

## Histone Deacetylase Inhibitors in the Treatment of Multiple Myeloma

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A Review of Selected Presentations  
from the 2007 Annual Meeting of  
the American Society of Hematology

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# Histone Deacetylase Inhibitors in the Treatment of Multiple Myeloma

A Review of Selected Presentations from the 2007 Annual Meeting of the American Society of Hematology

## 1167 Safety and Efficacy of the Combination of Bortezomib with the Deacetylase Inhibitor Romidepsin in Patients with Relapsed or Refractory Multiple Myeloma: Preliminary Results of a Phase I Trial<sup>1</sup>

HM Prince, H Quach, P Neeson, M Keegan, M Copeman, S Peinert, M Bishton, M Wolf, D Ritchie, JF Seymour, D Carney, D Westerman, S Harrison

Researchers from the Peter MacCallum Cancer Centre in Melbourne, Australia, investigated the safety and efficacy of a combination of the proteasome inhibitor bortezomib and the histone deacetylase (HDAC) inhibitor romidepsin (depsipeptide) in patients with relapsed or refractory multiple myeloma.<sup>1</sup> Bortezomib is currently approved by the US Food and Drug Administration (FDA) in combination with dexamethasone for the treatment of relapsed multiple myeloma, based on results from the Assessment of Proteasome Inhibition for Extending Remissions (APEX) trial.<sup>2</sup> Bortezomib acts by directly targeting myeloma cells and their interaction with the bone marrow microenvironment. Yu and colleagues reported synergistic activity between bortezomib and HDAC inhibitors, which induced apoptosis in *BCR/ABL*-

positive cells both sensitive and resistant to the tyrosine kinase inhibitor imatinib.<sup>3</sup> Combinations of bortezomib and the HDAC inhibitors sodium butyrate and suberoylanilide hydroxamic acid (SAHA, vorinostat) were then investigated by Pei and associates in preclinical research that demonstrated synergistic activity between the two classes of drugs in human multiple myeloma cells.<sup>4</sup> Cells preincubated with a subtoxic concentration of bortezomib markedly sensitized cells to sodium butyrate- and vorinostat-induced mitochondrial dysfunction, caspase 9, 8, and 3 activation, and poly(ADP-ribose) polymerase degradation, resulting in synergistic induction of apoptosis. It was noted that the combination was markedly lethal in primary CD138-positive, but not in CD138-negative, bone marrow cells taken from patients with multiple myeloma. Romidepsin, an HDAC inhibitor, was demonstrated to induce apoptosis in a myeloma cell line and primary patient myeloma cells by Khan and coworkers.<sup>5</sup> In a phase II trial, romidepsin administered as a single agent for treatment of aggressively relapsed multiple myeloma stabilized tumor masses in 9 of 12 patients after the first 4-week treatment cycle.<sup>6</sup> Furthermore, Sutheesophon and colleagues demonstrated that romidepsin-induced apoptosis and mitochondrial translocation of *Bax* were markedly enhanced by bortezomib in human myeloid leukemic cell lines.<sup>7</sup>

Based on these findings, the Australian researchers initiated a phase I/II open-label, single-center, single-

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arm, dose-escalation trial of bortezomib, dexamethasone, and romidepsin in patients with relapsed or refractory multiple myeloma; early results were presented at the 2007 annual meeting of the American Society of Hematology (ASH). Eight patients who had previously received a median of two therapies—either bortezomib (n=1), vincristine-containing therapy (n=3), or thalidomide (n=4)—received bortezomib 1.3 mg/m<sup>2</sup> on days 1, 4, 8, and 11 and dexamethasone 20 mg on days 1, 2, 4, 5, 8, 9, 11, and 12. Administration of romidepsin was commenced at 8 mg/m<sup>2</sup> intravenously on days 1, 8, and 15 of a 28-day cycle, and involved an initial accelerated dose-escalation phase with inpatient dose escalation. Seven patients were evaluable at the time of presentation for response and toxicity. The researchers assessed response according to M-protein response criteria, with complete responses (CRs) documented by EBMT (European Group for Blood and Marrow Transplantation) criteria. Dose-limiting toxicities (DLTs) were defined as platelets below 25 × 10<sup>9</sup>/L; grade 4 neutropenia despite support granulocyte colony-stimulating factor; grade 3 or 4 nausea, emesis, or diarrhea despite treatment; any other grade 3 or 4 nonhematologic toxicity; or a greater than 4-week suspension of treatment due to toxicity. No DLTs were observed in patients who received romidepsin doses of 8 mg (n=1) or 10 mg (n=3). At doses of 12 mg, three episodes of grade 4 thrombocytopenia and one episode of febrile neutropenia occurred. The researchers noted that 2 of the patients with grade 4 thrombocytopenia had platelet counts below 100 × 10<sup>9</sup>/L prior to commencing the combination (the minimum requirement at inclusion was 50 × 10<sup>9</sup>/L). Additional drug-related toxicities observed were grade 3 fatigue (n=1), neutropenia (n=1), and sepsis (n=1) and grade 2 fatigue (n=1), peripheral neuropathy (n=2), nausea (n=1), and diarrhea (n=1). Two patients required a bortezomib dose reduction because of peripheral neuropathy (coadministered with 12 mg/m<sup>2</sup> romidepsin).

As of August 2007 the median number of cycles delivered was three and a total of 3 patients had progressed—2 patients after cycle 1 and 1 patient after cycle 4. One immunofixation-negative CR, 3 partial responses (PRs), and 1 minor response were observed, for an overall response rate of 71%. Five of 8 patients remain on the combination therapy. The researchers concluded that these early findings demonstrate efficacy for this HDAC inhibitor/proteasome inhibitor combination in patients with multiple myeloma, with a promising response rate, some durable responses, and acceptable toxicity. Patient accrual continues at the doses of bortezomib 1.3 mg/m<sup>2</sup>, dexamethasone 20 mg, and romidepsin 10 mg/m<sup>2</sup>; however, future modifications to the scheduling of the regimen are planned in order to address dose-limiting transient thrombocytopenia.

## 1168 Phase I Trial of Suberoylanilide Hydroxamic Acid + Bortezomib in Relapsed Multiple Myeloma Patients<sup>8</sup>

A Badros, S Philip, R Niesvizky, O Goloubeva, C Harris, J Zweibel, J Wright, A Burger, S Grant, M R Baer, M J Egorin

The HDAC inhibitor vorinostat is indicated for the treatment of cutaneous manifestations of T-cell lymphoma in patients with progressive, persistent, or recurrent disease on or following two systemic therapies. Vorinostat, like other HDAC inhibitors, achieves its therapeutic effect by inducing cell cycle arrest and apoptosis. The effect of vorinostat on cell lines and patient cells from B-cell malignancies, including multiple myeloma, was first demonstrated by Mitsiades and associates,<sup>9</sup> who showed that vorinostat induced apoptosis in all tumor cells tested. In addition, vorinostat sensitized MM.1S cells to death receptor-mediated apoptosis and inhibited the secretion of interleukin 6 (IL-6) by bone marrow stromal cells induced by binding of multiple myeloma cells, suggesting that it can overcome cell adhesion-mediated drug resistance. Pei and colleagues built upon this research by combining bortezomib and vorinostat preclinically.<sup>4</sup> Badros and coworkers further noted that vorinostat affects cell growth by modifying the transcription of cellular proteins such as histones, transcription factors, E3 ubiquitin ligases, and stress response proteins such as heat-shock protein 90 (HSP90).<sup>8</sup> This phase I study was designed to determine the maximum tolerated dose, pharmacokinetics, pharmacodynamics, and level of activity of vorinostat plus bortezomib in patients with relapsed or refractory multiple myeloma.

At the time of the ASH annual meeting, 21 heavily pretreated patients (median age, 55 years; range, 38–79) had been treated. The median time from diagnosis of multiple myeloma to entry on the study was 5.3 years (range, 1.5–15 years). Patients had received a median of six prior regimens (range, 3–10), including tandem stem cell transplantations (n=11), single stem cell transplantations (n=8), thalidomide (n=21), and lenalidomide (n=14). Additionally, 19 patients had received a median of two prior bortezomib-based regimens (range, 1–5); of these patients, 14 progressed on their last bortezomib-based therapy. Overall, 19 patients' disease progressed on their most recent therapy, with a median of 20 days (range, 15–39 days) between their last therapy and study entry. Only 2 patients were in first relapse on thalidomide maintenance. The patients' isotypes included IgG (n=10), IgA (n=5), light chain (n=4), and nonsecretory (n=2). Twelve patients exhibited complex karyotypes.

The study design included five 3-patient cohorts administered bortezomib and vorinostat at escalating dose

levels (Table 1). Two DLTs were observed in the vorinostat 500 mg cohort (grade 4 prolonged QT interval and grade 4 fatigue). Several grade 3/4 toxicities were observed after cycle 2, including myelosuppression requiring transfusion and growth-factor support. Grade 2 or higher nonhematologic toxicities included fatigue (n=5), diarrhea (n=3), atrial fibrillation (n=1), shingles (n=1), and pneumonia (n=2, bacterial and viral).

Sixteen patients were evaluable for response; 1 near CR and 7 PRs were observed, for an overall response rate of 50%. Six patients had stable disease and 3 had progressive disease. At last follow-up, 3 patients remained in remission, 9 exhibited progressive disease, and 5 had died. Dexamethasone was administered to 4 patients in cycle 2, but there was no associated improvement in response. The maximum tolerated dose of vorinostat was 400 mg daily for 8 days in combination with bortezomib 1.3 mg/m<sup>2</sup> administered on days 1, 4, 8, and 11. Based on the responses observed, particularly in bortezomib-refractory patients, the authors recommended that the combination regimen be further evaluated in the phase II setting.

### 1172 Phase I Trial of Oral Vorinostat (Suberoylanilide Hydroxamic Acid, SAHA) in Combination with Bortezomib in Patients with Advanced Multiple Myeloma<sup>10</sup>

D M Weber, S Jagannath, A Mazumder, R Sobucks, G J Schiller, M Gavino, C Sumbler, C McFadden, C Chen, J L Ricker, S Rizvi, C Oerth, P Brownell, M A Hussein

Another phase I dose-escalation trial of vorinostat combined with bortezomib was reported at the 2007 ASH annual meeting by Weber and colleagues.<sup>10</sup> In comparison to the study by Badros and coworkers,<sup>8</sup> this study demonstrated less activity for vorinostat. A maximum tolerated dose had not yet been established at the time of presentation.

In this trial, which enrolled 20 patients (median age, 61 years; range, 52–76 years), vorinostat was administered orally in combination with intravenous bortezomib in 21-day cycles, for a maximum of eight cycles until progressive disease or intolerable toxicity (Table 1). Patients with active relapsed or refractory multiple myeloma who had not received bortezomib in the preceding 3 months were eligible; Eastern Cooperative Oncology Group performance status 0–2 and adequate hematologic, hepatic, and renal function were further entry requirements. The patients had received a median of three prior systemic therapies (range, 1–14), and 4 patients had previously received bortezomib-based therapy.

As of July 1, 2007, 20 patients had been enrolled, 18 of whom had received at least one dose and were evaluable for safety. One patient in cohort 3 experienced a DLT (transient aspartate aminotransferase [AST] elevation). The most common drug-related toxicities (all grades) were nausea (56%), thrombocytopenia (50%), diarrhea (39%), vomiting (39%), fatigue (39%), and anemia (22%). Grade 3 drug-related adverse events were thrombocytopenia (33%, none associated with bleeding), peripheral neuropathy (11%), neutropenia (11%, none febrile), diarrhea (6%), diverticulitis (6%), fatigue (6%), increased AST (6%), memory changes (6%), nausea (6%), vomiting (6%), and upper respiratory infection (6%). Eight patients discontinued treatment from the study due to progressive disease (n=3), fatigue (n=2), nausea (n=2), and diverticulitis (n=1). Among 17 patients evaluable for efficacy, all had measurable response or stable disease: 4 experienced a PR, 2 showed a minimal response, and 11 had stable disease. Among 3 evaluable patients previously treated with bortezomib, 1 achieved a PR and 1 achieved a minimal response. The study by Badros and associates<sup>8</sup> demonstrated greater activity even though its patient population was more heavily pretreated, including with a greater number of prior bortezomib-based therapies. However, 12 patients received the maximal dose of bortezomib (1.3 mg/m<sup>2</sup>) in the Badros study versus 3 patients in the Weber study. Nonetheless, like Badros and coworkers, Weber and colleagues concluded that the combination of vorinostat and bortezomib is well tolerated and efficacious in heavily pretreated patients with relapsed/refractory multiple myeloma.

### 1175 A Phase II Multiple Dose Clinical Trial of Histone Deacetylase Inhibitor ITF2357 in Patients with Relapsed or Progressive Multiple Myeloma: Preliminary Results<sup>11</sup>

M Galli, S Salmoiraghi, J Golay, A Gozzini, A Bosi, C Crippa, G Rossi, N Pescosta, S Cortelazzo, A Sechi, T Oldoni

ITF2357, an oral hydroxamate HDAC inhibitor, has been shown to reduce proinflammatory cytokine production in primary cells in vitro and exhibit anti-inflammatory effects in vivo at nonapoptotic concentrations.<sup>12</sup> Researchers investigated the activity of ITF2357 on multiple myeloma and acute myeloid leukemia (AML) cells in vitro and in vivo.<sup>13</sup> The drug was found to induce apoptosis and cell death in both multiple myeloma and AML cells. It was noted that ITF2357 was strongly cytotoxic in an IL-6–dependent multiple myeloma cell line, and

**Table 1.** Dose Levels and Responses in Two Phase I Studies of Bortezomib Plus Vorinostat

<i>Badros et al*</i>					<i>Weber et al</i>				
Cohort	Bortezomib, mg/m <sup>2</sup>	Vorinostat, mg	No. of cycles	Response	Cohort	Bortezomib, mg/m <sup>2</sup>	Vorinostat, mg	No. of cycles	Response
1	1.0	100 bid	5, 7, 5	SD ×3	1	0.7 <sup>†</sup>	200 bid	3, 3, 14	SD ×2, PD
2	1.3	100 bid	5, 6, 3	SD, PR, PD	2	0.9 <sup>†</sup>	200 bid	4, 5, 6	SD ×2, PD
3	1.3	200 bid	8, 3, 8	VGPR, SD, PR	3	0.9 <sup>‡</sup>	400 daily	2, 3, 5, 6, 6, 6	SD ×3, MR, PD ×2
4	1.3	400 daily	5, 3, 3	SD, PD, PR	4	1.1 <sup>‡</sup>	400 daily	3, 3, 4, 5, 11	SD ×4, MR
5	1.3	500 daily	7, 1, 1	PR, NE, NE	5	1.3 <sup>‡</sup>	400 daily	1, <sup>§</sup> 1, <sup>§</sup> 2	NE ×3
Maximum tolerated dose	1.3	400 daily	4, 3, 2, 1, 1, 1	PR ×3, too early for evaluation ×3	Maximum tolerated dose	—	—	—	—

\*Bortezomib administered on days 1, 4, 8, and 11 of a 21-day cycle; vorinostat administered on days 4–11. Patients received 8 cycles. Dexamethasone was added for nonresponders, cycle 2.

<sup>†</sup>Days 4, 8, 11, and 15.

<sup>‡</sup>Days 1, 4, 8, and 11.

<sup>§</sup>Treatment cycle in progress at time of presentation.

MR=minimal response; NE=not evaluable; PD=progressive disease; PR=partial response; SD=stable disease; VGPR=very good partial response.

the drug inhibited production of growth and angiogenic factors by bone marrow stromal cells, in particular IL-6 and vascular endothelial growth factor. Results of one pre-clinical study comparing the cytotoxic effects of ITF2357 versus vorinostat in human myeloma cell lines and freshly isolated multiple myeloma samples have been reported.<sup>14</sup> ITF2357 was found to be 2- to 10-fold more potent than vorinostat. It exhibited strong cytotoxic activity in 7 of 9 multiple myeloma cell lines and induced apoptosis starting at 24 hours (but was best measured at 48 hours). Parallel to the findings in cultured multiple myeloma cell lines, ITF2357 demonstrated more potent cytotoxic activity compared with vorinostat against freshly isolated purified multiple myeloma samples at both 24 and 48 hours of culture.

Galli and colleagues reported preliminary results from a phase II dose-finding study assessing ITF2357 in patients with relapsed or progressive multiple myeloma.<sup>11</sup> Fifteen patients (age 52–77 years) with multiple myeloma who had received at least two different lines of therapy were enrolled in the study. ITF2357 was admin-

istered every 12 hours for 4 consecutive days followed by 3 days of rest every week during the first cycle (ie, first 4 weeks); concomitant oral dexamethasone (up to 20 mg) was administered every week. The first 6 patients received ITF2357 at a dose of 150 mg every 12 hours for 4 consecutive days per week. Two of these patients experienced grade 3 diarrhea during the first cycle and, as a result, the subsequent 9 patients received a reduced ITF2357 dose of 100 mg every 12 hours with the same schedule. None of these 9 patients experienced a DLT during the first cycle of treatment. The median duration of treatment was 7 weeks (range, 2 [n=1] to 12 [n=5] weeks). Grade 2 or higher thrombocytopenia was the most common side effect (n=11), and grade 4 thrombocytopenia occurred in 4 patients. Grade 3/4 gastrointestinal toxicity was observed in 4 patients. No grade 4 neutropenia was observed. Three patients experienced four serious adverse events: pneumonia (n=1), severe deterioration of general condition requiring hospitalization (n=1), and both events (n=1). Two months after completing the 12 weeks of treatment 1 patient developed atrial fibrillation that

required therapy. Two other patients experienced transient electrocardiogram abnormalities that did not require hospitalization or therapy.

The researchers reported 1 PR, 4 patients with stable disease, and 10 patients with disease progression. At last follow-up, 6 patients remain alive (3 with stable disease and 3 with disease progression). Nine patients died due to progressive disease. ITF2357 was considered tolerable when administered orally at the dose of 100 mg every 12 hours for 4 consecutive days per week, but the researchers noted that this agent given as monotherapy is unlikely to have significant therapeutic effects in patients with advanced multiple myeloma. However, some evidence of antimyeloma activity was observed in a group of patients with deteriorated clinical conditions and advanced disease. As a result, they recommend that further clinical investigation of ITF2357 in combination with other drugs active against multiple myeloma be initiated.

### 1179 Final Results of a Phase I Trial of Oral Vorinostat (Suberoylanilide Hydroxamic Acid, SAHA) in Patients with Advanced Multiple Myeloma<sup>15</sup>

PG Richardson, CS Mitsiades, K Colson, E Reilly, L McBride, J Chiao, L Sun, J Ricker, S Rizvi, C Oerth, B Atkins, I Fearen, KC Anderson, DS Siegel

O'Connor and colleagues have reported on clinical experience with intravenous and oral formulations of single-agent vorinostat in patients with advanced hematologic malignancies, including multiple myeloma.<sup>16</sup> With the oral formulation, the following major adverse events were observed: fatigue, diarrhea, anorexia, and dehydration; with the intravenous formulation, myelosuppression and thrombocytopenia were observed. The hematologic toxicities tended to resolve shortly after vorinostat administration ceased. No febrile neutropenia or neutropenic sepsis was observed. Five patients demonstrated measurable reduction in tumor cells, but none of these patients had multiple myeloma.

Based on such reports, Richardson and colleagues conducted a phase I trial of single-agent oral vorinostat in 13 patients with measurable, relapsed and/or refractory multiple myeloma who exhibited adequate hematologic, hepatic, and renal function.<sup>15</sup> Vorinostat was administered at twice-daily doses of 200, 250, or 300 mg for 5 days each week of a 4-week cycle or 200, 300, or 400 mg for 14 days of a 3-week cycle until progressive disease or intolerable toxicity was observed. The objectives of the study were to determine the maximum tolerated dose on each of the schedules and assess activity and safety. The patients' median age was 63 years and they had received

a median of seven prior systemic therapies. One patient (250 mg bid 5 days/week) developed a DLT of grade 3 fatigue. There were no other DLTs and the maximum administered doses were 250 mg twice daily for 5 days each week of a 4-week cycle and 200 mg twice daily for 14 days of a 3-week cycle. The researchers observed mostly grade 2 drug-related adverse events, including fatigue (69%), anorexia (62%), dehydration (46%), diarrhea (46%), and nausea (38%). Of 10 evaluable patients, 1 exhibited a minimal response and 9 had stable disease. The researchers concluded that vorinostat administered orally was generally well tolerated at 250 mg twice daily for 5 days each week of a 4-week cycle or 200 mg twice daily for 14 days of a 3-week cycle. As a result of the modest activity seen, further research is warranted of combinations of vorinostat with other antimyeloma agents. It was noted that the maximum tolerated dose was not determined because the study was terminated early by the sponsor.

## Commentary

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HDAC inhibitors are a new class of chemotherapeutic agents shown to have potent anticancer activities in pre-clinical studies, which are currently in various stages of clinical development for a variety of malignancies.<sup>17</sup> Early clinical studies showed substantial activity in relapsed and refractory cutaneous T-cell lymphoma (CTCL), resulting in vorinostat becoming the first HDAC inhibitor approved for this indication by the FDA.

Increased acetylation of specific residues in histones H3 and H4 by HDAC inhibitors is associated with open and active chromatin and increased mRNA transcription. The exact final pathways that lead to the anticancer effects observed in various tumor types remain to be fully elucidated; however, there is clear evidence that key activities of HDAC inhibitors include 1) induction of tumor cell apoptosis and suppression of cell proliferation by activation of cell cycle checkpoints at G<sub>1</sub>/S or G<sub>2</sub>/M; 2) induction of cellular differentiation; 3) suppression of angiogenesis; and 4) enhancement of host immune surveillance. Induction of apoptosis by HDAC inhibitors has been linked to alterations in gene expression resulting in upregulation of proapoptotic (*Bax*, *Bak*, *Bim*, *TP2*, *Apaf-1*, *Trail*, *DR4*, *DR5*, *Bmf*) and downregulation of antiapoptotic (*Bcl2*, *Bcl-XL*, *XIAP*, *survivin*, *Akt*, *c-FLIP*, *c-RAF*, *MCL-1*) genes.<sup>18-20</sup> Moreover, HDAC inhibitors can induce a cell cycle checkpoint at the G<sub>1</sub>/S transition through transcriptional activation of *CDKN1A* encoding

the cyclin-dependent kinase inhibitor p21/WAF1/CIP.<sup>21</sup> Furthermore, the molecular basis of anticancer effects of HDAC inhibitors may go beyond inhibition of histone acetylation. A growing list of nonhistone targets—involved in the regulation of cell proliferation, cell death, and cell migration—have been identified, including p53, Ku70,  $\alpha$ -tubulin, and HSP90. Thus acetylation-dependent changes in the activities of such proteins may play an equally important role in mediating the anticancer effects of HDAC inhibitors.<sup>19,22,23</sup>

There are several HDAC inhibitor families divided by their chemical structure, which vary in their ability to inhibit the various classes of histone deacetylases. These compounds have demonstrated in vivo and in vitro activity against both hematologic and nonhematologic malignancies, either alone or in combination with traditional chemotherapeutic agents.<sup>17</sup>

Various investigators have demonstrated that, as single agents, HDAC inhibitors have activity against multiple myeloma cells in vitro, inducing growth arrest and apoptosis in both cell lines and primary patient cells in a dose-dependent manner.<sup>5,9,24-26</sup> This process is associated with downregulation of *BCL-2*, *BCL-XL*, and *MCL-1*, upregulation of *p21* and *p53*, with these effects seemingly IL-6-independent. Subsequently, several groups have studied HDAC inhibitors preclinically with the proteasome inhibitor bortezomib, demonstrating that this combination synergistically induces apoptosis, mitochondrial injury, radical oxygen species generation, and oxidative injury in multiple myeloma cells.<sup>4,7,27</sup> In addition, inhibition of aggresome formation (a key escape mechanism for malignant plasma-cell survival following bortezomib therapy) appears to be crucial. When the proteasome is inhibited by bortezomib, aggregates of misfolded proteins are directed along acetylated  $\alpha$ -tubulin fibrils toward a single perinuclear region, forming an aggresome. This process allows the cell to survive and requires tubulin deacetylase activity. Catley and associates have demonstrated that panobinostat (LBH589) induces  $\alpha$ -tubulin hyperacetylation.<sup>27</sup> Thus, when panobinostat is administered with bortezomib, hyperacetylated  $\alpha$ -tubulin forms bundles, with diminished aggresome formation, and the cell undergoes apoptosis. Moreover, Kawaguchi and colleagues have demonstrated that HDAC6, a microtubule-associated deacetylase, is a component of the aggresome and has the capacity to bind both polyubiquitinated misfolded proteins and dynein motors, thereby acting to recruit misfolded protein cargo to dynein motors for transport to aggresomes.<sup>28</sup> Cells deficient in HDAC6 fail to clear misfolded protein aggregates from the cytoplasm, cannot form aggresomes properly, and are rendered hypersensitive to the accumulation of misfolded proteins. Of note, HDAC inhibitors and bortezomib have been shown to interact synergistically in CD138-positive

primary human bone marrow multiple myeloma but not in their normal CD138-negative counterparts, indicating that the toxicity may be differential between malignant and normal cells.<sup>4</sup>

The first clinical trial of HDAC inhibition specifically in myeloma patients was by Niesvizky and coworkers, using the cyclic peptide romidepsin (depsipeptide).<sup>6</sup> In this phase II trial, romidepsin was well tolerated, with fatigue, nausea, and transient thrombocytopenia being the major adverse events. Twelve patients were treated at the same dose used successfully to treat CTCL and peripheral T-cell lymphoma: 14 mg/m<sup>2</sup> administered intravenously on days 1, 8, and 15 of a 28-day cycle.<sup>29</sup> Patients were generally heavily pretreated, with an average of three prior lines of therapy (range, 2–4) and a mean disease duration of 6.25 years (range, 2–9 years). In this trial, using a stringent response criteria, 2 patients were withdrawn after the first cycle with stable disease. Six patients progressed during the first two cycles and 4 patients remained stable after 2–7 cycles. There were no PRs observed, although minor response criteria (eg, 25–50% reduction in paraprotein) was not used in this trial. Of note, although stable disease was the best response, rapidly increasing paraprotein levels were generally observed during the prior treatment, with plateauing of the paraprotein with romidepsin therapy.

The second trial of an HDAC inhibitor alone, this time with vorinostat, was presented by Richardson and colleagues at the ASH Meeting in December 2007 and recently published in full.<sup>30</sup> In the published manuscript, Richardson et al reported on 13 patients with multiple myeloma treated with vorinostat in a dose-escalating phase I design. The study was prematurely terminated because of sponsor withdrawal, and the maximum tolerated dose was not determined. Of note, the dose intensity administered in the various cohorts was somewhat lower than that generally used for the treatment of CTCL (400 mg/day continuous dosing).

ITF2357 is an orally effective member of the hydroxamic family of HDAC inhibitors with in vivo activity against multiple myeloma cells.<sup>13</sup> Galli and associates described their phase II experience whereby they administered 150 mg or 100 mg of ITF2357 every 12 hours for four consecutive days followed by three days of rest every week of a 28-day cycle.<sup>11</sup> Up to 12 weeks of treatment with ITF2357 was scheduled. Concomitant oral dexamethasone was given at a maximum dose of 20 mg every week. Sixteen multiple myeloma patients with a median of three prior therapies (range, 2–8) were treated. Four patients achieved stable disease and 1 had a PR. The most common grade 3 or 4 toxicities were gastrointestinal side effects, neutropenia, and thrombocytopenia.

Panobinostat (LBH589), another orally available hydroxamic acid derivative, is also currently being evalu-

ated in phase I and II studies in multiple myeloma. In an ongoing phase I study of 7 evaluable patients treated to date, 3 heavily pretreated patients have had stable disease, with improvements in constitutional symptoms and plateauing of the monoclonal protein observed (personal communication, A. Spencer, Alfred Hospital, Melbourne, Australia).<sup>31</sup>

What is clear from the above studies is that the HDAC inhibitors are well tolerated and their side-effect profile is such that they appear suitable for combination therapy, allowing targeting of multiple biologic pathways and mechanisms. Consequently, investigators are now examining the combination of HDAC inhibitors—romidepsin<sup>1</sup> and vorinostat<sup>8,10</sup>—with bortezomib in clinical trials. These studies are still immature but do demonstrate that such combinations are tolerable with near maximum single-agent doses of both drugs deliverable, with promising response rates. It is also of interest that there are now preclinical data showing that the combination of bortezomib and HDAC inhibitors is synergistic in other hematologic malignancies such as chronic lymphocytic leukemia.<sup>32</sup>

Clearly, HDAC inhibitors have activity in myeloma. Given the preclinical demonstration of synergism with bortezomib and the recent evidence that the two drugs can be combined with acceptable toxicity, larger trials will likely be initiated to determine if these preclinical findings translate into a clinical benefit. Finally, combination therapy of HDAC inhibitors with standard chemotherapeutic agents such as liposomal doxorubicin and melphalan,<sup>33</sup> and with lenalidomide,<sup>34</sup> have been demonstrated, and so other combinations with HDAC inhibitors with or without bortezomib warrant testing in the clinic.

## References

- Prince M, Quach H, Neeson P, et al. Safety and efficacy of the combination of bortezomib with the deacetylase inhibitor romidepsin in patients with relapsed or refractory multiple myeloma: preliminary results of a phase I trial. *Blood*. 2007;110(11): Abstract 1167.
- Richardson PG, Sonneveld P, Schuster MW, et al; Assessment of Proteasome Inhibition for Extending Remissions (APEX) Investigators. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. *N Engl J Med*. 2005;352:2487-2498.
- Yu C, Rahmani M, Conrad D, Subler M, Dent P, Grant S. The proteasome inhibitor bortezomib interacts synergistically with histone deacetylase inhibitors to induce apoptosis in Bcr/Abl+ cells sensitive and resistant to ST1571. *Blood*. 2003;102:3765-3774.
- Pei XY, Dai Y, Grant S. Synergistic induction of oxidative injury and apoptosis in human multiple myeloma cells by the proteasome inhibitor bortezomib and histone deacetylase inhibitors. *Clin Cancer Res*. 2004;10:3839-3852.
- Khan SB, Maududi T, Barton K, Ayers J, Alkan S. Analysis of histone deacetylase inhibitor, depsipeptide (FR901228), effect on multiple myeloma. *Br J Haematol*. 2004;125:156-161.
- Niesvizky R, Ely S, DiLiberto M, et al. Multicenter phase II trial of the histone deacetylase inhibitor depsipeptide (FK228) for the treatment of relapsed or refractory multiple myeloma (MM). *Blood*. 2005;106(11): Abstract 2574.
- Sutheesophon K, Kobayashi Y, Takatoku MA, et al. Histone deacetylase inhibitor depsipeptide (FK228) induces apoptosis in leukemic cells by facilitating mitochondrial translocation of Bax, which is enhanced by the proteasome inhibitor bortezomib. *Acta Haematol*. 2006;115(1-2):78-90.
- Badros A, Philip S, Niesvizky R, et al. Phase I trial of suberoylanilide hydroxamic acid (SAHA) + bortezomib (bort) in relapsed multiple myeloma (MM) patients (pts). *Blood*. 2007;110(11):Abstract 1168.
- Mitsiades N, Mitsiades CS, Richardson PG, et al. Molecular sequelae of histone deacetylase inhibition in human malignant B cells. *Blood*. 2003;101:4055-4062.
- Weber DM, Jagannath S, Mazumder A, et al. Phase I trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) in combination with bortezomib in patients with advanced multiple myeloma. *Blood*. 2007;110(11):Abstract 1172.
- Galli M, Salmoiraghi S, Golay J, et al. A Phase II multiple dose clinical trial of histone deacetylase inhibitor ITF2357 in patients with relapsed or progressive multiple myeloma: preliminary results. *Blood*. 2007;110(11):Abstract 1175.
- Leoni F, Fossati G, Lewis EC, et al. The histone deacetylase inhibitor ITF2357 reduces production of pro-inflammatory cytokines in vitro and systemic inflammation in vivo. *Mol Med*. 2005;11(1-12):1-15.
- Golay J, Cuppini L, Leoni F, et al. The histone deacetylase inhibitor ITF2357 has anti-leukemic activity in vitro and in vivo and inhibits IL-6 and VEGF production by stromal cells. *Leukemia*. 2007;21:1892-900.
- Rambaldi A, Micò C, Cuppini L, et al. The new histone deacetylase inhibitor ITF2357 is a strong inducer of apoptosis in multiple myeloma cells. Presented at the 10th Congress of the European Hematology Association, Stockholm, Sweden, June 2-5, 2005. Abstract 0230.
- Richardson PG, Mitsiades CS, Colson K, et al. Final results of a phase I trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) in patients with advanced multiple myeloma. *Blood*. 2007;110(11):Abstract 1179.
- O'Connor OA, Heaney ML, Schwartz L, et al. Clinical experience with intravenous and oral formulations of the novel histone deacetylase inhibitor suberoylanilide hydroxamic acid in patients with advanced hematologic malignancies. *J Clin Oncol*. 2006;24:166-173.
- Rasheed WK, Johnstone RW, Prince HM. Histone deacetylase inhibitors in cancer therapy. *Expert Opin Invest Drugs*. 2007;16:659-678.
- Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. *Nat Rev Drug Discov*. 2006;5:769-784.
- Bhalla KN. Epigenetic and chromatin modifiers as targeted therapy of hematologic malignancies. *J Clin Oncol*. 2005;23:3971-3993.
- Bishton M, Kenealy M, Johnstone R, Rasheed W, Prince HM. Epigenetic targets in hematological malignancies: combination therapies with HDACis and demethylating agents. *Expert Rev Anticancer Ther*. 2007;7:1439-1449.
- Richon VM, Sandhoff TW, Rifkind RA, Marks PA. Histone deacetylase inhibitor selectively induces p21<sup>WAF1</sup> expression and gene-associated histone acetylation. *Proc Natl Acad Sci U S A*. 2000;97:10014-10019.
- Marchion D, Munster P. Development of histone deacetylase inhibitors for cancer treatment. *Expert Rev Anticancer Ther*. 2007;7:583-598.
- Zhang K, Dent SY. Histone modifying enzymes and cancer: going beyond histones. *J Cell Biochem*. 2005;96:1137-1148.
- Lavelle D, Chen YH, Hankewych M, DeSimone J. Histone deacetylase inhibitors increase p21<sup>WAF1</sup> and induce apoptosis of human myeloma cell lines independent of decreased IL-6 receptor expression. *Am J Hematol*. 2001;68:170-178.
- Catley L, Weisberg E, Tai YT, et al. NVP-LAQ824 is a potent novel histone deacetylase inhibitor with significant activity against multiple myeloma. *Blood*. 2003;102:2615-2622.
- Maiso P, Carvajal-Vergara X, Ocio EM, et al. The histone deacetylase inhibitor LBH589 is a potent antimyeloma agent that overcomes drug resistance. *Cancer Res*. 2006;66:5781-5789.
- Catley L, Weisberg E, Kiziltepe T, et al. Aggresome induction by proteasome inhibitor bortezomib and alpha-tubulin hyperacetylation by tubulin deacetylase (TDAC) inhibitor LBH589 are synergistic in myeloma cells. *Blood*. 2006;108:3441-3449.
- Kawaguchi Y, Kovacs JJ, McLaurin A, Vance JM, Ito A, Yao TP. The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress. *Cell*. 2003;115:727-738.
- Piekars RL, Frye R, Turner M, et al. Phase II trial of romidepsin, FK228, in cutaneous and peripheral T-cell lymphoma: clinical activity and molecular markers. *Blood*. 2006;108(11 suppl). Abstract 2479.
- Richardson PG, Mitsiades CS, Colson K, et al. Phase I trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) in patients with advanced multiple myeloma. *Leuk Lymphoma*. 2008;49:502-507.
- Spencer A, Prince M, DeAngelo DJ, et al. Phase IA/II study of oral LBH589, a novel deacetylase inhibitor, administered on 2 schedules, in patients with advanced hematologic malignancies. *Blood*. 2007;110(11 suppl). Abstract 907.
- Dai Y, Chen S, Kramer LB, Funk VL, Dent P, Grant S. Interactions between bortezomib and romidepsin and belinostat in chronic lymphocytic leukemia cells. *Clin Cancer Res*. 2008;14:549-558.
- Campbell RA, Sanchez E, Steinberg J, et al. The novel potent pan-deacetylase inhibitor, panobinostat (LBH589), markedly enhances the anti-myeloma effects of chemotherapy in vitro and in vivo. *Blood*. 2007;110(11 suppl). Abstract 2520.
- Ocio EM, Vilanova D, San-Segundo L, et al. Triple combinations of the HDAC inhibitor panobinostat (LBH589) + dexamethasone with either lenalidomide or bortezomib are highly effective in a multiple myeloma mouse model. *Blood*. 2007;110(11 suppl). Abstract 1514.