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Bench-to-bedside translation of targeted therapies in multiple myeloma

Kenneth C. Anderson, MD

Jerome Lipper Multiple Myeloma Center, Dana-Farber Cancer Institute, Harvard Medical School, Cambridge, Mass.

Multiple myeloma (MM) is characterized by excess monoclonal plasma cells in the bone marrow (BM), in most cases associated with monoclonal protein in blood or urine. Nearly 50 years ago, the use of combined melphalan and prednisone was shown to extend median survival of patients with MM to 2–3 years. In an approach pioneered by Prof. Tim McElwain in the 1970s, high-dose melphalan followed by BM transplantation in the 1980s and peripheral blood stem cell rescue in the 1990s further increased median survival to 3–4 years. Since 1998, MM has represented a new paradigm in drug development due to the remarkable therapeutic efficacy of targeting tumor cells in their microenvironment[1, 2]—an approach perhaps best exemplified by the use of the proteasome inhibitor bortezomib and immunomodulatory drugs (IMiDs) thalidomide and lenalidomide to target the MM cell in the BM microenvironment. This approach has rapidly translated from bench to bedside, producing six new Food and Drug Administration (FDA)-approved treatments in the past 7 years and a doubling of patient survival from 3–4 to 7–8 years as a direct result.[3] My colleagues and I have made contributions in the areas of identifying novel targets in the tumor and microenvironment, confirming the activity of inhibitors directed at these targets, and then leading clinical trials assessing the efficacy and safety of these agents. These collaborative efforts have included basic and clinical investigators, the pharmaceutical industry, the National Cancer Institute, FDA regulators, and patient advocacy groups, with the common focus and sole goal of improving MM treatments.[4] Indeed, the use of novel targeted inhibitors in relapsed refractory MM, relapsed MM, newly diagnosed MM and, most recently, consolidation and maintenance therapies has totally transformed MM therapy and patient outcome.

I have been carrying out bench to bedside research in MM now for 38 years, initially inspired by my mentor Dr. Richard L. Humphrey, who taught me the two most important

Correspondence to: Kenneth C. Anderson, MD, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02115-5450; kenneth_anderson@dfci.harvard.edu.

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lessons that have shaped my research and clinical practice ever since. When I was a medical student at Johns Hopkins, he instilled in me the desire to “make science count for patients” in MM 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 showed me the importance of treating patients as family. He has served as my inspiration and role model ever since.

Monoclonal antibodies and immune-based therapies

After an introduction to MM in both the laboratory and the clinic at Johns Hopkins during my medical school and internal medicine training, I moved to Dana-Farber Cancer Institute for training in medical oncology, hematology, and tumor immunology. There, Drs. George Canellos and Robert Mayer showed 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, since these MoAbs allowed for identification of the lineage and stage of differentiation of B cell malignancies, as well as permitting comparisons of the neoplastic B cell to its normal cellular counterpart. A panel of B cell MoAbs was very useful for complementing histopathologic diagnosis and identifying non-T 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]

From the outset, these MoAbs were also used in innovative treatment strategies in MM, and our efforts to develop immune-based MoAb and immunotoxin therapies, tumor vaccines, and mechanisms to abrogate host immunosuppression continue to the present. For example, given that high-dose therapy and autologous BM transplantation achieved remarkable extent and frequency of response, we early on examined whether cocktails of MoAbs (CD 10, CD20, PCA-1) could purge MM cells from autografts ex vivo prior to autologous BM transplantation.[7] Although effective at purging 2–3 logs of MM cells, this strategy had little impact on overall outcome, likely due to residual systemic tumor burden. T-cell (CD6)-directed MoAbs were 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 effect (GVM)[9] and develop clinical protocols of nonmyeloablative allografting in order to exploit GVM 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 evaluating MoAb-based immunotoxin therapies.[1, 10, 11] For example, we found CS-1 to be highly and uniformly expressed at the gene and protein level in patient MM cells, and then showed that targeting this antigen with elotuzumab was effective in preclinical models of MM in the BM milieu both in vitro and in vivo.[12] These promising data in turn motivated a clinical trial of elotuzumab, which showed that the agent achieved stable disease in relapsed refractory MM but did not induce responses sufficient to warrant new drug development. However, our preclinical studies showed that lenalidomide enhanced antibody-dependent cellular cytotoxicity triggered by elotuzumab,[12] providing the rationale for a combination clinical

trial with very promising results. This bedside to bench and back iterative process illustrates our translational focus. An example of an immunotoxin clinical trial is that of CD138 linked to maytansinoid toxin DM, which is currently ongoing based upon our promising data both in vitro and in xenograft models of human MM in mice.[13]

Our more recent focus in immune therapies has been on the development of vaccines. Avigan and coworkers have shown in both murine MM[14] and human MM[15] that vaccination with fusions of dendritic cells (DC) with tumor cells allows for generation of T and B cell tumor-specific responses in vitro and in vivo in preclinical models. Recent clinical trials of MM-DC vaccinations to treat minimal residual disease post-transplantation show that these vaccinations are triggering host anti-tumor T 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 CD 138 are functionally significant targets in MM cells, and we have gone on to derive peptides from these antigens that can be presented to trigger cytotoxic T-lymphocyte responses in HLA-A2 positive patients.[16] Ongoing clinical trials are evaluating vaccination with cocktails of these peptides in patients most likely to respond, with the goal of triggering clinically significant immune responses.

We have also characterized the underlying immunodeficiency in MM patients in order to design strategies to overcome it.[17] Our studies have demonstrated decreased helper and increased suppression pro-MM growth cytokines and dysregulated immune-homeostasis. And, for example, the demonstration of increased TH-17 cytokines promoting MM cell growth has set the stage for a related clinical trial of anti-IL-17 MoAb in MM.[17] In our studies of host accessory cells, we have shown that plasmacytoid DCs (pDCs) in MM patients do not induce immune effector cells as do normal pDCs, but instead promote tumor growth, survival, and drug resistance.[18] 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 related clinical trial.

The tumor in its microenvironment

Therapies targeting MM in the BM microenvironment

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. As a result, we have been able to take multiple single agents and combinations 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 the designs of single-agent and combination treatment clinical trials.

Specifically, we have developed models of MM in the BM microenvironment that have been useful in defining the roles of tumor cell-BM accessory cell contact, as well as the roles of cytokines in the BM milieu, in conferring growth, survival, and drug resistance in MM.[1, 19, 20] 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 the anti-tumor activity of dexamethasone is attenuated.[21] At both the gene transcript and proteasome activity levels, the ubiquitin proteasome cascade is upregulated by MM-BM binding, perhaps contributing to its enhanced activity in this context.[22] Bortezomib: (1) 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; (2) downregulates adhesion molecules on the tumor and in BM, thereby abrogating adhesion; and (3) targets the microenvironment to trigger anti-angiogenesis, as well as triggering apoptosis of osteoclasts while promoting osteoblast differentiation.[21, 23–27] This drug was rapidly translated from the bench to the bedside and received accelerated FDA approval in 2003 for treatment of relapsed refractory MM, followed by approval for relapsed MM and as initial therapy based upon its superiority in randomized phase III clinical trials.[28–30] Most recently, very promising data on the use of bortezomib as consolidation and maintenance therapy are emerging.

However, not all MM responds to bortezomib, and those tumors that do ultimately develop resistance. From the outset, we have therefore tried to identify gene signatures of response versus resistance to bortezomib in MM[33] as well as to develop functional assays to better predict whose cancer is 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.[32] It is remarkable that bortezomib has opened a whole new area of preclinical and clinical experimentation in cancer targeting the ubiquitin proteasome cascade; the strategies include targeting deubiquitinating enzymes upstream of the proteasome, selective and broad targeting of proteasome activity, and targeting the immunoproteasome. 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. The next-generation, more potent intravenous inhibitor of chymotryptic activity carfilzomib has overcome bortezomib resistance in preclinical and early clinical trials. Oral proteasome inhibitors targeting chymotryptic activity that translated from the bench to bedside in phase I clinical trials include Onx 0912, which triggers cytotoxicity against MM cell lines and patient cells, and MLN2238/9708, which demonstrates more potent preclinical activity against MM cells in vivo than bortezomib.[33–38] NPI-0052 targets chymotryptic, tryptic-like, and caspase-like activities, and similarly shows clinical promise.[37] 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.[39]

Since the empiric observation that thalidomide had anti-MM activity in 1998, we have studied the IMiDs thalidomide, lenalidomide, and pomalidomide 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 NK, T, and NK-T cell immunity to MM cells.[41–43] Like bortezomib, lenalidomide was rapidly translated from the bench to the bedside. Our preclinical studies demonstrated increased

responses when lenalidomide (triggers caspase 8-mediated apoptosis) was combined with dexamethasone (induces caspase 9-mediated apoptosis); our phase I and II clinical trials established the maximal tolerated dose and confirmed the enhanced clinical efficacy of combined lenalidomide and dexamethasone, informing the design of phase III clinical trials leading to its FDA/European Medicines Agency approval to treat relapsed MM.[28, 29, 43–47] Trials of lenalidomide as initial therapy in both the transplant candidate and elderly populations, as well as in consolidation and maintenance therapy, have yielded very promising results.[48, 49] For example, maintenance lenalidomide has been shown to add years of progression-free survival (PFS) in both newly diagnosed transplant and non-transplant candidates. We and others recently have shown that the second generation IMiD pomalidomide produces remarkable and durable responses, with a favorable side effect profile, even in the setting of MM resistant to lenalidomide and bortezomib.[50, 51]

Focus on the BM microenvironment: therapies targeting accessory cells with anti-MM activity

Bortezomib and lenalidomide are examples of targeting the tumor and also impacting the microenvironment, since both have a positive impact on bone disease in MM.[27, 52] We have also had a long-term interest in targeting the MM BM microenvironment with the goal of triggering MM responses. For example, MM cells secrete DKK-1, which downregulates osteoblast function via an effect on 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;[53] a clinical trial of BHQ880 MoAb is ongoing. We have also shown that B cell activating factor (BAFF) is elevated in the BM plasma of patients with MM and mediates osteoclastogenesis, as well as tumor cell survival and drug resistance; anti-BAFF MoAb can neutralize these effects,[54] and a clinical trial of this MoAb is ongoing. Most recently, we have shown that targeting BTK in our preclinical models not only blocks osteoclast formation and growth, thereby maintaining bone integrity, but also inhibits MM cell growth. These studies illustrate the principle that targeting cytokines or accessory cells in the tumor microenvironment can also impact MM cell growth, further validating the utility of our in vitro and in vivo model systems.

Preclinical studies to identify combination targeted therapies

We have used functional oncogenomics to inform the design of novel combination therapies. For example, bortezomib was shown to inhibit DNA damage repair in vitro,[27] providing the rationale for its combination with DNA damaging agents to enhance or overcome drug resistance. Indeed, a large randomized phase III trial of bortezomib versus bortezomib with pegylated doxorubicin showed prolonged PFS and overall survival and increased extent and frequency of response with the combination,[55] leading to FDA 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.[56] Hideshima and colleagues then showed that p38MAPK inhibitor decreased

downstream Hsp 27 and thereby overcame bortezomib resistance in MM cell lines and patient cells,[57] providing the rationale for a clinical trial of bortezomib and p38MAPK inhibitor.

In another example, based upon hallmark cyclin D abnormalities in MM, Raje and colleagues have studied cyclin D kinase inhibitors alone and in combination in MM.[58, 59] In addition, Ghobrial and coworkers have translated promising preclinical data on an mTOR inhibitor and bortezomib into clinical trials.[60] We also have shown that bortezomib triggers activation of Akt, and that bortezomib with the Akt inhibitor perifosine can overcome resistance to bortezomib in preclinical models.[61] Our phase I/II trials of this combination therapy showed durable responses even in the setting of bortezomib resistance, and a phase III trial of bortezomib versus bortezomib with perifosine in relapsed MM is ongoing.

Finally, we believe that protein homeostasis represents one of the most attractive novel therapeutic targets in MM. Specifically, 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.[62] Most important, blockade of aggresomal and proteasomal degradation of proteins by histone deacetylase (HDAC) inhibitors (vorinostat, panobinostat, tubacin) and proteasome inhibitors (bortezomib, carfilzomib), respectively, triggers synergistic MM cell cytotoxicity in preclinical studies.[62–64] We are leading international phase I/II trials combining the HDAC inhibitors vorinostat or panobinostat with bortezomib, which have thus far shown that responses are achieved in the majority of patients with relapsed bortezomib-refractory MM, as well as phase III trials for FDA registration of these combinations. A very promising finding is that an HDAC6-selective inhibitor causes acetylation of tubulin and more potently and selectively blocks aggresomal protein degradation, providing synergistic MM cytotoxicity when combined with bortezomib. This combination has rapidly translated from our laboratory to the bedside in clinical trials aimed at determining whether clinical efficacy can be achieved without the side effect profile of fatigue, diarrhea, thrombocytopenia, and cardiac abnormalities associated with the more broad type HDAC1 or 2 inhibitors.

To date, the most exciting combination emerging from our preclinical studies is that of lenalidomide and bortezomib, with the respective caspase 8-mediated apoptosis and caspase 9-mediated apoptosis inducing synergistic cytotoxicity in models of MM cells in the BM milieu.[65] Richardson and colleagues led efforts to translate these findings to clinical trials in advanced MM, which showed that lenalidomide, bortezomib, and dexamethasone achieved a response rate of 58% in relapsed MM that was often refractory to either agent.[66] Most important, our center has shown that lenalidomide, bortezomib, and dexamethasone combination therapy achieves a response rate of 100% in newly diagnosed MM, with 74% of patients having at least very good partial response and 52% having complete or near complete response.[45] Given these unprecedented results, a clinical trial is now evaluating whether high-dose chemotherapy and stem cell transplantation adds value in the context of this high extent and frequency of response to combined novel therapies.

The integration of novel combination therapy, predicated upon scientific rationale, has transformed and continues to transform the treatment of MM. Going forward and based

upon 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.

Oncogenomic studies

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 therapy 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 identifying gene and protein changes distinguishing patient MM cells from normal plasma cells in a syngeneic twin.[67] We have repeatedly used transcript profiling to identify signatures of response, initially with bortezomib and subsequently with multiple other single-agent and combination therapies,[31] and most recently showed 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 knock down experiments in our models of MM cells in the BM milieu, these may serve as potential therapeutic targets.[68]

Single nucleotide polymorphism (SNP) arrays have also identified CNAs and allowed for the development of novel prognostic models.[69] For example, recent SNP analyses of clinically annotated samples identified CNAs that may predict clinical outcome, including increased 1q and 5q as sites for putative MM oncogenes and decreased 12p as a site of putative MM suppressor genes.[69] Most important, 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, NF-κB signaling, IRF4 and Blimp-1, and histone methylating enzymes, all consistent with MM biology.[70] These studies also identified unexpected mutations, such as those in BRAF observed in melanoma, and these discoveries may have clinical application in the near future. Finally, we have now shown that there is 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.

Future directions and conclusions

Our ongoing efforts include identification and development of immune strategies (vaccines and adoptive immunotherapy), novel agents targeting the MM cell in the BM microenvironment, and rational multi-agent combination therapies and use of genomics to improve patient classification and allow for personalized medicine in MM. With continued rapid progress, MM will become a chronic illness with sustained complete responses in a significant proportion of patients.

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