where therapeutic options are currently limited. The use of DNA hypomethylation therapy has been found to be efficacious in patients with MDS leading to improvement in disease-associated cytopenias, delay in transformation to AML and improved OS [39, 40], however, the hypomethylating agent 5-azacytidine has limited efficacy in PMF [17, 41].

Histone deacetylases and inhibitors

Acetylation of proteins is modulated by the dynamic and antagonistic action of two classes of enzymes, histone deacetylases (HDACs) and histone acetyltransferases (HATs). HATs acetylate amino-terminal lysine residues whereas HDACs catalyse the removal of acetyl groups. The HDAC family comprises 18 genes, grouped into 4 classes based on their homology to yeast orthologues. Class I (HDACs 1–3 and 8), Class II A (HDAC 4,5,7 and 9), Class II B (HDAC6 and 11) and Class IV (HDAC11) require zinc for catalysing deacetylase activity, while Class III (Sirutins 1–7) utilise nicotinic adenine dinucleotide (NAD⁺) for their catalytic mechanisms [26, 42]. Moreover, there is growing evidence that the acetylation status of proteins plays a key role in the regulation of cellular signalling and disease development [27, 42–48].

HDACi represent a novel class of chemotherapeutic drugs that are able to regulate cellular functions through their ability to inhibit HDAC action and can be subdivided into six groups based on their structure: Hydroxamic acid-derived compounds, cyclic peptides, short-chain fatty acids, benzamides, electrophilic ketones and several agents that are not otherwise classifiable [49]. Their inhibitory effects on cell proliferation and survival can be mediated by both chromatin-dependent (acetylation of histone proteins) and chromatin-independent pathways (acetylation of non-histone proteins). Alterations in gene expression induced by histone acetylation results in changes in expression levels of pro- and anti-apoptotic genes and modulation of cell cycle genes (reviewed in [48, 50]). Amongst the non-histone acetylation targets that via HDACi induced acetylation could potentially impart anti-cancer effect are transcription factors (p53, c-Myc, Smad7, BCL6, GATA, STAT3, NF-κB, E2F family, MEF2, AML1, CREB), chaperone proteins (Hsp90), cytoskeletal proteins (α-tubulin), nuclear import proteins (Importin α), DNA repair enzymes (Ku70) and steroid receptors (androgen receptors, estrogen receptor) (reviewed in [46, 51, 52]). Furthermore, HDACi are
able to target both proliferating and non-proliferating transformed cells to induce growth arrest and apoptosis, although the molecular mechanisms underlying the apparent specificity of HDACi to induce cytotoxicity of neoplastic cells and not normal cells remains to be elucidated [53–57].

MPN and HDAC inhibitors

BCR-ABL1+ chronic myelogenous leukemia

Chronic Myeloid Leukemia (CML) is a MPN of myeloid progenitor cells characterised by the expression of a 210-KDa fusion oncoprotein, BCR-ABL, resulting from the juxtapositioning of chromosomes 9 and 22 [the Philadelphia chromosome translocation, t(9;22)]. The BCR-ABL kinase is constitutively active and via downstream signaling regulates multiple survival pathways, including Ras/Raf/mitogen-activated protein kinase (MEK)/extracellular signal-regulated kinase (ERK), PI3/AKT, NF-κB, JAK/STAT, anti- and pro-apoptotic proteins [58]. Monotherapy with the TKI imatinib mesylate is effective in chronic-phase CML patients, however, accelerated and blast phase patients are generally resistant to imatinib [59]. Imatinib resistance is usually characterized by amplification of the BCR-ABL gene, protein overexpression, missense mutations in BCR-ABL kinase domains or through mechanisms independent of BCR-ABL signalling [10–12, 60, 61]. Dasatinib and nilotinib are two novel second generation kinase inhibitors that are effective in imatinib-resistant patients, although patients with the T315I BCR-ABL mutation do not respond to these drugs [62, 63]. Therefore, additional therapies to combat emergent TKI resistance are required and are currently being explored.

Recent studies indicate that HDACi are capable of attenuating the expression of BCR-ABL and of inducing apoptosis in both imatinib sensitive and resistant cell lines [64–69]. Furthermore, the tumour-specificity of HDACi was seen with HDACi preferentially affecting the BCR-ABL positive clonal cells and not normal cells [70–73]. In addition, analyses of the expression and functional activity of HDACs and HATs in the imatinib resistant CML cell line K562 have indicated that Class I (HDAC1, 2 and 3), Class II A (HDAC4) and Class III (SIRT1) HDACs are all elevated and increased compared to imatinib sensitive cells, whereas the levels of HATs are decreased [12, 74]. To date, several HDACi that have been tested against CML cells include suberoylanilide hydroxamic acid (SAHA [Vorinostat]), LBH589 (Panobinostat), LBQ824, apicidin, FK-228 (Romidepsin [Depsipeptide]), valproate, sodium butyrate and AN-9 (Pivanex). In most of the studies performed the primary mechanism by which HDACi appeared to induce apoptosis in CML cells was through the reduction of BCR-ABL levels and therefore downregulation of the multiple signalling pathways controlling survival of the neoplastic cells. As such, while the expression of HDACs and HATs in imatinib-responsive and refractory patient samples has not been assessed it would appear that HDAC inhibition may represent a promising anti-leukemic approach for imatinib-refractory patients.

The pan-HDAC inhibitors SAHA, LBH589 and LAQ824 display inhibitory activity against both Class I and Class II HDACs and are therefore able to modulate a diverse range of proteins involved in growth arrest and apoptosis [26, 49, 57, 75]. SAHA, one of the most investigated compounds, has been used in preclinical studies of human imatinib-sensitive and –resistant cell lines and in CD34+ cells from imatinib-refractory patients either alone or in combination with kinase inhibitors [12, 68, 70, 76, 77], proteosome inhibitors [78] or a MEK/ERK pathway inhibitor [73]. LBH589 and LAQ824 also induce cytotoxicity in imatinib-refractory CML cell lines and/or patient samples both alone and in combination with other agents [64, 65, 67]. Apicidin overcomes resistance to TNF-related apoptosis inducing ligand (TRAIL) in K562 cells via inhibition of the PI3K/AKT pathway and nuclear translocation of NF-κB with a subsequent reduction in Bel-xL expression [79]. Likewise, FK228 induces cell cycle arrest and apoptosis of imatinib-resistant CML cells in primary patient samples, in contrast however, expression of Bel-xL, Bel-2 or Bax does not change [80, 81]. In addition, Okabe et al., demonstrated that FK228 treatment induces a diverse range of histone and non-histone protein acetylation in BCR-ABL transfected cell lines, including acetylation of p53, Hsp90 and BCR-ABL [81]. Valproate, commonly used as an anti-epileptic drug, enhances imatinib-induced growth arrest and apoptosis in CML cell lines and primary mononuclear cells with concomitant p2|C|IP1 upregulation, Bcl-2 downregulation and interference with NF-κB DNA binding [66, 82]. Sodium butyrate (SB) or AN-9 (Pivanex) alone or in combination with the MEK inhibitor PD184352, bortezomib or imatinib results in synergistic lethality of CML cell lines [73, 78, 83, 84]. Importantly, combination treatments of TKI-resistant neoplastic cells appear to overcome drug resistance compared to treatment with HDACi alone [64–66, 68, 70, 72, 73, 76–80, 84]. Overcoming drug resistance could be attributed to either the synergistic reduction of BCR-ABL protein levels and/or conceivably the elimination of leukemia stem cells by HDACi that are not affected by conventional TKI treatment [85].

In general, HDACi induce apoptosis in neoplastic cells by modulating multiple signalling pathways that orchestrate proliferation, differentiation and survival. Their anti-proliferative effect is mediated through increased production of cell cycle inhibitor proteins p2|C|IP1 [64–68, 72, 73,
77, 78] and in some instances, p27KIP1 [64, 67, 68], attenuation of cyclin D1 [73, 78] and hypophosphorylation of the retinoblastoma (Rb) protein [45]. While HDACi treatment alone induces chromatin-dependent transactivation of p21CIP1 expression resulting in growth arrest, combination treatment with a MEK1/2 inhibitor or the multikinase inhibitor Sorafenib abrogates p21CIP1 induction resulting in enhanced apoptosis [73, 77]. Indeed, p21CIP1 is known to oppose apoptosis through multiple mechanisms, including inhibition of caspase-3 activation [86, 87]. Therefore, whereas HDACi mediated p21CIP1 expression induces cell cycle arrest, cell lethality may require p21CIP1 downregulation. The expression of p27KIP1 appears to be related to a non-transcriptional mechanism and possibly a consequence of BCR-ABL mediated regulation through the PI3K/AKT pathway [67, 88]. Lethality results from activation of both the extrinsic and intrinsic apoptotic pathways [37, 40, 43, 44], through production of reactive oxygen species (ROS) [71, 73, 78] and by the induction of autophagy [71]. In particular, induction of pro-apoptotic Bim and in some instances, a decrease in pro-survival Bcl-xL is critical for HDACi mediated apoptosis [64, 67, 69, 76, 79]. Finally, the proteosome inhibitor bortezomib or the MEK pathway inhibitor PD184352 when used in combination with SAHA also affects multiple signalling pathways, including induction of Jun Kinase (JNK) phosphorylation, inactivation of AKT and abrogation of NF-κB DNA binding [73, 78, 79].

Treatment with HDACi also influences gene expression through chromatin-independent mechanisms by acetylating non-histone proteins. However, studies that specifically focus on chromatin-independent targets of HDACi in CML cells are limited. Some of the non-histone HDAC substrates that are known to be involved in the cytotoxicity of CML cells post HDACi exposure are Hsp90, p53, Ku70 and α-tubulin. Importantly, imatinib-resistant K562 cells display hypoacetylation of these non-histone proteins [12, 73]. Acetylation of Hsp90, a HDAC6 substrate, results in accelerated degradation of Hsp90 client proteins, including BCR-ABL, AKT and c-Raf, via the 26S proteosome [65, 76, 89–91]. Reduction of BCR-ABL levels post-HDACi treatment, observed in most cases of HDACi treated CML cells, appears to be a consequence of disrupted Hsp90 chaperone function and proteosomal degradation of BCR-ABL [12, 65, 67, 76]. Ku70, a DNA repair enzyme, is another known HDAC substrate and when deacetylated it sequesters the proapoptotic protein Bax in the cytoplasm rendering it inactive. Acetylation of Ku70 by HATs is integral to stress-induced lethality by Bax [92]. In imatinib-resistant K562 cell line, the expression of cytoplasmic Ku70 is increased, while the amount of acetylated Ku70 is markedly decreased, suggesting that Bax-mediated apoptosis is inhibited [12]. Transcriptional activation of the tumour suppressor p53 by acetylation also plays a key role in HDACi mediated cell cycle arrest and apoptosis. Deacetylation of p53 by HDAC1–3 or SIRT1 inhibits its transcriptional activity, and subsequently, the activation of its downstream molecules, Bax and p21 [93–95]. Therefore, acetylation of intracellular proteins by HDACi likely plays a critical role in orchestrating the cellular fate of leukemic cells.

‘Classic’ MPN

The safety and efficacy of HDACi in the treatment of patients with MPN has been evaluated in a small number of preclinical and phase I/II studies. The rationale for the use of HDACi in MPN stems from pre-clinical studies that have demonstrated enhanced sensitivity of JAK2V617F mutated versus non-mutated cells derived from patients with PV or PMF [96, 97]. Epigenetic therapy provides a promising platform for the treatment of PMF; evidence for this was provided by the preferential reduction in the number of JAK2V617F-positive progenitor cells when sequential administration of 5-aza-2′-deoxycytidine (a DNA methyltransferase inhibitor) and Trichostin A (a pan-HDAC inhibitor) was performed [97]. Furthermore, quantitative analysis of Class I-III HDACs mRNA expression and activity in CD34+ cells from PMF patients reveals that HDAC1, 2, 6, 8, 10 and SIRT1, 2, 3, 5 and 7 are overexpressed, while HDAC4, 5 and 11 and SIRT4 are downregulated [30]. The pattern of HDAC mRNA expression did not correlate with the JAK2V617F mutation status, furthermore, the relevance of the aberrant PMF CD34+ cell HDAC mRNA expression and activity and subsequent effects on biological function and pathogenesis of PMF are unknown.

Exposure of mutant cells to the Class I and II HDAC inhibitor, ITF2357, down-regulated JAK2V617F, p-STAT3 and p-STAT5 levels [96]. An ITF2357 Phase IIA study demonstrated efficacy in PV, ET and PMF patients with rapid improvement of constitutional symptoms, reduction in splenomegaly and improved haemopoietic function [97]. Treatment of CD34+ PMF-MPN cells with LBH589 induces apoptosis of PMF-MPN cells and inhibits the expression and activity of JAK2V617F with a subsequent reduction in p-STAT3, p-STAT5, p-AKT, p-GATA-1 and Bcl-xL [98]. In addition, the binding of HSP90 to JAK2 was partially inhibited by LBH589 treatment suggesting that acetylation of HSP90 could be playing a role in the degradation of JAK2 protein. Synergistic induction of MPN cell apoptosis was also observed when LBH589 and a JAK2 inhibitor (TG101209) were combined [98]. A Phase IA/II trial evaluating oral LBH589 in patients with PMF (n=10) and post-PV MF (n=3) that included 9 patients with JAK2V617F mutation exhibited clinical activity for up to 39 months including a reduction in spleen size and improvement in constitutional symptoms [99]. Similarly,
HDACi in the management of selected individuals with MPN. Phase I/II studies are required to ascertain the utility of epigenetic-directed MPN therapy. Further pre-clinical and linkage of genetic and epigenetic changes that promote MPN are still poorly understood. Increased knowledge of the epigenetic aberrations such as histone deacetylase activity in therapeutic benefit, however, the extent and specific role of not genomic changes have the potential to be modulated for (chromatin-independent) changes. Importantly, epigenetic but both epigenetic (chromatin-dependent) and non-epigenetic mechanisms that may at least in part be due to abnormal acetylation of non-histone proteins. Furthermore, abnormal histone deacetylase activity has been noted in PMF and provides a rationale for the treatment of MPN with HDACi, either alone or in combination with existing therapies. Preliminary studies indicate that HDACi when used in combination with TKIs or JAK2 inhibitors may overcome drug resistance and enhance the apoptotic effects on MPN cells. HDACi likely induce lethality through a combination of both epigenetic (chromatin-dependent) and non-epigenetic (chromatin-independent) changes. Importantly, epigenetic but not genomic changes have the potential to be modulated for therapeutic benefit, however, the extent and specific role of epigenetic aberrations such as histone deacetylase activity in MPN are still poorly understood. Increased knowledge of the linkage of genetic and epigenetic changes that promote tumorogenesis represents a prerequisite for successful epigenetic-directed MPN therapy. Further pre-clinical and phase I/II studies are required to ascertain the utility of HDACi in the management of selected individuals with MPN.

Conclusion

Therapeutic interventions for MPNs include the use of highly targeted and efficacious therapy with TKIs for the abnormal BCR-ABL fusion protein found in CML and the use of less specific agents, including JAK2 inhibitors, that target ubiquitously expressed proteins in JAKV617F mutated PV, ET and PMF. Despite TKIs revolutionising the management of CML, TKI resistance can occur through BCR-ABL-independent mechanisms that may at least in part be due to abnormal acetylation of non-histone proteins. Furthermore, abnormal histone deacetylase activity has been noted in PMF and provides a rationale for the treatment of MPN with HDACi, either alone or in combination with existing therapies. Preliminary studies indicate that HDACi when used in combination with TKIs or JAK2 inhibitors may overcome drug resistance and enhance the apoptotic effects on MPN cells. HDACi likely induce lethality through a combination of both epigenetic (chromatin-dependent) and non-epigenetic (chromatin-independent) changes. Importantly, epigenetic but not genomic changes have the potential to be modulated for therapeutic benefit, however, the extent and specific role of epigenetic aberrations such as histone deacetylase activity in MPN are still poorly understood. Increased knowledge of the linkage of genetic and epigenetic changes that promote tumorogenesis represents a prerequisite for successful epigenetic-directed MPN therapy. Further pre-clinical and phase I/II studies are required to ascertain the utility of HDACi in the management of selected individuals with MPN.

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References

1. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW (2008) World health organisation classification of tumours of haematopoietic and lymphoid tissues. International Agency for Research on Cancer (IARC) Press, Lyon
2. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ (2007) Cancer Statistics. 2007. CA Cancer J Clin 57:43–66
3. Geary CG (2000) The story of chronic myeloid leukaemia. Br J Haematol 110:2–11
4. Baccarini M, Russo D, Rosti G, Martiniell G (2003) Interferon-alfa for chronic myeloid leukaemia. Semin Hematol 40:22–33
5. Baccarini M, Saglio G, Goldman L et al (2006) Evolving concepts in the management of chronic myeloid leukaemia: recommendations from an expert panel on behalf of the European Leukemia Net. Blood 108:1809–1820
6. Gratwohl A, Brand R, Apperley J et al (2006) Allogeneic hematopoietic stem cell transplantation for chronic myeloid leukemia in Europe 2006: transplant activity, long-term data and current results. An analysis by the Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). Haematologica 91:513–521
7. Druker BJ, Guilhot F, O’Brien SG et al (2006) Five-year follow-up of patients receiving imatinib for chronic myeloid leukaemia. N Engl J Med 355:2408–2417
8. Kantarjian HM, Talpaz M, Giles F, O’Brien S, Cortes J (2006) New insights into the pathophysiology of chronic myeloid leukaemia and imatinib resistance. Ann Intern 145:913–923
9. Weissberg E, Manley PW, Cowan-Jacob SW, Hochhaus A, Griffin JD (2007) Second generation inhibitors of BCR-ABL for the treatment of imatinib resistant chronic myeloid leukaemia. Nat Rev Cancer 7:345–356
10. Dai Y et al (2004) A Bcr-Abl-independent, Lyn-dependent form of imatinib mesylate (STI-571) resistance is associated with altered expression of Bcl-2. J Biol Chem 279(33):34227–34239
11. Donato NJ et al (2004) Imatinib mesylate resistance through altered expression of Bc1-2. J Biol Chem 279(33):34227–34239
12. Lee SM et al (2007) Bcr-Abl-independent imatinib-resistant K562 cells show aberrant protein acetylation and increased sensitivity to histone deacetylase inhibitors. J Pharmacol Exp Ther 322(3):1084–1092
13. Vannucchi AM, Guglielmelli P, Rambaldi A, Bogani C, Barbui T (2009) Epigenetic therapy in myeloproliferative neoplasms: evidence and perspectives. J Cell Mol Med 13:1437–1450
14. Jones AV, Silver RT, Waghorn et al (2006) Minimal molecular response in polycythemia vera patients treated with imatinib or interferon alpha. Blood 107:3339–3341
15. Kiladjian JJ, Chomienne C, Fenaux P (2008) Interferon-alpha therapy in bcr-abl-negative myeloproliferative neoplasms. Leukemia 22:1990–1998
16. Kiladjian JJ, Cassinat B, Chevret S et al (2008) Pegylated Interferon-alfa-2a induces complete haematological and molecular responses with low toxicity in polycythaemia vera. Blood 112:3065–3072
17. Mesa RA, Verstovsek S, Rivera C et al (2009) 5-Azacitidine has limited therapeutic activity in myelofibrosis. Leukemia 23:180–182
18. Cervantes F, Mesa R, Barosi G (2007) New and old treatment modalities in primary myelofibrosis. Cancer J 13:377–383
19. Mesa RA, Camoriano JK, Geyer SM et al (2007) A phase II trial of ifosfamide in myelofibrosis: primary, post-polycythaemia vera and post-essential thrombocythemia. Leukemia 21:1964–1970
20. Mesa RA, Verstovsek S, Rivera C et al (2008) Bortezomib therapy in myelofibrosis: a phase II clinical trial. Leukemia 22:1636–1638
21. Tefferi A, Lasho TL, Mesa RA et al (2007) Lenalidomide therapy in del(5)(q31)-associated myelofibrosis: cyto genetic and JAK2V617F molecular remissions. Leukemia 21:1827–1828

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41. Quintas-Cardama A, Tong W, Kantarjian H et al (2008) A phase II study of 5-Azacitidine for patients with primary and post essential thrombocythemia/polycythemia vera myelofibrosis. Leukemia 22:965–970

42. Haberland M et al (2009) Genetic dissection of histone deacetylase requirement in tumor cells. Proc Natl Acad Sci USA 106(19):7751–7755

43. Haberland M et al (2009) Genetic dissection of histone deacetylase requirement in tumor cells. Proc Natl Acad Sci USA 106(19):7751–7755

44. Lehmann H et al (2002) Histone acetyltransferases and deacetylases in the control of cell proliferation and differentiation. Adv Cancer Res 86:41–65

45. Mai A et al (2005) Histone deacetylation in epigenetics: an attractive target for anticancer therapy. Med Res Rev 25(3):261–309

46. Spange S et al (2009) Acetylation of non-histone proteins modulates cellular signalling at multiple levels. Int J Biochem Cell Biol 41(1):185–198

47. Gloczak MA et al (2005) Acetylation and deacetylation of non-histone proteins. Gene 363:15–23

48. Gloczak MA et al (2007) Histone deacetylases and cancer. Oncogene 26(37):5420–5432

49. Miller TA et al (2003) Histone deacetylase inhibitors. J Med Chem 46(24):5097–5116

50. Bolden JE et al (2006) Anticancer activities of histone deacetylase inhibitors. Nat Rev Drug Discov 5(9):769–784

51. Minucci S et al (2006) Histone deacetylase inhibitors and the cancer cell. Mol Cell 21:505–511

52. Fernandez-Mercado M, Cebrian V, Euba B et al (2008) Methylation status of SOCS1 and SOCS3 in BCR-ABL-negative and JAK2V617F negative chronic myeloproliferative disorders. Br J Haematol 141(5):504–511

53. Bogani C, Ponziani V, Gugliemelli P et al (2008) Hypermethylation of CXCR4 promoter in the CD34+ cells from patients with primary myelofibrosis. Stem Cells 26:1920–1930

54. Wang X, Zhang W, Ishii T et al (2008) Correction of the DNA methyltransferase 1 gene (DNMT1) in malignant T cells. Blood 108:1058–1064

55. Tefferi A (2008) Epigenetic alterations and anti-epigenetic therapies in myelofibrosis. Leuk Lymphoma 49(23):2231–2232

56. Wang JC et al (2008) Enhanced histone deacetylase enzyme activity in primary myelofibrosis. Leuk Lymphoma 49(12):2321–2327

57. Fourouclas N, Li J, Gilby DC et al (2008) Methylation of the suppressor of cytokine signalling 3 gene (SOCS3) in myeloproliferative disorders. Haematologica 93:1635–1644

58. Capello D, Deambrogi C, Rossi D et al (2008) Epigenetic inactivation of suppressors of cytokine signalling in Philadelphia-negative chronic myeloproliferative disorders. Br J Haematol 141(5):504–511

59. Shi S, Calhoun HC, Xia F et al (2006) JAK signalling globally counteracts heterochromatic gene silencing. Nat Genet 38:1071–1076

60. Fraga MF et al (2005) Loss of acetylation at Lys16 and Lys23 of histone H3 in malignant T cells. Blood 108:1058–1064

61. Minucci S et al (2006) Histone deacetylase inhibitors trigger a G2 arrest in cancer cells. Proc Natl Acad Sci USA 103(7):2513–2518

62. Silverman LR, Demakos EP, Peterson BL et al (2002) Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome. Leukemia 16:965–970

63. Gloczak MA et al (2005) Acetylation and deacetylation of non-histone proteins. Gene 363:15–23

64. Gloczak MA et al (2007) Histone deacetylases and cancer. Oncogene 26(37):5420–5432

65. Miller TA et al (2003) Histone deacetylase inhibitors. J Med Chem 46(24):5097–5116

66. Bolden JE et al (2006) Anticancer activities of histone deacetylase inhibitors. Nat Rev Drug Discov 5(9):769–784

67. Minucci S et al (2006) Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. Nat Rev Cancer 6(1):38–51

68. Xu WS et al (2007) Histone deacetylase inhibitors: molecular mechanisms of action. Oncogene 26(37):5541–5552

69. Brinkmann H et al (2001) Histone hyperacetylation induced by histone deacetylase inhibitors is not sufficient to cause growth inhibition in human dermal fibroblasts. J Biol Chem 276(25):22491–22499

70. Burgess AJ et al (2004) Histone deacetylase inhibitors specifically kill non-proliferating tumour cells. Oncogene 23(40):6693–6701

71. Burgess AJ et al (2001) Histone hyperacetylation induced by histone deacetylase inhibitors is not sufficient to cause growth inhibition in human dermal fibroblasts. J Biol Chem 276(25):22491–22499
83. Grebennova D et al (2006) The proteomic study of sodium butyrate antiproliferative/cytodifferentiation effects on K562 cells. Blood Cells Mol Dis 37(3):210–217

84. Rabizadeh E et al (2007) Pivanex, a histone deacetylase inhibitor, induces changes in BCR-ABL expression and when combined with STI571, acts synergistically in a chronic myelocytic leukemia cell line. Leuk Res 31(8):1115–1123

85. Zhang B et al (2010) Effective targeting of quiescent chronic myelogenous leukemia stem cells by histone deacetylase inhibitors in combination with imatinib mesylate. Cancer Cell 17(5):427–442

86. Bissonnette N et al (1998) p21-induced cycle arrest in G1 protects cells from apoptosis induced by UV-irradiation or DNA polymerase II blockage. Oncogene 16(26):3461–3469

87. Suzuki A et al (1999) Caspase 3 inactivation to suppress Fas-mediated apoptosis: identification of binding domain with p21 and ILP and inactivation machinery by p21. Oncogene 18(5):1239–1244

88. Gesbert F et al (2000) BCR-ABL regulates expression of the cyclin-dependent kinase inhibitor p27Kip1 through the phosphatidylinositol 3-Kinase/AKT pathway. J Biol Chem 275(50):39223–39230

89. Bali P et al (2005) Inhibition of histone deacetylase 6 acetylates and disrupts the chaperone function of heat shock protein 90: a novel basis for antileukemia activity of histone deacetylase inhibitors. J Biol Chem 280(29):26729–26734

90. Rao R et al (2008) HDAC6 inhibition enhances 17-AAG-mediated abrogation of hsp90 chaperone function in human leukemia cells. Blood 112(5):1886–1893

91. Cohen HY et al (2004) Acetylation of the C terminus of Ku70 by CBP and PCAF controls Bax-mediated apoptosis. Mol Cell 13(5):627–638

92. Juan LJ et al (2000) Histone deacetylases specifically down-regulate p53-dependent gene activation. J Biol Chem 275(27):20436–20443

93. Kume S et al (2006) Silent information regulator 2 (SIRT1) attenuates oxidative stress-induced mesangial cell apoptosis via p53 deacetylation. Free Radic Biol Med 40(12):2175–2182

94. Wang LG et al (2008) De-repression of the p21 promoter in prostate cancer cells by an isothiocyanate via inhibition of HDACs and c-Myc. Int J Oncol 33(2):375–380

95. Guerini V et al (2008) The histone deacetylase inhibitor ITF2357 selectively targets cells bearing mutated JAK2(V617F). Leuke mia 22(4):740–747

96. Shi J et al (2007) Effects of chromatin-modifying agents on CD34+ cells from patients with idiopathic myelofibrosis. Cancer Res 67(13):6417–6424

97. Rambaldi A, Dellacasa CM, Salmoiraghii S et al (2008) A phase 2A study of the histone-deacetylase inhibitor ITF2357 in patients with Jak2V617F positive chronic myeloproliferative neoplasms. Blood 67(13):1196–1198

98. Park SJ et al (2009) Cotreatment with apicidin overcomes TRAIL resistance via inhibition of Bcr-Abl signaling pathway in K562 leukemia cells. Exp Cell Res 315(11):1809–1818

99. Kawano T et al (2004) Depsipeptide enhances imatinib mesylate-induced apoptosis of Bcr-Abl-positive cells and ectopic expression of cyclin D1, c-Myc or active MEK abrogates this effect. Anticancer Res 24(5A):2705–2712

100. Okabe S et al (2007) Depsipeptide (FK228) preferentially induces apoptosis in BCR-ABL-expressing cell lines and cells from patients with chronic myelogenous leukemia in blast crisis. Stem Cells Dev 16(3):503–514

101. Lee J (2009) Clinical efficacy of vorinostat in a patient with essential thrombocythemia and subsequent myelofibrosis. Ann Hematol 88(7):699–700