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Emerging treatments of multiple myeloma; beyond IMIDs and bortezomib

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Abstract

The successful clinical development of thalidomide, bortezomib and lenalidomide not only transformed the therapeutic management of multiple myeloma (MM), but also catalyzed a renewed interest in the development of additional classes of novel agents for this disease. This review focuses on a series of new therapeutics that have shown promising preclinical results, as well as encouraging safety profiles and early evidence of anti-MM activity in clinical studies, either alone or in combination with other, conventional or novel, anti-MM treatments. These agents include second-generation proteasome inhibitors and immunomodulatory agents, as well as members of other therapeutic classes, such as histone deacetylase inhibitors (HDAC), heat shock protein 90 inhibitors and the alkylphospholipid Akt inhibitor perifosine.

Introduction

The successful clinical development of thalidomide, bortezomib and lenalidomide has significantly changed the treatment landscape for multiple myeloma (MM) and contributed to improved overall survival of MM patients compared to patients diagnosed in the era prior to the development of these novel therapies [1]. These 3 new agents have catalyzed renewed interest in development of additional classes of novel agents for this otherwise incurable malignancy. Current investigational compounds include not only second-generation proteasome inhibitors and immunomodulatory agents, but also members of other therapeutic classes. This review focuses on the recent progress in the translational and clinical development of novel anti-MM agents beyond bortezomib, thalidomide and lenalidomide. Particular emphasis is placed on agents which have shown promising preclinical results, as well as encouraging safety profiles and early evidence of anti-MM activity in clinical studies, either alone or in combination with other, conventional or novel, anti-MM treatments.

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Second-generation proteasome inhibitors: carfilzomib (PR-171) and salinosporamide (NPI-0052)

The successful clinical development of bortezomib not only changed the natural history of MM patients, but also provided indisputable clinical validation of the role of proteasome as a therapeutic target for this disease. Specifically, the fact that bortezomib can be administered safely at doses and schedules which provide meaningful clinical benefit addressed previous concerns about the clinical feasibility of proteasome inhibition. It also created new interest in the development of other proteasome inhibitors that would hopefully exhibit improved properties compared to bortezomib. The efforts to develop second generation proteasome inhibitors aimed at achieving increased potency of inhibition of the intended target, but also attempted to address two important clinical needs, namely bioavailability via the oral route and decreased peripheral neuropathy, which is key dose limiting adverse event of bortezomib.

The two new proteasome inhibitors that have emerged so far from both preclinical and clinical studies in MM include the lactacystin-like agent NPI-0052 (Salinosporamide A) [2,3] and the epoxyketone carfilzomib (PR-171) [4,5]. Both compounds are considered irreversible inhibitors of the proteasome, in contrast to bortezomib which reversibly inhibits the chymotryptic-like activity of the 20S proteasome through non-covalent interaction with its β5 subunit. At the molecular level, the irreversible binding of carfilzomib to its target is attributed to its structural similarity to epoxomicin, a natural product which forms irreversible adducts only with the N-terminal threonine of the β5 subunit [6] and not the other proteasome subunits. Because of this substrate-selective adduct formation, the activity of carfilzomib is restricted to the chymotryptic-like activity of the 20S proteasome [5]. In contrast, pre-clinical reports indicate that NPI-0052 inhibits not only the chymotryptic-like activity of the 20S proteasome, but also its other 2 proteolytic activities (tryptic-like and caspase-like) [3]. In preclinical in vitro studies with MM cell lines and primary tumor cells, carfilzomib has exhibited anti-MM activity at nM concentrations 5, with IC_{50} values that are in the same order of magnitude as those for bortezomib [7]. Similarly to bortezomib, short exposures to carfilzomib can trigger irreversible activation of MM cell death[5], although this effect appears to require shorter exposures to carfilzomib than to equimolar concentrations of bortezomib. This may be due to the distinct irreversible binding of carfilzomib with the proteasome, as opposed to bortezomib's reversible binding to its target.

NPI-0052 is derived from fermentation of the marine gram-positive actinomycete *Salinospora* and although it is structurally distinct from bortezomib, it shares several of its properties. NPI-0052, like bortezomib can induce death of MM cells resistant to diverse conventional and novel anti-MM agents. Consistent with the effect on the chymotryptic-like activity of the 20S proteasome, NPI-0052 suppresses the transcriptional activity of NF-κB in MM cells, although it is likely that its anti-MM activity includes other mediators as well. NPI-0052 overcomes, similarly to bortezomib, the proliferative/anti-apoptotic effects conferred by BMSCs or by BM-derived cytokines (e.g. IL-6). These in vitro observations provide an understanding of the similarities between these proteasome inhibitors.

It is notable that both NPI-0052 and carfilzomib have been reported to have *in vitro* anti-MM activity against primary MM cells that are derived from patients relapsing from or refractory to bortezomib [3]. For NPI-0052, its ability to overcome bortezomib resistance has been ascribed, at least in part, to the fact that this agent blocks not only the chymotryptic activity of the proteasome, but also its other 2 proteolytic activities. It is plausible that this broader spectrum of activity of NPI-0052 allows it to more comprehensively block the degradation of ubiquitinylated proteins and thereby trigger pro-apoptotic pathways that are either not triggered by bortezomib-mediated suppression of the chymotryptic-like activity of the 20S proteasome or require higher concentrations/longer exposures to achieve this effect. In contrast, carfilzomib

selectively inhibits chymotryptic-like activity of the proteasome, similarly to bortezomib. Because the *in vitro* IC_{50} values for these 2 inhibitors are also comparable, it is plausible that the activity of carfilzomib against bortezomib-resistant MM cells is not due to a broader spectrum of activity or more potent inhibition of chymotryptic-like 20S proteasome function. Instead, it is more likely related to the irreversibility of the effect of carfilzomib, because irreversible inhibition of the proteasome could allow tumor cells less time to recover from the impact of treatment.

An attractive feature of both NPI-0052 and carfilzomib is the potential for oral bioavailability. Both agents have been administered orally in preclinical models and have shown that this route of administration is associated with anti-tumor activity. This aspect distinguishes these agents from bortezomib, which is currently available only for use as an injectable preparation. So far, however, the clinical development of carfilzomib and NPI-0052 is based on intravenous administration.

A multicenter phase I study was performed to evaluate the safety and clinical activity of carfilzomib in patients with relapsed or refractory hematological malignancies [8]. The trial enrolled 51 patients, including 21 MM patients, 17 of whom had previously been treated with bortezomib. Four of the MM patients in that trial responded to carfilzomib treatment (PR of 19%). Based on these early data, open-label, single-arm, phase II studies of carfilzomib were initiated in relapsed and refractory MM [9] as well as in relapsed MM [10], respectively. Preliminary results of these studies were presented at the 2008 meeting of the American Society of Hematology. In the first study, carfilzomib was administered as 20 mg/m^2 IV push on days 1, 2, 8, 9, 15 and 16 of each 28-day cycle (i.e. twice-weekly on the first 2 days of each week during the first 3 weeks of 4-week cycles). Enrolled patients had to have relapsed from at least 2 prior therapies (including bortezomib and thalidomide or lenalidomide) and also had to be refractory to their last treatment, defined as <25% response to their last treatment or progression while on therapy (or within 60 days of its completion). Patients were heavily pretreated, with a median number of prior lines of therapy of 5 (range of 2–15), while 80% of patients had received at least 4 prior lines of therapy. More than 50% of patients had received bortezomib as their last therapy. The overwhelming majority of patients had also previously received lenalidomide (89%), thalidomide (91%), alkylating agents (94%), anthracycline (80%), or stem cell transplant (SCT) (83%). In a preliminary analysis of 39 patients evaluable for response, the rates of partial response (PR), minor response (MR), and stable disease (SD) were 13% (5 patients), 13% (5 patients), and 41% (16 patients), respectively, for a combined rate of MR or better of 26% (95% CI 13%–42%.) In bortezomib-refractory patients, 1 PR and 4 MRs were observed, for a combined rate of MR or better of 5/26 (19%). Anemia, cytopenia or neutropenia at grade 3 or higher were reported in 33%, 24% and 4.3% of patients, respectively. Thrombocytopenia was cyclical, and similarly to bortezomib, platelet counts recovered during the end of each cycle, returning to their baseline levels at the beginning of the next. Grade 3 or higher non-hematologic side effects included fatigue, increases in creatinine levels, hypocalcemia, hyperkalemia and hyperuricemia, all of which were reported in <10% of patients. It is notable that the reported changes in creatinine levels were transient, reversible, and non-cumulative and did not appear to limit treatment duration or efficacy of carfilzomib [9]. It is also important to note that carfilzomib treatment was not associated with clinically significant peripheral neuropathy, or significant deterioration of it in patients with pre-existing neuropathy.

In the phase II trial of carfilzomib in relapsed MM patients[10], the drug was administered using the same schedule as in the trial for relapsed and refractory MM, i.e. at 20 mg/m^2 IV push (with dexamethasone (Dex) 4 mg PO premedication during cycle 1) on days 1,2, 8, 9, 15 and 16 of each 28-day cycle (QDx2 weekly for 3 weeks of each cycle) for up to 12 cycles. Enrollment was limited to patients with 1–3 prior lines of therapy. In this trial, 17 of 31 patients had been

previously exposed to bortezomib, while the percentage of patients previously exposed to thalidomide or lenalidomide was lower than in the study of Jagannath et al [9]. In bortezomib-naïve patients, the rates of complete response (CR), very good partial response (VGPR) and PR were 7%, 14%, and 36%, respectively, for an overall response rate of 57%. In patients previously exposed to bortezomib, the overall response (PR or better) rate was 18%, with an additional 6% of patients having MR. SD was observed in 29% of bortezomib-naïve patients and 59% of patients previously exposed to bortezomib. Grade 3 or 4 neutropenia, anemia and thrombocytopenia were observed in 10%, 6.5% and 6.5%, respectively, of patients in this trial. Grade 3 or 4 nonhematologic adverse events were limited to dyspnea (6.5% of patients). Importantly, in terms of peripheral neuropathy only one grade 1 event (3.3% of patients) was reported. As more mature data from these trials, as well as future ones, become available, the patterns of safety and anti-MM activity of carfilzomib will become more evident and will hopefully better define the role of carfilzomib in the management of MM.

The aforementioned preclinical data on NPI-0052 provided the framework for a phase I dose-escalation of NPI-0052 monotherapy in MM patients with relapsed/refractory disease, including patients that had previously received bortezomib and/or lenalidomide. NPI-0052 was administered as a weekly IV injection on Days 1, 8 and 15 of every 4 week-cycle with concomitant hydration. NPI-0052 dose was escalated in cohorts of 3 patients dependent on observed adverse events utilizing a 3+3 design. Preliminary results were presented at the Annual Meeting of the American Society of Hematology in 2008 [11]. The first 10 patients in the trial were treated with NPI-0052 doses ranging from 0.025 mg/m^2 to 0.075 mg/m^2 without reaching an MTD so far. From the safety standpoint, marked but reversible elevation in serum creatinine was observed in one patient (in the context of possible progression of underlying light chain nephropathy and with interval worsening of renal function noted prior to enrollment) who responded to drug discontinuation and glucocorticoids. No peripheral neuropathy or myelosuppression has been observed so far in this trial. Other drug-related adverse events were manageable at all dose levels tested. No major reductions in M-protein had been observed in this trial so far but two patients with relapsed/refractory MM had stable disease and remained on study for >6 months and 1 year, respectively, with no significant toxicity. Dose escalation continues in order to determine a phase 2 dose for further trial(s) in advanced MM.

Pomalidomide (CC-4047)

Despite its structural similarity with thalidomide and lenalidomide, pomalidomide (formerly known as CC-4047 or IMID-1) exhibits distinct properties in terms of the qualitative and quantitative pattern of its activity in different preclinical models and bioassays. The first preclinical documentation of anti-MM activity of pomalidomide came from *in vitro* studies conducted by Hideshima et al [12]. These studies showed that pomalidomide demonstrated a pleiotropic range of anti-MM actions, including direct anti-proliferative/pro-apoptotic effect on MM cell lines and primary MM patient tumor cells; modulation of MM cell adhesion to BMSCs; and suppression of proliferative/anti-apoptotic cytokines produced as a result of MM-BMSC interaction [12]. Additional studies also documented the ability of pomalidomide to enhance immune effector cell killing of MM cells [13]. Other studies showed that pomalidomide, similarly to lenalidomide, can have enhanced anti-MM activity when combined with bortezomib [14]. A phase I trial of pomalidomide (CC-4047) in relapsed or refractory MM [15] determined a maximum tolerated dose of 2 mg per day and showed that this compound was active in MM. Its side effects included deep venous thrombosis and neutropenia. MR or better was observed in 67% of patients in that trial, while 54% of patients achieved PR or better, including 4 of 24 patients (17%) with CR[15]. A second phase I trial conducted by Streetly et al. [16] determined that pomalidomide can be administered on alternate days at a MTD of 5 mg p.o. without thromboembolic events. PR or better was achieved in 10 of 20 (50%) of patients, while

complete remission was achieved in 2 of 20 patients (10%). Progression free survival was 10.5 months, with a median overall survival of 33 months.

These results provided the impetus for a phase II trial of pomalidomide plus Dex in relapsed or refractory MM [17]. Pomalidomide was administered at 2 mg p.o. daily on days 1–28 of each four-week cycle while Dex was administered at 40 mg p.o. on days 1, 8, 15 and 22 of each cycle, with aspirin (325 mg p.o. days 1–28) prophylaxis also administered. In the trial, 65% of patients had been previously treated with SCT and 60% of patients had prior use of either thalidomide or lenalidomide. In terms of the safety profile of the compound, 32% of patients had grade 3 or 4 neutropenia, while the rates of grade 3 or 4 thrombocytopenia or anemia were less than 5%. Nonhematologic adverse events included a fatal case of pneumonia in a neutropenic patient and grade 3 events (overall in 28% of patients) which included fatigue, constipation and hyperglycemia. It is notable that grade 1 or 2 peripheral neuropathy was observed in up to 30% of patients (23% of all patients had only grade 1 peripheral neuropathy). No cases of deep venous thromboses or pulmonary embolism were documented. Thirteen percent of patients had dose reductions of pomalidomide due to neutropenia or neuropathy, while 32% of patients had dose reductions of Dex due to edema, hyperglycemia, confusion, mood alteration or muscle weakness. In 60 evaluable patients, followed-up for a median of 4 months, stringent CR, VGPR, PR, and SD were observed in 1, 14, 20, and 11 patients, respectively (i.e. 2%, 23%, 33%, and 18%, respectively), for an overall response rate of 58% and a combined CR plus VGPR rate of 25%. Importantly, 13 out of the first 37 patients enrolled in the trial were lenalidomide-refractory and, in these patients, responses were observed in 29%. These results suggest that the combination of pomalidomide with Dex is active for the management of advanced MM and that its profile of adverse events predominantly consists of myelosuppression with neutropenia. Phase II clinical trials of this combination in patients with lenalidomide refractory and bortezomib refractory disease are expected to take place.

Deacetylase inhibition (Vorinostat, LBH589, and other DAC inhibitors)

The degree of acetylation of histones influences their physical interaction with DNA and how it is packaged in the nucleus, which can in turn have large scale impact on gene expression. Histone acetylation is regulated by the opposing effects of histone acetyltransferases (HATs) and histone deacetylases (HDACs). Inhibition of HDACs triggers accumulation of acetylated histones and induces differentiation and/or apoptosis of many types of malignant cells. Preclinical studies in our group had indicated that the HDAC inhibitor vorinostat (also known formerly as suberoylanilide hydroxamic acid, SAHA) is active in vitro against MM cells, including cells resistant to conventional and/or novel anti-MM agents, and overcomes protective effects of IL-6 and BMSCs [18]. Exposure of MM cells to vorinostat is associated with antiproliferative and/or proapoptotic molecular sequelae, which include suppression of transcripts for diverse growth factors and/or their receptors; caspase inhibitors; oncogenic kinases; DNA synthesis/repair enzymes; as well as proteasome subunits.

Previous studies by our group had shown that, when exposed to bortezomib treatment, MM cells exhibit distinct compensatory stress responses, which include up-regulation of transcripts for various members of the ubiquitin-proteasome pathway, such as proteasome subunits and ubiquitin conjugating enzymes[19]. This stress response can be interpreted as an attempt of the MM tumor cells to withstand the stress caused by bortezomib treatment. The production of more proteasome particles could conceivably compensate for the bortezomib-induced suppression of the enzymatic activity of the already existing proteasomes. In that context, our observation that HDAC inhibition suppresses the expression of proteasome subunits and other ubiquitin proteasome pathway members suggested that the combination of a proteasome inhibitor with an HDAC inhibitor could conceivably have synergistic anti-MM activity, not only through the broad spectrum proapoptotic/anti-proliferative activity of these two drug

classes, but also through a specific cooperation of these two agents to comprehensively suppress the activity of the ubiquitin proteasome pathway[20]. Indeed, preclinical experiments confirmed that vorinostat plus bortezomib exhibit enhanced in vitro anti-MM activity [20] and subsequent studies using different types of HDAC and/or proteasome inhibitors in MM [21,22], as well as other types of neoplasias [23–28], further validated these original observations. These findings coupled with the oral bioavailability of vorinostat and its manageable profile of adverse events in clinical trials in other neoplasias [29] provided the basis for a phase I trial of single agent oral vorinostat [30]. In that trial, vorinostat was administered orally at 200, 250 or 300 mg twice daily for 5 days per week in 4-week cycles or at 200, 300, or 400 mg twice daily for 14 days in 3-week cycles, until progressive disease or intolerable toxicity was observed. That trial enrolled 13 patients who had received a median number of 3 prior lines of therapy. One patient treated with vorinostat at 250 mg twice daily 5 days per week developed grade 3 fatigue as dose-limiting toxicity (DLT), but no other DLTs were observed. Due to early study termination, following sponsor's decision, MTD was not determined in that trial. Maximum administered doses were 250 mg twice daily for 5 days/week of 4-week cycles and 200 mg twice daily for 14 days of 3-week cycles. Drug-related side effects were mostly grade ≤2 and included fatigue, anorexia, dehydration, diarrhea, and nausea. Among 10 evaluable patients, 1 MR and 9 SDs were observed.

Although these observations might imply that vorinostat had only modest single-agent activity in relapsed/refractory MM, it should be emphasized that this monotherapy study did not intend to exclusively focus on single-agent activity, but was designed to become the basis for potential trials combining vorinostat with other anti-MM agents. Furthermore, vorinostat monotherapy was administered in this MM trial twice daily. This schedule was different from that used for the studies that led to the FDA approval of vorinostat for cutaneous T cell lymphoma (CTCL). The experience with vorinostat in the CTCL field has revealed that once daily dosing is more potent and better tolerated [31] than twice daily administration. This implies that the modest activity of vorinostat in that MM trial may have been a repercussion of a shorter duration of treatment and lower cumulative dose administered due to side effects related to the twice daily schedule. Taking all these parameters into consideration and in view of preclinical data on the anti-MM activity of bortezomib in combination with vorinostat, two separate multi-center phase I clinical trials for advanced MM patients were initiated to evaluate the safety and efficacy of this combination

Preliminary results from two separate multi-center phase I trials were presented at the 2007 Annual Meeting of the American Society of Hematology by Badros [32] and by Weber [33], and updated analyses of the 2 trials were presented by Weber et al at the 2008 ASH meeting [34]. In the first study [32], the combination of vorinostat with bortezomib was administered in 21 heavily pretreated patients who were resistant to multiple key anti-MM therapies: their median time from diagnosis was 5.3 yrs, and their median number of prior regimens was 6 (range 3–10). Nineteen of 21 patients were refractory to their last therapy prior to enrollment in that trial. Double autologous SCT and single SCT had been performed in 11 of 21 and in 8 of 21 patients, respectively. All 21 patients had previously received thalidomide-based treatment, while 14 of 21 patients had previously received lenalidomide; and 19 of 21 had received bortezomib-based treatment, with a median of 2 lines of prior bortezomib-based regimens (range: 1–5). It is notable that 14 of 21 patients had not responded to their last bortezomib-based therapy. Among 16 evaluable patients, 1 nCR, 7 PRs (50% nCR+PR rate) and 5 SDs were observed. Overall, stabilization or drop in M-protein was observed in 13 of 16 patients. These results were consistent with those reported in another multi-institution phase I clinical trial [33] which enrolled 17 patients with advanced MM: stabilization or decrease in M-protein was observed in all 17 patients (4 PRs, 2 MRs, 11 SDs) evaluable for response. These 2 trials indicate that combination of HDAC inhibitors with bortezomib, or perhaps other proteasome inhibitors, warrant further

evaluation in MM and a randomized clinical trial comparing the anti-MM activity of bortezomib plus vorinostat vs bortezomib alone is currently underway in MM.

The early preclinical results of SAHA (vorinostat) in MM stimulated further interest towards the study of other HDAC inhibitors, including hydroxamic acid-based HDAC inhibitors such as LAQ824 [35] and LBH589 [22]; as well as HDAC6-selective inhibitors, such as tubacin [36–38]. Tubacin primarily inhibits the activity of HDAC6 (e.g. tubulin deacetylation) in the cytosol [36,37]. Therefore, in contrast to hydroxamic acid inhibitors (vorinostat, LAQ824), tubacin functions more as a "cytoplasmic deacetylase" rather than as a "nuclear deacetylase", although reported preclinical data suggest a functional overlap between these 2 classes. For instance, LBH589 can inhibit deacetylase function in the cytoplasm [22,39]. LAQ824 and LBH589 have been shown to have direct anti-MM activity in preclinical models, while clinical trials of LBH589 have been initiated. Tubacin has limited, if any, single-agent in vitro anti-MM activity. However, it inhibits in preclinical in vitro studies the microtubule-associated deacetylase HDAC6, which supports the transport of misfolded protein aggregates to the aggresome. The formation of aggresomes, as a stress response to proteasome inhibition, and the inhibition of aggresome function by tubacin have been proposed to explain, at least in part, the enhanced in vitro activity of the combination of tubacin and bortezomib [38].

Perifosine – Akt inhibition

The PI-3K/Akt cascade mediates the proliferative/anti-apoptotic signaling events triggered by activation of upstream growth factor receptors [40–42], and consequently its components constitute promising therapeutic targets for MM. Extensive efforts have been made to develop small molecule inhibitors which have been shown in preclinical studies to target PI-3K (e.g. BEZ235) [43] or Akt (e.g. EXEL-6075) [44] and plans to translate these preclinical observations into clinical trials are in place. However, earlier in this decade, the development of these small molecule inhibitors was much further away from clinical trials. Therefore, the early focus on the clinical applications of Akt in MM was placed on perifosine, a synthetic novel alkylphospholipid which inhibits Akt activation. Perifosine inhibits constitutive and cytokine (IL-6, IGF-1)-induced Akt activation in MM cells and, therefore, induces MM cell death even when the MM cells are adherent to BMSCs cells. In addition, perifosine downregulates survivin levels [45]; augments Dex-, lenalidomide- or bortezomib-induced MM cell cytotoxicity; and has *in vivo* anti-MM activity in a human plasmacytoma mouse model, [46]. These preclinical data provided a platform for phase I/II studies of perifosine in combination with Dex, Bortezomib and/or lenalidomide in MM patients.

A phase II study of perifosine + low-dose (20 mg biw) Dex in relapsed/refractory MM patients (n=67) showed an overall response rate of 35% [47] which was encouraging given the heavily pre-treated patient population of this study (median of 4 prior lines of therapy, with 66% of patients being refractory to their last therapy). In addition, a phase I trial of the triplet of perifosine, lenalidomide and Dex in relapsed or refractory MM patients (n=30) has shown an overall response rate of 70%. This study involves a less heavily pre-treated patient population (1–4 lines of prior therapy, median of 2, 33% of patients being refractory to their last therapy) [48]

The combination of perifosine + bortezomib was evaluated in a phase I/II trial [49]. Bortezomib, administered at the standard dose (1.3 mg/m^2 dose, with dose reductions permitted to 1.0 and to 0.7 mg/m^2) and schedule (days 1, 4, 8 and 11 of 21-day cycles), was combined with oral perifosine (50 mg or 100 mg daily throughout each cycle). On progression, Dex was added (20 mg 4x/wk). This trial enrolled a heavily pre-treated population of patients: the median number of prior therapies was 5 (range 1 – 13, including a median of 2 lines of prior bortezomib-containing therapies, range 1–4). It is notable that among all patients in the trial, 83% were

relapsed and refractory, and 69% were bortezomib-refractory. Overall, prior treatment with bortezomib, Dex, lenalidomide, thalidomide or SCT had been administered in 100%, 98%, 75%, 74%, and 57% of patients respectively. No DLTs were reported in the phase I portion of the study, while the 50 mg qd of perifosine and 1.3 mg/m^2 of bortezomib were identified as being better tolerated, with longer duration of treatment beyond cycle 1. These doses were therefore selected for evaluation in the phase II part of the trial. Overall, no unexpected toxicities were observed in this trial. Thrombocytopenia, anemia, neutropenia, and pneumonia were the most frequent grade ≥3 adverse events, but were manageable with dose reduction and supportive care in this heavily pre-treated patient population. No grade 4 peripheral neuropathy was observed, only 1 patient had grade 3 neuropathy and treatment-emergent neuropathy of any grade was observed in only 16% of patients. In 72 evaluable patients (cutoff date of Nov 2008), best response analysis (according to EBMT/Uniform Criteria) showed that 3 patients (4%) achieved CR/nCR; 12 had PR (17%); and 12 had MR (17%), for an overall response rate (CR/nCR + PR + MR) of 38%, while 29 patients (40%) had SD. Among bortezomib-refractory patients, the combined CR+PR+MR rate to bortezomib plus perifosine was 15% and after addition of Dex was 31%. SD was maintained in 23% with bortezomib plus perifosine and in 44% of patients overall after addition of Dex. The median TTP in all evaluable patients was 6.3 months, and 6.2 months in bortezomib-refractory patients. These results indicated that this combination is active and well tolerated in previously bortezomib-treated pts with advanced disease [49].

Heat shock protein 90 inhibitors

Heat shock protein 90 (Hsp90) is expressed in both normal and neoplastic cells (as reviewed in [50,51]) and functions as a molecular chaperone for diverse client proteins. It helps them maintain their 3-dimensional structure in conformations essential to carry out their function. For example, various receptors for growth factors and cytokines; mutated or chimeric oncoproteins; and effectors of proliferative/anti-apoptotic signaling (e.g. Akt, IKK, Raf) are regulated by Hsp90 function. Therefore, Hsp90 has also been the topic of intense research in the field of oncology. Geldanamycin, the parent compound of the family of ansamycin inhibitors of Hsp90, as well as its various analogs, such as 17-allylamin-17-demethoxy-geldanamycin (17-AAG, or tanespimycin) bind to the ATP binding pocket of Hsp90 and perturb the ability of Hsp90 to execute its chaperoning function. As a result, the ansamycin Hsp90 inhibitors inhibit the function of several key anti-apoptotic/proliferative cascades in MM tumor cells and sensitize them to other therapeutics, including the proteasome inhibitor bortezomib [52]. These initial pre-clinical observations have been subsequently extended in vitro [53–58] and in vivo [53,55,59] by several groups in different experimental settings and with different Hsp90 inhibitors or formulations thereof.

The preclinical studies of Hsp90 inhibitors in MM led to a phase I trial of single-agent tanespimycin (17-AAG) administered in a cremophor-based formulation (KOS-953) [60]. That study showed that this formulation of 17-AAG can be administered safely, without the significant peripheral neuropathy associated with bortezomib or thalidomide. Importantly, although MTD for 17-AAG was not reached in that trial, signs of antitumor activity were observed, including durable clinical benefit (PR/MR or SD) in 9 out of 22 (41%) of the heavily pre-treated patients enrolled in the trial. Overall, the manageable profile of adverse events, especially the lack of peripheral neuropathy, as well as the evidence for anti-MM activity in some heavily pre-treated patients, coupled with the enhanced preclinical anti-MM activity of combinations of Hsp90 inhibitors with proteasome inhibitors provided the framework for a multicenter phase I trial of tanespimycin (17-AAG) and bortezomib [61]. In that trial, the profile of adverse events was manageable without significant cardiotoxicity, peripheral neuropathy or deep vein thrombosis. Clinical responses were observed at all dose levels of KOS-953 while the bortezomib dose was > 1.0 mg/m^2. The overall response rate (PR+MR+SD) was 71% for

bortezomib-naive patients. Among bortezomib-refractory patients (i.e. with progressive disease on bortezomib-containing regimen prior to study entry or no response to prior bortezomib exposure) 33% responded, while the response rate for bortezomib-pretreated, but not refractory, patients, was 38% [61]. The encouraging results from this phase I/II study of KOS953 + bortezomib, including the lack of additive adverse effects or pharmacokinetic interactions provided the basis for an ongoing randomized phase III trial of bortezomib plus KOS-953 vs. bortezomib alone.

The preclinical and early clinical experience with 17-AAG and other Hsp90 inhibitors of the ansamycin family has sparked new interest in the study of other Hsp90 inhibitors, such as the water soluble IPI-504 [55,62–65]; the resorcinylic isoxazole amide NVP-AUY922 [66]; and SNX-2112 [67].

Future directions

The treatment of MM has radically changed during the last decade, with the introduction of bortezomib, thalidomide and lenalidomide clearly improving the outcome of MM patients. However, curative treatments for MM patients are still not available and their development will require further research. The advances achieved in the last decade were largely driven by the development of novel classes of therapeutics, as opposed to incremental changes in the methods of using previously existing treatment paradigms. It is therefore logical to expect that major further progress will have to involve, at least to some extent, the development of new therapeutic drug classes that are currently not established for the treatment of MM. As the research efforts further intensify, additional drug classes will be developed, and hopefully some of them will receive FDA approval for the treatment of MM.

In the effort to develop combination regimens, it will be important to maintain a balance between the attempt to maximize the quality of response and avoiding additive or synergistic toxicity by the components of the combination. For example, the combination of lenalidomide, bortezomib and dexamethasone (RVD) showed encouraging safety and efficacy data in relapsed/refractory (MR or better of 73%, PR or better of 55%) [68] and newly diagnosed MM patients (PR or better of 100% so far, VGPR or better of 74% and CR/nCR of 44%) [69]. This prompted further studies of combination where additional agents are incorporated into the RVD backbone, such as the combination of RVD-cyclophosphamide [70]. While such combinations have high rates and pronounced depth of responses, as observed with RVD, it is important to emphasize the need to develop such combinations in a manner that does not lead to increased toxicity, a theme that has recently been highlighted by studies such as the ECOG trial of lenalidomide combination with low- vs. high-dose Dex [71] or the experience from other regimens combining proteasome inhibition, IMIDs, glucocorticoids and cytotoxic chemotherapy [72].

As more investigational agents are being tested preclinically and clinically in MM, it is tempting to compare the results of their early clinical studies with the historical experience of those initial clinical trials of thalidomide, lenalidomide, or bortezomib for MM treatment. An important cautionary note is that the early clinical development of these 3 currently established anti-MM agents (especially of thalidomide and bortezomib) took place at a time when the population of MM patients enrolled in clinical trials was less heavily pretreated than today. Indeed, the clinical trials reviewed in this article typically involved populations of patients of whom the majority had been previously exposed to bortezomib, thalidomide, and lenalidomide, including multiple combinations thereof. Therefore, it may not be realistic to expect that monotherapy with investigational anti-MM agents can match the early experience of thalidomide, bortezomib and lenalidomide, especially as resistance to not only glucocortioids

and/or conventional cytotoxics, but also multiple other recently established anti-MM agents and combinations has become a feature of the relapsed and refractory patient population.

Notwithstanding these considerations, it is important to note that the experience with "backbone" agents such as bortezomib, thalidomide and lenalidomide has already shown that it will be highly unlikely that any new drug class, no matter how potently active against MM, will be able to achieve curative responses in this disease as monotherapy. Combination regimens involving both novel and conventional anti-MM agents will likely remain a key strategy to improve the rates, depth, and durability of clinical benefit of any new drug classes that will be incorporated in the future therapeutic armamentarium against MM.

References

1. Kumar SK, Rajkumar SV, Dispenzieri A, Lacy MQ, Hayman SR, Buadi FK, et al. Improved survival in multiple myeloma and the impact of novel therapies. Blood 2008;111(5):2516–2520. [PubMed: 17975015]
2. Feling RH, Buchanan GO, Mincer TJ, Kauffman CA, Jensen PR, Fenical W. Salinosporamide A: a highly cytotoxic proteasome inhibitor from a novel microbial source, a marine bacterium of the new genus salinospora. Angew Chem Int Ed Engl 2003;42(3):355–357. [PubMed: 12548698]
3. Chauhan D, Catley L, Li G, Podar K, Hideshima T, Velankar M, et al. A novel orally active proteasome inhibitor induces apoptosis in multiple myeloma cells with mechanisms distinct from Bortezomib. Cancer Cell 2005;8(5):407–419. [PubMed: 16286248]
4. Paoluzzi L, Gonen M, Gardner JR, Mastrella J, Yang D, Holmlund J, et al. Targeting Bcl-2 family members with the BH3 mimetic AT-101 markedly enhances the therapeutic effects of chemotherapeutic agents in in vitro and in vivo models of B-cell lymphoma. Blood. 2008
5. Kuhn DJ, Chen Q, Voorhees PM, Strader JS, Shenk KD, Sun CM, et al. Potent activity of carfilzomib, a novel, irreversible inhibitor of the ubiquitin-proteasome pathway, against preclinical models of multiple myeloma. Blood 2007;110(9):3281–3290. [PubMed: 17591945]
6. Meng L, Mohan R, Kwok BH, Elofsson M, Sin N, Crews CM. Epoxomicin, a potent and selective proteasome inhibitor, exhibits in vivo antiinflammatory activity. Proc Natl Acad Sci U S A 1999;96 (18):10403–10408. [PubMed: 10468620]
7. Hideshima T, Richardson P, Chauhan D, Palombella VJ, Elliott PJ, Adams J, et al. The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. Cancer Res 2001;61(7):3071–3076. [PubMed: 11306489]
8. O'Connor OA, Orlowski RZ, Alsina M, Stewart K, Trudel S, Vallone MK, et al. Multicenter phase I studies to evaluate the safety, tolerability, and clinical response to intensive dosing with the proteasome inhibitor PR-171 in patients with relapsed or refractory hematological malignancies. Blood 2006;108 (11):687A–688A.
9. Jagannath S, Vij R, Stewart A, Somlo G, Jakubowiak A, Reiman R, et al. Initial Results of PX-171-003, An Open-Label, Single-Arm, Phase II Study of Carfilzomib (CFZ) in Patients with Relapsed and Refractory Multiple Myeloma (MM). Blood 2008;112(s)Abstract 864
10. Vij R, Wang M, Orlowski R, Stewart AK, Jagannath S, Kukreti V, et al. Initial Results of PX-171-004, An Open-Label, Single-Arm, Phase II Study of Carfilzomib (CFZ) in Patients with Relapsed Myeloma (MM). Blood. 2008
11. Richardson P, Hofmeister CC, Zimmerman TM, Chanan-Khan AA, Spear MA, Palladino MA, et al. Phase 1 Clinical Trial of NPI-0052, a Novel Proteasome Inhibitor in Patients with Multiple Myeloma. Blood. 2008;(112s)abstract 2770
12. Hideshima T, Chauhan D, Shima Y, Raje N, Davies FE, Tai YT, et al. Thalidomide and its analogs overcome drug resistance of human multiple myeloma cells to conventional therapy. Blood 2000;96 (9):2943–2950. [PubMed: 11049970]
13. Davies FE, Raje N, Hideshima T, Lentzsch S, Young G, Tai YT, et al. Thalidomide and immunomodulatory derivatives augment natural killer cell cytotoxicity in multiple myeloma. Blood 2001;98(1):210–216. [PubMed: 11418482]

14. Mitsiades N, Mitsiades CS, Poulaki V, Chauhan D, Richardson PG, Hideshima T, et al. Apoptotic signaling induced by immunomodulatory thalidomide analogs in human multiple myeloma cells: therapeutic implications. Blood 2002;99(12):4525–4530. [PubMed: 12036884]
15. Schey SA, Fields P, Bartlett JB, Clarke IA, Ashan G, Knight RD, et al. Phase I study of an immunomodulatory thalidomide analog, CC-4047, in relapsed or refractory multiple myeloma. J Clin Oncol 2004;22(16):3269–3276. [PubMed: 15249589]
16. Streetly M, Hunt BJ, Parmar K, Jones R, Zeldis J, Schey S. Markers of endothelial and haemostatic function in the treatment of relapsed myeloma with the immunomodulatory agent Actimid (CC-4047) and their relationship with venous thrombosis. Eur J Haematol 2005;74(4):293–296. [PubMed: 15777340]
17. Lacy MQ, Hayman SR, Gertz MA, Allred JB, Mandrekar SJ, Dispenzieri A, et al. Pomalidomide (CC4047) Plus Low-Dose Dexamethasone (Pom/dex) Is Highly Effective Therapy in Relapsed Multiple Myeloma. Blood 2008;112:866s.
18. Mitsiades N, Mitsiades CS, Richardson PG, McMullan C, Poulaki V, Fanourakis G, et al. Molecular sequelae of histone deacetylase inhibition in human malignant B cells. Blood 2003;101(10):4055–4062. [PubMed: 12531799]
19. Mitsiades N, Mitsiades CS, Poulaki V, Chauhan D, Fanourakis G, Gu X, et al. Molecular sequelae of proteasome inhibition in human multiple myeloma cells. Proc Natl Acad Sci U S A 2002;99(22):14374–14379. [PubMed: 12391322]
20. Mitsiades CS, Mitsiades NS, McMullan CJ, Poulaki V, Shringarpure R, Hideshima T, et al. Transcriptional signature of histone deacetylase inhibition in multiple myeloma: biological and clinical implications. Proc Natl Acad Sci U S A 2004;101(2):540–545. [PubMed: 14695887]
21. 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(11):3839–3852. [PubMed: 15173093]
22. Catley L, Weisberg E, Kiziltepe T, Tai YT, Hideshima T, Neri P, 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(10):3441–3449. [PubMed: 16728695]
23. Sutheesophon K, Kobayashi Y, Takatoku MA, Ozawa K, Kano Y, Ishii H, 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. [PubMed: 16424655]
24. Emanuele S, Lauricella M, Carlisi D, Vassallo B, D'Anneo A, Di Fazio P, et al. SAHA induces apoptosis in hepatoma cells and synergistically interacts with the proteasome inhibitor Bortezomib. Apoptosis 2007;12(7):1327–1338. [PubMed: 17351739]
25. Miller CP, Ban K, Dujka ME, McConkey DJ, Munsell M, Palladino M, et al. NPI-0052, a novel proteasome inhibitor, induces caspase-8 and ROS-dependent apoptosis alone and in combination with HDAC inhibitors in leukemia cells. Blood 2007;110(1):267–277. [PubMed: 17356134]
26. Baradari V, Hopfner M, Huether A, Schuppan D, Scherubl H. Histone deacetylase inhibitor MS-275 alone or combined with bortezomib or sorafenib exhibits strong antiproliferative action in human cholangiocarcinoma cells. World J Gastroenterol 2007;13(33):4458–4466. [PubMed: 17724801]
27. Nawrocki ST, Carew JS, Pino MS, Highshaw RA, Andtbacka RH, Dunner K Jr, et al. Aggresome disruption: a novel strategy to enhance bortezomib-induced apoptosis in pancreatic cancer cells. Cancer Res 2006;66(7):3773–3781. [PubMed: 16585204]
28. Place RF, Noonan EJ, Giardina C. HDAC inhibition prevents NF-kappa B activation by suppressing proteasome activity: down-regulation of proteasome subunit expression stabilizes I kappa B alpha. Biochem Pharmacol 2005;70(3):394–406. [PubMed: 15950952]
29. Kelly WK, Richon VM, O'Connor O, Curley T, MacGregor-Curtelli B, Tong W, et al. Phase I Clinical Trial of Histone Deacetylase Inhibitor: Suberoylanilide Hydroxamic Acid Administered Intravenously. Clin Cancer Res 2003;9(10):3578–3588. [PubMed: 14506144]
30. Richardson P, Mitsiades C, Colson K, Reilly E, McBride L, Chiao J, et al. Phase I trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) in patients with advanced multiple myeloma. Leuk Lymphoma 2008;49(3):502–507. [PubMed: 18297527]

31. Duvic M, Talpur R, Ni X, Zhang C, Hazarika P, Kelly C, et al. Phase II Trial of Oral Vorinostat (Suberoylanilide Hydroxamic Acid, SAHA) for Refractory Cutaneous T-cell Lymphoma (CTCL). Blood. 2006
32. Badros A, Philip S, Niesvizky R, Goloubeva O, Harris C, Zweibel J, et al. Phase I trial of suberoylanilide hydroxamic acid (SAHA) plus bortezomib (Bort) in relapsed multiple myeloma (MM) patients (pts). Blood 2007;110(11):354A–354A. [PubMed: 17376889]
33. Weber DM, Jagannath S, Mazumder A, Sobecks R, Schiller GJ, Gavino M, 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):355A–355A.
34. Weber D, Badros AZ, Jagannath S, Siegel D, Richon V, Rizvi S, et al. Vorinostat Plus Bortezomib for the Treatment of Relapsed/Refractory Multiple Myeloma: Early Clinical Experience. Blood. 2008;(112s)Abstract 871
35. Catley L, Weisberg E, Tai YT, Atadja P, Remiszewski S, Hideshima T, et al. NVP-LAQ824 is a potent novel histone deacetylase inhibitor with significant activity against multiple myeloma. Blood 2003;102(7):2615–2622. [PubMed: 12816865]
36. Haggarty SJ, Koeller KM, Wong JC, Butcher RA, Schreiber SL. Multidimensional chemical genetic analysis of diversity-oriented synthesis-derived deacetylase inhibitors using cell-based assays. Chem Biol 2003;10(5):383–396. [PubMed: 12770821]
37. Haggarty SJ, Koeller KM, Wong JC, Grozinger CM, Schreiber SL. Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6)-mediated tubulin deacetylation. Proc Natl Acad Sci U S A 2003;100(8):4389–4394. [PubMed: 12677000]
38. Hideshima T, Bradner JE, Wong J, Chauhan D, Richardson P, Schreiber SL, et al. Small-molecule inhibition of proteasome and aggresome function induces synergistic antitumor activity in multiple myeloma. Proc Natl Acad Sci U S A 2005;102(24):8567–8572. [PubMed: 15937109]
39. Bali P, Pranpat M, Bradner J, Balasis M, Fiskus W, Guo F, et al. Inhibition of histone deacetylase 6 acetylates and disrupts the chaperone function of heat shock protein 90: a novel basis for antileukemia activity of histone deacetylase inhibitors. J Biol Chem 2005;280(29):26729–26734. [PubMed: 15937340]
40. Tu Y, Gardner A, Lichtenstein A. The phosphatidylinositol 3-kinase/AKT kinase pathway in multiple myeloma plasma cells: roles in cytokine-dependent survival and proliferative responses. Cancer Res 2000;60(23):6763–6770. [PubMed: 11118064]
41. Hideshima T, Nakamura N, Chauhan D, Anderson KC. Biologic sequelae of interleukin-6 induced PI3-K/Akt signaling in multiple myeloma. Oncogene 2001;20(42):5991–6000. [PubMed: 11593406]
42. Mitsiades CS, Mitsiades N, Poulaki V, Schlossman R, Akiyama M, Chauhan D, et al. Activation of NF-kappaB and upregulation of intracellular anti-apoptotic proteins via the IGF-1/Akt signaling in human multiple myeloma cells: therapeutic implications. Oncogene 2002;21(37):5673–5683. [PubMed: 12173037]
43. McMillin DW, Negri J, Delmore J, Hayden P, Mitsiades N, Richardson PG, et al. Anti-myeloma activity of selective PI-3K/PDK/mTOR inhibitor BEZ235. Blood 2007;110(11):359A–359A.
44. McMillin DW, Negri JM, Delmore J, Ooi M, Hayden P, Mitsiades N, et al. Anti-myeloma activity of the dual Akt/p70(S6K) inhibitor EXEL-6075. Blood 2007;110(11):200A–200A.
45. Hideshima T, Catley L, Raje N, Chauhan D, Podar K, Mitsiades C, et al. Inhibition of Akt induces significant downregulation of survivin and cytotoxicity in human multiple myeloma cells. Br J Haematol 2007;138(6):783–791. [PubMed: 17760810]
46. Hideshima T, Catley L, Yasui H, Ishitsuka K, Raje N, Mitsiades C, et al. Perifosine, an oral bioactive novel alkylphospholipid, inhibits Akt and induces in vitro and in vivo cytotoxicity in human multiple myeloma cells. Blood 2006;107(10):4053–4062. [PubMed: 16418332]
47. Richardson PG, Lonial S, Jakubowiak A, Krishnan A, Wolf J, Singhal S, et al. Perifosine (KRX-0401) plus low dose dexamethasone is active in patients with relapsed and refractory multiple myeloma (MM): Perifosine MM investigator group phase II multicenter study update. Haematologica-the Hematology Journal 2007;92(6):155–155.
48. Jakubowiak A, Richardson P, Zimmerman TM, Alsina M, Kaufman JL, Harvey C, et al. Phase I Results of Perifosine (KRX-0401) in Combination with Lenalidomide and Dexamethasone in Patients with Relapsed or Refractory Multiple Myeloma (MM). Blood 2008;112:3691s.

49. Richardson P, Wolf J, Jakubowiak A, Zonder J, Lonial S, Irwin DH, et al. Phase I/II Results of a Multicenter Trial of Perifosine (KRX-0401) + Bortezomib in Patients with Relapsed or Relapsed/Refractory Multiple Myeloma Who Were Previously Relapsed from or Refractory to Bortezomib. Blood 2008;112(11s)Abstract 870
50. Powers MV, Workman P. Inhibitors of the heat shock response: Biology and pharmacology. FEBS Lett. 2007
51. Neckers L, Ivy SP. Heat shock protein 90. Curr Opin Oncol 2003;15(6):419–424. [PubMed: 14624223]
52. Mitsiades CS, Mitsiades NS, McMullan CJ, Poulaki V, Kung AL, Davies FE, et al. Antimyeloma activity of heat shock protein-90 inhibition. Blood 2006;107(3):1092–1100. [PubMed: 16234364]
53. Mitsiades CS, Mitsiades N, Rooney M, Hideshima T, Chauhan D, Munshi NC, et al. Anti-tumor activity of KOS-953, a cremophor-based formulation of the hsp90 inhibitor 17-AAG. Blood 2004;104(11):660A–661A.
54. Duus J, Bahar HI, Venkataraman G, Ozpuyan F, Izban KF, Al-Masri H, et al. Analysis of expression of heat shock protein-90 (HSP90) and the effects of HSP90 inhibitor (17-AAG) in multiple myeloma. Leuk Lymphoma 2006;47(7):1369–1378. [PubMed: 16923571]
55. Sydor JR, Normant E, Pien CS, Porter JR, Ge J, Grenier L, et al. Development of 17-allylamino-17-demethoxygeldanamycin hydroquinone hydrochloride (IPI-504), an anti-cancer agent directed against Hsp90. Proc Natl Acad Sci U S A 2006;103(46):17408–17413. [PubMed: 17090671]
56. Chatterjee M, Jain S, Stuhmer T, Andrulis M, Ungethum U, Kuban RJ, et al. STAT3 and MAPK signaling maintain overexpression of heat shock proteins 90alpha and beta in multiple myeloma cells, which critically contribute to tumor-cell survival. Blood 2007;109(2):720–728. [PubMed: 17003370]
57. Francis LK, Alsayed Y, Leleu X, Jia X, Singha UK, Anderson J, et al. Combination mammalian target of rapamycin inhibitor rapamycin and HSP90 inhibitor 17-allylamino-17-demethoxygeldanamycin has synergistic activity in multiple myeloma. Clin Cancer Res 2006;12(22):6826–6835. [PubMed: 17121904]
58. Davenport EL, Moore HE, Dunlop AS, Sharp SY, Workman P, Morgan GJ, et al. Heat shock protein inhibition is associated with activation of the unfolded protein response (UPR) pathway in myeloma plasma cells. Blood. 2007
59. Mitsiades CS, Mitsiades N, Rooney M, Negri J, Geer CC, Pink M, et al. IPI-504: A novel hsp90 inhibitor with in vitro and in vivo antitumor activity. Blood 2004;104(11):660A–660A.
60. Chanan-Khan AA, Richardson PG, Alsina M, Carroll M, Lonial S, Krishnan A, et al. Phase 1 clinical trial of KOS-953+Bortezomib (BZ) in relapsed refractory multiple myeloma (MM). Blood 2005;106(11):109a–110a.
61. Richardson, P.; Chanan-Khan, A.; Lonial, S.; Krishnan, A.; Alsina, M.; Carroll, M., et al. A multicenter phase 1 clinical trial of tanespimycin (KOS-953) + bortezomib (BZ): encouraging activity and manageable toxicity in heavily pre-treated patients with relapsed refractory multiple myeloma (MM). 2006 Annual Meeting of the American Society of Hematology; Orlando FL. 2006.
62. Patterson J, Palombella VJ, Fritz C, Normant E. IPI-504, a novel and soluble HSP-90 inhibitor, blocks the unfolded protein response in multiple myeloma cells. Cancer Chemother Pharmacol 2008;61(6):923–932. [PubMed: 17624530]
63. Siegel D, Jagannath S, Mazumder A, Vesole D, Borrello I, Sydor J, et al. Update on phase I clinical trial of IPI-504, a novel, water-soluble Hsp90 inhibitor, in patients with relapsed/refractory multiple myeloma (MM). Blood 2006;108(11):1022A–1022A.
64. Demetri GD, George S, Morgan JA, van den Abbeele A, Quigley MT, Fletcher JA, et al. Overcoming resistance to tyrosine kinase inhibitors (TKIs) through inhibition of Heat Shock Protein 90 (Hsp90) chaperone function in patients with metastatic GIST: results of a Phase I Trial of IPI-504, a water-soluble Hsp90 inhibitor. Ejc Supplements 2006;4(12):173–173.
65. Jagannath S, Siegel D, Richardson P, Mazumder A, Sydor J, Goddard J, et al. Phase I clinical trial of IPI-504, a novel, water-soluble Hsp90 inhibitor, in patients with Relapsed/Refractory multiple myeloma (MM). Blood 2005;106(11):719A–720A.
66. McMillin DW, Negri J, Delmore J, Hayden P, Ooi M, Mitsiades N, et al. Activity of new heat shock protein 90 (hsp90) inhibitor NVP-AUY922 against myeloma cells sensitive and resistant to conventional agents. Blood 2007;110(11):474A–474A.

67. Okawa Y, Hideshima T, Steed P, Vallet S, Hall S, Huang K, et al. SNX-2112, a selective Hsp90 inhibitor, potently inhibits tumor cell growth, angiogenesis, and osteoclastogenesis in multiple myeloma and other hematological tumors by abrogating signaling via Akt and ERK. Blood. 2008

68. Richardson P, Jagannath S, Raje N, Jakubowiak A, Lonial S, Ghobrial I, et al. Lenalidomide, bortezomib, and dexamethasone (Rev/Vel/Dex) in patients with relapsed or relapsed/refractory multiple myeloma (MM): Preliminary results of a phase II study. Blood 2007;110(11):797A–797A.

69. Richardson P, Lonial S, Jakubowiak A, Jagannath S, Raje NS, Avigan D, et al. Lenalidomide, bortezomib, and dexamethasone in patients with newly diagnosed multiple myeloma: encouraging efficacy in high risk groups with updated results of a phase I/II study. Blood 2008;112:92s.

70. Kumar S, Flinn IW, Noga SJ, Hari P, Rifkin RM, Callander N, et al. Safety and efficacy of novel combination therapy with bortezomib, dexamethasone, cyclophosphamide, and lenalidomide in newly diagnosed multiple myeloma: initial results from the phase I/II multi-center EVOLUTION Study. Blood 2008;112:93s.

71. Rajkumar SV, Jacobus S, Callander N, Fonseca R, Vesole D, Williams M, et al. A randomized trial of lenalidomide plus high-dose dexamethasone (RD) versus lenalidomide plus low-dose dexamethasone (Rd) in newly diagnosed multiple myeloma (E4A03): A trial coordinated by the eastern cooperative oncology group. Blood 2007;110(11):74. [PubMed: 17371947]

72. Bensinger W, Jagannath S, Vescio R, Camacho ES, Wolf JL, Irwin DH, et al. A Phase II Study of Bortezomib (Velcade ®), Cyclophosphamide (Cytoxan®), Thalidomide (Thalomid®) and Dexamethasone as First-Line Therapy for Multiple Myeloma. Blood 2008;112:94s.