

# Newer therapeutic molecules for multiple myeloma

Jain P, Gupta S, Parikh PM

Department of Medical Oncology, Tata Memorial Hospital, Dr. E. Borges Road, Parel, Mumbai - 400 012, India.

Correspondence to: Dr. Preetesh Jain, E-mail: drpreetesh@yahoo.co.in

## Abstract

Therapeutic management of multiple myeloma (MM) for the last several decades has mainly involved regimens based on use of glucocorticoids and cytotoxic chemotherapeutics. Despite progress in delineating the activity of such regimens, at either conventional or high doses, MM has remained an incurable disease. This has sparked major interest in the development of novel therapies that in part capitalize on recent advances in our understanding of the biology of MM, including the molecular mechanisms by which MM cell–host bone marrow (BM) interactions regulate tumor-cell growth, survival, and drug resistance in the BM milieu. Herein, we review the latest progress in the development of these novel anti-MM therapies, with major focus on therapies which have translated from preclinical evaluation to clinical application, including thalidomide and its more potent immunomodulatory derivatives (IMiD), the first-in-class proteasome inhibitor bortezomib (formerly known as PS-341). Search strategy included Medline using the terms ‘Myeloma and Newer Drugs’ citations relevant to treatment guidelines issued in 1999 and 2008 were screened.

**Key words:** Bortezomib, multiple myeloma, thalidomide

## Introduction

Multiple myeloma (MM) remains incurable with conventional treatments, with median survival duration of 3–5 years. The disease follows a relapsing course in the majority of patients, regardless of treatment regimen or initial response to treatment. Novel, more effective treatment approaches are required in order to improve outcomes and extend survival.<sup>1</sup> Newer therapies capitalize on recent advances in our understanding of the biology of MM, including the molecular mechanisms by which MM cell–host bone marrow (BM) interactions regulate tumor-cell growth, survival, and drug resistance in the BM milieu. The development of *in vitro* and *in vivo* models of MM stromal interactions has allowed not only for better characterization of these molecular phenomena but also for identification of specific therapeutic strategies to overcome these interactions and achieve an enhanced anti-MM effect, even against MM resistant to conventional therapies. Novel therapies are translating from preclinical evaluation to clinical application.

## Potential Targets

The signaling cascades involved in the cytokine-mediated proliferation, survival, drug resistance, and migration of MM cells have been well characterized. For example, certain cytokines – e.g., interleukin-6 (IL-6) and vascular endothelial growth factor (VEGF) – trigger proliferation of MM cells via mitogen-activated protein kinase (MAPK) signaling, whereas MM cell migration induced by cytokines (e.g., VEGF) is mediated via a protein kinase C-dependent, extra cellular signal-related kinase-independent pathway. MM cell apoptosis induced by Fas, gamma-irradiation (IR), and dexamethasone is mediated by distinct signaling cascades [Figures 1-3].<sup>[2]</sup>

Dexamethasone triggered (but not IR- or Fas-triggered) apoptosis is mediated via activation of related adhesion focal tyrosine kinase (RAFTK). While dexamethasone activates caspase 9, novel agents such as thalidomide and other immunomodulatory derivatives (IMiDs) deactivate caspase 8 and downstream cascades. This supports the utility of coupling novel and conventional therapies, such as thalidomide and dexamethasone.

Mechanisms of drug resistance induced by cytokines have been identified: IL-6 and Insulin-like growth factor 1 (IGF-1) inhibit apoptosis triggered by dexamethasone (but not IR) via Akt signaling and nuclear factor kappa B (NF- $\kappa$ B) activation, with downstream induction of intracellular inhibitors of apoptosis (IAPs), including FLICE (Fas-associated death domain-like interleukin-1 $\beta$ -converting enzyme) inhibitor protein (FLIP), survivin, cellular inhibitor-of-apoptosis protein (cIAP)-2, A1/bfl-1, and XIAP. These studies validate signaling cascades triggered by MM localization in the BM microenvironment mediating tumor-cell growth, survival, drug resistance, and migration as potential novel therapeutic targets.

## Novel Agents

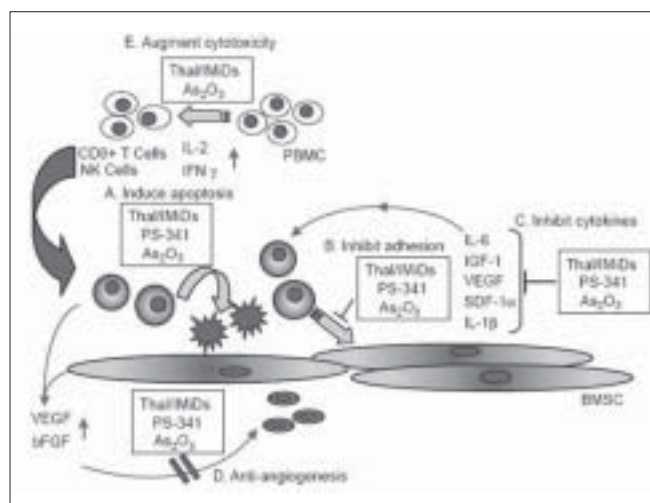
### Thalidomide and immunomodulatory derivatives

Thalidomide, or  $\alpha$ -N (phthalimido) glutarimide, is an orally bioavailable glutamic acid derivative which inhibits production of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and angiogenic cytokines such as basic fibroblast growth factor (bFGF) and/or VEGF. Reports of increased BM angiogenesis in MM, coupled with the antiangiogenic properties of thalidomide, provided the empiric rationale for administration of thalidomide on a compassionate basis to three patients with refractory MM. Following encouraging clinical responses in two of these patients, a larger phase II trial of single agent thalidomide (200 mg orally, starting daily dose, with inpatient dose escalation by 200 mg every two weeks

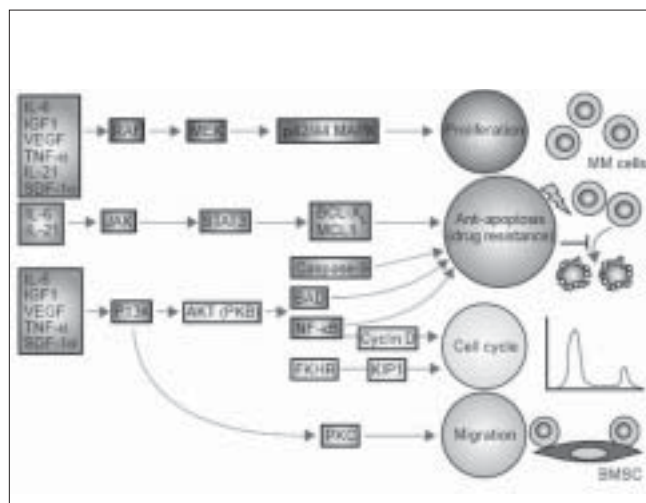
to a maximum daily dose of 800 mg) was conducted at the University of Arkansas in 84 patients with relapsed or refractory MM.

Of these patients, 76 had relapse after high-dose chemotherapy. Thalidomide treatment lasted for a median of 80 days (range 2–465 days) and objective responses were observed in 32% of cases. These responses included decrease in serum or urine levels of monoclonal paraprotein by >90% in eight patients (with complete remissions in two of them), >75% in six patients, >50% in seven patients, and >25% in six patients. However, no significant changes in BM microvascular density were reported. At least one-third of the patients had mild or moderate constipation, weakness, fatigue, or somnolence. More severe adverse effects were infrequent (occurring in <10% of patients), and hematologic toxicity was rare. Subsequent studies from other centers confirmed the activity of thalidomide in MM,<sup>[3]</sup> showing that the optimal dose of thalidomide is variable among patients and should be titrated to a level which is both well tolerated and achieves clinical response.

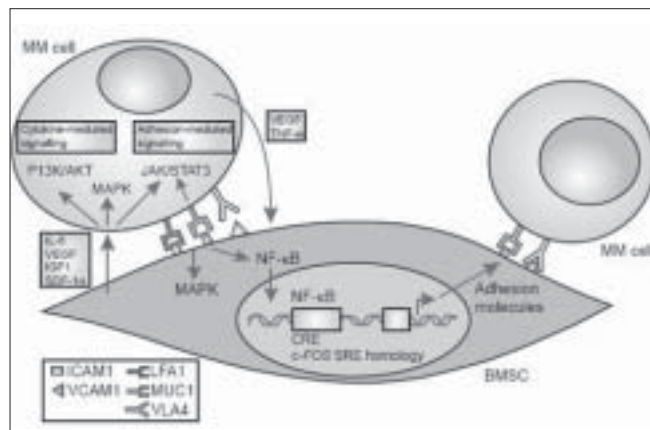
Thalidomide has also been administered in combination with dexamethasone, cytotoxic chemotherapeutics, or other agents such as clarithromycin. These combinations generally achieve higher response rates than separate administration of either thalidomide or the drugs with which it is partnered, and in some cases clinical responses are observed in patients resistant to each



**Figure 1:** Actions of novel agents against multiple myeloma (MM), including thalidomide and its immunomodulatory derivatives (Thal/ImiDs), the proteasome inhibitor PS-341, and arsenic trioxide (As<sub>2</sub>O<sub>3</sub>). Many of these agents can (a) induce G1 growth arrest and/or apoptosis in MM cell lines and patient cells resistant to conventional chemotherapy; (b) inhibit MM cell adhesion to BM stromal cells (BMSCs); (c) decrease cytokine production and sequelae; and (d) decrease angiogenesis in the BM microenvironment



**Figure 2:** Cascades mediating proliferation, survival, and migration in multiple myeloma (MM) cells. The RAS/RAF/mitogen-activated protein kinase (MAPK) kinase (MEK)/MAPK pathway triggers proliferation of MM cells. The Janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT3) pathways, as well as the upregulation of BCL-XL and MCL1, increase the resistance to apoptosis.



**Figure 3: Myeloma (MM) cells and their BM microenvironment.** The binding of MM cells to BM stromal cells (BMSCs) triggers adhesion- and cytokine-mediated MM cell growth, survival, and migration. MM cell binding to BMSCs induces the activation of p42/44 mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB) in BMSCs. The activation of NF-κB upregulates adhesion molecules on both MM cells and BMSCs, and cytokines secreted from MM cells induce interleukin 6 (IL-6) secretion, tumor necrosis factor alpha (TNF-α), and vascular endothelial growth factor (VEGF).

of these drugs individually. Indeed, in some cases the side-effects can be potentially very serious, for example, thromboembolic events in patients receiving thalidomide combined with dexamethasone or, mainly, anthracyclines. Dose reductions of thalidomide (or other components of combination therapies), as well as careful selection and comprehensive monitoring of patients receiving these regimens, may facilitate the prevention of such events.<sup>[4]</sup>

Seven phase I, II, and III studies of lenalidomide (also known as CC-5013 or CDC-5013 or revlimid) have been performed in patients with refractory or relapsed MM and have led to favorable clinical results.<sup>5</sup> A phase II multicenter randomized controlled open label study was conducted to select the lenalidomide regimen which, when used alone or in combination with dexamethasone, would provide the most promising evidence of efficacy in relapsed/refractory MM patients. In this trial, patients were randomized to oral lenalidomide at either 30 mg once daily or 15 mg twice daily for a total of six cycles, each comprising 3 weeks on therapy and 1 week off lenalidomide. The trial showed that the 30 mg once daily lenalidomide dose is better tolerated than the twice daily regimen, and that administration of lenalidomide in the context of a 3-weeks-on/1-week-off schedule is highly active with a response rate of approximately 35%. Importantly, the adverse event profile of lenalidomide is manageable and is hallmarked by very little neuropathy (< 5%). Incidence of thrombosis with lenalidomide therapy was analyzed. In a recent study, it was seen that there was an increasing trend when given with corticosteroids.<sup>6</sup> In patients in whom dexamethasone

was added, improved response rates were seen in about 40% of cases.

In the phase III setting, lenalidomide was combined with dexamethasone and is being compared with dexamethasone plus placebo in relapsed or refractory MM patients. This study has now completed accrual and final results are awaited. The precise molecular mechanisms mediating the anti-MM effects of thalidomide and the IMiDs have not been fully elucidated. This is mainly due to the enantiomeric interconversion of thalidomide and its spontaneous cleavage to multiple short-lived and difficult-to-characterize metabolites, as well as its species-specific metabolic activation, mainly by the liver, which confound the interpretation of preclinical *in vitro* and *in vivo* mechanistic studies.

At least four distinct, but potentially complementary, mechanisms have been proposed to account, at least in part, for the antitumor activity of thalidomide and its derivatives: (a) direct antiproliferative/proapoptotic effects against MM cells, which are probably mediated by one or more *in vivo* metabolites of thalidomide, and include inhibition of the transcriptional activity of NF-κB and its antiapoptotic target genes – e.g., the caspase inhibitors FLIP, cIAP-2, or the antiapoptotic Bcl-2 family member A1/Bfl;<sup>[18]</sup> (b) indirect targeting of MM cells by abrogation of the tumor cell protection conferred by their cell adhesion molecule or cytokine mediated (e.g., IL-6-mediated) interactions with BM stromal cells (BMSCs); (c) antiangiogenic effects; and (d) immunomodulatory effects, including enhanced natural killer (NK) cell-mediated cytotoxicity against tumor cells. Since NF-κB protects MM cells from the proapoptotic effects of steroids or cytotoxic chemotherapeutics, the inhibition of NF-κB activity by thalidomide/derivatives may account for the ability of combinations with dexamethasone or cytotoxic chemotherapeutics to achieve more potent *in vivo* antitumor responses than either agent alone.<sup>[7]</sup>

## Proteasome Inhibitors

### Bortezomib (formerly PS-341)

The ubiquitin-proteasome pathway is an intracellular proteolytic system which regulates the degradation of a broad spectrum of intracellular proteins, including diverse regulators of cell proliferation (e.g., cyclin-dependent kinase inhibitor proteins) or apoptosis. Bortezomib (pyrazylcarbonyl-Phe-Leu-boronate) represents the prototypic member of a class of peptide boronate proteasome inhibitors of 26S proteasome activity. Preclinical studies have shown cytotoxicity in various *in vitro* and *in vivo* preclinical models (prostate cancer, Burkitt's lymphoma, and adult T-cell leukemia),

with at least additive effects in combination with various chemotherapeutic agents against the Lewis lung carcinoma model. The major interest in proteasome inhibition as a therapeutic strategy in MM was fueled by the observation that MM cells were not only particularly sensitive to bortezomib *in vitro* but also those seven out of eight patients with MM achieved clinical responses to bortezomib therapy within the context of a phase I trial for patients with various hematologic malignancies.

Bortezomib directly inhibits proliferation and induces apoptosis of human MM cell lines and freshly isolated patient-derived MM cells, even in MM cells resistant to conventional therapies.<sup>[8]</sup> Importantly, the *in vitro* anti-MM activity of bortezomib is exerted even when MM cells are exposed to the drug in the presence of BMSCs, indicating that the protection afforded to MM cells by BMSCs against cytotoxic chemotherapeutics or steroids does not apply in the setting of treatment with proteasome inhibitors.

Consistent with this result, bortezomib does not confer, at the proteomic level, any global nonspecific accumulation of undegraded proteins, since the levels of many proteins remain unchanged. This can be attributed to the fact that bortezomib does not indiscriminately block all aspects of proteasome function, but instead selectively targets its chymotryptic activity. This also raises the notion that the therapeutic window associated with this class of agent may be due to the fact that, among the broad spectrum of proteolytic activities and corresponding targets of the proteasome, only a subset of them are modulated by bortezomib. A qualitative analysis of gene expression profiles of bortezomib-treated MM cells indicates that this agent causes an upregulation of proapoptotic regulators, such as the TNF- $\alpha$ -related apoptosis-inducing ligand (TRAIL) receptor, and suppression of antiapoptotic proteins such as survivin and Bcl-2. For many of these antiapoptotic regulators, e.g. caspase inhibitors, their decreased expression is consistent with the inhibitory effect of bortezomib on NF- $\kappa$ B activity; as shown with NF- $\kappa$ B DNA binding enzyme-linked immunosorbent assays (ELISAs), bortezomib suppresses the transcriptional activity of NF- $\kappa$ B due to the lack of degradation of ubiquitinated I $\kappa$ B.<sup>[9]</sup>

The accumulation of this negative regulator of NF- $\kappa$ B prevents its nuclear translocation and thus suppresses the expression of NF- $\kappa$ B-dependent antiapoptotic genes, such as the aforementioned caspase inhibitors. Furthermore, bortezomib causes a significant reduction in the expression of transcripts for molecules implicated in cell-cycle regulation and growth-factor-induced cell signaling pathways mediating proliferation, survival, and/

or drug resistance of MM cells, respectively, culminating in the induction of apoptosis via a dual activation of caspase-8 and caspase-9. In addition, bortezomib triggers significant reduction in several regulators of DNA synthesis and DNA repair. Proteasome inhibitors represent a second class of therapeutics targeting the MM cell in its BM microenvironment.

Based upon studies showing that IL-6 is the major growth and survival factor for human MM cells, and the observation that MM cell adhesion to BMSCs triggers the transcription and secretion of IL-6 in BMSCs via an NF- $\kappa$ B-dependent mechanism, it is hypothesized that the blockade of NF- $\kappa$ B using bortezomib may mediate anti-MM activity by inhibiting paracrine IL-6 production in BMSCs. Studies confirm that bortezomib acts directly to induce apoptosis of MM cells resistant to known conventional therapies, overcomes the protective effects of IL-6, and adds to the anti-MM effects of dexamethasone. In the BM microenvironment, bortezomib actively inhibits the binding of MM cells to BMSCs, the transcription and secretion of IL-6 triggered by the adhesion of MM cells to BMSCs, and BM angiogenesis.

These studies, coupled with phase I trials of bortezomib which demonstrated objective clinical responses in eight out of eight patients with refractory MM, provided the rationale for a multicenter phase II trial of bortezomib in MM. In that trial of bortezomib administration to 202 heavily pretreated patients with relapsed refractory MM, the overall response rate (minimal response plus partial response (PR) plus CR) was 35%, with an additional 24% experiencing stable disease. Importantly, according to Blade's criteria, complete responses were observed in 10% of bortezomib-treated patients – 4% were immunofixation-negative and the other 6% had residual MM paraprotein levels detectable only by immunofixation. Median time to progression was seven months and median duration of survival was 16 months, with a median duration of response of 12 months in those patients with PR and CR.

Paraprotein responses to bortezomib treatment were associated with improved hemoglobin levels, decreased blood transfusion requirements, improvement in renal function, and normalization of uninvolved immunoglobulins. Drug-related gastrointestinal toxicity and fatigue were manageable in most cases. Significant thrombocytopenia and neuropathy occurred primarily in patients in whom these conditions were pre-existent, and serious adverse events were uncommon. Importantly, recent data from a multicenter, international phase III trial in relapsed MM patients – which included 669 patients from 93 centers – indicate that bortezomib



is superior to high-dose dexamethasone therapy, in terms of both time to disease progression and overall survival, as well as response rate (45 versus 26%,  $P < 0.0001$ ).<sup>[10]</sup> Importantly, superiority for bortezomib versus high-dose dexamethasone was observed in second line (i.e. at first relapse) and as later salvage therapy, with toxicity profiles between the two treatment arms that were balanced and manageable. In combination with pegylated liposomal doxorubicin it significantly improved TTP compared with bortezomib alone in relapsed refractory MM.<sup>[11]</sup> Second generation proteasome inhibitors are under phase II trial, while PR-171, a novel epoxyketone-based irreversible proteasome inhibitor, is currently in clinical development. In comparison to bortezomib, PR-171 exhibits equal potency but greater selectivity for the chymotrypsin-like activity of the proteasome. PR-171 is well tolerated when administered for either 2 or 5 consecutive days at doses resulting in >80% proteasome inhibition in blood and most tissues. In human tumor xenograft models, PR-171 mediates an antitumor response that is both dose and schedule dependent. The antitumor efficacy of PR-171 delivered on 2 consecutive days is stronger than that of bortezomib administered on its clinical dosing schedule.<sup>[12]</sup>

#### VEGF inhibitors

Because of the increased microvascular density detected in the BM of MM patients compared to that of healthy donors, several studies have been undertaken during the last decade to identify the role that angiogenic cytokines such as VEGF play in MM. Subsets of MM and plasma-cell leukemia (PCL) patients do express the high-affinity VEGF receptor Flt-1 on their tumor cells. In turn, *in vitro* assays show that high levels of VEGF trigger Flt-1 phosphorylation with modest cell proliferation and robust migration of MM cells.<sup>[13]</sup> Such studies have suggested that inhibition of VEGF receptor signaling might be an interesting therapeutic strategy for MM. Ongoing clinical trials are evaluating the anti-MM activity of the VEGF receptor tyrosine kinase inhibitor PTK787/ZK222584 in patients with relapsed MM.

#### TRAIL/Apo2L

TRAIL/Apo2L (TNF-related apoptosis-inducing ligand/Apo2 ligand) is a member of the TNF superfamily of death-inducing ligands, which includes TNF-alpha and Fas ligand (FasL). TRAIL/Apo2L is expressed on the surface of immune effector cells, such as cytotoxic T-lymphocytes and NK cells, and has been proposed to contribute to their ability to destroy their targets (e.g., tumor cells) via ligation of its cell-surface receptors death receptor 4 (DR4) and death receptor 5 (DR5) expressed on the surface of tumor cells, including MM cells. Trimeric human recombinant forms of the

extracellular domain of TRAIL/Apo2L have been synthesized which have the ability to engage DR4 and DR5 molecules on the surface of tumor cells and potentially induce tumor-cell apoptosis. MM cells are particularly sensitive to TRAIL/Apo2L *in vitro* and *in vivo* and undergo caspase-8-dependent apoptosis, which circumvents resistance of MM cells to conventional (dexamethasone, anthracyclines, and alkylating agents) or novel (including thalidomide and its derivatives) anti-MM agents. Interestingly, although DR4 and DR5 are broadly expressed in a wide range of normal tissues, TRAIL/Apo2L selectively induces apoptosis of tumor cells but not their normal counterparts, for reasons which are incompletely understood.<sup>[14]</sup> These preclinical studies have provided the rationale for therapeutic strategies aiming at TRAIL receptor signaling, either with use of TRAIL/Apo2L constructs *per se* or with agonistic antibodies against the TRAIL receptors to overcome drug resistance in MM.

#### Farnesyl transferase inhibitors

During more advanced and biologically aggressive stages of this disease, activating mutations of members of the Ras family are more prevalent. *In vitro* studies confirm that these mutations contribute to MM cell resistance to conventional agents such as dexamethasone, doxorubicin, and melphalan. Furthermore, a wide range of BM-derived cytokines (e.g., IL-6, VEGF, and IGF-1) can modulate the proliferative rate of MM cells via the Ras/Raf/MAPK signaling cascade. These observations suggested that inhibition of Ras signaling could constitute an attractive therapeutic option for MM. Ras oncoproteins require membrane association in order to perform their signal transduction function. In turn, membrane localization of Ras requires a prenyl lipid modification, which is regulated by farnesyltransferase (FTase) and Geranylgeranyltransferase I (GGTase-I). These enzymes catalyze the prenyl post-translational modification of Ras by transferring farnesyl or geranylgeranyl groups to the cysteine (Cys) residue of its carboxyl terminal CAAX (CZcysteine, AZaliphatic, XZ any amino acid). All forms of Ras oncoproteins – H-, N-, and K-Ras – are subject to farnesylation. Therefore, instead of direct inhibition of Ras signaling function, pharmacological blockade of this cascade can be achieved by inhibition of the function of FTase or GGTase enzymes, for example, using CAAX peptidomimetics such as FTI-27773 and GGTI-2166. When FTase function is inhibited, K-Ras, but not N- or H-Ras, becomes geranylgeranylated. These FTase inhibitors have been shown to have *in vitro* activity against MM cells resistant to anthracyclines or alkylating agents.

Recently, the activity and tolerability of the FTase

inhibitor tipifarnib (Zarnestra) was evaluated in a phase II clinical trial in patients with relapsed MM. Forty three patients who had previously received a median of four (range 1–6) chemotherapy regimens received tipifarnib at 300 mg orally twice daily for 3 weeks every 4 weeks. The most common toxicity was fatigue, occurring in 66% of patients. Other adverse effects included diarrhea, nausea, neuropathy, anemia, and thrombocytopenia; 64% of patients had disease stabilization. Treatment with tipifarnib suppressed FTase, but not GGTase-I, in BM and peripheral-blood mononuclear cells. However, inhibition of farnesylation did not correlate with disease stabilization. These data suggested that FTase inhibitors can be tolerable and induce disease stabilization in relapsed MM patients.<sup>[15]</sup> Additionally, it was demonstrated that combination of a bortezomib and lonafarnib demonstrates synergistic myeloma-cell death and warrants further preclinical and clinical studies.<sup>[16]</sup>

#### Histone deacetylase inhibitors

Histone deacetylases (HDACs) and acetyltransferases (HATs) regulate gene expression by removal or addition, respectively, of acetyl groups to 3-amino groups in lysine residues of core nucleosomal histones. Increased histone acetylation attenuates their electrostatic interaction with the negatively charged DNA backbone, promotes unfolding of the histone–DNA complex, and modulates access of transcription factors to their sites of action and transcription of their target genes. Normal cell differentiation and function require coordinated gene transcription and appropriate balance of HAT and HDAC activities. Indeed, deletions or inactivating mutations of HATs have been implicated in the development of human neoplasms, whereas inhibition of HDAC activity and increased transcriptionally active chromatin promotes differentiation, growth arrest, and/or apoptosis of tumor cells *in vitro* and *in vivo*.

The therapeutic targeting of HDAC function has been translated into clinical trials with the use of hydroxamic-acid-based hybrid polar compounds, such as Vorinostat (suberoylanilide hydroxamic acid (SAHA)). SAHA induces accumulation of acetylated core nucleosomal histones, with related induction of differentiation and/or apoptosis in transformed and neoplastic cells. SAHA treatment of MM cells was associated with increased p21 and p53 protein levels, dephosphorylation of Rb, cleavage of Bid (which suggests a role for Bcl-2 family members in regulation of SAHA induced cell death), as well as a calpain-dependent, caspase-independent pathway for induction of apoptosis in MM cells. Importantly, SAHA sensitizes MM cells to a wide range of proapoptotic stimuli, including death receptor ligands such as FasL and TRAIL/Apo2L,<sup>79</sup> dexamethasone,

cytotoxic chemotherapeutics, thalidomide analogs, and bortezomib.<sup>[17,18]</sup>

Multifaceted anti-MM effects can be attributed to the constellation of antiproliferative and/or proapoptotic molecular events triggered by SAHA, including downregulation of transcripts for members of the IGF/IGF-1 receptor (IGF-1R) and IL-6 receptor (IL-6R) signaling cascades, antiapoptotic molecules (e.g., caspase inhibitors), oncogenic kinases, DNA synthesis/repair enzymes, and transcription factors (e.g., XBP-1, E2F-1) implicated in MM pathophysiology, as well as suppression of activity of the proteasome and expression of its subunits.

#### Heat shock protein 90 inhibitors

Heat shock protein 90 (Hsp90) is a molecular chaperone which functions to facilitate the intracellular trafficking, conformational maturation, and three-dimensional folding of a wide spectrum of intracellular proteins critically involved in cell proliferation, survival, and drug resistance. The ansamycin-based antibiotic geldanamycin (GA) and its analogs bind to the critical ATP-binding site of Hsp90, thereby abrogating its chaperoning activity. Because many of the client proteins of hsp90 are involved in transmitting molecular signals derived from the cytokine milieu of the MM BM, inhibition of hsp90 function deprives MM cells of their ability to respond to a broad range of antiapoptotic microenvironmental stimuli.

Hsp90 inhibition also blocks both constitutive and cytokine-induced activation of NF- $\kappa$ B and telomerase in the BM milieu. As a result of these properties, GA and other Hsp90 inhibitors induce apoptosis of MM cell lines and primary MM cells which are resistant to dexamethasone, anthracyclines, thalidomide, IMiDs, TRAIL/Apo2L, and bortezomib. Interestingly, bortezomib treatment of MM cells induces a major compensatory upregulation of Hsp90, which represents a stress response in tumor cells to withstand the toxic effects of intracellular accumulation of undegraded proteins. Therefore, blocking this response with hsp90 inhibitor (Tanespimycin) enhances MM cell apoptosis triggered by bortezomib, suggesting a possible role of combination regimens with these two classes of drugs in MM.<sup>[19]</sup>

#### References

1. Rajkumar SV, Kyle RA. Multiple myeloma: Diagnosis and treatment. *Mayo Clin Proc* 2005;80:1371–82.
2. Podar K, Tai YT, Davies FE. Vascular endothelial growth factor triggers signaling cascades mediating multiple myeloma cell growth and migration. *Blood* 2001;98:428–35.
3. Rajkumar SV, Fonseca R, Dispenzieri A. Thalidomide in the treatment

- of relapsed multiple myeloma. *Mayo Clin Proc* 2000;75:897-901.
4. Rajkumar SV, Hayman S, Gertz MA. Combination therapy with thalidomide plus dexamethasone for newly diagnosed myeloma. *J Clin Oncol* 2002;20:4319-23.
5. Richardson PG, Schlossman RL, Weller E. Immunomodulatory drug CC-5013 overcomes drug resistance and is well tolerated in patients with relapsed multiple myeloma. *Blood* 2002;100:3063-7.
6. Menon SP, Rajkumar SV, Lacy M, Falco P, Palumbo A. Thromboembolic events with lenalidomide based therapy in multiple myeloma. *Cancer* 2008;112:1522-8.
7. Hideshima T, Chauhan D, Shima Y. Thalidomide and its analogs overcome drug resistance of human multiple myeloma cells to conventional therapy. *Blood* 2000;96:2943-50.
8. Hideshima T, Richardson P, Chauhan D. The proteasome inhibitor PS-341 inhibits growth, induces apoptosis and overcomes drug resistance in human multiple myeloma cells. *Cancer Res* 2001;61:3071-6.
9. Mitsiades N, Mitsiades CS, Poulaki V. Molecular sequelae of proteasome inhibition in human multiple myeloma cells. *Proc Natl Acad Sci USA* 2002;99:14374-9.
10. Richardson P, Sonneveld P, Schuster M. Bortezomib demonstrates superior efficacy to high-dose dexamethasone in relapsed multiple Myeloma: final report of the APEX study. *Blood* 2004;104:336a.
11. Orlowski RZ, Zhuang SH, Parekh T; DOXIL-MMY-3001 Study Investigators. The combination of pegylated liposomal doxorubicin and bortezomib significantly improves time to progression of patients with relapsed/ refractory multiple myeloma compared with bortezomib alone: Results from a planned interim analysis of a randomized phase III study. *Blood* 2006;108:124a.
12. Demo SD, Kirk CJ, Aujay MA, Buchholz TJ, Dajee M, Ho MN, et al. Antitumor activity of PR-171, a novel irreversible inhibitor of the proteasome. *Cancer Res* 2007;67:6383-91.
13. Richardson PG. New Drugs for Myeloma. *Oncologist* 2007;12:664-89.
14. Mitsiades CS, Treon SP, Mitsiades N. TRAIL/Apo2L ligand selectively induces apoptosis and overcomes drug resistance in multiple myeloma: Therapeutic applications. *Blood* 2001;98:795-804.
15. Alsina M, Fonseca R, Wilson EF. Farnesyltransferase inhibitor tipifarnib is well tolerated, induces stabilization of disease, and inhibits farnesylation and oncogenic/tumor survival pathways in patients with advanced multiple myeloma. *Blood* 2004;103:3271-7.
16. David E, Sun SY, Waller EK, Chen J, Khuri FR, Lonial S. The combination of the farnesyl transferase inhibitor lonafarnib and the proteasome inhibitor bortezomib induces synergistic apoptosis in human myeloma cells that is associated with down-regulation of p-AKT. *Blood* 2005;106:4322-9.
17. 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. Program and abstracts of the 49th Annual Meeting of the American Society of Hematology; December 8-11, 2007; Atlanta, Georgia. Abstract 1172.
18. 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). Program and abstracts of the 49th Annual Meeting of the American Society of Hematology; December 8-11, 2007; Atlanta, Georgia. Abstract 1168.
19. Richardson PG, Chanan-Khan A, Lonial S, et al. Tanespimycin (T) + bortezomib (BZ) in multiple myeloma (MM): Confirmation of the recommended dose using a novel formulation. Program and abstracts of the 49th Annual Meeting of the American Society of Hematology; December 8-11, 2007; Atlanta, Georgia. Abstract 1165.

**Source of Support:** Nil, **Conflict of Interest:** None declared.

### Author Help: Online Submission of the Manuscripts

Articles can be submitted online from <http://www.journalonweb.com>. For online submission articles should be prepared in two files (first page file and article file). Images should be submitted separately.

- 1) **First Page File:**  
Prepare the title page, covering letter, acknowledgement, etc., using a word processor program. All information which can reveal your identity should be here. Use text/rtf/doc/pdf files. Do not zip the files.
- 2) **Article file:**  
The main text of the article, beginning from Abstract till References (including tables) should be in this file. Do not include any information (such as acknowledgement, your names in page headers, etc.) in this file. Use text/rtf/doc/pdf files. Do not zip the files. Limit the file size to 400 kb. Do not incorporate images in the file. If file size is large, graphs can be submitted as images separately without incorporating them in the article file to reduce the size of the file.
- 3) **Images:**  
Submit good quality color images. Each image should be less than **1024 kb (1 MB)** in size. Size of the image can be reduced by decreasing the actual height and width of the images (keep up to about 6 inches and up to about 1200 pixels) or by reducing the quality of image. JPEG is the most suitable file format. The image quality should be good enough to judge the scientific value of the image. Always retain a good quality, high resolution image for print purpose. This high resolution image should be sent to the editorial office at the time of sending a revised article.
- 4) **Legends:**  
Legends for the figures/images should be included at the end of the article file.