The 39th David A. Karnofsky Lecture: Bench-to-Bedside Translation of Targeted Therapies in Multiple Myeloma

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ABSTRACT

Multiple myeloma (MM) is a remarkable example of rapid bench-to-bedside translation in new drug development. The proteasome inhibitor bortezomib and immunomodulatory drug lenalidomide targeted MM cells in the bone marrow (BM) microenvironment to overcome conventional drug resistance in laboratory and animal models and were rapidly translated into clinical trials demonstrating their efficacy in patients with relapsed and then newly diagnosed MM, with a doubling of the median survival as a direct result. The future is even brighter. First, immune-based therapies are being developed (eg, elotuzumab monoclonal antibody [MoAb]; CD138DM immunotoxin; MM cell–dendritic cell vaccines; CD138, CS-1, and XBP-1 peptide vaccines; anti-17 MoAb; and other treatments to overcome causes of immune dysfunction). Second, promising next-generation agents target the MM cell in its microenvironment (eg, deubiquitinating enzyme inhibitors; chymotryptic [carfilzomib, Onyx 0912, MLN 9708] and broader [NPI-0052] proteasome inhibitors; immunoproteasome inhibitors; and pomalidamide). Moreover, agents targeting bone biology (eg, zoledronic acid, anti–DKK-1 MoAb, anti–B-cell activating factor MoAb and bortezomib, Btk inhibitor) show promise not only in preserving bone integrity but also against MM. Third, rationally based combination therapies, including bortezomib with Akt, mammalian target of rapamycin, or histone deacetylase inhibitors, are active even in bortezomib-refractory MM. Finally, genomics is currently being used in the definition of MM heterogeneity, new target discovery, and development of personalized therapy. Myeloma therefore represents a paradigm for targeting the tumor in its microenvironment, which has already markedly improved patient outcome in MM and has great potential in other hematologic malignancies and solid tumors as well.


INTRODUCTION

Multiple myeloma (MM) is characterized by excess monoclonal plasma cells in the bone marrow (BM), in most cases associated with monoclonal protein in blood and/or urine. With the use of combined melphalan and prednisone nearly 50 years ago, median patient survival of patients with MM was extended to 2 to 3 years. Originally pioneered by Tim McElwain in the 1970s, high-dose melphalan followed by BM transplantation in the 1980s and with peripheral blood stem-cell rescue in the 1990s further increased patient median survival to 3 to 4 years. Since 1998, MM has represented a new paradigm in drug development because of the remarkable therapeutic efficacy of targeting tumor cells in their microenvironments. In particular, the observation that proteasome inhibitor bortezomib and immunomodulatory drugs (IMiDs) thalidomide and lenalidomide target the MM cell in the BM microenvironment has rapidly translated from bench to bedside and six new US Food and Drug Administration–approved treatments in the past 7 years, with a doubling of patient survival from 3 to 4 to 7 to 8 years as a direct result. Our contributions have been in the areas of identifying novel targets in the tumor and microenvironment, validating inhibitors directed at these targets, and conducting clinical trials leading to their approval. These collaborative efforts have included basic and clinical investigators, the pharmaceutical industry, the National Cancer Institute, US Food and Drug Administration regulators, and patient advocacy groups, with a common focus and inspired by the sole goal of improving MM treatments. Indeed, the use of novel targeted inhibitors to treat relapsed refractory MM, relapsed MM, and newly diagnosed MM and most recently as consolidation and maintenance therapies has totally transformed MM therapy and patient outcome.

I have been carrying out bench-to-bedside research in MM for 38 years now, initially inspired by my mentor, Dr Richard L. Humphrey, who taught me the two most important lessons that have shaped my research and clinical practice. As a
medical student at Johns Hopkins, he instilled in me the opportunity in MM to “make science count for patients” by developing laboratory and animal models of disease and then rapidly translating promising leads from the bench to the bedside in clinical trials. Moreover, he impressed in me the importance of treating patients as family. He has served as my inspiration and role model ever since.

DEVELOPMENT OF IMMUNE-BASED THERAPIES

After an introduction to MM both in the laboratory and clinic at Johns Hopkins during my medical school and internal medicine training, I moved to the Dana-Farber Cancer Institute for training in medical oncology, hematology, and tumor immunology. There Drs George Canellos and Robert Mayer instilled in me the importance of clinical investigation. Under the tutelage of Drs Lee Nadler and Stuart Schlossman, I was part of a team that developed monoclonal antibodies (MoAbs) directed at B-cell malignancies, including MM.5,6 It was an extraordinary time, because these MoAbs allowed for identification of the lineage and stage of differentiation of B-cell malignancies as well as comparison of the neoplastic B cell with its normal cellular counterpart. A panel of B-cell MoAbs was useful to complement histopathologic diagnosis and identify non–T-cell acute lymphoblastic leukemia, chronic lymphocytic leukemia and lymphomas, and MM as tumors corresponding to pre–B cells, isotype diversity B differentiative stages, and plasma cells, respectively.5

Right from the outset, these MoAbs were also used in innovative treatment strategies in MM, and our efforts to develop immune–MoAb and immunotoxin therapies, tumor vaccines, and mechanisms to abrogate host immunosuppression continue to the present. Specifically, high-dose therapy and autologous BM transplantation achieved remarkable extent and frequency of response, and early on, we examined whether cocktails of MoAbs (eg, CD10, CD20, PCA-1) could purge MM cells from autografts ex vivo before autologous BM transplantation.7 Although effective at purging two to three logs of MM cells, impact on overall outcome was unaffected, likely because of residual systemic tumor burden. T cell (CD6)–directed MoAb was used to purge T cells from allogeneic BM grafts to abrogate graft-versus-host disease.8 However, the transplant-related mortality of allotransplantation in MM remains unacceptably high to the present, and we continue to carry out studies to identify targets of allogeneic graft-versus-myeloma effect8 and clinical protocols of nonmyeloablative allografting to exploit graft-versus-myeloma effect while avoiding attendant toxicity. Over many years, we have continued to carry out preclinical and clinical studies of MoAbs targeting MM cells, tumor-host interactions, and cytokines as well as evaluated MoAb-based immunotoxin therapies.1,10,11 (Fig 1). For example, we identified CS-1 to be highly and uniformly expressed at the gene and protein levels in corresponding to pre–B cells, isotype diversity B differentiative stages, and plasma cells, respectively.5

Fig 1. Monoclonal antibody therapeutic targeting of multiple myeloma (MM). Monoclonal antibodies evaluated in clinical trials mediate antibody-dependent cellular cytotoxicity (ADCC) or complement mediated cytotoxicity (CDC) as well as directly target growth or apoptotic signaling pathways. IL, interleukin. Data adapted.12

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development of vaccines. Vasair et al15 have shown in murine MM and Rosenblatt et al16 in human MM that vaccination with fusions of dendritic cells (DCs) with tumor cells allows for generation of T- and B-cell tumor–specific responses in vitro and in vivo preclinical models; derived recent clinical trials of MM-DC vaccinations to treat minimal residual disease posttransplantation are triggering host antitumor T-cell and humoral responses associated with high rates of complete response. An alternative strategy is the use of cocktails of peptides for vaccination. Specifically, we have shown that CS-1, XBP-1, and CD138 are functionally significant targets in MM cells and derived peptides from these antigens, which can be presented and trigger cytotoxic T lymphocyte responses in human leukocyte antigen A2–positive patients.17 Ongoing clinical trials are evaluating vaccination with cocktails of these peptides in patients most likely to respond, with the goal of triggering immune responses with clinical significance.

We have also characterized the underlying immunodeficiency in patients with MM to design strategies to overcome it.18 Our studies have demonstrated decreased help, increased suppression, pro-MM growth cytokines, and dysregulated immune-homeostasis, always with a view toward mechanism and clinical application. For example, the demonstration of increased TH-17 cytokines promoting MM cell growth set the stage for a related clinical trial of anti–interleukin-17 MoAb in MM.18 In our studies of host accessory cells, we have shown that plasmacytoid DCs (pDCs) in patients with MM do not induce immune effector cells, as do normal pDCs, but instead promote tumor growth, survival, and drug resistance.19 In preclinical studies, maturation of pDCs with CpG oligonucleotides both restores immune stimulatory function of pDCs and abrogates their tumor-promoting activity, setting the stage for a derived clinical trial.

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From the 1990s to the present, we have developed in vitro and in vivo models to define the role of MM-BM interactions in pathogenesis, identify novel targets, and validate novel targeted therapies. We have then gone on to translate multiple single and combination agents targeting the tumor and microenvironment from bench to bedside in clinical trials. We have also used oncogenomics to characterize pathogenesis, identify novel targets, predict response, and inform design of single-agent and combination clinical trials.

Specifically, we have developed models of MM in the BM microenvironment that have been useful in defining the role of tumor cell–BM accessory cell contact as well as cytokines in the BM milieu in conferring growth, survival, and drug resistance in MM. Importantly, these models have allowed for the identification of agents that can overcome cell adhesion–mediated drug resistance to conventional therapies. The proteasome inhibitor bortezomib, for example, triggers MM cell cytotoxicity in the BM, whereas antitumor activity of bortezomib can overcome cell adhesion–mediated drug resistance to conventional therapies. Both at gene transcript and proteasome activity levels, the ubiquitin proteasome cascade is enhanced activity in this context. Bortezomib directly targets chymotryptic proteasome activity, inhibits 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. It was rapidly translated from the bench to the bedside and received accelerated US Food and Drug Administration approval in 2003 for treatment of relapsed refractory MM, followed by approval for relapsed MM and as initial therapy based on its superiority in randomized phase III clinical trials.

Most recently, promising data supporting bortezomib as consolidation and maintenance therapy have been emerging. However, not all MMs respond to bortezomib, and some tumors ultimately develop resistance. From the outset, we have therefore tried to identify gene signatures of response versus resistance to bortezomib in MM as well as develop functional assays to better predict patients whose cancers are most likely to respond. For example, we developed a predictive model in which tumors like MM with high proteasome load and low proteasome capacity have high proteasome stress and are therefore susceptible to proteasome inhibition, whereas solid tumors with high proteasome capacity and low proteasome load are relatively resistant to proteasome inhibitors. Importantly, bortezomib has opened a whole new area of preclinical and clinical experimentation in cancer targeting the ubiquitin proteasome cascade upstream of the proteasome with deubiquitinating inhibitors, selectively or more broadly targeting proteasome activity, and targeting the immunoproteasome (Fig 2). For example, our preclinical studies show that inhibitors of deubiquitinating enzymes upstream of the proteasome, such as USP-7 inhibitor P5091, inhibit human MM cell growth, and prolong host survival in a murine xenograft model. Carfilzomib, a next-generation, more potent intravenous inhibitor of chymotryptic activity, can overcome bortezomib resistance in preclinical and early clinical trials. Oral proteasome inhibitors targeting chymotryptic activity that have translated from the bench to bedside in phase I clinical trials include Onyx 0912, which triggers cytotoxicity against MM cell lines and patient cells, and MLN2238/9708, which has shown more potent preclinical activity against MM cells in vivo than bortezomib. NPI-0052 targets chymotryptic, tryptic-like, and caspase-like activities and similarly shows clinical promise. Finally, 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.⁴⁰

Since the empiric observation that thalidomide had anti-MM activity in 1998, we have studied the IMiDs thalidomide, lenalidomide, and pomalidamide in our models of MM in the BM microenvironment. These agents directly trigger caspase-8–mediated apoptosis; decrease binding of tumor cells to BM; inhibit constitutive and MM cell binding–induced secretion of cytokines from BM; inhibit angiogenesis; and stimulate autologous natural killer, T, and natural killer–T cell immunity to MM cells.⁴¹–⁴⁳ Like bortezomib, lenalidomide was rapidly translated from the bench to the bedside. Our preclinical studies demonstrated increased responses when lenalidomide (which triggers caspase 8–mediated apoptosis) was combined with dexamethasone (which induces caspase 9–mediated apoptosis), and our phase I and II clinical trials both established the enhanced clinical efficacy of combined lenalidomide with dexamethasone, informing the maximum-tolerated dose and confirmed the enhanced clinical efficacy in both newly diagnosed transplantation and maintenance lenalidomide has been shown to add years of progression-free and overall survival as well as increased extent and frequency of response,⁵⁷ leading to the US Food and Drug Administration/European Medicines Agency approval to treat relapsed MM.⁴⁹,⁵⁰ Trials of lenalidomide as initial therapy in both transplantation candidate and elderly populations, as well as consolidation and maintenance therapy, are promising.⁴⁹,⁵⁰ For example, maintenance lenalidomide has been shown to add years of progression-free survival in both newly diagnosed transplantation and nontransplantation candidates, further improving patient outcome. More recently, we and others have shown that the second-generation IMiD pomalidamide achieves remarkable and durable responses, with a favorable adverse effect profile, even in the setting of MM resistant to lenalidomide and bortezomib.⁵¹,⁵²

**Therapies Targeting Accessory Cells with Anti-MM Activity**

Bortezomib and lenalidomide are examples of targeting the tumor and also affecting the microenvironment, because both positively affect bone disease in MM.²⁸,⁵³ Conversely, we have also had a long-term interest in targeting the MM BM microenvironment, with the goal of also triggering MM responses (Fig 4). For example, MM cells secrete DKK-1, which downregulates osteoblast function via targeting Wnt signaling. In our preclinical murine xenograft models of human MM, the neutralizing anti–DKK-1 BHQ880 MoAb not only triggers new bone formation but also inhibits MM cell growth,⁵⁵ and a derived clinical trial of BHQ880 MoAb is ongoing. We have also shown that B-cell activating factor is elevated in the BM plasma of patients with MM and mediates osteoclastogenesis as well as tumor cell survival and drug resistance; importantly, anti–B-cell activating factor MoAb can neutralize these effects,⁵⁶ and a related clinical trial is ongoing. Most recently, targeting BTK in our preclinical models has not only blocked osteoclast formation and growth, thereby maintaining bone integrity, but also inhibited MM cell growth. These studies illustrate the principle that targeting cytokines or accessory cells in the tumor microenvironment can also affect MM cell growth, further validating the utility of our in vitro and in vivo model systems.

We have also used functional oncogenomics to inform the design of novel combination therapies. For example, bortezomib was shown to inhibit DNA damage repair in vitro,²⁶ 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 showed prolonged progression-free and overall survival as well as increased extent and frequency of response,⁵⁷ leading to the US Food and Drug Administration approval of bortezomib with pegylated doxorubicin to treat relapsed MM. In a second example, we found heat shock protein 27 (Hsp 27) to be increased at transcript and protein levels in patient MM cells in the setting of bortezomib refractoriness. Our 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.66 Hideshima et al.69 then showed that p38 mitogen-activated protein kinase inhibitor decreased downstream Hsp 27 and thereby overcame bortezomib resistance in MM cell lines and patient cells, providing the rationale for a clinical trial of bortezomib and p38 mitogen-activated protein kinase inhibitor. Third, on the basis of hallmark cyclin D abnormalities in MM, Raje et al.60,61 have studied cyclin D kinase inhibitors, alone and in combination, in MM. Fourth, Ghobrial et al.62 have translated promising preclinical data of mammalian target of rapamycin inhibitor and bortezomib into derived clinical trials. Fifth, we showed that bortezomib triggers activation of Akt and that bortezomib with Akt inhibitor perifosine can sensitize or overcome resistance to bortezomib in preclinical models.63 Our derived phase I and II trials of combination therapy showed durable responses even in the setting of bortezomib resistance, and a phase III clinical trial of bortezomib versus bortezomib with perifosine in relapsed MM is ongoing for US Food and Drug Administration approval. Sixth, Richardson et al.68 led efforts to translate these findings into clinical trials in advanced MM, which showed that lenalidomide, bortezomib, and dexamethasone combination therapy for newly diagnosed and relapsed refractory MM, often refractory to either agent alone. Most importantly, our center has shown that lenalidomide, bortezomib, and dexamethasone combination therapy for newly diagnosed MM achieves 100% responses, with 74% at least very good partial and 52% complete or near-complete responses.46 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. Therefore, the integration of combination novel-agent therapy, predicated on scientific rationale, is transforming the treatment paradigm in MM. Going forward, on the basis of these exciting results, we are now carrying out high-throughput drug screening to identify novel agents active against MM cells bound to BM stromal cells reflective of their microenvironment.
From the 1990s to the present, we have used oncogenomics to characterize MM pathogenesis, identify novel targets, predict response, and inform the design of single-agent and combination clinical trials. Our earliest studies profiled transcriptional changes occurring with transition from normal plasma cells to monoclonal gammopathy of undetermined significance to MM as well as identified gene and protein changes distinguishing patient MM cells from normal plasma cells in a syngeneic twin.70 We have repeatedly used transcript profiling to identify signatures of response, initially with bortezomib and subsequently with multiple other single and combination therapies,32 and most recently shown that microRNA profiling can also identify prognostic subgroups. Our DNA-based array comparative genomic hybridization studies have identified copy number alterations (CNAs) and suggested novel MM oncogenes or suppressor genes; once validated using knock-in and knockdown experiments in our models of MM cells in the BM milieu, these may serve as potential therapeutic targets71 (Fig 6).

Single nucleotide polymorphism array has also identified CNAs and allowed for the development of novel prognostic models.72 For example, recent single nucleotide polymorphism analyses of clinically annotated samples have identified CNAs, including increased 1q and 5q as sites for putative MM oncogenes or suppressor genes, once validated using knock-in and knockdown experiments in our models of MM cells in the BM milieu, these may serve as potential therapeutic targets71 (Fig 6).

Most importantly, as one of the founding centers of the Multiple Myeloma Research Consortium, we have participated in MM genome sequencing studies that have revealed mutated genes involved in protein homeostasis, nuclear factor κB signaling, IRF4 and Blimp-1, and histone methylating enzymes, all consistent with MM biology.73 These studies have also identified unexpected mutations, such as those in BRAF observed in melanoma, which may have short-term clinical application. Finally, our early studies now show continued evolution of genetic changes with progressive MM, strongly supporting the view that personalized medicine in MM must include profiling patient tumor cells not only at diagnosis but also at time of relapse.

Our ongoing efforts include the development of immune (vaccine and adoptive immunotherapy) strategies, development of novel agents targeting the MM cell in the BM microenvironment, development of rationally based multiagent combination therapies, and use of

**FUTURE DIRECTIONS AND CONCLUSIONS**

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genomics to improve both patient classification and allow for person-valued medicine in MM. With this continued rapid evolution of progress, MM will be a chronic illness with sustained complete responses in a significant fraction of patients.

In closing, I want to gratefully acknowledge the laboratory and clinical researchers at our center and throughout the world with whom I have had the privilege to work over many years. Not only have we together had an impact on the natural history of MM, but the next generation of leaders in MM research is now in place to expedite progress even further. We not only share academic interests in MM but also treasure long-standing personal friendships. I am deeply grateful to the many funding organizations and individuals supporting our efforts over many years. None of this would have been possible without the loving support of my family. And most importantly, I have been honored to care for many extraordinary patients, who are truly my heroes and will always be the inspiration for all that we do.

REFERENCES


AUTHOR’S DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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451

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