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ORIGINAL PAPER

Targeting Bone as a Therapy for Myeloma

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Abstract Myeloma bone disease (BD) not only impairs quality of life, but is also associated with impaired survival. Studies of the biology underlying BD support the notion that the increased osteoclastogenesis and suppressed osteoblastogenesis, is both a consequence and a necessity for tumour growth and clonal expansion. Survival and expansion of the myeloma clone is dependent on its interactions with bone elements, thus targeting these interactions should have antimyeloma activities. Indeed both experimental and clinical findings indicate that bone-targeted therapies not only improve BD, but also create an inhospitable environment for myeloma cell growth and survival, favouring improved clinical outcome. This review summarizes recent progress in our understandings of the biology of myeloma BD, highlighting the role of osteoclasts and osteoblasts in this process and how they can be targeted therapeutically. Unravelling the mechanisms underlying myeloma-bone interactions will facilitate the development of novel therapeutic agents to treat BD, which as a consequence are likely to improve the clinical outcome of myeloma patients.

Keywords Multiple myeloma · Bone disease · Osteoclast · Osteoblast · Therapy

Introduction

Multiple myeloma (MM) is the result of a clonal expansion of malignant plasma cells primarily located within the bone marrow (BM). Despite considerable therapeutic advances, the disease remains largely incurable with median survivals of 4–5 years dependent upon biological subgroup. Myeloma is unique among haematological malignancies, being characterized by osteolytic bone lesions and the development of skeletal related events (SREs). At presentation 70% of patients have bone disease (BD) and 60% patients report a pathologic fracture over the course of their disease [1]. The presence of BD is a defining characteristic of myeloma requiring treatment; moreover the extent of BD and bone resorption activity has been shown to be an important risk factor for overall survival (OS) [2–4]. As BD is the major contributor to morbidity and mortality in MM, in addition to chemotherapy targeting the myeloma cells, bone-supportive treatment is also an essential component of the therapy, and accumulating evidence suggests that bone targeted therapies not only reduce skeletal complications but also improve survival.

In the normal BM microenvironment, bone is constantly undergoing remodelling with a delicate balance between bone resorption and bone formation. The mechanism underlying myeloma BD is an uncoupling of bone resorption from bone formation as a consequence of increased osteoclastic activity and inhibition of osteoblast function [5]. We are now beginning to understand that this dysregulation is not only responsible for the bone destruction, but also for the initiation, maintenance and expansion of the myeloma clone. This pro-survival effect is thought to be mediated via direct cell-cell contact between myeloma and bone cells, as well as via positive cytokine feedback loops set up during the bone resorption process, creating a vicious cycle of bone resorption and tumour growth.

The complex interactions present in the BM microenvironment bring with them the potential for their therapeutic targeting. In this respect, in addition to therapies targeting

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the myeloma cells directly, additional benefit could be achieved by combination with agents targeting myeloma cell interactions with the BM microenvironment. This approach may not only be beneficial during induction treatment, by increasing the sensitivity of the myeloma cells to cytotoxic agents, but may also be important during the maintenance phase by targeting the plasma cell niche in the BM, such that the biology of residual clonal cells is modified. The complex interactions in the BM can be targeted in different ways including targeting the cell-cell contact of the MM cells with stromal elements, targeting the growth factors produced by both the MM cells and the stromal elements, as well as targeting the factors released from bone modelling process. Such changes in the emphasis of the treatment reflect our increasing ability to induce complete responses and the consequent requirement to develop approaches able to maintain these responses long term.

In this review, we discuss recent progress in the understanding of the myeloma-BM microenvironment interaction. We highlight the role of normal BM constituents, particularly osteoclasts, osteoblasts and their precursors, in myeloma pathogenesis and drug resistance. We also describe the antimyeloma activity of bone targeting therapies and how this could improve clinical outcome for MM patients (Fig. 1).

Pathogenesis of Myeloma and its Interactions with the Bone Marrow Microenvironment

MM is currently viewed as a prototypical disease model for studying tumour–microenvironment interactions, based on the hypothesis that the biology of MM is highly dependent on the interactions with structural and soluble components within the microenvironment, which are also responsible for the generation of BD. Thus it should also be considered an excellent model in which we can understand whether targeting tumour-microenvironment interactions can have anticancer effects.

While the spectrum of genetic change in individual cases of myeloma is thought to determine the clinical behaviour of that case, this cannot be the whole story. The clonal plasma cells of all MM patients harbour genetic lesions, and these chromosome abnormalities are present in most cases of monoclonal gammopathy of undetermined significance

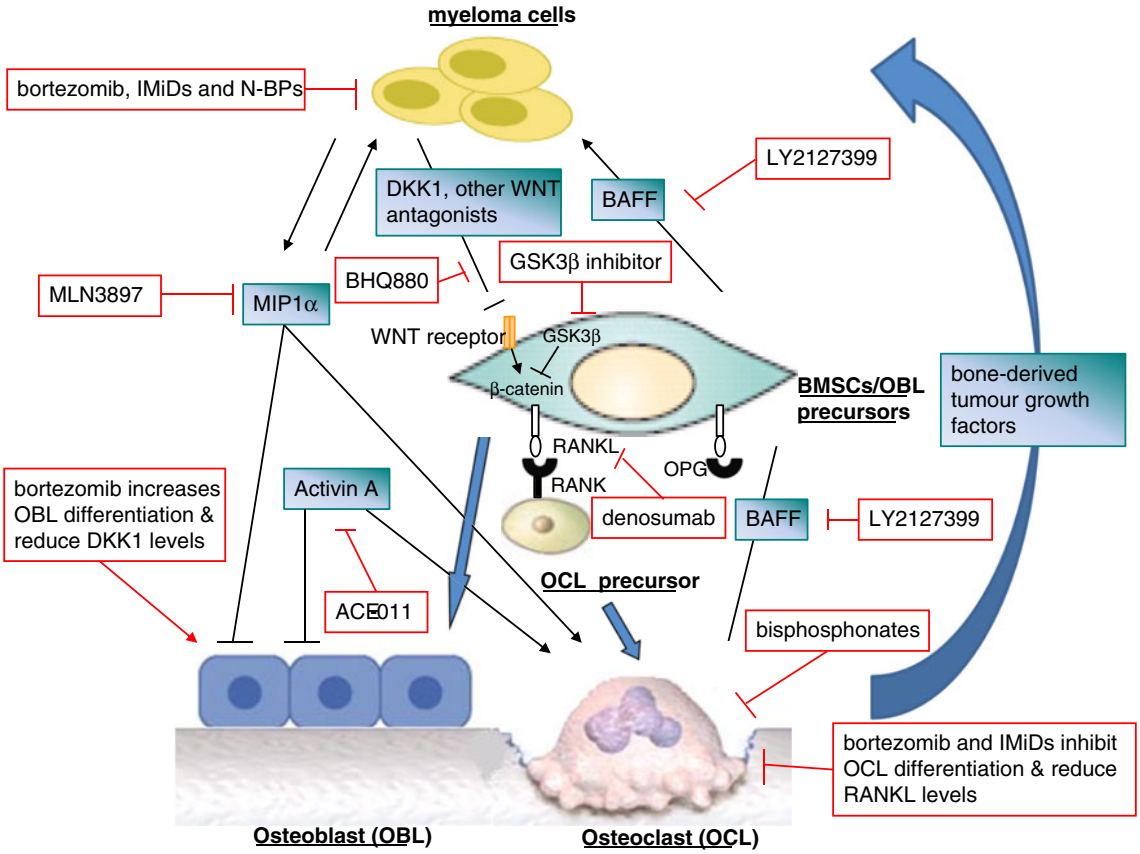

Fig. 1 Bone targeted therapies. Recent progress in the pathogenesis of bone disease and myeloma biology have accelerated our understanding of the effects of current treatment on myeloma and associated bone disease, more importantly they have translated into the development of novel bone-targeted agents

(MGUS) [6], which is not characterized by lytic bone lesions. Thus the biology of MM cells is not determined exclusively by genetic abnormalities, but is also influenced by interactions with the local microenvironment, as well as by other epigenetic features in the myeloma cell. Thus considering the biology of the plasma cell and its interaction with the stromal microenvironment becomes important.

On binding to their stromal environment myeloma cells have been shown to induce changes in several cell types that are intimately involved in the induction of bone lesions, including BM stromal cells (BMSCs), BM endothelial cells, immune cells, osteoblasts and osteoclasts. The induced changes, in turn, offer the MM cells a supportive stromal environment, access to vascular networks, and locally produced growth factors and cytokines [7], favouring their growth and survival. Interestingly, although microenvironmental interactions are important, the genetic lesions associated with subgroups of myeloma also seem to modulate rates of BD possibly by modifying the interaction of the myeloma cells with the BM milieu. For example, MM cells harbouring the t(14;16) translocation overexpress the transcription factor c-maf, which activates cyclin D2 expression and increases MM cell proliferation. In addition, c-maf up-regulates b7-integrin expression and potentiates MM cell adhesion to BMSCs [8]. It has been shown that cases characterized by MAF deregulation have less BD as do cases with the t(4;14) [9, 10]. In contrast MM cells characterized by hyperdiploid karyotypes have more BD and seem to depend on the BM microenvironment for the induction of cyclin D1 expression [10, 11]. Furthermore, the hyperdiploid subgroup and cases with cyclin D1 overexpression are under-represented in plasma cell leukaemia [12], where microenvironmental interactions are clearly less important. These examples support the notion that the genetics of MM cells and their interactions with the BM microenvironment are not completely independent of each other, but rather functionally interact to influence the biology and clinical behaviour of the disease.

Osteoclasts are the principal resorptive cells of bone and play a key role in the regulation of bone mass. They are multinucleated cells formed by the fusion of the mononuclear progenitors of the monocyte/macrophage family [13]. Myeloma cells either directly produce or induce other cells to produce “osteoclast activating factors”, which drive the differentiation of haematopoietic stem/precursor cells to mature osteoclasts as well as increasing their bone resorbing activity. Osteoclast activation significantly increases the growth and survival of the myeloma clone, an effect that is mediated both by direct cell-cell contact and indirectly via the release of soluble factors. Soluble growth factors and cytokines in the MM microenvironment, such as interleukin 6 (IL-6), osteopontin, B cell activating factor of the TNF family (BAFF) and a proliferation-inducing ligand (APRIL), have been implicated in osteoclast-induced myeloma cell survival [14].

Cell adhesion mediated drug resistance (CAMDR) is a feature of the myeloma cell interaction with osteoclasts in the BM. In this context it has been observed that co-culturing osteoclasts with myeloma cells is able to protect myeloma cells from drug-induced apoptosis [15]. Of interest in this respect is that after in vitro interaction with osteoclasts or stromal cells, myeloma cells acquire a more immature phenotype [16, 17]. Such observations are clinically relevant as high resolution imaging approaches have shown that plasma cells can persist in focal lesions in the BM of patients who have otherwise achieved a complete remission [18, 19]. Such a process could also be responsible for maintaining myeloma stem cells within a stromal cell niche in the BM, mediating chemo-resistance and subsequent disease relapse [20]. The effect of BMSCs to support proliferation, survival and drug resistance of MM should be viewed as pathological recapitulation of their natural role in supporting haematopoiesis.

Stromal cells are a heterogeneous assembly of mesenchymal stem cells (MSCs) that can differentiate into a variety of cell types including osteoblasts. MM cell adhesion to BMSCs (via adhesion molecules such as VLA-4 and VCAM-1) inhibits their differentiation into osteoblasts, at the same time, enhancing MM cell proliferation and survival through the direct activation of intracellular signal transduction pathways [7]. In addition, the increased production of pro-survival factors by stromal cells or myeloma cells, as consequence of their interaction, promotes myeloma cell growth in a paracrine or autocrine fashion. Important factors produced as a consequence of plasma cell-stromal cell interactions include IL-6, insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), tumour necrosis factor- α (TNF-α), stromal cell-derived factor 1α (SDF-1a), fibroblast growth factor (FGF), interleukin-1 (IL-1) and macrophage inflammatory protein 1 alpha (MIP-1α) [7]. In the myeloma cell, the cytokine stimulation and/or adhesion process activates diverse interdependent signalling cascades, including the Ras/Raf/MEK/MAPK pathway, the PI3K/Akt pathway, the JAK/Stat3 pathway, the nuclear factor-kappa B (NF-κB) pathway and the Wnt signalling pathway [21]. Taken together, these findings emphasize the role of protection provided by the BM microenvironment for myeloma cells, as well as providing a hint that modifying these interactions may improve the outcome of treatment.

A full understanding of the role of osteoblasts in mediating myeloma cell growth has been difficult to define, partially due to the use of ill-defined populations of osteoblasts [14]. Mature osteoblasts differ from BMSCs and immature osteoblasts by the expression of a different

pattern of cytokines and "osteoclast activating factors". Terminally differentiated osteoblasts produce high levels of OPG and reduced levels of RANKL, consequently reducing osteoclastogenesis and, therefore, bone resorption [22–24]. The production of pro-survival growth factors, such as IL-6 and IGF1, are reduced as BMSCs differentiate into osteoblast [25], also favouring an anti-myeloma effect. This effect may also be enhanced by the fact that mature osteoblasts can produce factors directly inhibiting the survival of myeloma cells as well as interfering with the pro-survival impact of osteoclasts [26]. One of the molecular mechanisms underlying such effects is mediated via decorin, a small leucine-rich proteoglycan. Decorin seem to directly induce MM cell apoptosis by activating caspase 3 and upregulating p21 [27]. In addition it indirectly inhibit tumour growth by degrading critical cell-surface growth-factor receptors such as MET and epidermal growth factor receptor (EGFR) [28] and by suppressing myeloma-induced osteoclastogenesis and angiogenesis [27]. These observations suggest that the inhibition of osteoblast differentiation in MM not only contributes to the induction of osteolytic lesions, but also creates favourable conditions for myeloma cell survival and proliferation. Thus therapeutic approaches to stimulate osteoblast differentiation may be able to reduce the levels of myeloma growth factors and osteoclastogenic factors in BM microenvironment, therefore, restraining myeloma clone growth and consequently improving clinical outcome.

Pathogenesis of Bone Disease in Myeloma

BD in myeloma is characterized by purely osteolytic lesions with no new bone formation, an effect that is mediated via increased osteoclastic activity and inhibition of osteoblast function. Interestingly in respect of the pattern of BD, myeloma cells lie in close proximity to the sites of active bone resorption and seem to play a key role in altering the balance of bone resorption and bone formation [29]. It is not surprising, therefore, that directly targeting the interactions of these local cellular components may have an impact on the growth of the myeloma clone with the potential for improving patients' survival.

Osteoclast Activating Factors

Several osteoclast activating factors have been found to be important in regulating bone resorption. The most significant system, in this respect, consists of the receptor activator of NF-κB (RANK), its ligand RANKL and a soluble decoy receptor, osteoprotegerin (OPG) [29] (Fig. 1). RANK is a transmembrane receptor of the TNF superfamily which is expressed on the surface of osteoclastic cells and their precursors, whereas its ligand, RANKL, is expressed by BMSCs, osteoblasts and T-lymphocytes. RANK-RANKL signalling leads to the activation of NF-κB and Jun N-terminal kinase (JNK) pathways, resulting in the differentiation of osteoclast from their precursor cells [30]. Moreover, RANKL is also involved in enhanced osteoclast survival through inhibition of Fas-mediated apoptosis [31]. OPG, the soluble decoy receptor for RANKL, is produced by osteoblasts as well as by other cell types within the BM and balances the interaction between RANKL and RANK, therefore, limiting its role in osteoclast activation.

In the myeloma BM microenvironment, the interaction between BMSCs and MM cells results in increased RANKL expression and decreased OPG production, favouring bone resorption [32, 33]. Circulating levels of OPG and RANKL have been shown to correlate with the clinical activity of MM, the severity of BD and poor prognosis [34, 35]. The critical role of RANKL-OPG axis in MM-induced BD has been further demonstrated in several mouse models, showing that recombinant OPG or a RANKL inhibitor (RANK-Fc) both prevented bone destruction and reduced tumour burden [33, 36]. The importance of this system would suggest that if a strategy targeting BD as a therapy for myeloma is to be effective, an effect should be seen if this system is targeted. The availability of denosumab, an antibody against RANKL, has given us the ability to address this question and is discussed below.

MIP1α, largely produced by myeloma cells and osteoclasts, belongs to the C-C chemokine subfamily. It binds to G-protein-coupled receptors, CCR1 and CCR5, to activate ERK and AKT signalling pathways. It is a potent inducer of osteoclast formation independent of RANKL by promoting osteoclast precursor cell migration and fusion [37] (Fig. 1). It also has multiple roles in myeloma cell, including promoting myeloma cell growth, survival and migration [38]. High serum levels of MIP1α correlate with osteolytic lesions and survival in MM patients [39], and targeting it directly could have important effects on improving patients' outcome. A clinical grade small molecule CCR1 antagonist, MLN3897, inhibits MIP1α-induced osteoclastogenesis and myeloma cell proliferation in vitro [40]. Recent report shows that MIP1α also inhibits osteoblast function (Fig. 1) [41], an effect mediated via downregulation of the osteogenic transcription factor osterix and downstream ERK signalling. MLN3897 blocks ERK phosphorylation and restores, at least partially, osteocalcin expression *in vitro* and *in vivo*. Evidence suggesting that targeting this molecule can improve clinical outcome comes from in vivo experiments showing that both antisense oligonucleotide and neutralizing antibody against MIP1α inhibit tumour growth and restore bone remodelling in a mouse model of MM BD [42, 43]. These observations highlight the

important pathogenic role of MIP1α in MM and associated BD and warrant the need to assess the targeted inhibitors in upcoming clinical trials.

Osteoblast Inhibitors

Myeloma BD seems to be related not only to the increased activity of osteoclast but also to a lack of an appropriate compensatory osteoblastic response. Bone formation is inhibited in myeloma via two distinct mechanisms, the first being the functional inhibition of already existing osteoblasts [44–46] and the second via the impaired differentiation of MSCs into new mature osteoblasts [44, 47, 48]. In addition to reducing new bone formation, the excess of immature osteoblasts provides a rich source of the osteoclastogenic factor RANKL, favouring bone resorption and myeloma cell survival [49]. The differentiation to a mature osteoblast is inhibited by factors secreted by both myeloma cells (e.g. DKK1, sFRP2-3, sclerostin, IL-7 and HGF) and microenvironmental cells (e.g. IL-3, activin A), as well as by direct cell-cell contact between MM cells and osteoblast precursors. A full understanding of the mechanisms underlying this process should provide a rich source of therapeutic targets able to treat BD and also to improve the survival of myeloma patients.

A positive signal delivered by the Wnt signaling pathway is crucial in osteoblast differentiation [50] (Fig. 1). Classically, Wnt binds to the coreceptors LRP5 and 6, and the complex then binds to the frizzled receptor. Signal transduction from the frizzled receptor results in stabilization of beta-catenin, which translocates to the nucleus and stimulates osteoblast differentiation [51]. A variety of secreted molecules can inhibit Wnt proteins by direct binding and associated inactivation (e.g. sFRP) or by competitive antagonism on Wnt surface receptors (e.g. DKK-1 and sclerostin). These antagonists may serve as potential therapeutic targets to increase bone formation, and consequently restrain the growth of the myeloma clone.

DKK1 binds to LRP5/6, thereby inhibiting downstream Wnt signalling. A role for DKK1 in the inhibition of osteoblast activity in MM was first suggested based on gene expression profiling (GEP) of myeloma patients [52], an observation which has been confirmed by various other detection methods (ELISA, qRT-PCR, western blot and immunostaining) [53, 54]. In addition, DKK1 has also been shown to enhance osteoclast activity via an increase in RANKL/OPG ratio [23, 24]. The serum DKK-1 levels have been shown increased in MM patients and correlate with the extent of BD [55, 56], and BM plasma, from MM patients, can inhibit osteoblast differentiation in vitro, an effect that is neutralized by an anti-DKK-1 antibody [52, 55]. Importantly in terms of targeting BD as a potential anti-myeloma therapy, it has been shown that administration of an anti-DKK-1 antibody in a mouse model of myeloma inhibits bone destruction, increases bone formation and also inhibits tumour growth [57].

The sFRP family proteins inhibit Wnt signalling via binding to Wnt proteins, resulting in reduced osteoblast function. Same as DKK1, FRZB is upregulated in MM [52, 58, 59], and a low BD group is characterized by its downregulation [4]. sFRP-2 has also been reported to inhibit osteoblast differentiation in myeloma patients [60], but conflicting results have also been found [61].

Sclerostin has, more recently, been identified as another major Wnt signalling pathway inhibitor via a mechanism similar to DKK1 [62]. Mutations of *SOST*, the gene encoding sclerostin, are linked to high-bone mass disorders [49], whereas transgenic mice overexpressing *SOST* are osteopenic [63]. Sclerostin levels are increased in the serum of patients with MM, correlating with advanced ISS stage, increased bone resorption, reduced osteoblast function and poor survival [64]. While sclerostin was thought to be exclusively expressed in osteocytes; it has recently been shown to be expressed in myeloma cells. Sclerostin has been demonstrated to reduce bone formation marker in an in-vitro co-culture system of BMSCs and myeloma cells and a neutralizing sclerostin antibody has been shown to improve bone formation markers [65]. These results suggest that anti-sclerostin represents a promising therapy for the anabolic treatment and warrant the assessment of this approach in myeloma BD.

Although there is compelling evidence that targeting Wnt signaling prevents myeloma BD in experimental models and that this may translate into improved survival outcome, concern has been raised over the implications for tumour growth. Activation of the Wnt signaling pathway through β-catenin has been linked to tumourigenesis [66] and expression of β-catenin has been demonstrated in myeloma cells [67]. Currently, published data are conflicting as to the role of Wnt signaling in myeloma cells [23, 67, 68]. Importantly, in all studies in vivo, when the tumour cells were present within the BM microenvironment, activation of Wnt signaling resulted in a reduction in tumour burden and prevention of myeloma BD [23, 68]. These data highlight the importance of the local microenvironment and demonstrate that, despite the potential to increase tumour growth at extramedullary sites, increasing Wnt signaling in the BM microenvironment seems to be able to prevent the development of myeloma BD and reduce tumour burden. Overall Wnt signalling pathway is a promising potential target for treatment of myeloma BD with the aim of not only to increase bone mass but also to improve patients' survival. However, further studies are necessary to clarify the role of Wnt signaling in myeloma growth, particularly at extramedullary sites.

Why Might There be a Survival Benefit for Targeting Bone

BD not only significantly impairs quality of life as consequence of skeletal complications such as bone pain and pathological fracture, but also has been linked to poor prognosis in myeloma patients. Bone resorption activity has been shown to be an independent risk factor for OS in MM patients [3]. Results from the MRC myeloma IX trial show that patients with presenting BD have a significantly shorter OS compared to those without BD, with a shorter survival from relapse being the main contributor to this effect (median 12.2 vs 23.4 months) [unpublished data]. In contrast, a low BD group has favourable survival [4]. These observations are not surprising as the dysregulated BM microenvironment components, which are responsible for BD in myeloma, have been demonstrated to contribute to disease progression and resistance to chemotherapy; although BD-associated poor quality of life may also contribute to the impaired outcome.

The important contributions of the BM microenvironment to disease progression can explain, to a certain extent, why the "novel drugs" that target the bone microenvironment as well as myeloma cells, have been more effective than conventional approaches. Apart from their direct anti-tumour activities, the immunomodulatory agents (IMiDs) thalidomide and lenalidomide and the proteasome inhibitor bortezomib, seem to affect osteoclast and osteoblast activity in myelomatous bones [69–72] (Fig. 1).

Bisphosphonates (BPs) are the current standard care for the prevention and treatment of malignant BD [73, 74], strong preclinical evidences from various models of MM suggest that nitrogen-containing BPs (N-BPs) such as zoledronic acid (ZOL) may have anticancer activity including inhibiting angiogenesis, enhancing antitumour immune responses, and directly or indirectly modulating the proliferation and survival of myeloma cells [74]. This has been confirmed by a number of clinical trials showing bisphosphonates improve both OS and PFS in myeloma patients [75–79]. These findings further support the notion that the interactions between myeloma cell and surrounding BM microenvironment constitutes an important factor that needs to be taken into account in the development of novel therapeutic strategies. Here we summarize the current therapies for myeloma BD and their possible anti-tumour activities.

Targeting Bone Resorption and the Osteoclast

Bisphosphonates (BPs)

BPs naturally bind to mineralized surfaces such as bone and inhibit osteoclast-mediated bone resorption (Fig. 1). The BPs pamidronate, ZOL, and clodronate (CLO; in the European Union but not in the United States) are approved for the treatment of patients with osteolytic lesions from MM for the prevention of skeletal-related events (SREs). The second generation N-BPs (e.g. ZOL, pamidronate) have been proven more effective at reducing SREs compared to the first generation BPs (e.g. CLO) [80]. Moreover strong preclinical evidence, from various models of MM, suggest that the N-BPs may have anticancer activity (Fig. 1) via the inhibition of angiogenesis, enhanced anti-tumour immune responses, inhibition of tumour cell migration and directly modulating the proliferation and survival of myeloma cells [81–86]. In vivo N-BPs may also affect MM progression by blocking the release of cytokines and growth factors from the bone matrix, thereby breaking the vicious cycle of bone destruction and cancer growth [74]. In addition, the anticancer effects of BPs have been demonstrated to have synergy with agents that are used in the treatment of myeloma, including dexamethasone, thalidomide, and bortezomib [84, 87, 88]. Preclinical mouse models of MM indicate that the anti-myeloma effect of N-BPs may be mediated via the inhibition of protein prenylation and consequent inhibition of the RAS-RAF-MAPK pathway [89], a mechanism of action not shared by non-N-BPs.

Although no significant differences in survival with BPs were observed in earlier clinical trials in MM, BPs seemed to improve survival in high-risk subsets of patients [76, 77, 90]. For example, in a trial of patients with newly diagnosed or relapsed/refractory MM (N=392), pamidronate significantly increased survival in the subset of MM patients receiving second-line antimyeloma therapy in comparison to placebo [77]. Based on the BP anticancer theory and promising early results, a large randomized trial (N=1960) was conducted to evaluate the role of BPs in newly diagnosed MM patients receiving either intensive or non-intensive regimens [78]. Results show that ZOL significantly reduces skeletal morbidity and significantly improves both PFS and OS (HR 1.19, 95% CI 1.04–1.35) versus CLO. Notably, the survival benefit with ZOL remained significant after adjustment for SREs, consistent with clinically meaningful antimyeloma activity.

Denosumab

As described above, bone resorptive osteoclastic activity is magnified by RANK/RANKL signalling and is inhibited by OPG, a soluble decoy receptor for RANKL. In the myeloma BM microenvironment the interactions between MM cells and BMSCs result in increased RANKL expression and decreased OPG production, and consequently enhances osteoclast differentiation by triggering NF-κB/JNK signalling in osteoclast precursors. In preclin-

ical studies, the critical role of RANKL in myeloma-induced BD was confirmed in mouse models of human MM bone using RANKL-specific inhibitors or OPG.

A human neutralizing antibody against RANKL, denosumab, which mimics the endogenous effect of OPG, has been tested in myeloma patients (Fig. 1). A single subcutaneous administration of denosumab induces a sustained inhibition of bone resorption markers lasting about 80 days versus 30 days of bisphosphonates [91]. Denosumab has been investigated in two phase II studies of myeloma patients previously treated with BPs, and both studies confirmed its efficacy in reducing SREs [92, 93]. In one of the trials using denosumab as a single agent in patients with plateau phase or progressive MM, although denosumab did not significantly decrease tumour burden, some patients with progressive disease experienced disease stabilization [93]. This observation raised the possibility that denosumab could influence MM growth through alteration of the microenvironment instead of via a direct cytotoxic effect. However, recently Henry et al. [94] reported the results of a phase III randomized trial that directly compared denosumab with ZOL on SRE development and survival in patients with myeloma. Consistent with the other studies denosumab was at least as effective as ZOL in reducing the time to first SREs; however, the ZOL treated group had a more favourable survival (HR 2.26; 95% CI 1.13 to 4.50). Therefore, current findings indicate that both BPs and denosumab can effectively reduce SREs, but ZOL was superior to denosumab in terms of survival benefit in MM patients. It is important, therefore, to be able to rationalize these differences given the importance of the RANK/RANKL system and the ability of denosumab to target it. The explanation for these differences may relate to the mode of action of these two agents. While they both target osteoclasts and consequently may indirectly restrain tumour growth, ZOL also has a range of both direct and indirect anti-myeloma activities, which seems to contribute to its survival benefit in MM patients over and above that which can be achieved by simply targeting one element of the known interactions. Alternatively as RANKL is also involved in dendritic cell maturation and the regulation of T cell-dependent immune response, the anti-RANKL strategy may have an effect on the immune system and a possible decreased T cell-mediated cytotoxic effect on myeloma cells.

Anti-BAFF-Neutralizing Antibody

Osteoclasts and myeloma cells interact by stimulating each others' growth and survival, and a critical mediator in this interplay is a TNF family member BAFF (Fig. 1). BAFF is a MM growth factor derived from osteoclast and BMSC that mediates both myeloma cell survival and myeloma cell-BMSC adhesion [95, 96]. A neutralizing antibody against BAFF has been shown to significantly inhibit tumour burden in vivo and, importantly, reduce the number of lytic lesions and osteoclast differentiation [97]. On the basis of these results, a phase I study of this BAFF-neutralizing antibody (LY2127399) combined to bortezomib is currently ongoing in drug resistant MM patients (NCT00689507) [98].

Immunomodulatory Drugs

Immunomodulatory drugs (IMiDs), such as thalidomide, pomalidomide and lenalidomide, are effective agents for treating MM. Apart from their well-known anti-tumour activity, these drugs may directly interfere with osteoclast differentiation via the reduction of PU.1 expression, a critical transcription factor during osteoclast development [70, 72]. Moreover, the exposure of BMSCs derived from MM patients to lenalidomide decreases their secretion of RANKL, consequently impairs osteoclastogenesis and favours myeloma cell suppression [72] (Fig. 1). These data were related to the clinical findings that in the serum of MM patients treated with lenalidomide, RANK/RANKL levels were reduced, whereas OPG levels were increased. However both thalidomide and lenalidomide have been shown to induce DKK1 expression of myeloma cells after 48 h of treatment in a GEP study, which could potentially impair osteoblast function [99].

Targeting Bone Formation and the Osteoblast

The N-BPs ZOL and pamidronate are the current gold standard for the treatment of MM BD; however, not all patients respond to the treatment. Despite being on ZOL up to 30% newly diagnosed MM patients still develop SREs within 2 years [78]. This observation has led researchers to focus activity on agents able to promote anabolic bone activity as a way of treating BD. The biological pathways outlined above provide a framework against which to describe evaluation of such agents.

Bortezomib

Possible informative data on the role of stimulating bone formation as a therapeutic strategy has come indirectly from the use of the proteosome inhibitor Bortezomib which was incidentally shown to have anabolic bone activities. Bortezomib is a proteasome and NF-κB signalling inhibitor with potent anti-MM activity. Since RANKL enhances osteoclast differentiation by activating NF-κB pathway, it is not surprising that bortezomib, by reducing NF-κB activity, can impair osteoclast survival and differentiation [100,

101]. In addition to decreasing osteoclast activity in MM, bortezomib also has "anabolic" bone effects by inducing osteoblast differentiation [69, 102] (Fig. 1). Although the mechanism(s) by which proteasome inhibition leads to increased osteoblast activity has not been firmly established, previous work suggests that proteasome inhibition can upregulate Runx2 and osterix, two critical transcription factors in osteoblast differentiation [102–104]. Bortezomib also appears to decrease the serum levels of DKK-1 and RANKL in patients with MM [105] (Fig. 1). Taken together, these preclinical data provide an explanation for the increased bone formation markers and reduced bone resorption markers seen in MM patients treated with bortezomib [106–109]. In a study of mouse MM model the effect of bortezomib treatment was compared with that of melphalan, which has direct anti-myeloma effects but no effect on bone [110]. Only bortezomib prevented myeloma BD suggesting that the effects of bortezomib on BD were not a consequence of reduced tumour burden but a direct effect on bone cells. Although these findings suggest that bortezomib has the capacity to prevent myeloma BD, at present there is only one report showing the effectiveness of bortezomib on SREs in myeloma patients [111], and interestingly radiological evidence of bone healing was observed in some of the bortezomib-treated patients.

Anti-DKK1 and Other Agents Targeting WNT Pathway

Canonical Wnt signalling has been identified as an important pathway in normal osteoblast differentiation and is tightly regulated by a combination of positive induction through the binding of the Wnt ligand and negative regulation through secreted Wnt inhibitors. The two basic therapeutic strategies for enhancing bone regeneration through the Wnt signalling pathways are adding agonists or blocking naturally occurring antagonists. The delivery of a canonical Wnt ligand, Wnt3a, to a SCID mouse model of human intramedullary MM can inhibit bone destruction and tumour growth, but has no effect when myeloma cells were grown subcutaneously [23]. However, Wnt ligands are glycoproteins that are difficult and expensive to produce and, therefore, the alternative strategy of blocking the effect of natural antagonists is a more feasible approach that is currently being explored.

The inhibition of GSK3β is necessary for effective canonical Wnt signalling and subsequent osteogenic differentiation, GSK3β inhibitors are, therefore, good candidates for bone anabolic therapy in MM (Fig. 1). Similar to the effect of Wnt ligand delivery, reduced bone destruction and tumour growth have been achieved in the 5TGM mouse model of MM treated with lithium chloride, a suppressor of GSK3β and activator of Wnt signalling [68]. An in-vitro study in myeloma cell lines has shown that GSK3β inhibition sensitizes myeloma cells to a histone deacetylase inhibitor [112]. GSK3β inhibition has also been shown to reduce myeloma–induced BD as well as inducing tumour cell death in a murine plasmacytoma model [113]. An orally active, small molecule GSK3β inhibitor, 603281-31-8, has been reported to increase bone mass and bone formation markers, lower adiposity and reduce fracture risk in ovariectimized mice [114, 115]. These promising results from preclinical studies warrant the testing of GSK3β inhibitors in clinical settings.

DKK1 is a soluble inhibitor of the Wnt pathway produced by MM cells. Recently, a DKK1 neutralizing antibody (BHQ880) has been tested in myeloma in the context of the bone microenvironment [116] (Fig. 1). This antibody was able to enhance osteoblast differentiation, inhibit osteoclast differentiation, as well as reduce IL-6 levels in a co-culturing system, which are potentially therapeutically relevant. While the antibody did not demonstrate direct cytotoxic effects on MM cells, it did inhibit MM cell growth when the MM cells were cocultured with BMSCs and this was associated with reduced IL-6 secretion by BMSCs, suggesting that it may have anti-myeloma effects in vivo. Indeed a few studies using murine MM models show that DKK1-neutralizing antibody increases osteoblast numbers and bone formation, as well as inhibits MM cell growth [57, 116]. BHQ880 is being tested in an ongoing phase I/II clinical trial for patients with relapsed and refractory MM who are receiving ZOL and anti-MM therapy (NCT00741377) [98].

Activin A Inhibitor

Activin A is a member of the transforming growth factor-β (TGF-β) superfamily and is released from BMSCs and osteoclasts. It signals through the activin type 2A receptor and has dual effects of stimulating osteoclast activity and inhibiting osteoblast differentiation (Fig. 1). Activin A levels have been demonstrated to be elevated in the BM of MM patients and correlate with the extent of osteolytic lesions [117]. Effects of Activin A inhibition in MM were investigated using a soluble receptor RAP-011. RAP-011 treatment leads to increased OB differentiation and inhibits OC development in vitro. It has also been shown to increase bone volume and decrease MM tumour burden in a number of murine models of MM [117, 118]. Furthermore, RAP-011 also increased bone formation in macaques, demonstrating the capacity of this agent to enhance bone formation in vivo [119]. As a result of these studies, a phase II trial of the humanized counterpart of RAP-011, ACE-011, in bisphosphonate naive MM patients with osteolytic lesions has been carried out, the results show that the bone formation markers are increased while the bone resorption markers are decreased in patients treated with the antagonist [120].

Conclusions

As we have increasingly recognized that tumour burden and BD are inextricably linked in MM, understanding the biology of MM BD and its roles in tumour growth and drug resistance is crucial for the development of novel anti-myeloma strategies. Some of the current therapies, such as N-BPs, IMiDs and bortezomib, have been shown being able to target both the tumour and bone cells (e.g. osteoblast and osteoclast), and consequently reduce the tumour burden and BD. More novel bone-targeted agents, such as BHQ880 (anti-DKK1), denosumab (anti-RANKL), ACE-011 (anti-activin A) and LY2127399 (anti-BAFF) are under development, and will significantly improve the care of MM patients in the future.

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Conflict of interest The authors declare that they have no conflict of interest.

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