Multiple myeloma bone disease: from mechanisms to next generation therapy

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SUMMARY

Multiple myeloma bone disease is a major cause of morbidity and mortality in multiple myeloma patients and persists even in patients in remission. Multiple myeloma bone disease is caused by an uncoupling of bone remodelling, with increased osteoclast activity and decreased osteoblast activity, culminating in lytic bone destruction. Bisphosphonates are the current standard-of-care but new therapies are needed. As the molecular mechanisms controlling multiple myeloma bone disease are increasingly understood, new therapeutic targets are extensively explored in the preclinical setting and initial clinical trials with novel compounds show promising results. In this review, we provide a comprehensive overview of the biology of multiple myeloma bone disease, summarise its current clinical management and discuss preclinical and clinical data on next generation therapies.

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INTRODUCTION

Multiple myeloma (MM) is a haematological malignancy characterised by the clonal proliferation and accumulation of malignant plasma cells in the bone marrow and associated end-organ damage.[1] Underlying MM are the oncogenic transformation of plasma cells and an altered bone marrow microenvironment that further contributes to MM development and progression. MM is the second most common haematological malignancy and has a yearly incidence of approximately 600 new cases in Belgium.[2] In the past decade, considerable therapeutic advances have been made by introducing hematopoietic stem cell transplantation and new targeted drugs such as immunomodulatory agents, proteasome inhibitors and monoclonal antibodies. Unfortunately, MM remains an incurable disease with a median overall survival of approximately six years for newly diagnosed patients.[3]

MM bone disease is a hallmark of MM and a major cause of morbidity and mortality in MM patients. It is characterised by the development of persistent lytic bone lesions and an uncoupling of the bone remodelling process. Pain related to so-called skeletal-related events (SREs) is the most frequent presenting symptom of MM patients.[4] In fact, up to 20% of patients present with a pathologic fracture.[5] More than 80% of MM patients develop MM bone disease and almost 60% develop a pathologic fracture during the course of the disease.[5,6]

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These fractures occur most often in the spine, which can result in spinal cord compression, and other common sites include the femur, pelvis, ribs and humerus.[7] Also, MM-induced bone loss underlies the hypercalcemia that is often observed in MM patients, which contributes to renal insufficiency and causes morbidities such as nausea, vomiting and confusion.[8] Aside from negatively impacting the quality of life and causing morbidity, MM bone disease has also been linked to patient survival, as the occurrence of a pathologic fracture increases the risk of death by more than 20%.[9,10] This is the result of a vicious cycle of MM expansion and bone destruction, which occurs via several mechanisms.

In this review, we will discuss the molecular mechanisms underlying MM bone disease, provide an overview of the current clinical management and discuss novel therapeutic strategies that are currently being developed.

UNCOUPLING OF BONE REMODELLING IN THE MULTIPLE MYELOMA BONE MARROW MICROENVIRONMENT

In physiologic conditions, the resorption of bone by osteoclasts and the deposition of new bone by osteoblasts are tightly coupled processes that occur in the bone remodelling compartment, a specialised structure lined by canopy cells which contains the bone remodelling unit, separating the latter from the rest of the bone marrow.[11] MM cells physically disturb this compartment, allowing the exchange of soluble factors and direct cell-cell interactions between MM cells and bone cells, i.e. osteoclasts, osteoblasts and osteocytes.[12] Furthermore, direct and indirect interactions between MM cells and other cells in the MM microenvironment, such as immune cells and stromal cells, result in the release of a wide range of factors that modulate the activity of bone cells.[13,14] Cumulatively, these mechanisms result in an uncoupled bone remodelling process, with an increased bone resorption by osteoclasts and a decreased bone formation by osteoblasts (*Figure 1*).

INCREASED BONE RESORPTION BY OSTEOCLASTS

The receptor activator of NF-κB (RANK) - RANK ligand (RANKL) - osteoprotegerin (OPG) axis plays a central role in the regulation of osteoclast activity and bone remodelling. RANK is expressed by osteoclast precursors and binding of RANKL to RANK induces osteoclast differentiation. OPG, a decoy receptor for RANKL, is secreted by osteoblasts and bone marrow stromal cells and the RANKL/OPG ratio is a critical regulator of the bone resorption rate.[15] RANKL/OPG is markedly increased in the MM microenvironment and serum RANKL/OPG negatively correlates with patient survival.[16] There is no consensus in the literature on whether MM cells themselves secrete RANKL. Farrugia *et al*. reported that patient-derived CD38+++ plasma cells express RANKL and can induce osteoclast differentiation.[17] Similarly, Sezer *et al*. reported RANKL expression in CD38++/CD138+ MM patient-derived plasma cells.[18] However, these findings are in contrast with multiple other studies, all indicating that MM cells themselves are not a source of RANKL but rather induce RANKL expression in the MM microenvironment, e.g. in stromal or immune cells.[19-22] In addition, osteocytes, which are embedded in the bone matrix and are the most abundant bone cell, are also a major source of RANKL. Interestingly, MM cells induce apoptosis in osteocytes which results in increased RANKL production by these cells, leading to increased osteoclast formation.[23,24]

MM cells secrete or induce the secretion of a range of other osteoclast activating factors in the MM microenvironment. This induction can be direct via cell-cell contact or occur via soluble factors. For example, the interaction between α4β1 integrin on MM cells and vascular cell adhesion molecule-1 (VCAM-1) on stromal cells induces RANKL production by the latter.[25] Also, direct interactions between MM cells and osteoclasts can lead to osteoclast activation and bidirectional jagged-notch signalling has been implicated in this process.[26,27] Moreover, MM cell-osteoclast interactions can enhance angiogenesis, resulting in increased osteoclastogenic activity by endothelial cells.[28] Finally, reports suggest that osteoclast differentiation from myeloid derived suppressor cells or fusion events of MM cells also contribute to bone resorption.[12,29,30]

Aside direct cell-cell interactions, many soluble factors that promote osteoclast differentiation have been identified in the MM microenvironment. Many of these act indirectly, i.e. by inducing the expression or potentiating the activity of RANKL or other osteoclast activating factors in the MM microenvironment. These include cytokines and growth factors such as interleukin-6 (IL-6), macrophage inflammatory protein -1α (MIP-1α), interleukin-3 (IL-3), growth differentiation factor 15 (GDF-15), parathyroid hormone related protein (PTHrP) and the glycosphingolipid GM3, which incorporates into lipid rafts on the osteoclast surface and ensures efficient RANKL-induced downstream signalling.[31-36] In addition, a number of cytokines in the MM

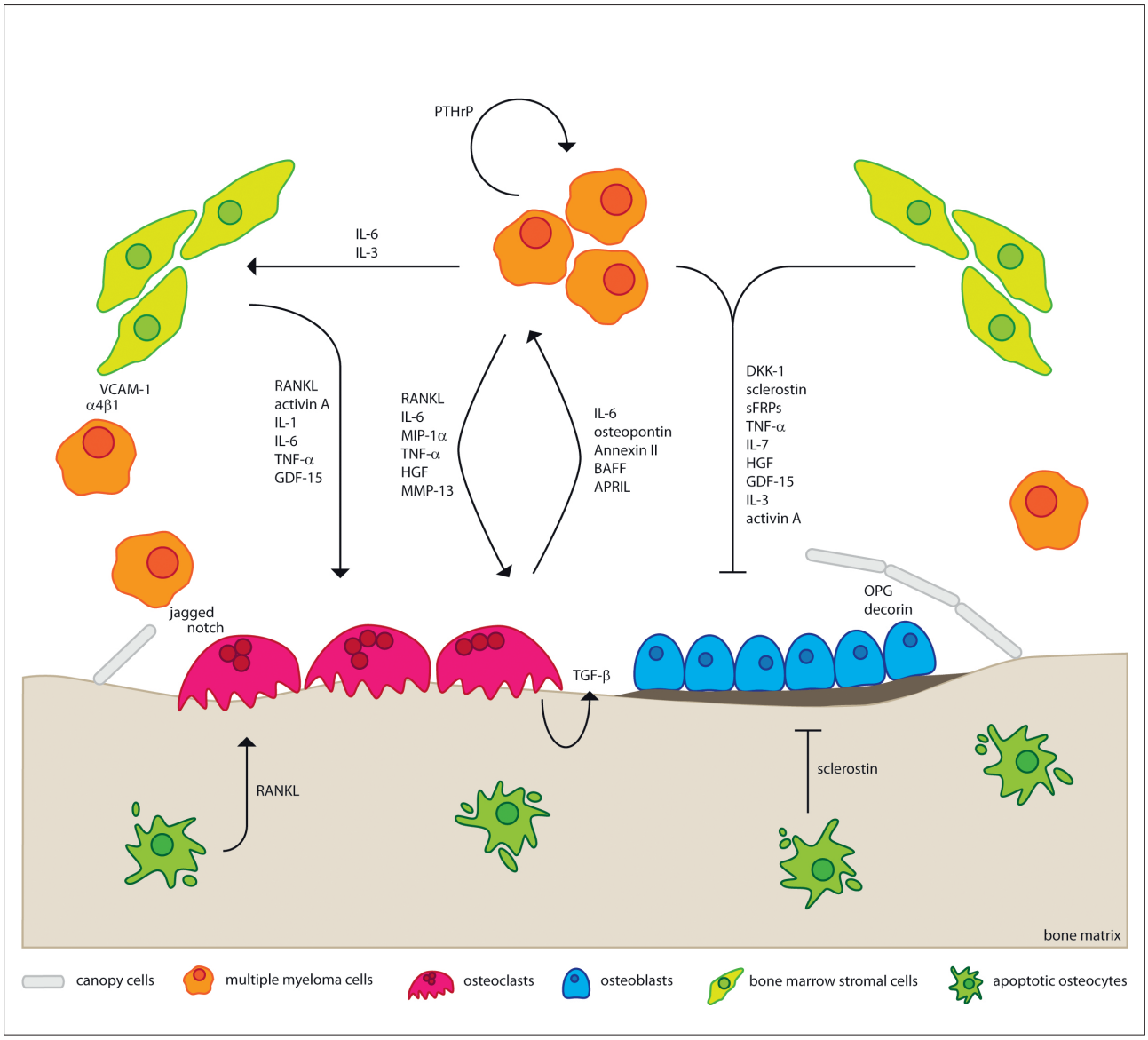

FIGURE 1. Extracellular factors involved in MM bone disease. MM cells physically disrupt the bone remodelling compartment and secrete a range of factors that stimulate osteoclast activity and inhibit osteoblast activity. In addition, direct and indirect interactions between MM cells and stromal cells and the induction of osteocyte apoptosis by MM cells leads to the release of factors that also contribute to MM bone disease. Increased osteoclast activity leads to the release of growth factors such as TGF-β from the bone matrix and also reciprocally stimulates MM tumour growth. Conversely, inhibition of osteoblast activity results in a decrease of OPG and decorin secretion by these cells, leading to enhanced bone resorption and MM tumour growth. Pointed arrows indicate stimulatory interactions while flat arrows indicate inhibitory interactions.

microenvironment have been shown to directly induce osteoclast differentiation, independent of RANKL signalling, or stimulate osteoclast activity. These include IL-6, MIP-1α, tumour necrosis factor-α (TNF-α), hepatocyte growth factor (HGF), activin A and matrix-metalloproteinase 13 (MMP-13).[37-43] Of note, many of these signalling cascades are interwoven and contain feedback loops. For example, MIP-1α is secreted by MM cells and stimulates osteoclast formation directly and via the potentiation of RANKL signalling.[32,44] Moreover, MIP-1α increases the expression of β1 integrin in MM cells leading to increased adhesion of these cells to stromal cells. This causes an increased secretion of RANKL, IL-6 and TNF-α by stromal cells, further enhancing tumour growth and bone resorption.[45] Of note, serum MIP-1α levels most strongly correlate with MM bone disease and patients survival.[46] A decrease of factors that normally hamper osteoclast differentiation

also contributes to increased osteoclastogenesis in the MM microenvironment. Pennisi *et al*. reported that bidirectional ephrin B2/EphB4 signalling between osteoclasts and stromal cells hampers osteoclast differentiation and that stromal expression of these factors is decreased in MM.[47] In addition, the inhibition of osteoblast differentiation in MM causes a decrease in the levels of OPG, which is produced by mature osteoblasts.[48]

Taken together, the increased osteoclastogenesis observed in MM is due to a complex signalling network consisting of direct and indirect pathways. MM cell-derived exosomes have been implicated in MM bone disease but their exact contribution remains to be elucidated.[49] Importantly, the increased osteoclast activity in MM not only causes exacerbated bone resorption, but also reciprocally stimulates tumour growth via multiple mechanisms, such as direct cell-cell contact, the production of MM growth factors like IL-6, osteopontin, annexin II, a proliferation inducing ligand (APRIL) and B cell activating factor (BAFF) by osteoclasts or the stimulation of bone marrow angiogenesis.[26,50-53] In addition, bone resorption could result in the release of growth factors such as transforming growth factor-β (TGF-β) from the bone matrix.[54]

DECREASED BONE FORMATION BY OSTEOBLASTS

Bone formation by osteoblasts is strongly and persistently inhibited in MM.[13,14,55] Even when patients are in complete remission for a long period of time, bone lesions due to MM bone disease rarely heal. This indicates that MM cells induce permanent changes in the bone marrow microenvironment that maintain osteoblast inhibition. Indeed, MM patient stromal cells retain an increased production of factors such as activin A, RANKL, IL6 and X-box binding protein 1 (XBP1s), even after weeks in culture.[13,40] In addition, a lack of mature osteoblasts further supports MM growth since these cells produce decorin, a proteoglycan that suppresses MM cell proliferation.[56] Interestingly, in the early phase of the disease there is an expansion of osteoblast precursors which secrete IL-3, IL-6 and granulocyte macrophage colony-stimulating factor (GM-CSF) and thereby stimulate MM cell growth and osteoclast differentiation.[57] However, at later stages osteoblast formation and function are inhibited which, together with increased osteoclast activity, results in bone destruction.

Runt-related transcription factor 2 (runx2) is a key transcriptional regulator of osteoblast differentiation from mesenchymal progenitor cells and inhibition of runx2 in osteoblast precursors has been observed in the MM microenvironment.[58] The mechanism underlying this inhibition is not completely understood, but MM cell-induced overexpression of the transcriptional repressors E4BP4 and growth factor independent 1 (gfi1) in osteoblast progenitors seems to play a role.[59,60] Similar to osteoclast activating factors, stromal- or MM cell-derived soluble factors have been identified that inhibit osteoblast differentiation or activity. Key mediators of osteoblast suppression in the MM microenvironment are inhibitors of the Wnt signalling pathway, including dickkopf-1 (DKK-1), sclerostin and secreted frizzled related proteins (sFRPs).[61-64] Wnt signalling leads to activation and nuclear translocation of β-catenin and this pathway plays a pivotal regulatory role in osteoblast differentiation. DKK-1 is highly expressed by MM cells and its expression correlates with the extent of MM bone disease.[65] However, the exact mechanism by which this factor contributes to osteoblast suppression remains unclear, as MM patients with high DKK-1 levels show equal levels of β-catenin compared to patients without MM bone disease.[63] Interestingly, DKK-1 disrupts Wnt3a-regulated expression of OPG and RANKL in osteoblasts, which further contributes to osteoclast formation and bone resorption.[66] In addition to RANKL, apoptotic osteocytes also release sclerostin, indicating that osteocyte apoptosis has both an osteoclast stimulatory and an osteoblast inhibitory effect.[23] Other pathways contribute to osteoblast suppression in MM bone disease as well. Tumour necrosis factor α (TNF-α) is secreted by MM cells, induces apoptosis in mature osteoblasts and suppresses osteoblast differentiation by downregulating key transcription factors such as TAZ, a transcriptional co-activator of runx2.[67] Suppression of runx2 in osteoblast progenitors is further potentiated by IL-7 and appears to occur via the induction of gfi1. Also, IL-7 suppresses runx2 activity rather than transcription.[58,60] TGF-β is released from resorbed bone matrix and thought to mediate osteoblast suppression, as treatment with a TGF-β type 1 receptor inhibitor restores osteoblast function in MM.[68] In addition, several osteoblast inhibitory factors, including DKK-1, sclerostin, MIP-1α, activin A, HGF, IL-3, IL-7 and GDF15, display increased serum or bone marrow plasma levels in patients with MM bone disease.[34,69-74] Also, osteoblast stimulatory factors, such as adiponectin, can be reduced in the MM microenvironment.[75]

DIAGNOSIS AND MANAGEMENT OF MULTIPLE MYELOMA BONE DISEASE

Pain related to SREs is the most frequent presenting symptom of MM patients. MM bone disease negatively impacts patient survival, is a major cause of morbidity resulting in a decreased quality of life and increases treatment costs.[9,10,76,77] In fact, SREs are a so-called myeloma-defining event, differentiating MM from its precursor diseases monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM (SMM).[78,79] Thus, early detection and optimal management of MM bone disease is of utmost importance.

A radiographic skeletal survey is routinely performed during the initial diagnostic workup, as recommended by the International Myeloma Working Group (IMWG).[80] However, a wide range of studies have now shown improved sensitivity and specificity for the diagnosis and monitoring of MM bone disease when using modern techniques such as whole-body low-dose computed tomography (WBLDCT), magnetic resonance imaging (MRI) and positron emission tomography (PET)-based techniques.[81] In fact, in many European institutions WBLDCT is now the standard technique used for MM patients.[82] In addition, bone turnover markers such as serum c-terminal telopeptide of type 1 collagen (CTX-1) or urinary n-terminal telopeptide (uNTx) can be used to monitor MM bone disease progression, response to treatment or relapse.[83] Of note, interpretation of bone turnover marker levels should be done with caution because of confounding factors such as the renal dysfunction often observed in MM patients, which interferes with the clearance of these markers, or non-malignant causes of altered bone turnover. A number of alternative serum biomarkers have recently been suggested including GDF15, decorin, bone specific alkaline phosphatase (BSALP), complement C4, miR-214 and miR-135b.[34,84-86]

Bisphosphonates are the cornerstone of current MM bone disease therapy.[87] Bisphosphonates are inorganic pyrophosphate analogues with a high affinity for calcium, causing these molecules to bind to hydroxyapatite and accumulate in the bone matrix. During bone resorption, bisphosphonates are released from the bone matrix and internalised by osteoclasts and their precursors via endocytosis.[88] Once internalised, bisphosphonates prevent osteoclast differentiation, activation and induce apoptosis. Their mechanism of action depends on the type of bisphosphonate. First generation non-nitrogenous bisphosphonates, such as clodronate, are incorporated into non-hydrolysable analogues of ATP that accumulate and result in apoptosis. Second and third generation nitrogenous bisphosphonates, such as pamidronate and zoledronic acid, inhibit farnesyl diphosphate synthase in the mevalonate pathway, leading to inhibition of protein prenylation and ultimately to apoptosis.[89] Intravenous pamidronate and zoledronic acid, and oral clodronate are effective for the prevention of SREs in MM and it is recommended that bisphosphonate therapy is initiated in MM patients with or without detectable osteolytic bone lesions on conventional radiography.[80] Moreover, bisphosphonate therapy should be considered for patients with MM precursor diseases, but only if these patients suffer from osteoporosis. Interestingly, a recent study by Raje *et al*. demonstrated the feasibility of dosing bisphosphonate therapy based on the monitoring of bone turnover makers.[90] In this study, 4 mg zoledronic acid was given every twelve weeks instead of every four weeks if patients had uNTx levels lower than 50 nmol/mmol creatinine and this resulted in a maintained low SRE rate. Pamidronate and zoledronic acid have comparable efficacy in reducing SREs in MM patients. However, zoledronic acid is recommended over clodronate because the former is more efficacious in preventing SREs and because its use is associated with a survival benefit.[91] The mechanism by which bisphosphonates exert anti-tumour effects is not completely understood and mechanisms such as decreased angiogenesis, induction of MM cell apoptosis and increased anti-tumour immunity have been suggested.[92-94] Based on these data, treatment with zoledronic acid or pamidronate is recommended for symptomatic MM patients with a recommended dose of 4 mg zoledronic acid or 90 mg pamidronate at 3- to 4-week intervals.[95] The advantage of bisphosphonates is not clear for patients without bone involvement on MRI or PET/CT.[96] In smoldering MM, bisphosphonates are not recommended and in cases of osteoporosis or vertebral fractures that are not due to myeloma, bisphosphonates should be given in asymptomatic patients with doses as given for osteoporosis, i.e. 5 mg zoledronic acid per year.[79,96] For symptomatic MM patients, the IMWG recommends that bisphosphonates should be administered for at least twelve months. After 24 months, it is at the physician's discretion whether to continue with bisphosphonate therapy. In patients not achieving complete response or very good partial response, zoledronic acid improved overall survival and reduced SREs after receiving treatment for more than two years. Whether this beneficial effect also occurs in patients achieving at least a very good partial response is not

TABLE 1. Major clinical trials on MM bone disease (excluding bisphosphonates).

Target	Drug	Study design	MM pts	Outcome/Results	Status/Reference
RANKL	denosumab	phase 2, denosumab vs. PAM/ZA	9	more frequent ↓uNTx, less on-study SREs	Fizazi *et al.* 2009
	denosumab	phase 2, denosumab	96	↓sCTx in plateau/relapsed pts	Vij *et al.* 2009
	denosumab	phase 3, denosumab vs. ZA	180	noninferior, trend to less on-study SREs, greater ↓uNTx, possible worse OS	Henry *et al.* 2011
	denosumab	phase 3, denosumab vs. ZA	1718	estimated completion: July 2016, primary outcome: time to on-study SRE	NCT01345019: active, not recruiting
cereblon, …	thalidomide	Td+ZA	35	↓sRANKL/OPG, ↓multiple resorption markers	Terpos *et al.* 2005
	thalidomide	Td+ZA	40	↓uNTX/crosslaps in pts obtaining > or = partial response	Tosi *et al.* 2006
cereblon, …	enalidomide	retrospective, Rd	106	↓sCTx and DKK-1 in responders	Terpos *et al.* 2014
	lenalidomide/ bortezomib	Rd or VRd	99	Rd: ↓sCTx in responders; VRd: ↓sCTx, sRANKL/OPG and DKK-1 and ↑bALP and OC irrespective of response	Terpos *et al.* 2014
	lenalidomide	phase 2, Rd + doxorubicin	45	estimated completion: September 2016, other outcome: change in multiple bone markers	NCT02471820: active, not recruiting
26S proteasome	bortezomib	restrospective, VTd	523	↑ALP in pts with at least partial response	Zangari *et al.* 2005
	bortezomib	V or Vd	34	↓sRANKL, cCTx, TRAP and DKK-1; ↑bALP and OC irrespective of response	Terpos *et al.* 2006
	bortezomib	V or Vd or non-V therapy	83	V or Vd: ↑bALP and OC irrespective of response	Heider *et al.* 2006
	bortezomib	V or Vd	21	↑osteoblasts on biopsy of pts with at least partial response, trend to ↓cCTx	Giuliani *et al.* 2007
	bortezomib	Vd+ZA	27	↑BMD in subset of relapsed pts	Terpos *et al.* 2010
	bortezomib	phase 3, VMP vs. MP	682	↑ALP correlating with response, ↓DKK-1	Delforge *et al.* 2011
	bortezomib	Vd+ZA	17	primary outcome: BMD	NCT00972959: completed
	bortezomib	Vd + doxorubicin + ASCT	19	estimated completion: December 2016, primary outcome: change in multiple bone markers	NCT01852799: active, not recruiting
20S proteasome	ixazomib	phase 2, ixazomib	20	estimated completion: September 2017, primary outcome: change in serum osteocalcin	NCT02499081: recruiting
20S proteasome	carfilzomib	phase 2, Cd	10	estimated completion: October 2016, secondary outcome: bone remodeling	NCT02020941: active, not recruiting

TABLE 1. Continuation.

Target	Drug	Study design	MM pts	Outcome/Results	Status/Reference
DKK-1	BHQ880	phase 1, BHQ880+ZA	28	primary outcome: time to on-study SRE, change in bone markers	NCT00741377: completed
	BHQ880	phase 2, BHQ880+Vd vs. Vd	9	primary outcome: time to on-study SRE in pts with renal insufficiency	NCT01337752: completed
	BHQ880	phase 2, BHQ880	41	secondary outcome: bone markers and BMD in high risk SMM pts	NCT01302886: completed
	DKN-01	phase 1, DKN-01	39	secondary outcome: multiple bone markers	NCT01457417: complete
activin A	sotatercept	phase 2, sotatercept +MPT	30	pts off BPs: ↑BMD and bALP	Abdulkadyrov *et al.* 2014
	sotatercept	phase 1, sotatercept +Rd	34	secondary outcome: multiple bone markers	NCT01562405: recruiting
	sotatercept	phase 2, sotatercept	20	primary outcome: change in bone markers	NCT02230917: recruiting

Major completed and ongoing (highlighted in red) clinical trials on MM bone disease with next generation therapies. References or clinicaltrials.gov identifier numbers are provided where available. Up arrows indicate an increase while down arrows indicate a decrease compared to baseline, placebo or control therapies (indicated in study design).

Pts: patients, PAM: pamidronate, ZA: zoledronic acid, T: thalidomide, d: dexamethasone, R: lenalidomide, V: bortezomib, M: melphalan, P: prednisolone, C: carfilzomib, uNTx: urinary N-terminal telopeptide of collagen type 1, SRE: skeletal related event, sCTx: serum C-terminal telopeptide of collagen type 1, OS: overall survival, RANKL: receptor activator of NF-κB ligand, OPG: osteoprotegerin, DKK-1: dickkopf-1, (b)ALP: (bone specific) alkaline phosphatase, OC: osteocalcin, TRAP: tartrate-resistant acid phosphatase, BMD: bone mineral density, SMM: smoldering multiple myeloma, BPs: bisphosphonates.

clear. In these patients, discontinuation of bisphosphonate therapy may be considered to prevent adverse effects. Bisphosphonates can be used to control bone pain and in a palliative setting radiotherapy can be considered to this end.[80] Balloon kyphoplasty can be considered for symptomatic vertebral compression fractures although its effectiveness remains disputed. As a result, balloon kyphoplasty is no longer reimbursed in Belgium (at a cost per level of ~6000 euro). Orthopaedic consultation should be sought in case of impending fractures or spinal cord complications.[80]

Bisphosphonates are generally well tolerated but serious adverse effects such as renal impairment or osteonecrosis of the jaw can occur. Therefore, preventive strategies should be adopted. In addition, bisphosphonate use is associated with a number of side effects such as atypical fractures, musculoskeletal pain, fever and hypocalcaemia.[97] These adverse effects may limit bisphosphonate use in some patients. Also, bisphosphonates have no bone anabolic effect and as such do not allow for healing of skeletal lesions. Together, these arguments underline the need to develop alternative and more potent therapies for MM bone disease.

NOVEL THERAPEUTIC STRATEGIES

Numerous therapeutic strategies are being explored in MM bone disease in both preclinical studies in murine MM models and in clinical trials (*Table 1*). Because of its central role in osteoclast differentiation, targeting the RANK/RANKL/OPG axis holds great potential in MM bone disease. Initial studies showed that administration of recombinant OPG prevents the development of MM bone disease and reduces tumour burden in a murine MM model and has a similar efficacy as pamidronate in MM patients.[98,99] In addition, a human monoclonal antibody targeting RANKL has been developed,

denosumab, which hampers osteoclast differentiation and survival and as a result decreases cancer-induced bone destruction, including in MM.[100] After promising initial clinical studies in different cancer types, phase III trials in MM patients comparing denosumab with zoledronic acid were performed and found that denosumab was superior to zoledronic acid in preventing skeletal events.[101] Although denosumab received FDA approval for the prevention of SREs in patients with solid tumours, this was not the case for MM, as the mortality rate was higher in the denosumab arm compared to the control arm in these patients.[101] However, Raje *et al*. recently raised valid concerns about differences in the baseline patient risk characteristics between the two arms.[102] To resolve this issue, a confirmatory phase III trial of denosumab and zoledronic acid in MM patients including adequate randomisation is currently underway (NCT01345019).

In order to increase osteoblast differentiation and activity, compounds targeting the Wnt signalling pathway have been developed. Treatment with a DKK-1 neutralising antibody, BHQ880, resulted in increased osteoblast numbers and trabecular bone as well as an inhibition of MM cell growth in murine MM models.[103] This led to the evaluation of BHQ880 and an alternative anti-DKK-1 antibody, DKN-01, in a number of clinical trials of which the complete results have yet to be reported. Similarly, neutralising anti-sclerostin antibodies, including romosozumab, show promise in increasing bone formation after pathological bone loss. Recently, Eda *et al*. reported that inhibition of sclerostin reversed MM bone disease in a murine xenograft MM model.[104]

Finally, blockade of MM cell-derived TNF-α and IL-7 prevented gfi1 induction in osteoblasts *in vitro*, relieving the suppression of runx2 and restoring osteoblast function.[60] These results warrant further exploration of targeting this pathway *in vivo*.

Cytokines that have a stimulatory effect on osteoclasts as well as an inhibitory effect on osteoblasts are therapeutic targets with great potential for MM bone disease. Activin A is a candidate for such an approach and treatment with RAP-011, a soluble activin A receptor prevented the development of bone disease in MM-bearing mice.[42] A similar compound, sotatercept, was tested in a phase II trial and partially repaired bone lesions in MM patients.[105] Additional clinical trials with sotatercept are currently recruiting patients. Other dual effect cytokines for which further exploration as therapeutic targets is warranted include MIP-1α, TNF-α, HGF, IL-3 and GDF15. Finally, many of the previously described factors involved in the biology of MM bone disease have been targeted via different means in different murine MM models, including ephrinB2/ephB4, adiponectin, MIP-1α and its receptor C-C motif chemokine receptor 1 (CCR1), BAFF, notch and TGF-β.[47,75,106-110] In all these studies, an inhibition of MM bone disease was observed.

Recently, a number of preclinical studies explored the therapeutic potential of small-molecule inhibitors of intracellular signal transduction pathways in MM bone disease. Bruton's tyrosine kinase (BTK) is expressed by MM cells and osteoclasts and regulates osteoclast differentiation.[111] BTK inhibition with ibrutinib inhibits MM growth and osteoclast activation in murine MM models, resulting in a decrease in MM bone disease.[112] Similar to BTK, SRC kinase is also involved in osteoclast activation and negatively regulates osteoblast function. Targeting of SRC kinase with saracatinib resulted in a prevention of MM bone disease in different murine models and initial reports suggest dasatinib treatment has a similar effect.[113,114] Similar to cytokine-targeted compounds, a wide range of small molecules targeting many cellular processes have been explored in preclinical studies as therapies for MM bone disease, with varying degrees of success. Amongst others, positive results were obtained by inhibiting phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) and mammalian target of rapamycin (mTOR), glycosphingolipid synthesis, sequestosome1/p62, p38 mitogen activated protein kinase (MAPK), AKT kinase and nicotinamide phosphoribosyltransferase (NAMPT).[36,115-120]

Drugs that are part of standard MM care not only reduce tumour load and thereby decrease the effect of MM cells on the bone marrow microenvironment, leading to less bone destruction, but also directly affect bone cell function. Proteasome inhibition leads to apoptosis of MM cells and several proteasome inhibitors have been approved for the treatment of MM patients, i.e. bortezomib, carfilzomib and ixazomib. Proteasome inhibition has a bone anabolic effect by promoting osteoblast differentiation.[121] In murine MM models, bortezomib induced an increase in bone formation and mineral density and similar results have recently been reported with ixazomib.[122,123] Also, bortezomib decreased DKK-1 levels in bone cells and in MM patients and inhibits osteoclast function.[65,124,125] This anabolic effect is clinically important as a healing of lytic lesions has been observed in some MM patients treated with these agents, with increased serum markers of osteoblast

KEY MESSAGES FOR CLINICAL PRACTICE

1 MM bone disease is major cause of morbidity and mortality in MM patients and persists even in patients in remission.

2 Diagnosis was routinely done with a skeletal survey, but modern imaging techniques such as WBLDCT are currently proposed as the new standard-of-care.

3 Intravenous pamidronate and zoledronic acid, and oral clodronate are effective for the treatment of MM bone disease. Zoledronic acid (4 mg/3-4 weeks) is preferred because it is associated with a survival benefit. Biomarkers such as uNTx might be useful to tailor treatment.

4 As the molecular mechanism is increasingly understood, next generation therapies are tested in ongoing clinical trials. Denosumab is in a confirmatory phase III trial while bone anabolic agents are in phase I/II trials.

activity such as alkaline phosphatase.[126] Therefore, further studies are needed on how proteasome inhibitors should be used optimally.[127] Immunomodulatory drugs (IMiDs) also directly affect bone cell function. IMiDs reduce osteoclastic resorption by inhibiting different factors such as PU.1 and BAFF.[128,129] In addition, IMiDs hamper the interactions between MM cells and other cells in the MM microenvironment such as stromal cells, osteoclasts and immune cells, interrupting the vicious cycle of bone destruction.[130] Similar to proteasome inhibitors, this is reflected in a number of clinical trials that show decreased bone turnover markers, DKK-1 levels and RANKL/OPG ratios in patients treated with IMiDs.

CONCLUSION

MM patients are benefiting from novel therapies that are being developed at a fast rate and markedly increase survival rates. However, MM bone disease persists in the vast majority of these patients and is a major cause of morbidity. Also, MM bone disease is involved in a vicious cycle of bone destruction and MM growth and thus directly contributes to increased mortality. Therefore, prediction, early detection and monitoring of MM bone disease are of great importance, warranting continued optimisation and exploration of imaging techniques and reliable biomarkers. Also, the development of new therapies is needed to prolong patient survival and improve their quality of life. In recent years, a large amount of studies have explored new therapeutic targets for MM bone disease, many of which show promising results in a preclinical setting. Of particular interest are those compounds that have a combined effect on osteoclasts and osteoblasts or compounds with a strong bone anabolic effect, as bone lesions in MM patients rarely heal. Also, new insights in the biology of MM bone disease resulted in the identification of important processes which have yet to be explored in a therapeutic setting, such as osteocyte apoptosis or the signalling pathways that suppress runx2 in osteoblast progenitors. Clinical trials with denosumab showed promising results and trials with new compounds or combinations are ongoing with the goal to make these next generation therapies available for MM, and possibly MGUS and SMM, patients.

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For the complete list of references, we refer to the electronic version of this article which can be downloaded from ariez.com

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