Myeloma bone disease: pathogenesis, current treatments and future targets

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Abstract

Introduction: Patients with myeloma develop localized and generalized bone loss leading to hypercalcaemia, accelerated osteoporosis, vertebral wedge fractures, other pathological fractures, spinal cord compression and bone pain. Bone loss is mediated by a variety of biological modifiers including osteoclast-activating factors (OAF) and osteoblast (OB) inhibitory factors produced either directly by malignant plasma cells (MPCs) or as a consequence of their interaction with the bone marrow microenvironment (BMM). Raised levels of OAFs such as receptor activator of nuclear factor-kappa B ligand (RANKL), macrophage inflammatory protein 1 alpha, tumour necrosis factor-alpha and interleukin 6 stimulate bone resorption by recruiting additional osteoclasts. Via opposing mechanisms, increases in OB inhibitory factors, such as dickkopf-1 (Dkk-1), soluble frizzled-related protein-3 and hepatocyte growth factor (HGF), suppress bone formation by inhibiting the differentiation and recruitment of OBs. These changes result in an uncoupling of physiological bone remodelling, leading to myeloma bone disease (MBD). Moreover, the altered BMM provides a fertile ground for the growth and survival of MPCs. Current clinical management of MBD is both reactive (to pain and fractures) and preventive, with bisphosphonates (BPs) being the mainstay of pharmacological treatment. However, side effects and uncertainties associated with BPs warrant the search for more targeted treatments for MBD. This review will summarize recent developments in understanding the intimate relationship between MBD and the BMM and the novel ways in which they are being therapeutically targeted.

Sources of data: All data included were sourced and referenced from PubMed.

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Areas of agreement: The clinical utility of BP therapy is well established. However, there is general acknowledgement that BPs are only partially successful in the treatment of MBD. The number of skeletal events attributable to myeloma are reduced by BPs but not totally eliminated. Furthermore, existing damage is not repaired. It is widely recognized that more effective treatments are needed.

Areas of controversy: There remains controversy concerning the duration of BP therapy. Whether denosumab is a viable alternative to BP therapy is also contested. Many of the new therapeutic strategies discussed are yet to translate to clinical practice and demonstrate equal efficacy or superiority to BP therapy. It also remains controversial whether reported anti-tumour effects of bone-modulating therapies are clinically significant.

Growing points: The potential clinical utility of bone anabolic therapies including agents such as anti-Dkk-1, anti-sclerostin and anti-HGF is becoming increasingly recognized.

Areas timely for developing research: Further research effectively targeting the mediators of MBD, targeting both bone resorption and bone formation, is urgently needed. This should translate promptly to clinical trials of combination therapy comprising anti-resorptives and bone anabolic therapies to demonstrate efficacy and improved outcomes over BPs.

Key words: myeloma, bone disease, therapeutic targets

Introduction

Multiple myeloma is predominantly a cancer of terminally differentiated B-lymphocytes, i.e. malignant plasma cells (MPCs). It accounts for 1% of all malignancies and 10% of all haematological malignancies1,2 and is almost always incurable. Current advances have led to increased survival,3,4 although this is still much shorter than for other mature B-cell malignancies, such as follicular lymphoma and chronic lymphocytic leukaemia. This discrepancy may be due to the development of myeloma bone disease (MBD), which affects approximately 60% of patients, rising to 80–90% at some stage of their disease.5,6 MBD-related morbidities include pain, pathological fractures, spinal cord compression and hypercalcaemia5,7,8 (Fig. 1). Skeletal-related events (SREs) including pathological or vertebral fractures, hypercalcaemia or severe bone pain9 compromise mobility and day-to-day independence and decrease quality of life.10–13 Moreover, SREs increase the risk of mortality and decrease survival, with pathological fractures, which occur in 40% of patients, being associated with a 23–32% increased risk of mortality.14 The spinal cord compression occurring in 5% of all patients with myeloma often leads to disability and a profound impact on prognosis, even if myeloma is otherwise contained.15,16 It is reasonable to speculate that if MBD can be eliminated or controlled, the prognosis of patients with myeloma may be extended to that commonly associated with other B-cell malignancies, such as follicular lymphoma and chronic lymphocytic leukaemia, where patients frequently survive beyond a decade, even with active disease.

This review aims to summarize recent developments in our understanding of pathways in MBD and the bone marrow microenvironment (BMM) and how they may be targeted. Improved understanding of MBD may not only lead to better prevention and control of bone disease, but also to treatments that provide an anti-tumour effect in this incurable cancer.
Myeloma bone disease

Under physiological conditions, skeletal health is maintained by a dynamic balance between bone formation and resorption, leading to re-mineralization of the skeleton approximately every 7 years and the ability of the skeleton to respond appropriately to physiological stress. In myeloma, this balance is uncoupled, with an increase in the number and activity of osteoclasts (OCs) and a decrease in the number of osteoblasts (OBs) leading to accelerated osteoporosis and the development of lytic lesions. These processes are intimately related to interactions between MPCs and cells of the BMM. For example, interactions between MPCs and bone marrow stromal cells (BMSCs) increase OC activity and differentiation, with the production of a group of mediators referred to originally by Mundy et al. over 40 years ago as osteoclast-activating factors (OAFs). In recent years, a number of OAF have been identified including interleukin-6 (IL-6), interleukin 1β (IL-1β), interleukin-3 (IL-3), macrophage inhibitory protein 1 alpha (MIP-1α), tumour necrosis factor-alpha (TNFα), hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF). Many of these OAF work through the pathway of receptor activator of nuclear factor kappa B (RANK), its ligand (receptor activator of nuclear factor-kappa B ligand, RANKL) and its inhibitor osteoprotegerin (OPG) (Fig. 2).

More recent studies have focussed on the interactions mediated between MPCs, the BMM and BMSCs, which decrease OB differentiation and function. Although MPC infiltration initially up-regulates the production of OB precursors, osteoblastic differentiation is subsequently inhibited resulting in reduced numbers and activity of OBs. Various molecules have been implicated as inhibitors of OB differentiation including HGF, transforming growth factor beta-1 (TGF-β1) and the Wnt signalling inhibitors, dickkopf-1 (Dkk-1), soluble frizzled-related protein-3 (sFRP-3) and sclerostin (Fig. 2).

The role of adhesion molecules

Recently, it has also been suggested that MPCs reside in and exploit specific osteogenic and angiogenic niches similar to those proposed to exist supporting the quiescent state maintenance and subsequent differentiation of haematopoietic stem cells (HSCs). These interactions are likely to be mediated by an array of chemotactic factors and adhesion molecules. Many cytokines, growth factors and adhesion molecules have been implicated and proposed to have various roles in the pathogenesis of myeloma survival, chemoresistance, quiescence and proliferation. Additional roles for these molecules have also been suggested, directly related to OB and OC activity, thus implicating adhesion molecules as contributors to MBD and identifying them as interesting potential therapeutic targets, which may prove effective not just as anti-tumour strategies but also as attenuators of MBD.
It has been suggested that, within the BMM, adhesion molecules mediate interactions between MPCs, BMSCs and the extracellular matrix. This may provide a proliferative advantage by activating the NFκB pathway, producing the growth factor IL-6, which promotes MPC survival and by conferring a level of drug.

Fig. 2 Mechanisms of MBD; cellular interactions. (a) Schematic diagram of the cellular interactions in myeloma. MPCs secrete OAFs including RANKL, MIP-1α, TNFα, IL-6 and IL-3. These OAFs stimulate bone resorption by recruiting additional OCs. (i) MPCs also secrete OB inhibitory factors, such as Dkk-1, sFRP3 and HGF, and sclerostin suppresses bone formation by inhibiting the differentiation and recruitment of OBs. Increased OC numbers and decreased OB numbers lead to net bone destruction, the loss of internal trabecular structure and the formation of focal lytic lesions. (b) Close up image of mouse tibiae in which haematopoietic tissue has been totally effaced by myeloma also leading to loss of internal trabecular structure and the formation of focal lytic lesions (arrowed). (ii) (c) Close up of a lytic lesion in evolution with prominent tartrate resistant acid phosphatase (TRAP) positive resorbing OCs (arrowed).
resistance. Other molecules are thought to be involved in the adherence of MPCs to BMSCs and OBs; the most thoroughly investigated adhesion molecules are integrins, in particular very late antigen-4 (VLA-4), vascular cell adhesion molecule-1 (VCAM-1) and syndecans. VLA-4, a β1-integrin expressed by MPCs, binds to fibronectin and VCAM-1 present on BMSCs. Integrins induce MPC proliferation through the production of growth factors and also confer cell adhesion-mediated drug resistance (CAMDR). Drug-resistant myeloma cell lines overexpress VLA-4, and cell lines expressing VLA-4 and VLA-5 exhibit antia apoptotic effects following melphalan or doxorubicin treatment. Studies blocking VLA-4 adhesion suggest that this molecule plays a vital role in the development of myeloma and osteolytic bone disease.

The role of the adhesion molecules Notch-1/Jagged-1 and N-cadherin have also been investigated. Notch is expressed on HSC and by binding to its receptor Jagged on BMSC it plays a role in HSC survival and differentiation. It has also been shown to be highly expressed in B-cell neoplasms, where its interaction with BMSC promotes tumour cell growth and survival. A similar role is proposed in myeloma. Notably, Notch and Jagged expression is greatly increased in patients with myeloma and in some patients with monoclonal gammopathy of undetermined significance (MGUS) compared with healthy individuals. Its binding induces myeloma growth factors IL-6, VEGF and insulin-like growth factor 1 (IGF-1), suggesting that it provides a proliferative role in early myeloma.

Cadherins are adhesion molecules that mediate cell migration and invasion in normal embryogenesis. N-Cadherin is involved in cell invasion but may have multiple roles in malignancy such as cell adhesion, invasion and migration. N-cadherin mediates MPC localization and adhesion in the bone marrow, residing close to N-cadherin expressing OB cells. It is expressed in about half of patients with myeloma and is linked to poor prognosis. Perhaps even more importantly than its cell adhesion and localization properties, it may reduce osteoblastogenesis, through inhibition of the Wnt signalling pathway, by binding Lrp5, thereby increasing β-catenin degradation. This induces OBs to overexpress N-cadherin, which may increase adhesion of MPCs, hence creating a vicious cycle of MPC adhesion and OB inhibition, which increases tumour burden and net osteolysis.

### Osteoclastic activation

**RANK/RANKL/OPG**

Most OAFs induce OC activation through the production of RANKL. Expressed by activated T cells, BMSC and OBs, RANKL binds to its receptor RANK expressed on the surface of OC progenitor cells. OPG, expressed by BMSC, also binds to RANK, thereby inhibiting OC differentiation and function by blocking the binding of RANKL. The interactions of these three members of the tumour necrosis factor superfamily are pivotal to bone remodelling, both physiologically and in MBD.

The high rate of bone resorption in myeloma is characterized by an increase in RANKL and a decrease in OPG, and this increased ratio is considered a potent driver of osteoclastogenesis. Myeloma patients have raised levels of RANKL in their serum compared with healthy individuals and those with monoclonal gammopathy of undetermined significance (MGUS). A positive correlation is seen between increasing levels of serum RANKL and the number of osteolytic lesions, and a significantly longer progression-free survival (PFS) is seen with lower levels of total RANKL in patients with myeloma. Blocking RANKL with OPG reduces bone destruction and tumour burden in animal models.

The interaction of MPCs and BMSCs induces the production of RANKL. It is not certain whether MPCs produce RANKL directly or if it is expressed by the BMSCs: some studies suggest that patient-derived MPC lines express RANKL whereas others suggest the opposite. The general consensus is that OC activation is induced by increased RANKL expression from BMSCs, owing to adhesion interactions between the MPCs and BMSCs resulting in the production of OAFs. A concomitant reduction in OPG expression may also contribute to increased bone loss.
IL-6

Studies suggest that dysregulation of IL-6 expression may contribute to MBD in a number of ways. Data suggest that IL-6 acts as a potent growth factor for MPCs, and its production is increased by tumour cell interaction with the BMM. In addition, IL-6 has potentiating effects on the osteoclastogenic factors MIP-1α, IL-3 and RANKL and induces RANKL production in the BMM. Levels of IL-6 have been correlated with increased bone turnover. IL-6 stimulated increases in survival and growth of MPCs lead to up-regulation of its own production, as well as the production of other osteoclastogenic factors; this results in increased tumour burden and MBD through creation of an autocrine cycle.

TNFα

TNFα, a potent upregulator of IL-6, is produced by MPCs, and this contributes to their proliferation and survival. TNFα also induces OC development by acting synergistically with RANKL. TNFα can also induce OB apoptosis and reduce the differentiation of mesenchymal stem cells (MSC) to mature OBs via down-regulation of RUNX2 and TAZ, which are both bone-specific transcription factors supporting OB development. The dual osteolytic effect of TNFα may provide a critical target for therapeutic intervention in myeloma.

MIP-1α

MIP-1α is a chemotactic cytokine (CC) chemokine, also known as CCL-3, which acts as a chemoattractant for macrophage–monocyte lineage cells, including OCs, by binding expressed receptors CCR1, CCR5 and CCR9. Produced by MPCs, it can induce their migration, proliferation and survival in the bone marrow via signalling pathways, such as AKT/protein kinase B (PKB) pathway and mitogen-activated protein kinase (MAPK) pathway, and triggers terminal stages of OC differentiation and activation, thus leading to increased bone resorption. Moreover, it drives osteoclastogenesis as it enhances the production of IL-6 and RANKL by increasing MPC and BMSC adhesion. Addition of an antibody to MIP-1α reduces OC number and lytic lesions in vivo models of myeloma and reduces adherence and homing of MPCs to BMSCs. High serum levels of MIP-1α in myeloma patients have been correlated with lytic lesions and reduced survival. Increased IL-6 production not only increases levels of RANKL, but it is also one of the most important factors in myeloma cell growth and survival, further increasing tumour burden. The dual effect of MIP-1α makes it a promising therapeutic target for control of both MBD and tumour burden.

HGF

HGF is emerging as a key target in the pathophysiology of myeloma tumour progression and MBD. Derksen et al. have described HGF production by BMSCs and by some MPCs. These authors have shown that MPC cells also express the HGF receptor Met. Thus, binding of HGF derived from BMSC and MPC cells, to Met, expressed on the surface of MPC cells initiates downstream signalling via the RAS pathway, which, in turn, drives proliferation of MPC cells and inhibits apoptosis. HGF has also been implicated as a cytokine regulating both OC development and inhibition of OB differentiation. Grano et al. have identified HGF production by OCs and expression of the HGF receptor by both OCs and OBs. These data suggested HGF-mediated autocrine regulation of OCs and paracrine regulation of OBs and that HGF therefore acted as a coupling factor between OC and OB activity. Subsequently, a number of studies noted expression of HGF by myeloma cell lines and purified primary myeloma cells. Standal et al. have recently demonstrated that HGF inhibits BMP-induced expression of alkaline phosphatase (ALP) in human MSCs and the murine OB.
precursor cell line C2C12. Considering the above, targeting HGF may well have substantial anti-tumour potential and help to prevent MBD.

IL-3

IL-3 is significantly increased in the plasma of patients with myeloma and stimulates MPC growth independently of the mechanisms of action of IL-6. It also increases osteoclastogenesis and bone destruction, possibly in combination with MIP-1α and RANKL. In addition, IL-3 plays a role in OB suppression through BMP-induced OB differentiation. Recent research has shown that IL-3 expression induces Activin A, which may be the mechanism by which it stimulates OC activity and production.

Osteoblastic inhibition

Recently, the importance of the role of OBs and bone formation in MBD has become more apparent and has been the subject of increased investigation. Interestingly, in the early phase of myeloma there appears to be an up-regulation of OB precursors. The interaction of MPCs with the BMM initiates the production of IL-1 and TNFα; these cytokines recruit OBs, which produce IL-6, a potent myeloma cell growth factor and bone resorption factor. OBs also produce the growth factors IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF), which further stimulate early myeloma cell growth and bone resorption. Both early OC and OB recruitment stimulate MPCs inducing apoptosis, growth and survival by the production of a number of cytokines and factors, predominantly IL-6. However, as disease progresses, OB maturation and function are inhibited and the reducing bone formation rate fails to counter increased resorption, resulting in net osteolysis. A number of different effectors are implicated in this process, many of which act as inhibitors of the Wnt signalling pathway.

The Wnt signalling pathway and its inhibitors

The Wnt signalling pathway, involved in embryogenesis and development in most animals, is an important regulator of stem cells and central nervous system patterning. Studies have shown that Wnt signalling regulates stem cell and cancer cell involvement in epidermal, intestinal and haematopoietic systems. Wnt signalling influences osteoblastogenesis through a canonical pathway involving extracellular and intracellular interactions, initiated by Wnt proteins binding cell surface receptors made from a complex of lipoprotein-related (Lrp) 5/6 and Frizzled (Fzd) transmembrane proteins. Once initiated, the complex induces an intracellular cascade involving dishevelled (Dsh), Axin and GSK-3 among others, which prevents phosphorylation of β-catenin, therefore preventing its breakdown. This increases cellular levels of β-catenin, thereby up-regulating the transcription of genes involved in OB development. There are a number of natural inhibitors of this pathway, which include dickkopfs (Dkk), secreted frizzled-related proteins (Sfrp) and Wnt inhibitory factor-1 (Wif-1).

Dickkopfs

Dkk are a multigene family consisting of two distinct cysteine-rich molecules with 10 cysteine residues, highly conserved between family members ranging from Dkk-1 to -4. Studies have shown that Dkk-1 and -4 inhibit the Wnt signalling pathway, preventing the intracellular interaction, which protects β-catenin from phosphorylation and its subsequent breakdown. The mechanism of inhibition is through competitive binding and removal of transmembrane receptors. Mao et al. demonstrated that Dkk-1 inhibits the function of the canonical Wnt signalling pathway by binding Lrp6 and transmembrane proteins Kremens 1 and 2. This trimeric complex is then endocytosed, internalizing the receptor for Wnt proteins and thus inhibiting the initiation of the Wnt signalling cascade.

The importance of Dkk in bone formation and density is demonstrated by the physiological effects of high and low serum levels of Dkk-1. In patients with Lrp5 transmembrane protein mutations, the membrane receptor is unable to bind Dkk-1 and this results in uninhibited Wnt signalling and increased bone mass density. On the other hand, in vitro studies have shown that Dkk-1 overexpression by MPC results in inhibition of the differentiation of OB
precursors and this results in development of focal lytic bone lesions in vivo. Dkk-1 inhibition of Wnt signalling may also indirectly stimulate osteoclastogenesis and increase bone resorption as well as decrease bone formation. Because Dkk-1 production inhibits the maturation of OBs, which produce OPG, an inhibitor of osteoclastogenesis, this results in decreased OC inhibition. In addition, increased numbers of immature OB, which produce RANKL, thereby stimulating OC differentiation, no longer differentiate and this results in a net increase in RANKL expression and subsequent OC differentiation.

Tian et al. demonstrated that myeloma cells express higher than normal levels of Dkk-1 and suggested an association between these elevated levels and MBD. Their data identified a significant increase in Dkk-1 expression in the plasma cells of patients with multiple myeloma, and they correlated this with increased levels of Dkk-1 protein in the bone marrow and peripheral blood. Expression of Dkk-1 is significantly increased in some patients with myeloma compared with the healthy population and MGUS and also correlates with the stage of disease, showing increased levels of Dkk-1 at more advanced stages. Data also suggest that Dkk-1 levels also correlate with the extent of lytic bone disease present.

Blocking Dkk-1 in vivo, through the use of monoclonal antibodies, results in significant increases in OB bone formation and bone mineral density in both murine and human models of myeloma. Yaccoby et al. demonstrated significantly increased numbers of OBs and increased bone mineral density in the SCID-rab mouse model, using primary human myeloma cell lines. Their data also show a significant decrease in OC number and reduced MPC tumour burden. Heath et al. also demonstrated significantly increased bone formation (25%) and increased bone mineralization (28%) using an anti-Dkk-1 antibody in the 5T2MM murine model of myeloma. Fulciniti et al. used a SCID-hu model of myeloma and the anti-Dkk-1 antibody BHQ880. Their study showed a significant increase in OB and trabecular bone. However, this effect was only observed in the presence of BMSC, suggesting that interactions between BMSC and MPCs are involved in Wnt-induced inhibition of OB maturation, or that there is additional interaction between BMSC, which promotes osteoblastogenesis and which is prevented by the use of this antibody. Interestingly, neither Heath et al. nor Fulciniti et al. demonstrated an OC effect or demonstrated the decreased bone option described by Yaccoby et al.

Both studies using MPC lines also showed a significant reduction of tumour burden when Dkk-1 was inhibited. Fulciniti et al. demonstrated a significant reduction in IL-6 with Dkk-1 antibody treatment, which, as a potent MPC growth factor, may be responsible for this anti-tumour effect. They also suggest the reduction in BMSC and MPC adhesion from this interaction, further reduces IL-6 production and increases the anti-myeloma effect. The mechanism of how Dkk-1 may enhance tumour cell growth has been suggested by Gunn et al. By co-culturing MPCs and MSCs from the BMME, they propose a feedback loop whereby MPC and MSC interact through Dkk-1 causing MSCs to differentiate into OB precursors that produce IL-6. This promotes MPC growth, which results in further production of Dkk-1 and inhibits the differentiation of precursor cells into OBs. Heath et al. pose the intriguing question: does increased bone formation result from reduced inhibition of Wnt signalling and decreased tumour burden from anti-Dkk-1 treatment. Despite this uncertainty, blocking Dkk-1 remains a tantalizing treatment option, aimed at reducing tumour burden and decreasing the extent of MBD.

Secreted frizzled-related proteins

sFRP are a family of cysteine-rich glycoproteins, structurally similar to Fzd proteins, which make up a cell membrane surface complex via combination with Lrp 5/6, although this complex lacks a transmembrane region. Once present at the cell surface, this complex inhibits Wnt signalling through interception and binding of Wnt proteins, preventing their interaction with the Lrp 5/6 and Fzd transmembrane compound, which would initiate the canonical Wnt signalling cascade. They are expressed by a number of cells involved in bone formation and regulation, including primary human OB. Studies using recombinant
sFRP-1 and -4 have shown that these proteins have a negative effect on OB differentiation and also reduce bone formation. To date, any corresponding inhibitory effect in vivo remains unreported.\textsuperscript{74} sFRP-1 is an important inhibitor of the canonical Wnt signalling pathway. It is consistently highly expressed by OBs and suppresses Wnt signalling by 70\%. sFRP-1 messenger RNA accumulates as MSC form pre-OBs. mRNA levels decline upon maturation of the pre-OB population.\textsuperscript{87} Thus, increased numbers of OB precursors and reduced differentiation to mature OBs lead to increased production of sFRP-1. Increased levels of sFRP-1 reduce bone mineral density, trabecular volume and biomechanical properties and increase osteochondral apoptosis.\textsuperscript{88} Inhibition of sFRP-1 using N-substituted piperidinyl diphenylsulfonyl sulphonamides results in OB activation and increased bone formation in an ex vivo murine model of myeloma.\textsuperscript{89} Data also suggest that over expression of sFRP-4 by OB decreases their proliferation and results in decreased bone formation in vivo.\textsuperscript{90}

Data suggest that sFRP-2 is overexpressed specifically by MPC derived from patients with advanced bone disease, whereas other Wnt inhibitors, such as Dkk-1 and sFRP-1, are not. Conditioned media from both human myeloma cell lines and primary cells reduced OB alkaline phosphatase activity and bone mineralization in vitro.\textsuperscript{91} Overexpression of sFRP-3 has also been linked with the progression of MGUS to myeloma, suggesting that this too may play a role in the development of MBD.\textsuperscript{92}

### Other regulators of the Wnt signalling pathway

Several other inhibitory factors have been identified that regulate the Wnt signalling pathway, and these could also play a role in the inhibition of OB in myeloma.

#### Wnt inhibitory factor-1

Wif-1 interacts with Wnt molecules at the cell surface, thereby inhibiting the interaction of Wnt with the cell surface co-receptors, which initiate Wnt signaling.\textsuperscript{71} Wif-1 is induced by bone morphogenetic protein-2 (BMP-2) signalling during OB differentiation and changes in its expression have therefore been implicated in the regulation of bone formation.\textsuperscript{74}

### Sclerostin

The gene SOST encodes the protein sclerostin. Mutations to this gene resulting in its down-regulation produce a phenotype characterized by excessive bone formation.\textsuperscript{93} The development of disease in its absence suggests that it plays an important role in normal bone homeostasis.\textsuperscript{94} Normally, sclerostin interacts with Lrp-5 and 6 and prevents the binding of Wnt, inhibiting the canonical Wnt pathway, thereby reducing bone formation. This idea is supported by the observation that mineralizing OB and central regulatory osteocytes highly express SOST.\textsuperscript{95} Its overexpression in transgenic mice results in low bone mass due to reduced bone formation by reduced OB activity.\textsuperscript{96} Studies aimed at discovering novel therapies for osteoporosis have revealed that inhibition of sclerostin production, using neutralizing antibodies, reduces bone loss and these findings suggest that this may be an effective treatment for bone disease in patients with myeloma.\textsuperscript{97} Furthermore, sclerostin levels were found to be elevated in patients with myeloma compared with patients with MGUS. Sclerostin levels also correlated negatively with bone-specific ALP further validating sclerostin as a potentially important therapeutic target.\textsuperscript{98} Anti-sclerostin antibodies are currently undergoing preclinical and clinical trials.

#### SOSTDC-1

Sclerostin domain containing 1 (SOSTDC-1), also called Wise, Usag-1 and Ectodin,\textsuperscript{74} functions as an inhibitor of Wnt signalling via competitive binding of Lrp-6.\textsuperscript{99} Studies have also shown that it also acts as an antagonist to BMP-induced OB differentiation, which has an important role in tissue homeostasis and normal regulation of bone formation.\textsuperscript{100,101} Given its ability to disrupt two separate pathways involved in OB formation and regulation, study of
the potential cross talk between these pathways may benefit the search for new treatments for MBD.74

**TGFβ superfamily**

The TGFβ superfamily includes transforming growth factor-β (TGFβ), BMP and the activin/inhibin molecules. Its interactions with other signalling pathways feature in the regulation of tissue homeostasis and embryogenesis. In addition, it is implicated in the pathogenesis of a number of diseases.100 Interactions of the TGFβ superfamily have been implicated in the regulation bone formation because TGFβ-1 knockout is associated with decreased bone mineralization and formation in mice.101 The interactive elements of the TGFβ superfamily comprise ligands such as activin, TGFβ itself and BMP, all of which bind a variety of type two receptors which, in turn, recruit type 1 receptors called activin-like kinases (ALK) thus forming a transmembrane complex comprising ligand, type 11 receptor and type 1 receptor. There are seven type 1 receptors and five type 2 receptors, and their interaction results in the phosphorylation of receptor-regulated Smad molecules (R-smad). R-smad interact with other family members prior to nuclear translocation, where they act as a transcription factor. The interactions of Smad are tightly regulated by the TGFβ family signalling cascade, and these interactions result in the biological functionality of this group of cytokines.102,103

The OC-related increase in bone resorption observed in myeloma is associated with an increase in TGFβ. While this increase is thought to up-regulate early OB recruitment, further differentiation and bone formation appear to be inhibited. While TGFβ is released during breakdown of the bone mineral matrix, any subsequent increase in bone formation is suppressed by MPCs. Production also stimulates the release of other MPC growth-stimulating cytokines, such as IL-6 and IGF-1.104 Data suggest that inhibition of the TGFβ type 1 receptor improves parameters of bone quality, such as trabecular architecture, via stimulation of OB differentiation and decrease of OC differentiation, and these findings provide a rationale for treating TGFβ as a therapeutic target.105

**Activin A**

Activin and closely related inhibins are members of the TGFβ superfamily whose actions antagonize one-another. First identified through involvement in control of follicle-stimulating hormone (FSH) release and inhibition from the pituitary gland,106 activin plays a role in bone modulation, particularly activin A that inhibits OB mineralization.107,108 By binding the activin type 2A receptor, activin A signals result in reduction of bone formation and increased bone resorption.48 Circulating activin A is significantly increased in MPC patients compared with healthy controls, and its increase correlates with disease stage and increased severity of bone disease.109 This study demonstrates that activin A blockade via a soluble activin receptor type IIA significantly increases osteoblastogenesis, increases bone formation and decreases bone destruction.110 Data from a small phase II clinical trial suggest that this soluble decoy receptor is well tolerated, increases markers of bone formation, has an anti-tumour effect and decreases bone pain in patients with myeloma.111

Activin A may also be an essential element of the process of osteoclastogenesis, because it appears to exert a synergistic effect with RANKL. OC formation is almost entirely ablated following treatment with soluble activin receptor type II A, findings that suggest its role are integral to OC formation.112 This dual effect makes it an appealing target for reversing the effects of MBD seen in patients.

**IL-3 and IL-7**

In addition to osteoclastic stimulation, IL-3 also plays a role in OB suppression through BMP-induced OB differentiation.65 Recent research has shown that IL-3 expression induces activin A, and this may result in increased osteoclastic activation and function, thus creating a dual target effect.66

It has been proposed that IL-7 plays a key role in the inhibition of OB differentiation.113 Giuliani et al. found that OB differentiation was inhibited in co-cultures of myeloma cells and OB precursors. In their study, they associated down-regulation of RUNX2 and CBFA1, transcription factors involved
in OB differentiation, with increased IL-7 expression. They also noted that this inhibitory effect was ameliorated by direct cell contact through VLA-4.\textsuperscript{113} Recent data suggest that IL-7 induced growth factor independence-1 (GFi1) represses RUNX2 and inhibits OB function.\textsuperscript{114} GFi1 expression was blocked with anti-IL-7 and anti-TNF\textsubscript{α} antibodies, a potentially useful observation for treatment of MBD.\textsuperscript{114}

**Current management of MBD and future directions**

Management of MBD requires a multimodality approach and should be both preventative and reactive. Radiotherapy is useful as palliative treatment for bone pain, along with appropriate analgesic and supportive care measures. Pre-emptive surgery may be considered in patients with lytic lesions with at high risk of fracture. Vertebroplasty and kyphoplasty are specialized orthopaedic interventions for pain associated with vertebral fractures.\textsuperscript{115}

**Radiotherapy for the treatment of MBD**

MPCs are highly sensitive to radiotherapy. For this reason, radiotherapy alone, i.e. with no systemic chemotherapy administered, is the mainstay of treatment of solitary plasmacytomas without evidence of disease elsewhere. Although recurrence does occur, radiotherapy alone is usually effective at eliminating plasmacytomas.\textsuperscript{116} Where there is evidence of disease elsewhere, i.e. multiple plasmacytomas or bone marrow disease, radiotherapy alone is considered sub-optimal as a monotherapy and hence systemic chemotherapy is given. Nevertheless, for active bone lesions, especially if bone pain is refractory, focal radiotherapy is frequently an extremely effective method of reducing bone pain.\textsuperscript{117} If fractures occur in long bones, such as the humerus or femur, orthopaedic stabilization and subsequent radiotherapy at a dose of 8 Gy, single fraction, are useful to improve pain control and may promote healing of the fracture site. Previously, hemibody irradiation has been used as a palliative treatment for advanced myeloma.\textsuperscript{117} However, this intervention was associated with significant toxicities including mucositis and prolonged cytopenias and is now seldom used. Total body irradiation has been used as part of a conditioning regimen prior to allogeneic stem cell transplantation in patients with myeloma and although this also increases the incidence of mucositis and diarrhoea during the engraftment period, there is some evidence to suggest that total body irradiation increases the likelihood of inducing a graft-versus-myeloma effect post-transplant.\textsuperscript{117}

**Bisphosphonates**

Pharmacologically, the mainstay of prevention of MBD has been treatment with BPs. Featuring a core P-C-P, BPs bind avidly to hydroxyapatite. BPs, when released from hydroxyapatite as a result of local bone resorption, are potent inhibitors of OC recruitment and substantially reduce OC activity and longevity. BPs are absorbed by macrophages, from which OCs are derived, and by mature OCs. BPs induce apoptosis in macrophages and OCs via, in the case of the non-nitrogen-containing compounds, accumulation of non-hydrolysable ATP metabolites and in the case of nitrogen-containing BPs via inhibition of the mevalonate pathway and in particular, the inhibition of the enzyme inhibits farsenyl pyrophosphate synthase (FFP synthase), which prevents the formation of isoprenoid lipids, e.g. geranylgeranyl pyrophosphate (GGPP); these are required for the post-translational prenylation (i.e. transfer of long-chain isoprenoid lipids) of proteins especially Ras, Rho, Rab and Rac. Reduction in these almost certainly interferes with cellular function and induces apoptotic cascades and may well decrease concentrations of functional cell cycle proteins inhibiting proliferation. BPs also reduce OC activity via disruption of the cytoskeleton and the OC ruffled border. Rates of acid extrusion and functional activity of enzymes critical to OC activity are also reduced.\textsuperscript{118}

A series of landmark studies trialling various BPs established the evidence for the use of BPs in patients with myeloma. Some BPs demonstrated efficacy while others did not. For example, in 1991, etidronate compared with placebo did not significantly
improve skeletal health as measured by vertebral index. Similarly, ibandronate compared with placebo evaluated in 2002 did not significantly reduce the number of SREs experienced. The first clear evidence of benefit came from the Finnish study comparing clodronate with placebo in 1992. In this study, incidence of vertebral fractures was lower but fell short of statistical significance. However, pain scores in patients treated with clodronate were significantly lower. Following on from this, the UK MRC Myeloma VI trial, 1998, which compared clodronate with placebo, reported significantly fewer vertebral fractures, less back pain and improved performance status in patients receiving clodronate. Further analysis of this trial evaluated whether there was a survival advantage in patients receiving clodronate and while this was not the case in all patients considered, a survival advantage was noted in patients who did not have evidence of vertebral disease at onset. In the USA, the nitrogen-containing BP, pamidronate was evaluated versus placebo also in 1998 and was found to reduce SREs but did not show a survival advantage. In 2001, zoledronic acid, given as a short, 5-min infusion, at a dose of 2–4 mg, was shown to be just as effective as pamidronate 90 mg given as a 2-h infusion. In a further head-to-head comparison in patients with breast cancer and myeloma in 2003, zoledronic acid was shown to be more effective than pamidronate in reducing SREs. The UK-based Myeloma IX trial demonstrated the significant benefit of treating with zoledronic acid, rather than sodium clodronate, in the reduction of SRE, in overall survival (OS) and PFS.

The use of BPs is associated with a number of pros and cons. They are relatively cheap especially now that zoledronic acid, previously significantly more expensive than pamidronate, is off-patent. However, intravenous administration is inconvenient for patients and has a major impact on day caseload within health services. Sodium clodronate is poorly and variably absorbed from the gastrointestinal tract. It is recommended that it should be taken without food and is associated with gastrointestinal side effects. The nitrogen-containing BP, pamidronate and zoledronic acid may threaten the sometimes precarious and unpredictable renal function in myeloma patients and require careful monitoring and dose adjustment. They are also associated with side effects at the time of administration, including flu-like symptoms, and are frequently associated with hypocalcaemia. All BP, especially the nitrogen-containing intravenous preparations, are associated with varying risks of osteonecrosis of the jaw (ONJ), e.g. up to 3.5% in the Myeloma IX trial. Once established, ONJ presents a chronic problem and there is little clarity regarding ongoing administration of BP, which may be important in myeloma patients with MBD. Atypical femoral and metatarsal fractures have also been reported and attributed to disruption of bone remodelling caused by the use of BPs.

Although there may be a logic to starting BP treatment earlier, for example in smouldering/asymptomatic myeloma, there is little evidence to support this idea and little consensus regarding duration of BP therapy, with patients often receiving indefinite BP treatment, or having a treatment break after 2 years. There is no conclusive evidence supporting either strategy and practice has varied worldwide between these two options influenced by local consensus and relative concerns including, on the one hand, the incidence of adverse effects of BPs, e.g. ONJ that has encouraged clinicians to discontinue BP treatment especially in quiescent disease states and, on the other hand, the proposed survival advantage of patients on long-term BP treatment, which has encouraged ongoing therapy.

**Is zoledronic acid now the bisphosphonate of choice?**

The UK MRC Myeloma IX trial has demonstrated that zoledronic acid is superior to clodronate not only in the reduction of myeloma SRE (27% versus 35%) in all patients regardless of bone status at onset, but also reported a survival advantage in patients who had evidence of bone disease at onset. Even after a relatively short median follow-up of 3.7 years, patients receiving zoledronic acid survive 5.5 months longer than those receiving clodronate. PFS is also increased by 2 months (19.5 months versus 17.5 months).
Zoledronic acid is associated with a survival advantage

Intriguing evidence, dating back over the last 10 years from animal models and in vitro studies, suggests that BPs exert an anti-tumour effect. The MRC Myeloma IX trial is the first major randomized controlled trial to show an apparent anti-tumour effect in human subjects with myeloma. However, despite these findings, the mechanism of this anti-tumour effect is still not entirely clear.

Potential anti-tumour mechanisms

Various mechanisms have been proposed to account for the anti-tumour effects of BP ranging from relatively simple to more complex interactions. Perhaps most simply, it has been proposed that because less bone is destroyed following BP treatment, there is less volume available for tumour expansion. Furthermore, bony barriers to tumour expansion remain intact, i.e. tumour is less likely to break out of its locale, expand and spread. Similarly, the BMM has often been perceived as a hospitable environment for tumour growth especially when enriched by growth factors such as TGFβ, ILGF liberated from the bone matrix during bone resorption. This has often been referred to as the so-called ‘seed and soil’ concept, a term first used by Paget. Because treatment with BP inhibits bone resorption, this favourable environment would be rendered relatively more inert. It has also been suggested that BP treatment can inhibit tumour cell adhesion to mineralized surfaces. Similar to their effects on OC, BPs appear to have a pro-apoptotic and anti-proliferative effect on myeloma cells. Anti-angiogenic effects have also been proposed. More complex interactions have also been proposed including enhanced γδ T-cell-mediated immunosurveillance.

Intriguing data have also emerged suggestive of a synergy between BP and various chemotherapeutic agents including doxorubicin, paclitaxel and cyclophosphamide. Most recently, sequential administration of doxorubicin followed 24 h later by zoledronic acid has been shown to decrease tumour burden in a mouse model of breast cancer. The authors propose that this is mediated by a complex interaction of increased pro-apoptotic factors and decreased cell cycle proteins. Further studies are required to confirm which of these mechanisms are most important.

Most studies have focussed on the nitrogen-containing members of the BP family. It has been proposed that the anti-tumour effect of BP can also be attributed to the inhibition of the mevalonate pathway with subsequent increase in apoptosis, as described above. Thus, these pro-apoptotic and anti-proliferative properties first proposed as mechanisms of OC inhibition may also act directly or indirectly on tumour cells.

Implications for clinical practice, including notes of caution

Rajkumar makes interesting observations in his editorial in *The Lancet* concerning the anti-tumour effects of zoledronic acid reported in the MRC MPC IX trial, including some words of caution. First, he observes that in most countries other than the UK, the alternative BP to zoledronic acid is pamidronate not clodronate and that pamidronate that was 10 times less expensive than zoledronic acid (even though this is now no longer the case with zoledronic acid recently coming off-patent) has not been associated with a survival disadvantage and may also have a lower incidence of ONJ. Second, he points out that it remains unclear whether patients without bone disease at presentation have a more favourable prognosis. However, the probability of an anti-tumour effect does add weight to the argument to treat early plasma cell dyscrasias, such as asymptomatic myeloma, with BP (e.g. yearly zoledronic acid in order to retard tumour progression and the development of MBD). Third, although the observation that most survival benefits appear to be derived early (within the first 4 months of treatment) is important, mortality at this juncture is cited as 8%. This compares less favourably with mortality at this juncture in patients treated with lenalidomide or bortezomib, which is quoted as 1% or less. Finally, he
observes that the median duration of treatment with zoledronic acid is 12 months. Given that the greatest benefit appears to be achieved by early treatment, he suggests that this should not be used to justify indefinite treatment with zoledronic acid.

**Non-bisphosphonate treatment modalities—denosumab and the future**

In addition to BP, recombinant OPG and OPG mimetics, as well as anti-RANKL constructs, are under development.\(^4\)\(^0\),\(^1\)\(^4\)\(^5\),\(^1\)\(^4\)\(^6\) An OPG mimetic has demonstrated anti-resorptive effects in the ST2MM murine model of myeloma, and there are no adverse effects in a preliminary phase 1 trial.\(^1\)\(^4\)\(^6\) However, more attention has focused on the anti-RANKL monoclonal antibody, denosumab (Amgen). In 2006, a comparison between treatment of myeloma-induced bone disease and breast cancer-induced bone disease using pamidronate and denosumab demonstrated prompt and substantial reduction in urinary and serum N-telopeptide, a marker of bone resorption, in both treatment groups. The reduction in urinary and serum N-telopeptide was sustained for 84 days in the denosumab-treated patients while the reductions were not sustained in the pamidronate-treated patients. The authors concluded that denosumab compared favourably given the lack of adverse effects and a sustained reduction in bone turnover for at least 84 days following a single subcutaneous dose.\(^1\)\(^4\)\(^5\) However, apparent benefits indicated by markers of bone resorption are of limited significance until they are backed up by clear evidence of a reduction in hard clinical endpoints, e.g. actual SRE. Demonstrable clinical advantage has yet to be unequivocally demonstrated for denosumab. Furthermore, in a recent study by Henry et al., 2011,\(^1\)\(^4\)\(^7\) although denosumab was shown to be non-inferior to zoledronic acid, an apparent increased death rate was noted in the denosumab arm requiring further corroboration. A large international study comparing denosumab and zoledronic acid is currently ongoing and should yield further evidence. Notwithstanding this, denosumab should be considered in patients with renal impairment given its apparent lack of nephrotoxicity.

**Bruton tyrosine kinase inhibition**

The Bruton tyrosine kinase (BTK) is a non-receptor tyrosine kinase expressed in maturing B-cells and implicated in B-cell maturation.\(^1\)\(^4\)\(^8\) BTK is expressed in MPCs and also OCs, at least those seen in murine models.\(^1\)\(^4\)\(^9\) Blockade of BTK using BTK inhibitors such as ibrutinib has been shown to inhibit tumour growth via down-regulation of NFκB, STAT3, ERK1/2 and AKT signaling.\(^1\)\(^5\) A direct inhibition of OC activity has also been demonstrated in a SCID-hu murine model of myeloma.\(^1\)\(^4\)\(^9\) This, in turn, is proposed to have an anti-tumour effect via reduction of OC-derived tumour growth factors. BTK inhibition is also proposed to prevent the adhesion of MPCs to BMSCs and to reduce the release of BMSC-derived growth factors including IL-6, SDF-1, M-CSF and MIP-1α.\(^1\)\(^4\)\(^9\)

**Radionuclides used in the treatment of MBD**

A number of radionuclides or radiopharmaceuticals have been trialled in the treatment of myeloma and other cancers that metastasize to bone. They have an affinity for bone undergoing active remodelling and are able to deliver a localized radiotherapeutic effect. In the treatment of prostate cancer, positive effects on the reduction of bone metastases and bone pain have been reported after treatment with samarium-153 ethylene diamine tetramethylene phosphonate.\(^1\)\(^5\)\(^1\),\(^1\)\(^5\)\(^2\) This agent has also been trialled in patients with myeloma and bone pain with substantial improvements in bone pain.\(^1\)\(^5\)\(^3\) Strontium ranelate\(^1\)\(^5\)\(^4\) has also demonstrated safety and efficacy in the treatment of osteoporosis and has been shown to increase bone formation rate. This agent may prove effective as a treatment for myeloma-induced bone disease although clinical data so far are limited.

**Recombinant parathyroid hormone**

Similarly, treatment with recombinant parathyroid hormone (rPTH ‘teraparatide’) demonstrates reductions in vertebral and appendicular fractures in patients with osteoporosis,\(^1\)\(^5\)\(^5\) as well as increased bone mineral
density in animal models of myeloma.\textsuperscript{156} The actions of rPTH are controversial because there is conflicting evidence that rPTH can also stimulate osteoclastogenesis.\textsuperscript{157} There have been isolated reports of malignancies occurring in patients on rPTH including a case report detailing the emergence of myeloma in a patient with osteoporosis treated with rPTH.\textsuperscript{158} Although these reports are exceptional, they have halted, for now at least, exploration of rPTH therapy in patients with MBD.

**Proteasome inhibitors also exhibit bone anabolic effects**

The addition of the highly effective proteasome inhibitor bortezomib (‘Velcade’) as an anti-tumour agent in myeloma has become a mainstay of treatment worldwide.\textsuperscript{159–161} One fortunate consequence of proteasome inhibition is reduced degradation by the proteasome of $\beta$-catenin, a common mediator of the osteoblastogenic Wnt signalling pathway. Specifically, indices of bone remodelling appear to normalize in patients treated with bortezomib.\textsuperscript{162–164} Novel proteasome inhibitors, with improved side effect profiles, such as carfilzomib, are also achieving positive effects on bone remodelling via stimulation of the Wnt signalling pathway.\textsuperscript{165,166}

**The effects of immunomodulatory drugs such as thalidomide and lenalidomide on MBD**

The effects on bone of immunomodulatory drugs (IMiDs) have been studied by several investigators with some conflicting evidence. Recent \textit{in vitro} studies have demonstrated an inhibition of OB activity as shown by reduced mineralization and ALP activity.\textsuperscript{167} However, clinical studies have demonstrated positive effects on bone disease, which may be independent of their anti-tumour effect. Anderson \textit{et al.}\textsuperscript{168} have shown that thalidomide attenuates RANKL-induced osteoclastic bone formation via down-regulation of the transcriptional factor PU.1. Furthermore, treatment of patients with thalidomide and dexamethasone for relapsed and refractory myeloma led to normalization of the sRANKL/OPG axis in addition to substantial anti-tumour activity.\textsuperscript{169} Similarly, lenalidomide has also been associated with reduced osteoclastic bone resorption due to inhibition of the osteoclast-activating factors APRIL (a proliferation inducing ligand) and BAFF (B-cell activating factor).\textsuperscript{170}

**Conclusion**

In recent years, survival outcomes for patients with myeloma have substantially improved.\textsuperscript{171–173} However, the disease remains almost always incurable. Many patients continue to suffer severe skeletal morbidity which, despite current treatments, is associated with chronic pain, dependency on analgesia and substantially compromises quality of life. Skeletal complications also contribute to a shortened life expectancy. Better treatments for MBD are needed, including more targeted inhibition of osteoclastogenesis beyond the standard of care with BP. Added benefits may come from the addition of bone anabolic agents such as anti-Dkk-1, anti-TGF$\beta$-1 and anti-HGF. Improvement in survival may be achieved not only from the direct benefits of maintaining and restoring skeletal health, but also from additional anti-tumour effects associated with bone-targeted therapies.

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