

Oncogenomics to Target Myeloma in the Bone Marrow Microenvironment

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Abstract

Multiple myeloma (MM) is an example of rapid bench-to-bedside translation in new drug development. Bortezomib and lenalidamide target the tumor cell in the bone marrow microenvironment to overcome drug resistance in laboratory and animal models; each is effective to treat relapsed and/or refractory, relapsed, and newly diagnosed MM, and both are now showing promise as maintenance therapy. Major ongoing translational research efforts include improved classification and personalized therapies; identification and validation of next-generation agents targeting the tumor cell in its microenvironment; novel immune therapies; rationally based combination therapies; and use of novel agents to delay or prevent development of active MM. This paradigm of targeting the tumor in its microenvironment has already extended median survival in MM from 3 to 7 to 8 years and has great potential to improve patient outcome in other hematologic malignancies and solid tumors as well. *Clin Cancer Res*; 17(6); 1225–33. ©2011 AACR.

Introduction

Over the past decade, progress in the treatment of multiple myeloma (MM) has transformed our therapeutic approaches and improved the outcome of affected patients (1). Melphalan and prednisone was used to treat this disease in 1962, and median patient survival increased to 2 to 3 years. High-dose melphalan followed by bone marrow (BM) transplantation, in the 1980s, and peripheral blood stem cell grafting in the 1990s increased median survival to 3 to 4 years. Since the recognition that thalidomide overcomes conventional drug resistance in 1998, MM has become a remarkable example of rapid bench-to-bedside translation in new drug development. The novel proteasome inhibitor bortezomib and immunomodulatory drug (IMiD) lenalidomide target the MM cell in the BM microenvironment to overcome cell adhesion drug resistance (CAM-DR) to conventional therapy in laboratory and animal models (2). For example, lenalidomide directly triggers caspase 8–mediated apoptosis; decreases binding of tumor cells to BM; inhibits constitutive and MM cell binding–induced secretion of cytokines from BM; inhibits angiogenesis; and stimulates autologous natural killer (NK), T-, and NK–T-cell immunity to MM cells (3–5). Similarly, binding of MM cells to BM upregulates the ubiquitin proteasome cascade at a transcriptional and activity level; therefore, MM cell binding to BM confers

sensitivity to bortezomib. Bortezomib directly targets chymotryptic proteasome activity, inhibits tumor growth and survival, induces apoptosis, upregulates heat shock proteins, inhibits DNA damage repair, and induces endoplasmic reticulum stress in MM cells; downregulates adhesion molecules on tumor and BM, thereby abrogating adhesion; and, importantly, targets the microenvironment to trigger antiangiogenesis, as well as apoptosis of osteoclasts, while promoting osteoblast differentiation (6–11).

Both bortezomib and lenalidomide were rapidly translated from the bench to the bedside. Each was first shown to be effective to treat relapsed refractory MM, and then relapsed MM; each was then combined with melphalan and prednisone to treat the elderly patient with newly diagnosed MM or with dexamethasone to treat the transplant candidate (12–21). This integration of lenalidomide or bortezomib into initial therapies has achieved unprecedented extent and frequency of response, progression-free survival (PFS), and overall survival (OS) in patients with newly diagnosed MM. To date there have been 6 U.S. Food and Drug Administration (FDA) approvals incorporating novel agents in the last 7 years, and median survival of MM patients has extended from 3 to 7 years as a direct result (22). Most recently, lenalidomide maintenance therapy has been shown to add years of PFS in both newly diagnosed transplant and nontransplant candidates, even further improving patient outcome. Most importantly, combination therapy of newly diagnosed MM with lenalidomide, bortezomib, and dexamethasone achieves 100% responses, with 74% at least very good partial and 52% complete or near complete responses (19). Given these unprecedented results, a clinical trial is now evaluating whether high-dose therapy and stem cell transplantation adds value in the context of this high extent and frequency of response to combined novel therapies. Table 1 lists a number of ongoing clinical trials in which the outcome is likely to further shift treatment paradigms in this disease.

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Table 1. Selected High Impact Clinical Trials Ongoing or Nearing Completion in Both Newly Diagnosed Multiple Myeloma and in the Relapsed Setting

Newly diagnosed MM	Clinical trial status, Institution
A randomized phase III trial of CC-5013 (lenalidomide, NSC-703813) and low-dose dexamethasone (LLD) versus bortezomib (PS-341, NSC-681239), lenalidomide, and low-dose dexamethasone (BLLD) for induction, in patients with previously untreated multiple myeloma without an intent for immediate autologous stem cell transplant. NCT00644228	Recruiting, SWOG, NCI
A phase III, randomized, open-label, 3-arm study to determine the efficacy and safety of lenalidomide (REVLIMID) plus low-dose dexamethasone when given until progressive disease or for 18 4-week cycles versus the combination of melphalan, prednisone, and thalidomide given for 12 6-week cycles in patients with previously untreated multiple myeloma who are either 65 years of age or older or not candidates for stem cell transplantation. NCT00689936	Recruiting, Celgene
A trial of single autologous transplant with or without consolidation therapy versus tandem autologous transplant with lenalidomide maintenance for patients with multiple myeloma (BMT CTN 0702). NCT01109004	Recruiting, NHLBI
A phase III randomized, double-blind study of maintenance therapy with CC-5013 (NSC # 703813, IND #70116) or placebo following autologous stem cell transplantation for multiple myeloma. NCT00114101	Fully enrolled, CALGB
A randomized phase III study to compare bortezomib, melphalan, prednisone (VMP) with high-dose melphalan followed by bortezomib, lenalidomide, dexamethasone (VRD) consolidation and lenalidomide maintenance in patients with newly diagnosed multiple myeloma. NCT01208766	Not yet recruiting, Stichting Hemato-Oncologie voor Volwassenen Nederland
Safety and efficacy of lenalidomide as maintenance therapy in patients with newly diagnosed multiple myeloma following a tandem autologous-allogeneic transplant. NCT01264315	Recruiting, Fondazione Neoplasie Sangue Onlus
A randomized, open-label, phase I study of CNTO 328 (anti-IL-6 monoclonal antibody) and VELCADE-melphalan-prednisone compared with VELCADE-melphalan-prednisone for the treatment of previously untreated multiple myeloma. NCT00911859	Recruiting, Centocor, Inc.
A randomized, phase III study comparing conventional-dose treatment using a combination of lenalidomide, bortezomib, and dexamethasone (RVD) to high-dose treatment with peripheral stem cell transplant in the initial management of myeloma in patients up to 65 years of age. NCT01208662	Recruiting, Dana-Farber Cancer Institute
Phase III study of lenalidamide, dexamethasone followed by melphalan, prednisone, lenalidamide versus high-dose melphalan and stem cell transplantation twice followed by maintenance lenalidomide or no maintenance.	Fully enrolled GEMIMA
Phase III study of high-dose melphalan and stem cell transplantation followed by lenalidamide versus no consolidation followed by lenalidomide versus no maintenance.	Fully enrolled IFM
A phase III, multicenter, randomized open label study of velcade, melphalan, prednisone, and thalidomide (V-MPT) versus velcade, melphalan, prednisone (V-MP) in elderly untreated multiple myeloma patients. NCT01063179	Fully enrolled GEMIMA Fondazione Neoplasie Sangue Onlus
Phase III trial of melphalan prednisone versus melphalan, prednisone, lenalidomide versus melphalan, prednisone, lenalidomide, and lenalidomide maintenance.	Fully enrolled
Relapsed Setting	
A randomized, multicenter, phase III study comparing carfilzomib, lenalidomide, and dexamethasone (CRd) versus lenalidomide and dexamethasone (Rd) in subjects with relapsed multiple myeloma. NCT01080391	Recruiting, Onyx Therapeutics, Inc.
A phase III randomized study to assess the efficacy and safety of perifosine added to the combination of bortezomib and dexamethasone in multiple myeloma patients. NCT01002248	Recruiting, Keryx-AOI Pharmaceuticals, Inc.
A phase III, randomized, open label trial of lenalidomide-dexamethasone with or without elotuzumab in relapsed or refractory multiple myeloma. NCT01239797	Not yet recruiting, Bristol-Myers Squibb
An international, multicenter, randomized, double-blind study of vorinostat (MK0683) or placebo in combination with bortezomib in patients with multiple myeloma. NCT00773747	Ongoing, Merck
A multicenter, randomized, double-blind, placebo controlled phase III study of panobinostat in combination with bortezomib and dexamethasone in patients with relapsed multiple myeloma. NCT01023308	Recruiting, Novartis Pharmaceuticals

Abbreviations: SWOG, Southwest Oncology Group; NCI, National Cancer Institute; NHLBI, National Heart, Lung, and Blood Institute; CALGB, Cancer and Leukemia Group B; IFM, Intergroupe Francophone du Myélome.

Major ongoing translational research goals that are likely to generate further progress in the field include the following: determining the underlying genetics and epigenetics; improved classification and development of personalized novel agents in MM; development of next-generation novel therapies targeting MM cells in the BM microenvironment; development of immune therapies; and development of rationally based combination therapies. The first critical goal is to understand the genetic basis of myelomagenesis. DNA-based array comparative genomic hybridization (aCGH) and single nucleotide polymorphism (SNP) array studies are both identifying copy number alterations and suggesting novel MM oncogenes or suppressor genes, which serve as potential therapeutic targets (23, 24). Moreover, DNA-based classification of uniformly treated patients is now also possible. Most importantly, recent genome sequencing studies have revealed mutated genes involved in protein translation, histone methylation, and blood coagulation. Importantly, personalized medicine must include profiling patients over time, as early studies now show continued evolution of genetic changes with progressive MM.

Second, functional validation of novel targets and directed inhibitors using our preclinical *in vitro* and *in vivo* models of MM cells in the BM milieu has allowed rapid translation to clinical trials (Fig. 1), and further investigation is likely to generate additional targets. Novel therapies

include those targeting the MM cell surface [elotuzomab monoclonal antibody (MoAb), cytokines in the BM milieu [anti-B-cell activating factor (BAFF) MoAb], or both tumor and the microenvironment [novel proteasome inhibitors and IMiDs, plasmacytoid dendritic cell (pDC) differentiation agents, and anti-dickkopf-1 (DKK-1) MoAb]. CS-1 is universally expressed at the gene and protein level in patient MM cells, and bedside-back-to-bench studies validated its role in MM cell survival (25). A derived clinical trial of elotuzomab (anti-CS-1) MoAb in relapsed refractory MM revealed stable disease, but did not trigger clinical responses to warrant its further development as a single agent. However, our studies showed that lenalidomide augments antibody-dependent cellular cytotoxicity (25), and a derived clinical trial of this combination now shows great promise. BAFF is elevated in the BM plasma of patients with MM and mediates tumor cell survival and drug resistance. Our preclinical studies show that anti-BAFF MoAb can neutralize these effects (26), and a related clinical trial is ongoing. Bortezomib has stimulated great interest in targeting protein homeostasis as a novel treatment strategy in MM. Moreover, although bortezomib rapidly moved from the bench to the bedside and FDA and European Medicine Agency (EMA) approval first in relapsed and refractory, then in relapsed, and most recently in newly diagnosed MM (16–18), not all patients have disease that responds to bortezomib, and those with

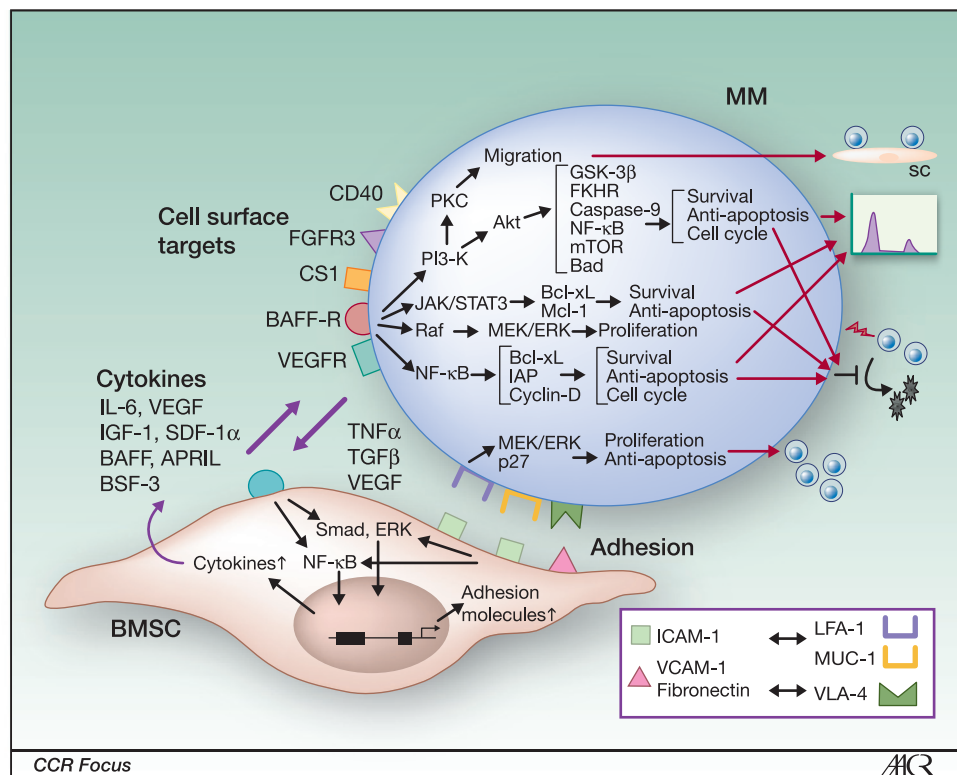


Figure 1. Genetic validation of novel targets mediating growth of the MM cell in the BM microenvironment. BMSC, bone marrow stem cell; IGF, insulin like growth factor; TNF, tumor necrosis factor; PI3K, phosphoinositide 3-kinase; JAK, Janus activated kinase; MEK, MAP/ERK kinase; ERK, extracellular signal regulated kinase. Figure adapted with permission from Hideshima et al. (80).

response ultimately develop resistance. Our preclinical studies show that inhibitors of deubiquitinating enzymes upstream of the proteasome, such as USP-7 inhibitor P5091, have anti-MM activity. More potent inhibitors of chymotryptic activity (CEP-18770, carfilzomib, onyx 9412; refs. 27–29) can overcome bortezomib resistance in preclinical and early clinical trials (1, 2, 30, 31). NPI-0052 targets chymotryptic, tryptic-like, and caspase-like activities, and, similarly, shows clinical promise (30). Inhibitors of the immunoproteasome, such as the PR-924 inhibitor of the LMP-7 immunoproteasome subunit, also block MM growth *in vitro* and *in vivo* (32). Next-generation IMiD pomalidomide shows more potent activity than lenalidomide against tumor cells in laboratory and animal models of MM cells in the BM microenvironment (3), and phase I-II clinical trials now show responses even in patients whose MM is resistant to lenalidomide and/or bortezomib. Finally, 2 additional strategies illustrate the potential of novel agents targeting the tumor in its microenvironment, namely that targeting the microenvironment can have indirect anti-MM activity as well. First, MM cells secrete DKK-1, which downregulates osteoblast function via targeting Wnt signaling. A derived clinical trial of BHQ880 anti-DKK-1 MoAb is ongoing, directed to improving MM bone disease; importantly, however, in preclinical murine xenograft models of human MM, the BHQ880 MoAb not only triggers new bone formation, but also inhibits MM cell growth (33). Second, our recent studies show that pDCs in the MM BM microenvironment promote tumor cell growth, survival, and drug resistance, and that treatment strategies targeting pDCs can inhibit tumor cell growth (34).

Third, novel strategies are now attempting to deliver on the promise of immune-based therapies. Humoral and cellular immune defects characteristic of MM include decreased TH1 and increased TH2 cells; increased TH17-associated cytokines; and increased T-regulatory cells (35). Importantly, therapies are now being directed at these abnormalities, such as, anti-interleukin-17 (IL-17) MoAb (36). Moreover, our studies show that IMiDs can augment T-, NK-, and NK-T-cell autologous anti-MM immunity (5, 37, 38). Indeed, personalized immune-based therapy may now enhance the likelihood of success. Specifically, genomic studies can now define cell surface targets on MM cells for vaccination and/or adoptive immunotherapy. Peptides from these validated antigens, chosen because they can be processed and presented in patients of specific human leukocyte antigen (HLA) types, are then used to vaccinate appropriate patients. In this way, genomics is used to define the targets, and genetics to define the appropriate host likely to respond. Ultimately, vaccination with cocktails of such peptides may be applicable to treat minimal residual disease more broadly in patients across a spectrum of HLA types.

Fourth, preclinical studies are informing the design of combination novel agent clinical trials in MM, thereby markedly enhancing the likelihood of success. Several examples can be cited. For example, bortezomib was

shown to inhibit DNA damage repair *in vitro* (9), providing the rationale for its combination with DNA-damaging agents to enhance or overcome drug resistance. Indeed, a large randomized phase III clinical trial of bortezomib versus bortezomib with pegylated doxorubicin in patients with relapsed and/or refractory MM increased extent and frequency of response, and prolonged PFS by 3 months and OS (39), leading to the FDA approval of bortezomib with pegylated doxorubicin to treat relapsed MM. In a second example, Hsp 27 was found to be increased at a transcript and protein level in patient MM cells in the setting of bortezomib refractoriness. Bedside-back-to-bench studies showed that overexpression of Hsp 27 conferred bortezomib resistance, whereas knockdown of Hsp 27 in bortezomib-resistant MM cells restored sensitivity (40). Laboratory studies showed that p38 mitogen activated protein kinase (MAPK) inhibitor decreased downstream Hsp 27 and overcame bortezomib resistance in MM cell lines and patient cells (41), providing the rationale for a clinical trial of bortezomib and p38 MAPK inhibitor. A third example is derived from laboratory observations that bortezomib triggers activation of Akt, and that the combination of bortezomib with the Akt inhibitor perifosine can sensitize or overcome resistance to bortezomib in preclinical models (42). In derived phase I-II trials, combination therapy acted similarly, and a phase III clinical trial of bortezomib versus bortezomib with perifosine in relapsed MM is currently ongoing for FDA approval. As a fourth example, we have shown that inhibition of the proteasome upregulates aggresomal degradation of protein and, conversely, that blockade of aggresomal degradation induces compensatory upregulation of proteasomal activity (43). Most importantly, blockade of aggresomal and proteasomal degradation of proteins by histone deacetylase inhibitors (vorinostat, panbinostat, tubacin) and proteasome inhibitors (bortezomib, carfilzomib), respectively, triggers synergistic MM cell cytotoxicity in preclinical studies (43–45). The histone deacetylase inhibitors vorinostat or panbinostat with bortezomib have already achieved responses in the majority of patients with relapsed bortezomib-refractory MM, and phase III clinical trials for FDA registration of these combinations are currently ongoing. We have shown synergistic cytotoxicity induced by combined lenalidomide (caspase 8-mediated apoptosis) and bortezomib (caspase 9-mediated apoptosis) in models of MM cells in the BM milieu (46); remarkably, bench-to-bedside translation to clinical trials shows 58% responses in relapsed refractory MM, often refractory to either agent alone (47). These examples show the great promise of scientifically informed, combination clinical trials. Finally, we have recently developed a new high-throughput tumor cell-specific bioluminescence platform to identify stroma-induced changes to anticancer drug activity, which is useful for identifying combinations of multiple drugs across dose ranges and in various sequences to inform design of multiple targeted agent clinical trials (48).

This issue of *CCR Focus* is aimed at understanding the major paradigm shifts that have characterized our

understanding and influenced the treatment of MM. Marked genetic heterogeneity has been shown in MM, which has implications for tumor pathogenesis, prognosis, and treatment. Here, Munshi and Avet-Loiseau update current information on genetic alterations, including amplifications and deletions in the MM genome and their clinical implications (49). For conventional therapy, hyperdiploid and t(11;14) have defined standard-risk MM with superior outcome, whereas nonhyperdiploid, t(4:14), del(17p), and del(13q14) have defined high-risk MM with inferior outcome. Importantly, however, novel therapies, such as bortezomib, can overcome the adverse outcome conferred by some, t(4:14), but not other, del(17p), abnormalities (50); the latter, in particular, continues to define high-risk disease. Currently, mRNA (microarray profiling), DNA (aCGH and SNP), and microRNA (miRNA) profiling studies of clinically annotated samples from uniformly treated patients are allowing the development of refined patient stratification and personalized medicine in MM. Munshi and Avet-Loiseau describe the utility of microarray profiling to define transcriptional changes that occur with evolution from monoclonal gammopathy of undetermined significance (MGUS), to smoldering multiple myeloma (SMM), to active MM (51), as well as transcript-based prognostic classification systems that have evolved to most recently include up to 10 subgroups of MM (52–55). These genetic and molecularly distinct subgroups of MM differ biologically, which also has important treatment implications; for example, fibroblast growth factor receptor 3 (FGFR3) inhibitor therapy may be useful in t(4:14) MM and rituxan therapy in CD 20–positive MM. Indeed, microarray profile–based models have now been proposed to define standard versus high-risk MM (56, 57), which is required not only to better stratify patients but also to allow comparison of outcomes in patient subgroups following novel targeted agents. DNA profiling is similarly defining disease heterogeneity with clinical implications. For example, aCGH has provided the basis for the first DNA-based prognostic classification of MM (23). In addition, recent SNP analyses of clinically annotated samples identified chromosome copy number changes, including increased 1q and 5q as sites for putative MM oncogenes, as well as decreased 12p as a site of putative MM suppressor genes, which predict clinical outcome (24). Most recently, miRNA profiling studies have been shown to distinguish normal plasma cells from MM cell lines; patients whose tumors resemble the former have improved outcome versus patients with tumors resembling cell lines. Finally, recent sequencing of MM patient samples has not only identified both known and novel mutations in newly diagnosed MM, but has also delineated changes with progression of disease. These advances have great potential to advance the promise of personalized medicine in MM.

Landgren, Kyle, and Rajkumar next describe clinical, as well as genetic and molecular, studies characterizing the evolution of MGUS to SMM to active myeloma (58). MGUS is characterized by <3 g/L monoclonal protein

and <10% BM plasma cells, with no associated hypercalcemia, renal dysfunction, anemia, or bone disease (CRAB; ref. 59). SMM has ≥ 3 g/L monoclonal protein or $\geq 10\%$ BM plasma cells, again without CRAB. Active MM has $\geq 10\%$ BM plasma cells, usually with associated monoclonal protein, and at least 1 or more features of CRAB. Although Dr. Kyle has carefully followed individuals with MGUS and identified clinical features predicting for progression (60), it has only recently been appreciated that all MM patients likely go through this precursor MGUS stage (61, 62). Given that the risk of progression of MGUS to MM or lymphoproliferative disorder is only 1% per year, and most patients, therefore, die of something else, the recommended approach has been expectant follow-up with no therapy (63, 64). Recent analyses, however, suggest that monoclonal protein >1.5 g/L, non-immunoglobulin G (IgG), and abnormal kappa to lambda ratio can predict for likelihood of progression, with an adjusted risk of progression of 27% at 20 years if all 3 are present. High-risk SMM likely to progress to active MM can similarly be defined on the basis of type and amount of protein, abnormal kappa to lambda ratio, as well as presence of immunophenotypically abnormal BM plasma cells, identified using multiparameter flow cytometry (65–67). Excitingly, the ability to define individuals at high risk for progression, coupled with the availability of novel agents with very favorable side effect profiles, is now allowing for clinical trials directed to delay or prevent progression to active MM. For example, Mateos and colleagues randomized patients with high-risk SMM to the combination of lenalidomide and dexamethasone for 9 4-week cycles or to a control cohort: median time to progression in the control arm was 19 months versus not reached in the treated cohort, with 16 of 47 patients progressing in the control versus no patients in the treated group. Given that patients with SMM have greater immune repertoire maintained than those with active MM, immune-based therapies, such as vaccination, also hold great promise for such prevention trials. Eventually, the spectrum of patients eligible for therapy may expand to include high-risk SMM.

Palumbo, Attal, and Roussel next describe up-to-date clinical trial results integrating novel therapies into the initial management of MM, which has transformed MM treatment (68). The use of thalidomide, lenalidomide, or bortezomib with dexamethasone as induction therapy in transplant candidates has achieved high response rates both before and after high-dose therapy. For example, the bortezomib and dexamethasone combination is superior to vincristine, adriamycin, and dexamethasone (VAD); high very good partial response after induction portends superior outcome, and at least very good partial response rates after a single high-dose therapy and transplant have decreased the use of second autologous transplantation (69). Three-drug regimens, such as bortezomib and dexamethasone with thalidomide or lenalidomide (19, 70, 71), are superior to 2-drug regimens, but, to date, 4-drug regimens have not resulted in further improvement (20). Either lenalidomide or bortezomib consolidation therapy

has been shown to upgrade the extent of response. Importantly, lenalidomide maintenance has been shown to prolong PFS posttransplant in 2 large randomized trials (72, 73). Ongoing trials are, indeed, now testing whether high-dose therapy and stem cell transplant adds to the high extent and frequency of responses attained with combination novel agents. Similar progress has been made with the addition of novel therapies to melphalan and prednisone (MP) therapy for the newly diagnosed nontransplant candidate. In particular, lenalidomide used with MP followed by lenalidomide maintenance has markedly extended PFS, further supporting lenalidomide maintenance therapy in MM (74). The combination of bortezomib and MP has achieved high overall and extent of response, and bortezomib maintenance is similarly under evaluation (18), which may become even more feasible owing to the recently shown efficacy of bortezomib administered via subcutaneous injection.

Lonial, Mitsiades, and Richardson update current treatment options for relapsed and refractory MM, reviewing a number of promising future targeted agents (75). Approved agents for relapsed and/or refractory MM include bortezomib (12, 13, 18), lenalidomide and dexamethasone (14, 15), as well as bortezomib with pegylated doxorubicin (39), each of which has achieved high response rates and both prolonged PFS and OS in the setting of relapsed and relapsed and/or refractory MM. Indeed the improved OS of MM patients today is related not only to improvements in induction therapies described above, but also to the ability to now effectively treat relapsed disease. Advances continue to evolve in several areas. First, we are learning to better use available novel therapies. For example, bortezomib used weekly (20), and most recently used via subcutaneous administration, can achieve comparable efficacy with less toxicity, especially neuropathy. Second,

next-generation IMiDs, such as pomalidamide (3), and proteasome inhibitors, such as carfilzomib (28) or NPI-0052(30), can overcome resistance to current therapies in preclinical studies and ongoing clinical trials. And importantly, combination therapies predicated upon science can achieve responses even in relapsed refractory MM. For example, the addition of Akt inhibitor perifosine (42) or histone deacetylase inhibitors panabinstat or vorinostat (43–45) to bortezomib can overcome bortezomib resistance; and the combination of lenalidomide with bortezomib achieves 58% responses even when MM is resistant to either agent alone (47). Ongoing studies are now defining combinations of 3 or more novel agents and hold great promise.

Finally, Raje and Roodman describe parallel advances in the management of bone-related complications in MM (76). The advent of aminobisphosphonate therapy in the early 1990s, for the first time, allowed effective therapy to delay and decrease bone-related complications (77), the major cause of morbidity and compromise of quality of life in MM patients. Enhanced understanding of pathogenesis of MM bone disease now offers multiple other therapeutic options, based upon either inducing osteoclast apoptosis or enhancing osteoblast function, that is, activin A (78). As noted above, for example, inhibiting DKK-1 can augment osteoblast function and restore bone integrity in MM (33). Excitingly, such bone-directed therapies can also have anti-MM activities, best illustrated by the prolongation of survival attributable to aminobisphosphonate use in the recent Myeloma Research Council trial, evaluating zoledronic versus clodronic acid treatment in patients receiving either initial intensive or nonintensive therapy (Fig. 2; ref. 79). Conversely, novel agents developed to target MM also have beneficial effects on bone, best illustrated by bortezomib, which induces osteoclast death and promotes

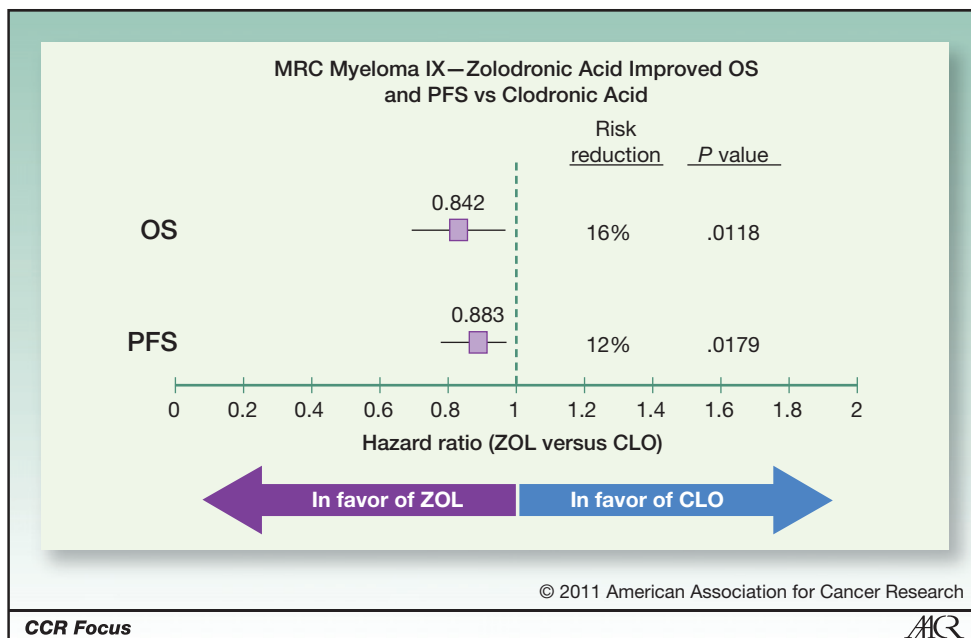


Figure 2. Therapies targeting bone prolong PFS and OS. Patients with newly diagnosed MM received either intensive versus nonintensive therapy. They were also randomized to receive either zoledronic (ZOL) or clodronic acid (CLO). ZOL significantly reduced relative risk of death by 16% versus CLO (hazard ratio = 0.842; 95% confidence interval = 0.736-0.963; $P = 0.0118$). ZOL prolonged OS by 5 months ($P = 0.04$). Cox model adjusted for chemotherapy and minimization factors.

osteoblast activity, thereby resulting in new bone formation in treated patients (11, 13). Finally, current studies are identifying those MM patients with ongoing bone resorption, in order to selectively treat those patients most likely to benefit from bone-targeted agents, while avoiding attendant complications in those patients not requiring these therapies.

In conclusion, MM is an example of rapid bench-to bedside translation of novel agents and improved patient outcome. This new treatment paradigm targeting the tumor

cell in its microenvironment has great promise to improve patient outcome not only in MM, but also in hematologic malignancies and solid tumors as well.

Disclosure of Potential Conflicts of Interest

K.C. Anderson, advisory board, Celgene, Novartis, Bristol-Myers Squibb, Onyx, Merck, Millennium; ownership interest, Acetylon.

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References

- Laubach JP, Richardson PG, Anderson KC. The evolution and impact of therapy in multiple myeloma. *Med Oncol* 2010; 27 [Suppl 1]: S1–6.
- Hideshima T, Mitsiades C, Tonon G, Richardson PG, Anderson KC. Understanding multiple myeloma pathogenesis in the bone marrow to identify new therapeutic targets. *Nat Rev Cancer* 2007;7:585–98.
- 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; 96:2943–50.
- Lentzsch S, LeBlanc R, Podar K, Davies F, Lin B, Hideshima T, et al. Immunomodulatory analogs of thalidomide inhibit growth of Hs Sultan cells and angiogenesis *in vivo*. *Leukemia* 2003;17:41–4.
- Görgün G, Calabrese E, Soydan E, Hideshima T, Perrone G, Bandi M, et al. Immunomodulatory effects of lenalidomide and pomalidomide on interaction of tumor and bone marrow accessory cells in multiple myeloma. *Blood* 2010;116:3227–37.
- 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;61:3071–6.
- 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;99:14374–9.
- LeBlanc R, Catley LP, Hideshima T, Lentzsch S, Mitsiades CS, Mitsiades N, et al. Proteasome inhibitor PS-341 inhibits human myeloma cell growth *in vivo* and prolongs survival in a murine model. *Cancer Res* 2002;62:4996–5000.
- Hideshima T, Mitsiades C, Akiyama M, Hayashi T, Chauhan D, Richardson P, et al. Molecular mechanisms mediating antimyeloma activity of proteasome inhibitor PS-341. *Blood* 2003;101:1530–4.
- Chauhan D, Hideshima T, Anderson KC. Targeting proteasomes as therapy in multiple myeloma. *Adv Exp Med Biol* 2008;615:251–60.
- Mukherjee S, Raje N, Schoonmaker JA, Liu JC, Hideshima T, Wein MN, et al. Pharmacologic targeting of a stem/progenitor population *in vivo* is associated with enhanced bone regeneration in mice. *J Clin Invest* 2008;118:491–504.
- Richardson P, Barlogie B, Berenson J, Singhal S, Jagannath S, Irwin D, et al. A phase 2 study of bortezomib in relapsed, refractory myeloma. *N Engl J Med* 2003;348:2609–17.
- Richardson PG, Sonneveld P, Schuster MW, Irwin D, Stadtmauer EA, Facon T, 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; 352:2487–98.
- Weber DM, Chen C, Niesvizky R, Wang M, Belch A, Stadtmauer EA, et al. Multiple Myeloma (009) Study Investigators. Lenalidomide plus dexamethasone for relapsed multiple myeloma in North America. *N Engl J Med* 2007;357:2133–42.
- Dimopoulos M, Spencer A, Attal M, Prince HM, Harousseau JL, Dmoszynska A, et al. Multiple Myeloma (010) Study Investigators. Lenalidomide plus dexamethasone for relapsed or refractory multiple myeloma. *N Engl J Med* 2007;357:2123–32.
- Richardson P, Schlossman R, Weller E, Hideshima T, Mitsiades C, Davies F, et al. The Immunomodulatory drug CC 5013 overcomes drug resistance and is well tolerated in patients with relapsed multiple myeloma. *Blood* 2002;100:3063–7.
- Richardson P, Blood E, Mitsiades CS, Jagannath S, Zeldenrust SR, Alsina M, et al. A randomized phase 2 trial of lenalidomide therapy for patients with relapsed or relapsed and refractory multiple myeloma. *Blood* 2006;15:3458–64.
- San Miguel JF, Schlag R, Khuageva NK, Dimopoulos MA, Shpilberg O, Kropff M, et al. VISTA Trial Investigators. Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. *N Engl J Med* 2008;359:906–17.
- Richardson PG, Weller E, Lonial S, Jakubowiak AJ, Jagannath S, Raje NS, et al. Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma. *Blood* 2010;116:679–86.
- Palumbo A, Bringhen S, Rossi D, Cavalli M, Larocca A, Ria R, et al. Bortezomib-melphalan-prednisone-thalidomide followed by maintenance with bortezomib-thalidomide compared with bortezomib-melphalan-prednisone for initial treatment of multiple myeloma: a randomized controlled trial. *J Clin Oncol* 2010;28:5101–9.
- Palumbo A. First-line treatment of elderly multiple myeloma patients. *Clin Adv Hematol Oncol* 2010;8:529–30.
- 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;111:2516–20.
- Carrasco DR, Tonon G, Huang Y, Zhang Y, Sinha R, Feng B, et al. High-resolution genomic profiles define distinct clinico-pathogenetic subgroups of multiple myeloma patients. *Cancer Cell* 2006;9:313–25.
- Avet-Loiseau H, Li C, Magrangeas F, Gouraud W, Charbonnel C, Harousseau JL, et al. Prognostic significance of copy-number alterations in multiple myeloma. *J Clin Oncol* 2009;27:4585–90.
- Tai YT, Dillon M, Song W, Leiba M, Li XF, Burger P, et al. Anti-CS1 humanized monoclonal antibody HuLuc63 inhibits myeloma cell adhesion and induces antibody-dependent cellular cytotoxicity in the bone marrow milieu. *Blood* 2008;112:1329–37.
- Neri P, Kumar S, Fulciniti MT, Vallet S, Chhetri S, Mukherjee S, et al. Neutralizing B-cell activating factor antibody improves survival and inhibits osteoclastogenesis in a severe combined immunodeficient human multiple myeloma model. *Clin Cancer Res* 2007;13:5903–9.
- Parlati F, Lee SJ, Aujay M, Suzuki E, Levitsky K, Lorens JB, et al. Carfilzomib can induce tumor cell death through selective inhibition of the chymotrypsin-like activity of the proteasome. *Blood* 2009; 114:3439–47.
- Zhou HJ, Aujay MA, Bennett MK, Dajee M, Demo SD, Fang Y, et al. Design and synthesis of an orally bioavailable and selective peptide epoxyketone proteasome inhibitor (PR-047). *J Med Chem* 2009;52:3028–38.
- Chauhan D, Singh AV, Aujay M, Kirk CJ, Bandi M, Ciccarelli B, et al. A novel orally active proteasome inhibitor ONX 0912 trigger *in vitro* and *in vivo* cytotoxicity in multiple myeloma. *Blood* 2010;116:4906–15.
- 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;8:407–19.
- O'Connor OA, Stewart AK, Vallone M, Molineaux CJ, Kunkel LA, Gerecitano JF, et al. A phase 1 dose escalation study of the safety

- and pharmacokinetics of the novel proteasome inhibitor carfilzomib (PR-171) in patients with hematologic malignancies. *Clin Cancer Res* 2009;15:7085–91.
32. Singh AV, Bandi M, Aujay MA, Kirk CJ, Hark DE, Raju N, et al. PR-924, a selective inhibitor of the immunoproteasome subunit LMP-7, blocks multiple myeloma cell growth both in vitro and in vivo. *Br J Haematol* 2011;152:155–63.
 33. Fulciniti M, Tassone P, Hideshima T, Vallet S, Nanjappa P, Ettenberg SA, et al. Anti-DKK1 mAb (BHQ880) as a potential therapeutic agent for multiple myeloma. *Blood* 2009;114:371–9.
 34. Chauhan D, Singh AV, Brahmandam M, Carrasco R, Bandi M, Hideshima T, et al. Functional interaction of plasmacytoid dendritic cells with multiple myeloma cells: a therapeutic target. *Cancer Cell* 2009;16:309–23.
 35. Prabhala RH, Neri P, Bae JE, Tassone P, Shammas MA, Allam CK, et al. Dysfunctional T regulatory cells in multiple myeloma. *Blood* 2006;107:301–4.
 36. Prabhala RH, Pelluru D, Fulciniti M, Prabhala HK, Nanjappa P, Song W, et al. Elevated IL-17 produced by TH17 cells promotes myeloma cell growth and inhibits immune function in multiple myeloma. *Blood* 2010;115:5385–92.
 37. Davies FE, Raju 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;98:210–6.
 38. Hayashi T, Hideshima T, Akiyama M, Podar K, Yasui H, Raju N, et al. Molecular mechanisms whereby immunomodulatory drugs activate natural killer cells: clinical application. *Br J Haematol* 2005;128:192–203.
 39. Orlowski RZ, Nagler A, Sonneveld P, Bladé J, Hajek R, Spencer A, et al. Randomized phase III study of pegylated liposomal doxorubicin plus bortezomib compared with bortezomib alone in relapsed or refractory multiple myeloma: combination therapy improves time to progression. *J Clin Oncol* 2007;25:3892–901.
 40. Chauhan D, Li G, Shringarpure R, Podar K, Ohtake Y, Hideshima T, et al. Blockade of Hsp27 overcomes Bortezomib/proteasome inhibitor PS-341 resistance in lymphoma cells. *Cancer Res* 2003;63:6174–7.
 41. Hideshima T, Podar K, Chauhan D, Ishitsuka K, Mitsiades C, Tai YT, et al. p38 MAPK inhibition enhances PS-341 (bortezomib)-induced cytotoxicity against multiple myeloma cells. *Oncogene* 2004;23:8766–76.
 42. Hideshima T, Catley L, Yasui H, Ishitsuka K, Raju 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;107:4053–62.
 43. Hideshima T, Bradner J, Wong J, Chauhan D, Richardson P, Schreiber SL, et al. Small molecule inhibition of proteasome and aggresome function induces synergistic anti-tumor activity in multiple myeloma. *Proc Natl Acad Sci U S A* 2005;102:8567–72.
 44. Mitsiades CS, Mitsiades NS, MuMullan 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;101:540–5.
 45. 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;108:3441–9.
 46. 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;99:4525–30.
 47. Richardson PG, Weller E, Jagannath S, Avigan DE, Alsina M, Schlossman RL, et al. Multicenter, phase I, dose-escalation trial of lenalidomide plus bortezomib for relapsed and relapsed/refractory multiple myeloma. *J Clin Oncol* 2009;27:5713–9.
 48. McMillin DW, Delmore J, Weisberg E, Negri JM, Geer DC, Klippel S, et al. Tumor cell-specific bioluminescence platform to identify stroma-induced changes to anticancer drug activity. *Nat Med* 2010;16:483–9.
 49. Munshi NC, Avet-Loiseau H. Genomics in multiple myeloma. *Clin Cancer Res* 2011;17:1234–42.
 50. Jagannath S, Richardson PG, Sonneveld P, Schuster MW, Irwin D, Stadtmauer EA, et al. Bortezomib appears to overcome the poor prognosis conferred by chromosome 13 deletion in phase 2 and 3 trials. *Leukemia* 2007;21:151–7.
 51. Davies FE, Dring AM, Li C, Rawstron AC, Shammas MA, O'Connor SM, et al. Insights into the multistep transformation of MGUS to myeloma using microarray expression analysis. *Blood* 2003;102:4504–11.
 52. Zhan F, Hardin J, Kordsmeier B, Bumm K, Zheng M, Tian E, et al. Global gene expression profiling of multiple myeloma, monoclonal gammopathy of undetermined significance, and normal bone marrow plasma cells. *Blood* 2002;99:1745–57.
 53. Magrangeas F, Nasser V, Avet-Loiseau H, Loriod B, Decaux O, Granjeaud S, et al. Gene expression profiling of multiple myeloma reveals molecular portraits in relation to the pathogenesis of the disease. *Blood* 2003;101:4998–5006.
 54. Zhan F, Huang Y, Colla S, Stewart JP, Hanamura I, Gupta S, et al. The molecular classification of multiple myeloma. *Blood* 2006;108:2020–8.
 55. Broyl A, Hose D, Lokhorst H, de Knegt Y, Peeters J, Jauch A, et al. Gene expression profiling for molecular classification of multiple myeloma in newly diagnosed patients. *Blood* 2010;116:2543–53.
 56. Shaughnessy JD Jr, Zhan F, Burington BE, Huang Y, Colla S, Hanamura I, et al. A validated gene expression model of high-risk multiple myeloma is defined by deregulated expression of genes mapping to chromosome 1. *Blood* 2007;109:2276–84.
 57. Decaux O, Lodé L, Magrangeas F, Charbonnel C, Gouraud W, Jézéquel P, et al. Intergroupe Francophone du Myélome. Prediction of survival in multiple myeloma based on gene expression profiles reveals cell cycle and chromosomal instability signatures in high-risk patients and hyperdiploid signatures in low-risk patients: a study of the Intergroupe Francophone du Myélome. *J Clin Oncol* 2008;26:4798–805.
 58. Landgren O, Kyle RA, Rajkumar SV. From myeloma precursor disease to multiple myeloma: New diagnostic concepts and opportunities for early intervention. *Clin Cancer Res* 2011;17:1243–52.
 59. Berenson JR, Anderson KC, Audell RA, Boccia RV, Coleman M, Dimopoulos MA, et al. Monoclonal gammopathy of undetermined significance: a consensus statement. *Br J Haematol* 2010;150:28–38.
 60. Kyle RA, Therneau TM, Rajkumar SV, Offord JR, Larson DR, Plevak MF, et al. A long-term study of prognosis in monoclonal gammopathy of undetermined significance. *N Engl J Med* 2002;346:564–9.
 61. Weiss BM, Abadie J, Verma P, Howard RS, Kuehl WM. A monoclonal gammopathy precedes multiple myeloma in most patients. *Blood* 2009;113:5418–22.
 62. Landgren O, Kyle RA, Pfeiffer RM, Katzmann JA, Caporaso NE, Hayes RB, et al. Monoclonal gammopathy of undetermined significance (MGUS) consistently precedes multiple myeloma: a prospective study. *Blood* 2009;113:5412–7.
 63. Kyle RA, Durie BG, Rajkumar SV, Landgren O, Blade J, Merlini G, et al. International Myeloma Working Group. Monoclonal gammopathy of undetermined significance (MGUS) and smoldering (asymptomatic) multiple myeloma: IMWG consensus perspectives risk factors for progression and guidelines for monitoring and management. *Leukemia* 2010;24:1121–7.
 64. Kyle RA, Therneau TM, Rajkumar SV, Larson DR, Plevak MF, Offord JR, et al. Prevalence of monoclonal gammopathy of undetermined significance. *N Engl J Med* 2006;354:1362–9.
 65. Rajkumar SV, Lacy MQ, Kyle RA. Monoclonal gammopathy of undetermined significance and smoldering multiple myeloma. *Blood Rev* 2007;21:255–65.
 66. Kyle RA, Remstein ED, Therneau TM, Dispenzieri A, Kurtin PJ, Hodnefield JM, et al. Clinical course and prognosis of smoldering (asymptomatic) multiple myeloma. *N Engl J Med* 2007;356:2582–90.
 67. Pérez-Persona E, Vidrales MB, Mateo G, García-Sanz R, Mateos MV, de Coca AG, et al. New criteria to identify risk of progression in monoclonal gammopathy of uncertain significance and smoldering multiple myeloma based on multiparameter flow cytometry analysis of bone marrow plasma cells. *Blood* 2007;110:2586–92.

68. Palumbo A, Attal M, Roussel M. Shifts in the therapeutic paradigm for patients newly diagnosed with multiple myeloma: maintenance therapy and overall survival. *Clin Cancer Res* 2011;17:1253–63.
69. Harousseau JL, Attal M, Avet-Loiseau H, Marit G, Caillot D, Mohty M, et al. Bortezomib plus dexamethasone is superior to vincristine plus doxorubicin plus dexamethasone as induction treatment prior to autologous stem-cell transplantation in newly diagnosed multiple myeloma: results of the IFM 2005-01 phase III trial. *J Clin Oncol* 2010;28:4621–9.
70. Cavo M, Attal M, Gertz MA, Giralt S, Ludwig H, Morgan GJ, et al. The current landscape of multiple myeloma treatment. *Leuk Res* 2008;32 [Suppl 1]: S1–24.
71. Attal M, Harousseau JL. The role of high-dose therapy with autologous stem cell support in the era of novel agents. *Semin Hematol* 2009;46:127–32.
72. McCarthy P, Owzar K, Anderson K. Phase III intergroup study of lenalidomide versus placebo maintenance therapy following single autologous stem cell transplan for multiple myelo: CALGB 100104. *J Clin Oncol* 2010;28:577s.
73. Attal M, Crisini C, Marit G. Lenalidomide maintenance after transplantation for myeloma. *J Clin Oncol* 2010;28:577s.
74. Palumbo A, Dimopoulos M, Delforge M. A phase III study to determine the efficacy and safety of lenalidomide in combination with melphalan and prednisone in elderly patients with newly diagnosed multiple myeloma. *Blood* 2009;114 [Suppl]: 253.
75. Lonial S, Mitsiades C, Richardson P. Treatment options for relapsed and refractory multiple myeloma. *Clin Cancer Res* 2011;17:1264–77.
76. Raje N, Roodman GD. Advances in the biology and treatment of bone disease in multiple myeloma. *Clin Cancer Res* 2011;17:1278–86.
77. Berenson J, Lichtenstein A, Porter L, Dimopoulos MA, Bordoni R, George S, et al. Myeloma Aredia Study Group. Efficacy of pamidronate in reducing skeletal events in patients with advanced multiple myeloma. *N Engl J Med* 1996;334:488–93.
78. Vallet S, Mukherjee S, Vaghela N, Hideshima T, Fulciniti M, Pozzi S, et al. Activin A promotes multiple myeloma-induced osteolysis and is a promising target for myeloma bone disease. *Proc Natl Acad Sci U S A* 2010;107: 5124–9.
79. Morgan GJ, Davies FE, Gregory WM, Cocks K, Bell SE, Szubert AJ, et al. National Cancer Research Institute Haematological Oncology Clinical Study Group. First-line treatment with zoledronic acid as compared with clodronic acid in multiple myeloma (MRC Myeloma IX): a randomised controlled trial. *Lancet* 2010;376: 1989–99.
80. Hideshima T, Bergsagel PL, Kuehl WM, Anderson KC. Advances in biology of multiple myeloma: clinical applications. *Blood* 2004;104: 607–18.

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