Focus on multiple myeloma

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Introduction

Multiple myeloma (MM) is a neoplasia of plasma cells (PCs), hallmarked by tumor cell tropism for the bone marrow (BM) and production of monoclonal immunoglobulin (Ig) detectable in serum and/or urine. Although MM is the 2nd most commonly diagnosed hematologic malignancy in the Western world, it is often viewed inaccurately as a rare disease because the short survival (compared to many other hematologic neoplasias) and uniformly fatal outcome of patients markedly decreases the prevalence of MM in the population. MM incidence is higher in men than women and is higher in African and lower in Asian populations, relative to Caucasians. Despite extensive epidemiological studies, specific modifiable risk factors for MM have not yet been conclusively identified.

Multistep temporal evolution of MM

MM pathophysiology encompasses a multistage evolution through monoclonal gammopathy of undetermined significance (MGUS), smoldering (asymptomatic) MM, symptomatic (intramedullary) MM, and extramedullary MM/plasma cell leukemia (PCL). MGUS, an asymptomatic premalignant condition present in 1% and 3% of individuals age >50 and >70, respectively, can stochastically progress to MM or related plasma cell dyscrasias, with ~1% annual risk and 25% cumulative probability of progression over 20 years (Kyle et al., 2002). Smoldering MM, an intermediate entity between MGUS and active MM, lacks MM-related symptoms, but often progresses, after variable periods of time, to overt symptomatic MM. The latter can be manifested clinically by anemia, lytic bone lesions (predominantly in axial skeleton) and diffuse osteoporosis, hypercalcemia, renal dysfunction (due to monoclonal Ig deposition), and increased risk for infection. In advanced disease, malignant PCs can form extramedullary lesions (e.g., soft tissue plasmacytomas) and be detected in the circulation as PCL.

Genetic basis of MM

An accumulating compendium of data acquired with diverse techniques (reviewed in Fonseca et al., 2004) reveals marked interpatient heterogeneity in the genetic background of MM cells. Unlike other hematologic malignancies (e.g., chronic myelogenous leukemia with Philadelphia chromosome), MM lacks pathognomonic genetic lesion(s) that could account, either per se or in concerted fashion, for all cases of this disease. MM cells can harbor (1) hyperdiploid karyotypes with infrequent translocations (<30%) or other structural chromosomal abnormalities, or (2) nonhyperdiploid (e.g., hypodiploid, hypotetraploid, or pseudodiploid) karyotypes with high prevalence of translocations (>85%) of Ig gene. These translocations juxtapose potent Ig gene enhancers next to highly diverse, but nonrandom, oncogene-harboring, partner loci: 11q13 (cyclin D1), 4p16 (FGFR3 and MMSET), 16q23 (c-maf), 6p21 (cyclin D3), and 20q11 (maf-B) account for ~40% of cases harboring Ig translocations. The rest involves a multitude of other, less frequent, partner loci (Avet-Loiseau et al., 2002; Fonseca et al., 2004). A recent hypothesis proposes that at least one of cyclins D1, D2, and/or D3 is overexpressed in MM, either through direct (11q13-cyclin D1 and 6p21-cyclin D3) or indirect (4p16, 16q23, other cyclin D2) effect of primary Ig translocations or through yet undefined mechanism(s) in hyperdiploid MM cells (Hideshima et al., 2004). Upregulation of cyclin D genes is proposed to render PCs more responsive to proliferative stimuli, e.g., BM-derived cytokines, resulting in selective expansion of a PC population further susceptible to additional genetic events (e.g., trisomies of chromosomes 3, 5, 7, 9, 11, 15, 19, and 21; loss of 13q14, often present in premalignant MGUS; and p16INK4a methylation/inactivation) that can lead to MGUS/MM (Avet-Loiseau et al., 2002; Fonseca et al., 2004; Rasillo et al., 2003).

To date, no known transcriptional (Davies et al., 2003; Zhan et al., 2003), cytogenetic (Fonseca et al., 2004), or mutational markers can clearly distinguish MGUS from MM. However, a multitude of adjunct genetic changes, which are absent or rare in MGUS, are increasingly prevalent in aggressive intramedullary MM or extramedullary MM and conceivably contribute to further progression of MM to its more aggressive stages, including secondary chromosomal translocations (e.g., involving c-*myc*), mutually exclusive activating mutations of K- or N-Ras (or FGFR3 in cases of t[4;14] translocation), mutations and/or monoallelic p53 deletion, Rb or p18INK4c inactivation, or PTEN deletions/inactivating mutations (Bezieau et al., 2002; Chesi et al., 2002; Fonseca et al., 2004; Shou et al., 2000). Univariate analyses of clinical outcome suggest improved survival after high-dose chemotherapy for patients with t(11;14)(q13;q32) or adverse prognosis in chemotherapy-treated patients harboring t(4;14)(p16.3;q32), t(14;16)(q32;q23), or chromosome 13 deletion(s) (Fonseca et al., 2004; Moreau et al., 2002; Shaughnessy et al., 2003). However, the biological significance of individual cytogenetic abnormalities remains elusive, e.g., due to different detection sensitivity of various cytogenetic techniques and confounding effects of concomitant presence of multiple genetic abnormalities and their evolving complexity during the course of the disease. The biological behavior of MM cells is determined by the composite effect of coexisting genetic lesions rather than as exclusive repercussion of any individual one. To address this limitation of univariate prognostic analyses, the multivariate capabilities of cDNA or oligonucleotide microarrays have been recruited to identify genes differentially expressed in malignant PCs versus normal counterparts or transcriptional signatures associated with MM patient subgroups which partially overlap with proposed cytogenetic-based classifications (Davies et al., 2003; Hideshima et al., 2004; Zhan et al., 2002). More studies will be needed to identify the most biologically relevant of these

transcripts and decipher the clinical applicability of these proposed classifications, especially in the face of ongoing changes in the therapeutic management of MM.

The role of BM microenvironment in MM pathophysiology

The biological and clinical behavior of MM cells is not exclusively determined by their genetic background. It is also influenced by their intricate bidirectional relationship with their local bone microenvironment: MM cells perturb normal skeletal homeostasis, causing debilitating osteolytic lesions, while the BM milieu provides MM cells with a multifaceted network of protective effects against proapoptotic insults.

Under physiological conditions, the skeleton undergoes ongoing structural remodeling to optimize its stress-bearing capacity, via precisely coordinated cycles of osteoclast-mediated resorption of old bone and subsequent compensatory bone formation by osteoblasts. In MM, however, these 2 opposing processes are uncoupled, due to (1) concomitant upregulation of multiple positive regulators of osteoclast formation and function, and (2) suppression of negative regulators of osteoclastogenesis and/or positive regulators of bone formation (Ashcroft et al., 2003). RANK (receptor activator of nuclear factor-κB [NF-κB]), a TNF receptor superfamily member expressed on osteoclasts and their precursors, engages RANKL (RANK ligand) expressed on BMSCs and osteoblasts, thereby triggering osteoclast differentiation and resorptive activity. It remains controversial whether MM cells themselves express RANKL or not; nonetheless, their presence potentiates osteoclast activity by (1) upregulation of RANKL expression in BMSCs (Roux et al., 2002); (2) decreased levels of osteoprotegerin (OPG), which functions as decoy against RANKL; (3) upregulation of multiple pro-osteoclastogenic cytokines (e.g., IL-6, IL-1α, IL-1β, IL-11, MIP-1α, M-CSF, TNF-α, PTHrP, and VEGF, which are either produced by MM cells or by host BM cells [e.g., BMSCs] due to paracrine/juxtacrine stimulation by MM cells [Ashcroft et al., 2003; Han et al., 2001]); and/or (4) increased levels, in subsets of MM patients with extensive bone lesions, of receptor for hyaluronan-mediated motility (RHAMM) (Maxwell et al., 2004) or of Dickoppf-1 (DKK-1) (Tian et al., 2003), an inhibitor of Wnt signaling, which inhibits differentiation of osteoblast precursor cells, conceivably contributing to uncoupling of bone formation from excessive resorption in MM.

The BM microenvironment constitutes a sanctuary for MM cells: their adhesion to extracellular matrix, BMSCs, and other cells of the BM milieu activates in MM cells a pleiotropic cascade of proliferative/antiapoptotic signaling pathways, including PI-3K/Akt/mTOR/p70S6K, IKK-α/NF-κB, Ras/Raf/MAPK, and JAK/STAT3; as well as downstream effectors, including cytoplasmic sequestration of proapoptotic Forkhead transcription factors; upregulation of D-type cyclins, caspase inhibitors, antiapoptotic Bcl-2 family members; and increased activity of the proteasome, telomerase and HIF-1α (Hideshima et al., 2001a; Mitsiades et al., 2002b, 2004a). These molecular events are triggered either directly, via cell adhesion molecule-mediated interactions of MM cells with BMSCs, osteoblasts, other BM cellular compartments, or the extracellular matrix (Landowski et al., 2003), or indirectly, via cytokines/growth factors released by BMSCs and/or MM cells and engaging their respective receptors on the MM cell surface. The expanding list of such paracrine/autocrine proliferative/survival factors includes interleukin-6 (IL-6), insulin-like growth factors (IGFs), IL-1α, IL-1β, HGF, VEGF, SDF-1, TNF-α, or Notch family members (Chauhan et al., 1996; Hideshima et al., 2004; Mitsiades et al., 2002a, 2004a; Nefedova et al., 2004). Many of these factors also induce pleiotropic indirect effects on MM cells by stimulating osteoclastogenesis (IL-6, IL-1, VEGF), modulating adhesion molecule profiles on MM cells and BMSCs (TNF-α), or promoting MM cell homing to the BM milieu (e.g., SDF-1) (Hideshima et al., 2004). Recent data highlight that IGFs and their receptor IGF-1R (CD221) play a more prominent role in MM pathophysiology than previously appreciated (Mitsiades et al., 2004a). Levels of IGFs are high in serum and even higher locally in the BM milieu due to paracrine release by osteoblasts and BMSCs (Mitsiades et al., 2004a), and IGFs confer more pronounced and sustained activation of proliferative/antiapoptotic signaling (e.g., PI-3K/Akt, IKK/NF-κB) than other BM-derived cytokines, e.g., IL-6 (Mitsiades et al., 2002a). IGF-1R signaling therefore emerges as a key regulator of MM cell biological behavior: it activates multiple proliferative/antiapoptotic cascades (including proteasome and telomerase activities), attenuates anti-MM activity of several antitumor drugs (including dexamethasone, cytotoxic chemotherapy, and proteasome inhibitors), primes MM cell responsiveness to other cytokines (e.g., IL-6), and stimulates production of angiogenic cytokines (Mitsiades et al., 2004a). Importantly, even though PCL cells can proliferate and survive independently of many BM-derived cytokines (e.g., IL-6), they maintain significant responsiveness to selective inhibition of IGF-1R signaling (Mitsiades et al., 2004a).

The composite clinical impact of these bidirectional MM cell-BM microenvironment interactions is detrimental not only as a direct result of osteolytic lesions, but also as indirect repercussion of the resistance that the BM milieu confers to MM cells against conventional chemotherapeutics or glucocorticoids, even in the absence of genetic lesions that would confer constitutive resistance (Hideshima et al., 2001a, Mitsiades et al., 2004a). This may explain, at least in part, the lack of curative outcome with conventional therapies, raising intriguing hypotheses about putative interpatient variation in the intrinsic biological behavior of BM microenvironment per se, and also underscores the significance of MM studies in preclinical models which recapitulate the complex circuitry of tumor-BM interactions, in order to define novel therapies to specifically target their entire spectrum. This is critical since MM cell-driven osteolysis and BM microenvironment-determined drug resistance are highly multifactorial, with substantial potential for redundancy and ensuing resistance to treatments targeting only individual mediators of these processes.

The intricate tumor-microenvironmental intreractions and their role in in vivo drug resistance are not restricted to MM, but are increasingly recognized as critical features of other neoplasias (Allinen et al., 2004). The molecular basis for distinct differences in the tropism and clinical phenotype of these interactions in MM versus other diseases remains to be determined. It is possible that those genetic lesions, which contribute to establishment of MGUS/MM, not only confer enhanced proliferative capacity and/or increased resistance to apoptosis, but also modulate the ability of MM cells to interact with their BM stromal milieu: MM cells with t(14;16) translocations overexpress the transcription factor c-maf, which transactivates the cyclin D2 promoter, enhancing myeloma cell proliferation, but also drives β7-integrin expression and enhanced MM cell adhesion to BMSCs (Hurt et al., 2004). Hyperdiploidy is proposed to render MM cells uniquely dependent on the BM environment, which possibly drives their cyclin D1 overexpression despite

absence of Ig translocations (Hideshima et al., 2004). These cases exemplify putative associations between specific genetic lesions and particular pathways mediating MM-microenvironmental interactions.

Recent advances in the therapeutic management of myeloma

For decades, MM treatment was based on cytotoxic chemotherapy, e.g., conventional dose melphalan-prednisone as front-line therapy for elderly patients and high-dose chemotherapy (HDT) with hematopoietic (autologous or allogeneic) stem cell support, aiming at higher rate and magnitude of responses in patients with favorable prognostic features. Recent data indicate modest, but consistent, benefits of single HDT with autologous stem cell support over standard-dose chemotherapy (Child et al., 2003), and, mainly in patients with suboptimal response to 1st HDT, of tandem double HDT over single HDT (Attal et al., 2003). An important role for bisphosphonates in management of MM bone complications is also established (Berenson et al., 2002). Yet a radical recent shift in MM therapeutics was the application of agents e.g., thalidomide (Thal), immunomodulatory Thal derivatives (IMiDs), and proteasome inhibitors (Richardson et al., 2002, 2003; Singhal et al., 1999), which have different molecular targets than chemotherapeutics.

Thal was proposed for MM treatment based on its antiangiogenic properties (D'Amato et al., 1994) and successfully applied by Singhal et al., leading to objective clinical responses (>50% decrease in monoclonal Ig levels) in ~1/3 of MM patients with relapsed refractory MM (Singhal et al., 1999), with higher response rates when combined with other agents, e.g., dexamethasone (Rajkumar et al., 2002; Weber et al., 2003). The immunomodulatory Thal derivative (IMiD) CC-5013 (Revlimid, lenalidomide) can be clinically active even in MM patients resistant to Thal, is not teratogenic in in vivo preclinical models, and exhibits manageable adverse event profile, devoid of the potential of Thal for peripheral neuropathy, somnolence, and constipation (Richardson et al., 2002). The diverse proposed mechanisms of anti-MM action of Thal and CC-5013 include not only antiangiogenic effects, but also direct antiproliferative and proapoptotic effects on MM cells, through inhibition of NF-κB transcriptional activity and activation of death receptor/caspase-8-mediated death signaling (Mitsiades et al., 2002d); modulation of MM-BMSC adhesive interactions and abrogation of secretion of prosurvival cytokines (Hideshima et al., 2000); and stimulation of NK cell number and cytotoxic activity against MM cells (Davies et al., 2001).

MM is also a prototypic disease model for antitumor activity of proteasome inhibitors, such as the boronic dipeptide bortezomib (PS-341). The proteasome functions in both normal and neoplastic cells to degrade ubiquitinated intracellular proteins destined for recycling of their building blocks (Hershko and Ciechanover, 1998). However, MM cells appear to be highly dependent on 20S proteasome function, since inhibition of chymotryptic activity of its β5 subunit by bortezomib triggers (1) concomitant activation of caspase-8 (via c-*myc*- and AP-1-mediated upregulation of Fas/FasL), caspase-9, and endoplasmic reticulum stress response, via inhibition of IRE1 activity (Lee et al., 2003; Mitsiades et al., 2002c), and (2) suppression of intracellular antiapoptotic pathways, e.g., intracellular accumulation of IκB and suppression NF-κB-dependent antiapoptotic proteins (e.g., FLIP, cIAP-2, and other caspase inhibitors) (Mitsiades et al., 2002c). Consequently, bortezomib is active even against drug-resistant MM cells in vitro and in vivo (Hideshima et al., 2001b) and offers objective clinical responses in approximately 1/3 of heavily pretreated MM patients with relapsed refractory disease (Richardson et al., 2003), as well as superior clinical outcome, compared to high-dose dexamethasone, in relapsed MM (P.G. Richardson et al., 2004, Proc. Am. Soc. Clin. Oncol., abstract).

While these new therapies target, at least in part, the responsiveness of MM cells to BM-determined antiapoptotic stimuli, their administration is not considered curative: not all patients are responsive, and drug resistance eventually develops even in responders, conceivably due to interpatient heterogeneity and intrapatient evolution in the genetic makeup and microenvironmental interactions of MM cells. Two distinct but mutually complementing ongoing approaches to address this challenge are the development of new classes of antitumor agents and the combinatorial use of existing and novel anti-MM drugs.

Many novel therapies developed for MM (Figure 1) either directly deprive MM cells of their ability to respond to proliferative and antiapoptotic microenvironmental cues, and/or target the sources of these stimuli, i.e., MM-stromal adhesion, bone resorption, or neoangiogenesis. The former goal can be achieved through agents which selectively inhibit (1) cell surface receptors or downstream effectors in signaling cascades mediating MM cell proliferation/drug resistance in the BM milieu (e.g., IGF-1R, FGFR-3, IKK-α, farnesyltransferase, mTOR) (Bolick et al., 2003; Chesi et al., 2002; Hideshima et al., 2004;

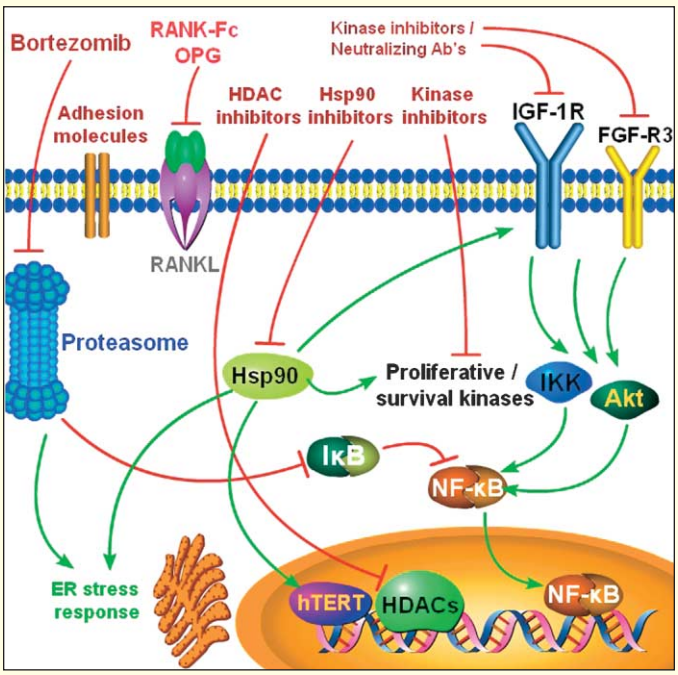

Figure 1. Schematic overview of emerging therapeutic targets in MM

Many emerging therapies for MM target the ability of MM cells to respond to proliferative/antiapoptotic microenvironmental cues. The levels of actions of these therapies are diverse, and include cell surface growth factors receptors (e.g IGF-1R), their downstream signaling effectors (e.g., Akt, IKK-α), cell adhesion molecules, posttranslational regulators of protein expression (e.g., proteasome) or 3-dimensional conformation and function (e.g., hsp90), regulators of transcriptional responses (e.g., HDACs) or replicative potential (e.g., hTERT), and inhibitors of bone resorption (e.g., RANK-Fc decoys or OPG constructs).

Mitsiades et al., 2004a; Shi et al., 2002), (2) pathways regulating MM cell adhesion (e.g., c-met) (Hov et al., 2004), (3) molecular chaperones, such as hsp90, which simultaneously regulates the 3-dimensional conformation and function of multiple proliferative/antiapoptotic kinases in MM cells (Mitsiades et al., 2002a), (4) histone deacetylases (HDACs), which regulate transcription of oncogenic/antiapoptotic genes differentially expressed in MM cells (Mitsiades et al., 2003c, 2004b), or (5) telomerase (Shammas et al., 2004), which regulates long-term replicative potential of MM cells and is functionally modulated by BM cytokine signaling (Mitsiades et al., 2004a). Therapies with direct microenvironmental focus may target adhesion molecules mediating MM-stromal interactions (e.g., α_4-integrin) (Mori et al., 2004), counteract bone resorption, e.g., RANK-Fc decoys or OPG constructs (Body et al., 2003; Sordillo and Pearse, 2003; Vanderkerken et al., 2003), or aim at disrupting tumor-associated endothelial cells (e.g., VEGF receptor signaling inhibitors) (Lin et al., 2002). The immunomodulatory properties of thalidomide also rekindled the interest of the MM field in immune-based therapies, e.g., DNA or peptide vaccines (Heslop et al., 2003) or adoptive immunotherapy with T cells (Kwak et al., 2004). Following its successful clinical use in acute promyelocytic leukemia, arsenic trioxide appears to have biological activity against MM, with diverse proposed mechanisms of action (Hussein et al., 2004; McCafferty-Grad et al., 2003).

Many of these therapies (e.g., inhibitors of IGF-1R kinase, hsp90, or HDACs) (Mitsiades et al., 2002a, 2003c, 2004a) trigger pleiotropic molecular sequelae via simultaneous targeting of multiple pathways and/or versatile biological functions of their individual molecular targets. The latter are not necessarily overexpressed or mutationally activated in MM cells (compared to normal cells), highlighting the importance of functional screenings for identification of novel therapeutic targets.

For the design of more effective anti-MM combination regimens, a consistent effort is made to rationally partner treatments that neutralize potential mechanisms of resistance to each other, simultaneously target multiple distinct critical pathways for tumor cell survival, and/or affect different functional levels within the same pathway. Using molecular profiling and functional studies of drug-treated MM cells in vitro and in vivo to define regimens which satisfy these criteria, several such combinations have translated into clinical applications, including combinations of thalidomide or its derivatives with dexamethasone or the proteasome inhibitor bortezomib, or combinations of bortezomib with cytotoxic chemotherapy or hsp90 inhibitors (Hideshima et al., 2000; Mitsiades et al., 2002a, 2002c, 2002d, 2003d). The synergy of bortezomib with chemotherapeutics (e.g., alkylating agents or antracyclines) is confirmed both in vitro and in vivo, even in MM cases resistant to both therapies administered separately (Mitsiades et al., 2003d; Orlowski, 2004), and involves concomitant abrogation of distinct molecular determinants of MM cell chemoresistance, including NF-κB transcriptional activity, inhibitors of apoptosis (IAPs), and genes involved in DNA damage repair (Mitsiades et al., 2003d).

Both the development of novel classes of anti-MM agents and their rational incorporation into combination regimens have been bolstered by drug testing in preclinical models recapitulating the intricate MM-BM milieu interaction, either in vitro (MM-BMSC coculture assays) or in vivo (SCID-NOD models of diffuse MM bone lesions [Mitsiades et al., 2003b, 2004a] or SCID-hu model of syngeneic MM-BMSC in vivo interaction [Urashima et al., 1997; Yaccoby et al., 1998]). These models are particularly important for assessment of in vivo drug efficacy, since subcutaneous xenograft models, commonly used in other disease settings, do not recapitulate the osteotropism of MM and the multifactorial protection it receives (even in advanced disease) by the BM milieu (Mitsiades et al., 2004a). The difficulty in assessing tumor response in models of diffuse lesions is addressed by use of MM cells transduced with luciferase and/or green fluorescent protein constructs, allowing for sensitive and noninvasive real-time quantification of tumor burden with whole-body fluorescence and/or bioluminescence imaging, respectively (Mitsiades et al., 2003b, 2004a).

Contributions of MM research to conceptual progress in cancer research: Future challenges

The notion of MM as a rare disease with little broader relevance for cancer research has been replaced by its emergence as a prototypic disease model for development of key concepts in cancer pathophysiology and therapeutics. The study of tumor-stromal interactions and their role in in vivo drug resistance, the development of proteasome inhibition, thalidomide and its derivatives, or IGF-1R kinase inhibition to specifically overcome microenvironmentally determined drug resistance, and the streamlined drug testing in in vivo models with clinically relevant distribution of diffuse tumor lesions are some of the critical contributions by MM research. The lack of curative treatments for MM has also transformed it into a critical testing ground for an expanding armamentarium of new therapeutics. The genetic complexity and heterogeneity of MM cells and/or BM microenvironment severely limit the likelihood of curative outcome with any single class of agents. A critical challenge will be to interdigitate the expanding knowledge on molecular profiling, novel in vivo models, and developmental therapeutics with clinical practice and offer clinically applicable combination therapies matching the molecular features of the disease in individual patients, in order to effectively counteract drug resistance and/or prevent disease- or treatment-related complications (Mitsiades et al., 2003a). If these challenges are successfully met, the MM field will also provide a valuable blueprint for overcoming these critical hurdles in other disease-specific oncological fields.

Acknowledgments

Supported by the Multiple Myeloma Research Foundation (C.S.M., N.M.), Leukemia and Lymphoma Society (C.S.M.), International Waldenstrom's Macroglobulinemia Foundation (C.S.M.), Fund to Cure Myeloma (K.C.A.), and NCI SPORE grant Career Developmental Award (C.S.M.). The authors apologize in advance for the inability, due to space limitations, to reference all studies relevant to the scope of this article.

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