The potential of panobinostat as a treatment option in patients with relapsed and refractory multiple myeloma

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***Abstract*:** Panobinostat is an investigational and potent histone deacetylase inhibitor (HDACi) that has shown promise as an antimultiple myeloma agent in the preclinical setting. In this review, we discuss the rationale for the use of panobinostat as a combination therapy for multiple myeloma and provide an overview of recent and ongoing clinical trials testing the safety and efficacy of panobinostat for the treatment of the disease.

Keywords: histone deacetylase inhibitor, multiple myeloma, panobinostat

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Introduction

During the past decade, significant progress has been made in the management of multiple myeloma due to the introduction of new therapeutic agents such as proteasome inhibitors (PIs) and immunomodulatory agents (IMiDs) [Mateos *et al*. 2013]. Despite this, multiple myeloma remains incurable and patients eventually become refractory to their treatment regimens. Thus, there is a clear need for the development of new therapeutic options. Histone deacetylase inhibitors (HDACis) are a relatively new class of agents that have demonstrated effective anticancer activity in the preclinical setting, and two HDACis have been approved by the US Food and Drug Administration (FDA) for the treatment of specific hematological malignancies [Ververis *et al*. 2013]. Panobinostat is an investigational and potent HDACi that has shown activity against multiple myeloma at nanomolar concentrations in preclinical studies [Atadja, 2009; Sanchez *et al*. 2011]. In this review, we discuss the rationale for the use of panobinostat as a combination therapy for multiple myeloma and provide an overview of recent and ongoing clinical trials testing the safety and efficacy of panobinostat for the treatment of this disease.

Multiple myeloma

Multiple myeloma (MM), a plasma cell dyscrasia, is the most common primary malignancy of the bone marrow [Morgan, 1999; Smith and Newland, 2000]. It is estimated that 24,050 new cases of MM (13,500 in men and 10,550 in women) will be diagnosed in the United States and that 11,090 men and women will die from the disease during 2014 [Siegel *et al*. 2014]. MM patients treated with conventional chemotherapy have an average overall survival (OS) of 4 years as these therapies are not curative. In recent years, new and more effective drugs, including IMiDs and PIs, have become available for the treatment of MM. Such drugs have been evaluated alone and in combination with established anti-MM agents, rapidly increasing the number of therapeutic options available to MM patients. As a result, the 5-year survival rate for MM patients is currently 44% [Brenner *et al*. 2008; Kumar *et al*. 2008; Pulte *et al*. 2014]. Unfortunately, even with these newer agents, responses to therapy are transient, and MM remains an incurable disorder with an eventual fatal outcome. Therefore, there is an urgent need to find novel therapeutic targets and develop new therapeutic strategies that are more effective and well-tolerated, particularly in the relapsed/refractory (RR) setting.

Histone acetylases and histone deacetylases

Protein acetylation is a dynamic post-translational modification that is controlled by two groups of enzymes with opposite activities: histone acetylases (HATs) and histone deacetylases (HDACs)

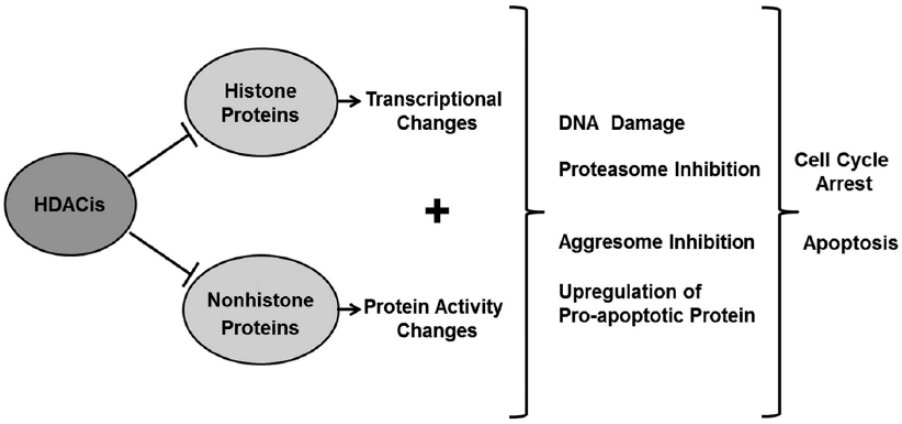

Figure 1. Histone deacetylase inhibitors (HDACis) block the deacetylation of both histone and nonhistone proteins, thereby causing transcriptional and protein activity changes. In multiple myeloma cells, such changes have been shown to lead to proteasome and aggresome inhibition, DNA damage and the upregulation of proapoptotic proteins, resulting in cell cycle arrest and apoptosis.

[Khan and La Thangue, 2012]. HATs and HDACs regulate gene transcription, cell differentiation, cell cycle progression and apoptosis by targeting both histone and nonhistone proteins [Maes *et al*. 2013] (Figure 1). Hyperacetylation of histone proteins results in a relaxed chromatin configuration which is compatible with gene transcription, whereas hypoacetylation of histones leads to chromatin compaction and gene silencing [Maes *et al*. 2013]. The activity of many chaperones and transcriptional factors, as well as that of tumor suppressor and structural proteins, depends on their acetylation status [New *et al*. 2012]. Therefore, alterations in HATs or HDACs can affect a myriad of cellular processes.

HDACs are categorized by their homology to yeast HDACs and based on their requirement for Zn^{2+} as a cofactor. Zn^{2+}-dependent enzymes include: class I HDACs (1–3 and 8), which localize to the cell nucleus and are ubiquitously expressed; class II a/b HDACs (4–7, 9 and 10), which can shuttle between the cell nucleus and cytoplasm and have tissue-specific expression; and class IV HDACs, of which HDAC11 is the sole member, a predominantly nuclear HDAC with limited tissue distribution (kidney, brain, heart, skeletal muscle and testis) [Gao *et al*. 2002; Ropero and Esteller, 2007; Khan and La Thangue, 2012]. HDACs in class III (sirtuins, SIR 1–7) are Zn^{2+}-independent/ NAD^{+}-dependent enzymes, for which their pattern of expression and tissue distribution remain poorly characterized [Ropero and Esteller, 2007; Khan and La Thangue, 2012].

The balance between acetylation and deacetylation is critical for normal cell function, and loss of protein acetylation has been shown to play a role in cancer initiation and progression [Ropero and Esteller, 2007; New *et al*. 2012]. Indeed, aberrant recruitment of HDACs to gene promoters has been shown to occur in hematological malignancies and HDAC deregulated expression has been reported in tumors of various origins including blood, colon, lung, bladder, pancreas, prostate, breast, cervix, brain, kidney, liver and stomach [Ropero and Esteller, 2007; Van Damme *et al*. 2012; Müller *et al*. 2013; Niegisch *et al*. 2013; Petta *et al*. 2013; Stenzinger *et al*. 2013; West and Johnstone, 2014). Because of their role in tumorigenesis, HDACs have long been considered an attractive therapeutic target.

Histone deacetylase inhibitors

HDACis are a diverse group of compounds that can be classified by chemical structure as short chain fatty acids (valproic acid, sodium butyrate and phenyl butyrate), hydroxamic acids (trichostatin A (TSA), vorinostat (SAHA), panobinostat, belinostat, dacinostat, resminostat, givinostat, suberohydroxamic acid (SBHA), rocilinostat, abexinostat, quisinostat, CHR-3996, AR-42 and pracinostat), mercaptoketones (KD5170), cyclic peptides (apicidin and romidepsin), benzamides (mocetinostat, entinostat, chidamide and tacedinaline), sirtuin inhibitors (niacinamide and sirtinol) and tubacin (Table 1) [Maes *et al*. 2013; West and Johnstone, 2014]. The majority of HDACis interfere with the Zn^{2+} ion in the catalytic site of one or

Table 1. Histone deacetylase inhibitors (HDACis) currently being tested in clinical studies for hematological malignancies.

Chemical class	HDACi	Clinical status (highest phase for hematological malignancies)	Hematological malignancy	ClinicalTrials.gov identifier
Short chain fatty acids	Valproic acid	II	AML, MDS, CLL, non-Hodgkin's and Hodgkin's lymphoma	NCT00414310; NCT00382590; NCT00339196; NCT00439673; NCT00326170; NCT01016990; NCT01356875
	Butyrate	*In vitro*	N/A	
	Phenylbutyrate	II	AML, MDS, non-Hodgkin's lymphoma, MM	NCT00006019
Hydroxamic acids	Trichostatin A	*In vitro*	N/A	
	Vorinostat	Approved	CTCL	NCT01554852; NCT00773747
		III	MM	
	Panobinostat	III	MM	NCT01023308
	Belinostat	II	AML, MM, MDS,, T-cell lymphomas, PTCL, B-cell non-Hodgkin's lymphoma	Cashen *et al.* [2012]; NCT00131261; NCT00131261; NCT00431340*; NCT00303953; NCT00274651; NCT00865969
	Dacinostat	I	N/A	
	Resminostat	II	Hodgkin's lymphoma	NCT01037478
	Givinostat	II	Polycythaemia vera, myeloproliferative neoplasms, MM, Hodgkin's lymphoma	Finazzi *et al.* [2013]; NCT01761968; NCT00792506;* NCT00606307; NCT00496431;$ NCT00792467
	Suberohydroxamic acid (SBHA)	Preclinical	N/A	
	Rocilinostat	I/II	MM, lymphoid malignancies	NCT01323751; NCT01997840; NCT02091063; NCT01583283
	Abexinostat	I/II	Non-Hodgkin's and Hodgkin's lymphoma	NCT00724984
	Quisinostat	II	CTCL	NCT01486277
	CHR-3996	I	N/A	NCT00697879
	AR-42	I	MM, CLL, lymphoma, AML	NCT01129193; NCT01798901
	Pracinostat	II	MDS, AML, myelofibrosis	Quintás-Cardama *et al.* [2012]; NCT01112384; NCT01075308; NCT01873703; NCT01993641; NCT01912274

Continued

Table 1. Continued

Chemical class	HDACi	Clinical status (highest phase for hematological malignancies)	Hematological malignancy	ClinicalTrials.gov identifier
Mercaptoketones	KD5170	Preclinical	N/A	
Cyclic peptides	Apicidin	Preclinical	N/A	
	Romidepsin	Approved II	CTCL and PCTL MM	 NCT00066638; NCT00765102
Benzamides	Mocetinostat	II	MDS, CLL, lymphoma	NCT00324220; NCT00431873; NCT00359086
	Entinostat	II	Hodgkin's lymphoma, AML, MDS, ALL	NCT00866333; NCT01305499; NCT00313586; NCT00462605; NCT00466115
	Chidamide	II	N/A	NCT01836679
	Tacedinaline	II	MM	NCT00005624
Sirtuin inhibitors	Niacinamide	I	Non-Hodgkin's and Hodgkin's lymphoma	NCT00691210
	Sirtinol	Preclinical	N/A	
1,3-dioxanes	Tubacin	Preclinical	N/A	

*The study was terminated due to dose-limiting toxicities.
$The study was terminated due to limited activity of the drug.
ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; CTCL, cutaneous T-cell lymphoma; HDAC, histone deacetylase; HDACi, histone deacetylase inhibitor; MDS, myelodysplastic syndrome; MM, multiple myeloma; N/A, not applicable; PCTL, peripheral T-cell lymphoma.

more specific HDACs or multiple HDAC classes (pan-HDACi).

A direct consequence of HDAC inhibition is the hyperacetylation of proteins, which results in a wide variety of responses including induction of cell cycle arrest, apoptosis, senescence and differentiation, as well as DNA damage, immunogenicity, downregulation of members of the aggresome pathway, and inhibition of angiogenesis [Maes *et al*. 2013; West and Johnstone, 2014] (Figure 1).

Based on their *in vitro* and *in vivo* preclinical activity, HDACis have undergone rapid clinical development. HDACis have been shown to exert effects in several types of cancers, although responses to treatment with single agent HDACis have primarily been observed in advanced hematologic malignancies and in thyroid, lung and prostate tumors [Rasheed *et al.* 2008; Prince *et al.* 2009].

Currently, vorinostat and romidepsin are the only two HDACis approved by the FDA for the treatment of cutaneous T-cell lymphoma (CTCL). Romidepsin has also been approved for the treatment of peripheral T-cell lymphoma (PTCL) [Treppendahl *et al.* 2014]. In *in vitro* studies, vorinostat has also displayed activity against MM cell lines. TSA, sodium butyrate and dacinostat (NVP-LAQ824) have been shown to inhibit proliferation and induce apoptosis in MM cell lines, patient-derived MM cells and cells resistant to various anti-MM therapies [Lavelle *et al.* 2001; Catley *et al.* 2003]. Significant decreases in tumor growth and increases in survival were also observed in response to dacinostat in a MM xenograft mouse model [Catley *et al*. 2003].

Other HDACis, including valproic acid, have been and continue to be evaluated in the clinical setting with mixed results [West and Johnstone, 2014]. Efforts to improve efficacy have led to both the assessment of existing compounds in combination therapies and the development of newer compounds, such as panobinostat and belinostat among others. The clinical status of the currently available HDACis is shown in Table 1.

Histone deacetylase inhibitors as therapy for MM

Malignant plasma cells produce large quantities of misfolded or unfolded immunoglobulins [Cenci, 2012] and rely heavily on their protein handling machinery, which includes both the proteasome and the aggresome, to circumvent cytotoxicity [Aronson and Davies, 2012]. Peptide degradation is also regulated by the aminopeptidase enzyme system, which catalyzes the hydrolysis of proteins and peptides from the NH_2-terminus [Botbol and Scornik, 1991]. Clearly, the inhibition of any of these pathways will have a detrimental effect on cell viability.

High expression levels of proteins involved in the proteasome pathway are often observed in hematopoietic malignancies [Jankowska *et al.* 2013]. A s a result, malignant plasma cells are particularly sensitive to proteasome inhibition [Aronson and Davies, 2012]. Proteasome inhibition has been shown to lead to cell death in malignant cells; however, an undesirable consequence of treatment with PIs is the compensatory induction of autophagy *via* the aggresome pathway [Hideshima and Anderson, 2012; Kale and Moore, 2012; Mateos *et al.* 2013]. Degradation of misfolded proteins *via* the aggresome requires both the presence of intact microtubules for protein transportation and the activity of HDAC6, which targets acetylated tubulin [Simms-Waldrip *et al.* 2008]. Inhibitors of HDAC6 such as tubacin interfere with the activity of the aggresome pathway and cause misfolded proteins to accumulate [Simms-Waldrip *et al.* 2008]. Inhibition of aminopeptidases disrupts protein turnover and leads to peptide accumulation and reduced amino acid availability, which in turns causes cytotoxicity. Treatment of MM cells with the aminopeptidase inhibitor tosedostat has been shown to induce cell cycle arrest, apoptosis and autophagy, and to synergize with the PI bortezomib [Moore *et al.* 2009].

The combination of HDACis and other anti-MM therapies has also been evaluated in preclinical studies. For instance, tubacin, vorinostat, romidepsin, belinostat, rocilinostat and panobinostat (see below) have all demonstrated synergistic cytotoxicity with bortezomib in MM cell lines, and primary cells from MM patients that are sensitive or resistant to bortezomib [Pei *et al.* 2004; Hideshima *et al.* 2005; Maiso *et al.* 2006; Feng *et al.* 2007; Simms-Waldrip *et al.* 2008; Campbell *et al.* 2010; Santo *et al.* 2012]. Conversely, bortezomib has been shown to downregulate the expression of class I HDACs in MM cells, thereby affecting gene transcription [Kikuchi *et al.* 2010]. For instance, basal expression of Kruppel-like family factor 9 (KLF9), a transcription factor that regulates pro-apoptotic genes, has been shown to be higher in MM cells from patients who respond to bortezomib, and treatment of MM cell lines with this PI has shown to upregulate KLF9 [Mannava *et al.* 2012]. HDACis have also been shown to potentiate the anti-MM activity of IMiDs such as lenalidomide and thalidomide, chemotherapeutic agents and steroids [Sanchez *et al.* 2011; Hajek *et al.* 2014]. Together, these studies have provided support for the use of HDACi as anti-MM therapy, especially when combined with other active anti-MM agents.

Panobinostat

Panobinostat (LBH589) is a potent cinnamic hydroxamic acid analogue capable of inhibiting class I, II and IV HDACs at nanomolar concentrations [Atadja, 2009]. Panobinostat was originally formulated for both intravenous (IV) and oral administration. This HDACi has demonstrated potent antiproliferative and cytotoxic activities in a variety of cell lines derived from hematological malignancies, including CTCL, chronic myelogenous leukemia (CML), acute myeloid leukemia (AML), Hodgkin lymphoma and MM, and cell lines derived from breast, prostate, colon and pancreatic cancers, while displaying minimal toxicity on normal cells [Catley *et al.* 2006; Maiso *et al.* 2006; Atadja, 2009; Bruzzese *et al.* 2013].

Panobinostat and MM preclinical studies

Panobinostat causes cell cycle arrest and caspase dependent and independent apoptosis in MM cell lines [Catley *et al.* 2006; Maiso *et al.* 2006]. Panobinostat has been shown to have cytotoxic effects on MM cell lines and tumor cells derived from MM patients known to be refractory to anti-MM drugs, including the anthracycline doxorubicin, anthracenedione antineoplastic agent mitoxantrone, alkylating agent melphalan, glucocorticosteroid dexamethasone and bortezomib. [Catley *et al.* 2006; Maiso *et al.* 2006]. Recently, it has been suggested that the inhibition of class I HDACs is sufficient to induce significant MM cell death and therefore that pan-HDACis such as panobinostat are more effective as single agents than inhibitors that target only HDAC6 such as tubacin [Mithraprabhu *et al.* 2013]. In MM cells, panobinostat can also reactivate the expression of genes which silencing is thought to enable the

Table 2. Histone deacetylase inhibitors (HDACis) with activity in multiple myeloma cell lines.

Chemical class	HDACi	References
Short chain fatty acids	Sodium butyrate	Lavelle *et al.* [2001] Pei *et al.* [2004]
Hydroxamic acids	Trichostatin A	Lavelle *et al.* [2001]
	Vorinostat	Pei *et al.* [2004] Matthews *et al.* [2013]
	Panobinostat	Catley *et al.* [2006] Maiso *et al.* [2006] Ocio *et al.* [2010] Sanchez *et al.* [2011] Matthews *et al.* [2013]
	Belinostat	Feng *et al.* [2007]
	Dacinostat	Catley *et al.* [2003]
	Rocilinostat	Santo *et al.* [2012]
	Romidepsin	Matthews *et al.* [2013]
1,3-dioxanes	Tubacin	Hideshima *et al.* [2005] Mithraprabhu *et al.* [2013]

HDACi, Histone deacetylase inhibitor.

proliferation of differentiated B cells, thereby inducing cell death [Kalushkova *et al.* 2010].

Panobinostat has been shown to increase the anti-MM activity of the bisphosphonate zoledronic acid, the insulin-like growth factor type 1 receptor tyrosine kinase inhibitor picropodiphyllin, the aminopeptidase inhibitor tosedostat, dexamethasone, bortezomib, doxorrubicin, and melphalan [Maiso *et al.* 2006; Moore *et al.* 2009; Sanchez *et al.* 2011; Lemaire *et al.* 2012; Bruzzese et al. 2013]. Similar to tubacin, panobinostat was shown to induce α-tubulin hyperacetylation, decrease the 20S chymotryptic activity of the proteasome, and reduce bortezomib-induced aggresome formation, which may help explain, at least in part, panobinostat's activity in bortezomib-resistant cells and the synergism observed between this HDACi and bortezomib [Catley *et al.* 2003, 2006].

Our previous studies in various MM cell lines demonstrated induction of tubulin and histone acetylation as well as caspase-dependent apoptosis in response to treatment with panobinostat, and these effects were potentiated when the HDACi was combined with either melphalan or doxorubicin [Sanchez *et al.* 2011]. We also observed significant decreases in human paraprotein levels (a measurement of MM burden) and tumor size after treatment with panobinostat (once daily for 5 days) in our human MM xenograft mouse model LAGλ-1, which carries uncultured, patient-derived MM cells. Similar to our *in vitro* studies, the anti-MM effect was shown to be enhanced when this HDACi was combined with melphalan (once weekly) or pegylated liposomal doxorubicin (PLD; three consecutive days a week) in this *in vivo* model [Sanchez *et al.* 2011].

Synergistic effects have also been observed in triple combinations of newer anti-MM drugs. *In vitro* treatment with panobinostat, dexamethasone and bortezomib or lenalidomide showed more cytotoxic activity than each anti-MM agent used alone or in dual combinations [Ocio *et al.* 2010]. In these xenograft mouse models of disseminated and extramedullary MM, the triple combinations also conferred a significant survival advantage compared with double agent combinations or single agent treatment. A summary of HDACis with activity in MM cells is shown in Table 2.

These findings provided support for the clinical development of panobinostat in combination with alkylating agents, IMiDs and/or PIs for the treatment of MM patients.

Panobinostat and MM clinical studies

The initial phase I studies evaluating single-agent panobinostat were carried out in solid tumors and hematological malignancies using the IV formulation [Giles *et al.* 2006; Sharma *et al.* 2013]. QTc prolongation and cardiac arrhythmias reported in these trials led to the discontinuation of the IV administration route [Khot *et al.* 2013; Sharma *et al.* 2013].

The safety and efficacy of single-agent panobinostat administered orally on Monday, Wednesday and Friday (thrice weekly) of every week or every other week was evaluated in a phase Ia/II study for patients with hematological malignancies, including MM [DeAngelo *et al.* 2013]. The maximum tolerated dose (MTD) of panobinostat was dependent on the indication and one partial response (PR) was observed in a MM patient [DeAngelo *et al.* 2013]. Following this, the activity of single-agent oral panobinostat administered at 20 mg thrice weekly for 2 weeks of a 21-day cycle was investigated in heavily pretreated RRMM patients. Panobinostat demonstrated durable, albeit modest, responses in two (one PR, one minimal response) of the 38 evaluable patients [Wolf *et al.* 2012]. As a result, the focus of clinical studies with oral panobinostat has shifted to combination therapies.

The vast majority of the clinical studies examining the safety and efficacy of panobinostat as combination therapy for MM patients have been carried out in the RR setting (Table 3). To date, the most promising combination appears to be that of panobinostat and bortezomib. The combination of oral panobinostat and IV bortezomib was investigated in a phase Ib trial [San-Miguel *et al.* 2013]. Panobinostat was administered on Monday, Wednesday and Friday for 3 consecutive weeks and bortezomib was administered at 1.0 mg/m^2 on days 1, 4, 8 and 11 of a 21-day cycle. In the dose escalation phase of the study, the MTD of panobinostat in combination with bortezomib was established at 20 mg [San-Miguel *et al.* 2013]. Thrombocytopenia was the most frequent hematological event and QTc prolongation was only observed in one patient. In the expansion phase of the trial, the schedule of panobinostat was changed and the drug was administered at the MTD thrice weekly but for only the first two weeks of a 21-day cycle to allow for platelet recovery. Dexamethasone at 20 mg administered after bortezomib was also allowed after cycle 2 because, as stated above, the triple combination of panobinostat, bortezomib and dexamethasone was shown to have greater anti-MM activity than any dual combination [Ocio *et al.* 2010]. The overall response rate (ORR) was 51.5% (n=62) and the ORR of the expansion phase was 73.3% (n=11). Responses (26.3%) were also observed in bortezomib-refractory patients [San-Miguel *et al.* 2013].

On the basis of these early studies, PANORAMA 2 (PANobinostat ORAl in Multiple MyelomA), a phase II, single arm, two-stage trial, evaluated the triple combination of panobinostat, bortezomib and dexamethasone for bortezomib-refractory MM patients [Richardson *et al.* 2012]. In the stage 1 of the trial, panobinostat was administered at 20 mg three times a week, on weeks 1 and 2 of a 21-day cycle for a total of 8 cycles. Bortezomib was given IV at 1.3 mg/m^2 on days 1, 4, 8 and 11, and oral dexamethasone was given at 20 mg on the day of, and the day after each bortezomib administration. Patients showing clinical benefit were eligible to continue therapy as part of the stage 2 of the trial. In stage 2, panobinostat was given three times a week on weeks 1, 2, 4 and 5 of a 6-week cycle, whereas bortezomib was administered once a week on weeks 1, 2, 4 and 5, and dexamethasone was given the day of, and the day after bortezomib administration. Responses were observed in 19 out of 55 evaluable patients, including one near complete response (CR) and 18 PRs; the ORR was 34.5% [Richardson *et al.* 2012]. Median progression-free survival (PFS) was 5.4 months and the median OS was 17.5 months [Schlossman *et al.* 2013]. The triple combination displayed manageable toxicities, with thrombocytopenia being the most common grade 3/4 hematological adverse event. Treatment emergent peripheral neuropathy was mild and observed in 27.3% of patients, with only one grade 3/4 event reported [Richardson *et al.* 2013]. A phase III randomized trial, PANORAMA 1, is comparing the efficacy of bortezomib and dexamethasone *versus* panobinostat, bortezomib and dexamethasone for MM patients who have previously received but were not refractory to bortezomib. Preliminary safety data from the first 525 evaluable patients enrolled in the trial have been reported and suggest that the safety profile of the triple combination is similar to that shown in the PANORAMA 2 trial [San-Miguel *et al.* 2012].

The promising results achieved with the combination of an HDACi and a PI have provided rationale for a phase I/Ib study testing panobinostat in combination with carfilzomib, a second generation PI that is very active in MM and has an improved safety profile compared with bortezomib [Shah *et al.* 2012]. Panobinostat was administered 3 times a week for the first 2 weeks of every 28-day cycle and carfilzomib was given as an infusion over 30 minutes on days 1, 2, 8, 9, 15 and 16. Dose levels started with panobinostat at 15 mg and carfilzomib at 20/27 mg/m^2 and escalated to 20 mg or 30 mg (panobinostat) and 20/36 or 20/45 mg/m^2 (carfilzomib) using a classic 3+3 schema based on dose-limiting toxicities. For the 17 evaluable patients, the ORR was 35%, including 2 very good partial responses (VGPRs) and 1 PR. Grade 3/4 toxicities included

Table 3. Panobinostat clinical trials for relapsed/refractory multiple myeloma.

Combination with	Phase	Number of patients	Efficacy	Adverse events (G3/G4)
BTZ	I	11	ORR = 36.4%	Leukopenia = 54.0% Neutropenia = 45.0% Thrombocytopenia = 36.0%
LEN/DEX	Ib	46 (30 evaluated for response)	ORR = 56.66%	Thrombocytopenia = 44.0% Neutropenia = 37.0%
BTZ	Ib	47 (dose escalation) 15 (expansion)	ORR = 52.9% (dose escalation) ORR = 73.3% (expansion)	*Dose escalation*: Thrombocytopenia = 85.5% Neutropenia = 63.8% Asthenia = 29.8% *Expansion*: Thrombocytopenia = 66.7% Neutropenia = 46.7% Fatigue = 20.0%
CFZ	I/Ib	17	ORR = 35.0%	Thrombocytopenia = 59% Fatigue = 59.0% Anemia = 41.0% Neutropenia = 35.2% Pneumonia-23.5%
CFZ	I/II	10 enrolled, 9 evaluated	ORR = 64%	Thrombocytopenia = 30.0% Neutropenia = 20.0% Fatigue = 11.0%
MEL	I/II	40	ORR = 7.5%	Thrombocytopenia = 30.8% Neutropenia = 23.1%
MEL/PRED/THAL	I/II	24	ORR = 38.5%	Neutropenia = 71.0% Thrombocytopenia = 35.5%
BTZ/ DEX (PANORAMA 2)	II	55	ORR = 38.5% CBR = 49.0%	Neutropenia = 53.0% PN = 2.0%
LEN/DEX	II	5	ORR = 40.0% CBR = 60.0%	Neutropenia = 60.0% Thrombocytopenia = 60.0% Febrile neutropenia = 20.0% Pulmonary embolism = 20.0%
BTZ/DEX (PANORAMA 1)	III	536 enrolled 525 evaluated		Thrombocytopenia = 36.2% Anemia = 13.0% Diarrhea = 14.5% Fatigue = 12.2% Neutropenia = 11.4% Peripheral neuropathy = 5.3%

BTZ, bortezomib; CBR, clinical benefit rate; CFZ, carfilzomib; DEX, dexamethasone; LEN, lenalidomide; MEL, melphalan; ORR, overall response rate; PRED, prednisone; THAL, thalidomide.

thrombocytopenia, fatigue, anemia, neutropenia and pneumonia [Shah *et al.* 2012]. An ongoing phase I/II study is also evaluating this combination [Berdeja *et al.* 2012]. Panobinostat was administered thrice weekly on weeks 1 and 3 of a 28-day cycle and carfilzomib was given as an infusion over 30 minutes on days 1, 2, 8, 9, 15 and 16 of weeks 1–3 of a 28-day cycle. Four dose levels were evaluated: panobinostat 20 mg and carfilzomib 20 (first cycle)/27 (subsequent cycles) mg/m^2; panobinostat 20 mg and carfilzomib 20/36 mg/m^2; panobinostat 20 mg and carfilzomib 20/45 mg/m^2; and panobinostat 30 mg and carfilzomib 20/45 mg/m^2 [Berdeja *et al.* 2013]. No dose limiting toxicities were observed in the dose-escalating phase of the study; and, therefore, the expansion phase opened at the maximum administered dose (MAD). In 9 evaluable patients, the ORR of this drug combination was 64% with responses observed among both bortezomib and IMiD refractory patients. A total of 61%

of patients experienced ⩾ grade 3 hematological toxicities, whereas 34% of patients experienced nonhematological toxicities. Peripheral neuropathy was infrequent (5% of patients) and no grade 3/4 cases were reported. A total of 59% of patients who received the MAD required panobinostat dose reductions [Berdeja *et al.* 2013]. Because of the observed toxicity, two additional dose levels are currently being evaluated.

Panobinostat in combination with lenalidomide and dexamethasone was also tested based on promising preclinical studies [Ocio *et al.* 2010]. A phase Ib clinical trial evaluated the MTD of the triple combination. Oral panobinostat was administered at 5, 10, 20 and 25 mg thrice weekly for 3 weeks, lenalidomide was given by mouth (PO) at 25 mg daily on days 1–21 and dexamethasone was administered PO 40 mg daily on days 1–4, 9–12 and 17–20 of a 21-day cycle [Mateos *et al.* 2010]. The MTD of panobinostat was 20 mg in this combination. Out of 30 evaluable patients, 17 showed responses, including 1 stringent CR, 1 CR, 7 VGPRs and 8 PRs. A subsequent phase II study evaluated panobinostat administered at the previously determined MTD (20 mg) [Mateos *et al.* 2010] on a thrice weekly schedule but on only weeks 1 and 3 of a 21-day cycle, and lenalidomide administered at the same dose and schedule used in the phase Ib trial [Biran *et al.* 2013]. Dexamethasone was administered at 40 mg once a week and a reduced dose of dexamethasone (20 mg) was administered to older patients (⩾75 years old). At the time of the report, only five lenalidomide-refractory patients were enrolled in the study. The regimen showed hematological toxicities and produced durable responses in 3 patients (1 VGPR, 1 PR and 1 minor response (MR), including lenalidomide-refractory patients [Biran *et al.* 2013].

Finally, our group has evaluated panobinostat in combination with the alkylating agent melphalan in RRMM patients. Based on preclinical results from our severe combined immune deficient human (SCID-hu) MM model [Sanchez *et al.* 2011], we evaluated the safety and efficacy of melphalan and panobinostat in a phase I/II trial [Berenson *et al.* 2014]. Due to tolerability issues, including grade 4 thrombocytopenia ($n=2$), grade 3 fatigue ($n=1$) and grade 4 neutropenia ($n=1$), the drug dosing and schedule was changed three times during the trial, resulting in four different schedules. The MTD was established at 20 mg of panobinostat and only 0.05 mg/kg of oral melphalan, both administered only during the first week (on days 1, 3 and 5) of a 28-day cycle. Using this schedule, ⩾ grade 3 neutropenia and thrombocytopenia were observed in 25 and 10% of patients, respectively. Both efficacy and toxicity appeared to have a direct correlation with the cumulative panobinostat exposure per cycle. Despite its tolerability, 20 mg of panobinostat and 0.05 mg/kg of melphalan, administered during the first week of each cycle produced no responses. Overall, responses were observed in only 3 (2 VGPRs, 1 PR) of the 45 patients evaluated for efficacy receiving panobinostat and melphalan, and this combination was associated with significant hematological and nonhematological toxicities including neutropenia (75%), thrombocytopenia (72.5%), anemia (52.5%), fatigue (58%) and nausea (55%) [Berenson *et al.* 2014]. Similar tolerability issues were observed when panobinostat was used in combination with melphalan, thalidomide and prednisone [Offidani *et al.* 2012]. In that phase II trial, oral melphalan was administered at 0.18 mg/kg on days 1–4, oral prednisone at 1.5 mg/kg on days 1–4, thalidomide at 50 mg/day continuously, and panobinostat at doses ranging from 10 to 20 mg three times a week for 3 weeks of each 28-day cycle. The ORR was 38.5%; however, the MTD of the drug combination could not be established due to the number of dose-limiting toxicities (grade 3 atrial fibrillation ($n=1$), fatigue ($n=1$), gastrointestinal toxicity ($n=2$), and febrile neutropenia ($n=2$) as well as grade 4 neutropenia ($n=10$) and thrombocytopenia ($n=2$)) observed in patients receiving 10 mg or 15 mg of panobinostat [Offidani *et al.* 2012]. Overall, the panobinostat–melphalan combination appears to be both too ineffective and toxic.

Other drug combinations are currently being tested for the treatment of MM. For instance, a phase I/II trial is evaluating panobinostat in combination with dexamethasone and the mammalian target of rapamycin (mTOR) inhibitor everolimus [ClinicalTrials.gov identifier: NCT00918333]. In addition, a phase I trial is assessing panobinostat in combination with the oral PI ixazomib and dexamethasone [ClinicalTrials.gov identifier: NCT02057640].

Side effects associated with panobinostat

The most common side effects observed after treatment with panobinostat include thrombocytopenia, neutropenia, anemia, diarrhea, and fatigue, which have been observed in all clinical studies and

across a variety of diseases [Rasheed *et al*. 2008; Khot *et al.* 2013]. Platelet count nadir occurs during the second week of therapy and is self-limited. [Rasheed *et al*. 2008; DeAngelo *et al*. 2013]. Electrolyte and biochemical disturbances including hypokalemia and hypocalcemia have also been reported [Rasheed *et al*. 2008]. Cardiac effects include prolonged QT interval on day 3 of treatment and nonspecific ST-T electrocardiogram (ECG) changes have been reported in patients receiving intravenous panobinostat; however, the incidence of QT prolongation is substantially reduced among subjects receiving the oral formulation [Rasheed *et al*. 2008; Khot *et al.* 2013]. The toxicity profile of panobinostat shows similarities with that of the FDA-approved HDACis vorinostat and romidepsin. Common adverse events reported for vorinostat used as a single agent were fatigue, anorexia, dehydration, nausea and diarrhea, whereas QT interval prolongation, fatigue and hematological toxicities (thrombocytopenia, anemia and neutropenia) were observed with the vorinostat-bortezomib combination treatment [Orlowski, 2013]. Thrombocytopenia, nausea, fatigue and reversible QT prolongation were also observed with single agent romidepsin [Niesvizky *et al.* 2011]. Thrombocytopenia and fatigue were also common in MM patients treated with romidepsin, bortezomib and dexamethasone [Harrison *et al.* 2011].

Challenges and future directions

Despite advances in the development of new anti-MM agents during the past decade, MM remains an incurable disease. Therefore, there is a constant search for newer and better therapies. HDACs regulate a plethora of cellular functions and HDACis have shown potent anticancer activity in preclinical studies [Neri *et al.* 2012], and thus their use as multitarget therapeutic agents is appealing.

Studies demonstrating the dynamic interplay between protein acetylation status, cell cycle progression and apoptosis in MM cell lines have provided a strong rationale for the use of panobinostat as a therapeutic option for MM. Despite the promising preclinical data, the clinical responses achieved after treatment of MM patients with single-agent panobinostat have been disappointing. Panobinostat is a nonselective HDACi and its wide spectrum of inhibition is associated with significant toxicities including thrombocytopenia, fatigue and gastrointestinal symptoms [Rasheed *et al*. 2008; Khot *et al.* 2013], which can limit exposure. The use of suboptimal doses and schedules, such as those used in the melphalan–panobinostat trials [Offidani *et al.* 2012; Berenson *et al*. 2013], has helped minimize untoward side effects but, unfortunately, it has also compromised efficacy. Thus far, the panobinostat–bortezomib–dexamethasone triple combination appears to be the most effective, with predictable and manageable toxicities [Richardson *et al*. 2013; San-Miguel *et al.* 2013]. The eagerly awaited results from the PANORAMA 1 trial may shed some light on whether or not this particular panobinostat combination produces clinical benefit.

Results from ongoing clinical studies evaluating panobinostat in combination with other anti-MM agents such as lenalidomide, carfilzomib and ixazomib [Mateos *et al.* 2010; Biran *et al.* 2013] may demonstrate broader therapeutic windows than those observed with melphalan and bortezomib. However, it is likely that further trials will be required to fine tune the best dose and schedule for each particular drug combination. A better understanding of the molecular pathways targeted by panobinostat in MM cells may provide a better rationale for the selection of new drug combinations with synergistic potential.

The FDA approval of vorinostat and romidepsin has propelled the use of currently available HDACis and the development of new ones. However, a number of issues remain unresolved. For instance, there is a need for good response and prognostic biomarkers to both assess HDAC inhibition and to help physicians make informed decisions about the therapeutic value of HDACis for their patient population [Hajek *et al*. 2014; Treppendahl *et al.* 2014]. Current biomarkers for HDACi activity, such as histone acetylation and gene expression changes, show correlation between dose and histone hyperacetylation; however, they have no prognostic value and/or are tissue- and, likely, HDACi-specific [Prince *et al.* 2009; Treppendahl *et al.* 2014].

In the context of MM, the relative contribution of each HDAC to the disease is still unknown and elucidating it will allow the use of specific HDACis which, in turn, may improve tolerability. Newer, HDAC-specific or class-specific inhibitors are being developed [West and Johnstone, 2014], and these compounds may prove to be more effective and to have better toxicity profiles than panobinostat.

In conclusion, panobinostat is a potent new HDACi with a potential role for the treatment of

MM. Current clinical data suggest that the panobinostat–bortezomib–dexamethasone combination is the most promising in the RRMM setting. Cumulative toxicity is still a main concern and it remains to be seen whether other panobinostat combinations are effective with acceptable tolerability profiles.

Conflict of interest statement

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