Deacetylase Inhibition in Haematological Malignancies – Advanced T-cell Lymphoma, Hodgkin's Lymphoma, Multiple Myeloma, Acute Myelogenous Leukaemia and Myelodysplastic Syndrome

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Abstract

One of the groups of novel targeted drugs that has been most extensively studied in the last few years are histone deacetylase inhibitors (HDACis). These compounds inhibit the deacetylation induced by deacetylase (DACs) in both histone and non-histone proteins, resulting in an antitumour effect. The first HDACi to obtain approval by the US Food and Drug Administration (FDA) for an oncological indication was vorinostat for the treatment of cutaneous T-cell lymphoma (CTCL). Other HDACis have also shown activity in this disease. The activity of these agents in monotherapy in highly refractory Hodgkin's lymphoma (HL) patients also shows clear therapeutic potential. Regarding acute myeloid leukaemia (AML), myelodysplastic syndrome (MDS) and multiple myeloma (MM), the antitumour activity in monotherapy, despite showing hints of activity, has been limited; however, based on pre-clinical studies, some combinations with other novel or well-established agents, such as anthracyclins or 5-azacytidine in the case of AML/MDS or with bortezomib or lenalidomide for MM, are ongoing, with promising signs of activity. Therefore, the use of these agents alone or in combination with other treatments seems to be an attractive approach for patients with haematological malignancies.

Although some haematological malignancies such as Hodgkin's lymphoma (HL) or acute myeloiud leukaemia (AML) are curable with conventional treatments, many patients still relapse, and these relapses are more resistant to therapy than the original disease. Moreover, other diseases, such as multiple myeloma (MM) or myelodysplastic syndrome (MDS), are considered incurable with the currently available therapeutic options. Therefore, there is a need for novel treatments in order to improve the remission rate and the survival of these patients. Important progress has been made in the last few years in the search for drugs with novel mechanisms of action directed against specific targets of the tumoral cells that could help in this task. Histone deacetylase inhibitors (HDACis) are among the targeted drugs that have raised hopes. This article reviews the mechanism of action of HDACis and shows some of the most recent clinical results obtained with these compounds in haematological malignancies (see *Table 1* for a summary).

Concept of Deacetylases

Deacetylases (DACs) are enzymes that specialise in the removal of acetyl groups from their target proteins. Formerly, histones were considered the main client proteins for DACs, which is why these enzymes are usually known as histone deacetylases (HDACs).[1,2] This activity is the basis for the epigenetic regulatory function of HDACs, which is exerted by controlling the delicate balance between acetylation and deacetylation of histones that control the

accessibility of the chromatin to transcription factors.[1] Nevertheless, many other non-histone proteins are also deacetylated by HDACs, such as α-tubulin, p53, p73, retinoblastome, several steroid receptors, E2F family members, Bcl-6, Hsp90, HIF-1α or Nur77, among others; in fact, more than 50 non-histone proteins have been described to be substrates of HDACs.[2]

Classification of Histone Deacetylase Inhibitors

HDACs have been classified into four groups based on their homology with yeast proteins.[3,4] Class I, II and IV HDACs are referred to as the classic HDACs, whereas class III HDACs are called sirtuins due to their homology to yeast Sir2; the latter group display characteristic features, such as the requirement of NAD+ as an essential co-factor for their activity and the absence of zinc in the catalytic site.[1,3] This article will focus on the classic HDACs as they are the ones that have been implicated in oncogenesis and are targets of the HDACis currently used in the clinic. Class I HDACs are usually localised within the nucleus of the cells[5–7] and comprise HDAC1, HDAC2, HDAC3 and HDAC8. Important substrates of this group of HDACs are proteins such as p53, MEF2D, Stat3 or E2F. Class II is further subdivided into classes IIA and IIB. Class IIA are both nuclear and cytoplasmic HDACs and include HDAC4, HDAC5, HDAC7 and HDAC9. More important seems to be class IIB, which comprises the cytoplasmic HDAC10 and, mainly, HDAC6, which is responsible for the acetylation of two relevant

Table 1: Summary of the Most Relevant Studies of Histone Deacetylase Inhibitors in Haematological Malignancies

Indication	Agent	Study	Phase	n	Results
CTCL	Vorinostat	Duvic et al.[27]	II	31	8 PR
	Vorinostat	Olsen et al.[28]	IIb	74	1 CR, 21 PR
	Panobinostat	Ellis et al.[29]	I	9	2 CR, 4 PR, 1 SD
	Panobinostat	Duvic et al.[30]	II	95	2 CR, 13 PR
	Romidepsin	Bates et al.[31]	II	63	4 CR, 21 PR, 19 SD
	Romidepsin	Kim et al.[32]	IIb	72	6 CR, 24 PR, 26 SD
PTCL	Romidepsin	Piekarz et al.[33]	II	31	7 CR, 10 PR
MM	Vorinostat	Richardson et al.[40]	I	10	1 MR, 9 SD
	Panobinostat	Wolf et al.[41]	II	38	1 PR, 1 MR, 3 SD
	ITF2357	Galli et al.[42]	II	15	1 PR, 4 SD
	Vorinostat + lenalidomide + dexamethasone	Siegel et al.[45]	I	8	1 CR, 1 PR, 2 MR, 2 SD
	Panobinostat + lenalidomide + dexamethasone	Spencer et al.[46]	I	13	1 sCR, 1 CR, 6 PR, 1 MR
	Vorinostat + bortezomib	Badros et al.[47]	I	16	1 nCR, 7 PR, 6 SD
	Vorinostat + bortezomib	Weber et al.[48]	I	34	9 PR, 7 MR, 18 SD
	Vorinostat + bortezomib	Weber et al.[48]	I	21	9 PR, 10 SD
	Panobinostat + bortezomib	Sezer et al.[50]	I	19	3 CR, 8 PR
	Romidepsin + bortezomib + dexamethasone	Harrison et al.[51]	I/II	18	4 CR/nCR, 8 PR, 5 MR
AML/MDS	Vorinostat	Garcia-Manero et al.[52]	I	31	2 CR, 2 CRi, 5 HI
	Panobinostat	Ottmann et al.[53]	I	26	2 CR, 1 CRi, 2 PR
	Romidepsin	Byrd et al.[54]	I	10	None
	Romidepsin	Klimek et al.[55]	I	11	1 CR, 6 SD
	MGCD0103	Garcia-Manero et al.[56]	I	29	3 CR
	MGCD0103	Lancet et al.[57]	I	19	4 SD
	MGCD0103 + 5-azacytidine	Garcia-Manero et al.[59]	I/II	24	3 CR, 3 CRi, 1 PR
	Vorinostat + 5-azacytidine	Silverman et al.[60]	I/II	11	5 CR, 1 CRi, 3 HI, 2 SD
	Entinistat + 5-azacytidine	Gore et al.[61]	I	27	2 CR, 4 PR, 6 HI
	Phenylbutyrate + 5-azacytidine	Maslak et al.[62]	I	10	5 PR/SD
HL	Vorinostat	O'Connor et al.[63]	I	12	1 PR, 3 SD
	Panobinostat	Ottmann et al.[53]	I	28	1 CR, 17 PR by CT, 10 PR by PET

AML = acute myeloid leukaemia; CR = complete response; CRi = complete response with incomplete platelet recovery; CT = computed tomography; CTCL = cutaneous T-cell lymphoma; HI = haematological improvement; HL = Hodgkin's lymphoma; MDS = myelodysplastic syndrome; MM = multiple myeloma; MR = metabolic response; nCR = near complete response; PET = positron emission tomography; PR = partial response; PTCL = peripheral T-cell lymphoma; sCR = stringent complete response; SD = stable disease.

substrates, α-tubulin[8,9] and Hsp-90,[10] which play a significant role in tumour pathogenesis. Finally, HDAC11 is the only representative of class IV and its function has not yet been well characterised.

Importance of Histone Deacetylases and Their Inhibition in Cancer

The rationale for the use of HDACis in oncology resides in the importance of epigenetic modifications in these diseases and in the changing role and activity that the already mentioned non-histone substrates play in oncogenesis. This is also supported by the fact that significant changes in the expression of HDACs have been described in different tumours. As an example, class I HDACs, principally HDAC1, 2 and 3, are overexpressed in gastrointestinal and prostatic tumours,[11–14] and this is associated with an adverse prognosis. Class IIA HDAC expression has been shown to be upregulated in different tumours: HDAC4 in breast and HDAC5 and 7 in colorectal cancer.[15] HDAC6 is significantly overexpressed and is associated with advanced stages of oral squamous cell carcinoma.[16] By contrast, the upregulation of its expression in breast cancer has been described to confer a better prognosis.[17,18]

Another important factor that specifically supports the use of HDACis in haematological malignancies is that the functional changes in these HDACs have been described in several leukaemias and lymphomas. In this regard, HDACs are frequently involved in some oncogenic translocations present in these diseases. The AML-1-ETO translocation, characteristic of M2 acute myeloid leukaemia, modulates repressive co-factors such as HDACs and DNA methyltransferases (DNMTs).[2,19–21] Both HDAC3 and HDAC4 are recruited by the PML/RARα translocation in acute promyelocytic leukaemia, and this contributes to the repression of transcription that provokes the loss of differentiation characteristic of this type of leukaemia.[22,23]

Classification of Histone Deacetylase Inhibitors

The above data have stimulated the investigation of HDACis as potential anticancer drugs, and several of these molecules are already at different stages of pre-clinical and clinical development. These HDACis can be divided into different groups according to their chemical structure and their pattern of HDAC inhibition.[2,4,5,24,25] Hydroxamates are derivatives of the hydroxamic acid and are characteristically pan-HDACi because they inhibit both class I and II HDACs. This group includes molecules such as vorinostat (SAHA), panobinostat (LBH589), TSA (trichostatin A), belinostat (PDX-101) and ITF2357. Due to its potency and breadth of activity, this is the group with more extensive development. A second class of HDACis is constituted by cyclic peptides, which are specific inhibitors of class I HDACs, with very scarce or no effect over class II HDACs. The most significant representative of this group is romidepsin (FK-228). Benzamides such as MS-275 or MGCD0103 are also class-I-specific, whereas aliphatic acids can inhibit class I HDACs and may have some effect on class IIA HDACs. This last group includes agents that have previously been used in the clinic for other indications, such as valproic acid, phenylbutyrate or butyrate, and have now been discovered to have HDACi activity. Nevertheless, their potency of

inhibition of HDAC is quite low. A different small molecule that does not fit into any of the previous classes is tubacin, which was recently described as an specific inhibitor of HDAC6[26] and induces acetylation of tubulin and Hsp-90, without affecting histone acetylation.

Histone Deacetylase Inhibitors in Cutaneous T-cell Lymphoma and Peripheral T-cell Lymphoma

The first HDACi to obtain approval by the US Food and Drug Administration (FDA) for an oncological indication was vorinostat, for the treatment of cutaneous T-cell lymphoma (CTCL). This approval was based on two main studies: a single-centre phase II dose-finding study, which resulted in an overall response rate (ORR) of 24%,[27] and the subsequent phase II trial in which 74 patients with persistent, progressive or refractory CTCL were administered oral vorinostat daily until disease progression. Thirty per cent of the patients achieved an objective response, independent of the status of the disease, with a median time to response of 55 days.[28] Toxicity was manageable, with gastrointestinal, constitutional and haematological symptoms the most frequent adverse effects. Other HDACis, such as panobinostat, are also being explored in CTCL: based on the promising results of a phase I dose-escalating study that showed two complete responses (CRs) and four partial responses (PRs) in nine heavily pre-treated patients,[29] a phase II trial of oral panobinostat is being conducted in patients with refractory CTCL. Preliminary results indicate that panobinostat used as single agent has activity in both bexarotene-naïve and bexarotene-exposed patients with a similar toxicity profile to that observed with vorinostat.[30] The results of two different phase II trials of romidepsin in 63 and 72 pre-treated CTCL patients have also recently been reported. Both trials showed very similar results, with response rates of 6–8% CR, 35–42% PR and 27–36% stable disease (SD).[31,32] Interestingly, patients with relapsed peripheral T-cell lymphoma (PTCL), a disease with a similar origin to CTCL, also benefit from the use of romidepsin. Piekarz recently reported a phase II study that enrolled 43 PTCL patients, achieving an ORR of 39% (16% CR and 23% CR) with a duration of response of 8.3 months.[33]

Histone Deacetylase Inhibitors in Multiple Myeloma

Several studies have demonstrated the *in vitro* and *in vivo* activity of different HDACis as single agents in MM.[34–39] The rationale for this activity resides in the acetylation of proteins important in the pathogenesis of this disease, such as HIF-1α, heat shock proteins, tubulin or p53, which results in the inhibition of their oncogenic function. The activity of some HDACis (vorinostat,[40] panobinostat,[41] ITF2357[42]) as single agents in relapsed/refractory MM have been tested in phase I/II trials and, despite their pre-clinical activity, the clinical efficacy of these drugs has been quite modest, achieving PR/MR in fewer than 10% of patients in all studies. Nevertheless, pre-clinical studies prompted the analysis of combination of HDACis with some currently used antimyeloma agents, such as the immunomodulatory drug lenalidomide[43] or the proteasome inhibitor bortezomib.[35,43,44] Vorinostat and panobinostat have been combined with lenalidomide and dexamethasone in two ongoing trials with good preliminary results (more than 50% ORR, including several CRs) and an acceptable safety profile.[45,46] More consolidated data exist for the clinical combination of HDACis with bortezomib. The rationale for this combination is the simultaneous targeting of several proteins involved in the unfolded protein response, a key mechanism for the

survival of myeloma cells due to the huge amount of proteins (especially monoclonal immunoglobulins) that need to be correctly managed. Proteasome inhibitors block the degradation of the ubiquitinated misfolded proteins by the proteasome, whereas the use of HDACis interferes with the activity of heat shock proteins, necessary for the correct folding of proteins, and with formation of aggressome (through the inhibition of HDAC6), which is also important in the elimination of toxic misfolded proteins. This simultaneous action results in a synergistic cytotoxic effect. Two phase I trials with vorinostat in combination with bortezomib have been reported,[47,48] showing an ORR of around 40–50%. Interestingly, the combination was able to induce responses in patients who had relapsed or had been refractory to bortezomib.[47,49] Panobinostat and romidepsin have also been combined with bortezomib, achieving an ORR of 58[50] and 95%,[51] respectively; similarly to vorinostat, responses were also observed in bortezomib-refractory patients. The main toxicity for this combination was thrombocytopenia, but overall it was well tolerated. It is interesting to note the promising activity in patients previously exposed to bortezomib, which highlights the synergy of the combination and the possibility of overcoming bortezomib refractoriness.

Histone Deacetylase Inhibitors in Acute Myeloid Leukaemia and Myelodysplastic Syndromes

As already mentioned, the role that HDAC abnormalities play in the development of cancer is particularly obvious in the case of leukaemias, because some of the genetic alterations that give rise to these diseases also interfere with HDAC expression.[22,23] Moreover, the importance of epigenetic deregulation in the pathogenesis of leukaemias and myelodysplastic syndromes has also been described. This is the reason why at least four HDACis have been tested as single agents in AML patients. Vorinostat in monotherapy, in a phase I dose-escalating study, achieved a 16% response rate, including two CRs and two CRs with incomplete platelet recovery (CRi).[52] In the phase I study conducted with panobinostat in advanced haematological malignancies, three patients out of the 36 who received the drug in a three-times-weekly schedule achieved a CR or CRi, and two more patients displayed PR. By contrast, no responses were observed in the group of patients who received the drug every other week.[53] Regarding romidepsin, two studies have been conducted in AML and MDS. In the first study, no responses were obtained from the 10 patients included, although some signs of antileukaemic activity were observed.[54] The second study enrolled 11 patients, with one CR and six SD.[55] MCGD0103 has also been explored in AML/MDS in two different trials, with some responses in both studies.[56,57] These hints of antileukaemic activity support the use of combinations of HDACis with other agents in order to more rapidly and effectively control the growth of the tumour clone. Pre-clinical studies could help in the search for synergistic combinations for these patients. This is the case with the combination of panobinostat with anthracyclins, which has recently been demonstrated by our group to be highly synergistic.[58] These results have led to the activation of a clinical trial in which panobinostat will be combined in a sequential manner with the classic idarubicin–cytarabine schedule in newly diagnosed elderly AML patients. A second attractive combination in AML/MDS is the association with hypomethylating agents in order to simultaneously target two different epigenetic mechanisms. Several studies have already shown interesting results in AML/MDS patients with combinations of different HDACis with 5-azacytidine.[59–62]

Histone Deacetylase Inhibitors in Hodgkin's Lymphoma

Despite the good prognosis of HL, when relapse occurs, results of salvage therapies are usually rather dissapointing. Preliminary results from phase I dose-escalating studies performed with different HDACis have shown promising activity in heavily pre-treated HL patients. Vorinostat induced SD or PR in four out of 12 HL patients.[63] The phase I study of panobinostat included 28 HL patients in two different schedules of treatment. Patients had been very heavily pre-treated with a median of five prior lines of treatment, and 80% of them had received a previous autologous stem cell transplant (ASCT). Single-agent panobinostat, administered orally, resulted in one CR, 17 metabolic responses by positron-emission tomography (PET) and 10 PR by computed tomography (CT).[53] The main toxicity in these patients was thrombocytopenia. Based on these results, an international phase II trial has already started, with favourable preliminary results. MGCD0103 has also been explored in 33 patients with HL. Among the 26 evaluable patients, two achieved CR, seven achieved PR and three enjoyed a durable SD. Overall, these results are very promising, particularly as they were obtained with oral drugs in monotherapy in patients who had been refractory to several combinations of chemotherapeutic agents, including ASCT in the majority of them. The search for optimal combinations of HDACis with other classic agents is warranted in order to further improve the outcome of both relapsed and, eventually, newly diagnosed HL patients.

Conclusion

Both the pathophysiological rationale and the currently available clinical results clearly support the use of HDAC inhibition as a strategy in the treatment of haematological malignancies. Moreover, to date, most of these promising clinical results have been obtained from dose-escalating studies, and therefore some patients probably received suboptimal doses. In addition, the majority of these studies were performed in refractory patients and with the drugs as monotherapy. Therefore, it would be reasonable to speculate that the administration of these compounds at optimal doses and in combination with other well-established or novel agents could result in a significant improvement of the results obtained to date, resulting in new hope for patients. ■

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