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REVIEW

Novel therapeutic targets in myeloma bone disease

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Multiple myeloma is a neoplastic disorder of plasma cells characterized by clonal proliferation within the bone marrow. One of the major clinical features of multiple myeloma is the destructive osteolytic bone disease that occurs in the majority of patients. Myeloma bone disease is associated with increased osteoclast activity and suppression of osteoblastogenesis. Bisphosphonates have been the mainstay of treatment for many years; however, their use is limited by their inability to repair existing bone loss. Therefore, research into novel approaches for the treatment of myeloma bone disease is of the utmost importance. This review will discuss the current advances in our understanding of osteoclast stimulation and osteoblast suppression mechanisms in myeloma bone disease and the treatments that are under development to target this destructive and debilitating feature of myeloma.

Abbreviations

BTK, Bruton's tyrosine kinase; DKK1, dickkopf 1; Gfi1, growth factor independent-1; IMiDs, immunomodulatory drugs; MIP-1α, macrophage inflammatory protein-1α; OPG, osteoprotegerin (TNFRSF11B); RANK, receptor activator of NF-κB (TNFRSF11A); RANKL, RANK ligand (TNFSF11)

Myeloma bone disease

Multiple myeloma is a neoplastic disorder of plasma cells in the bone marrow. It is characterized by clonal proliferation within the bone marrow, osteolytic bone disease and secretion of a monoclonal protein in the blood and/or urine of the patient. The accumulation of this protein is often the cause of organ dysfunction such as renal failure in patients with multiple myeloma (Kuehl and Bergsagel, 2002). Myeloma is the second most common of all haematological cancers (10–15%). It has a global incidence of approximately 120 000 cases per year and accounts for around 1% of all cancers (Ludwig *et al.*, 2013). Survival rates have improved in recent years and although myeloma remains incurable, patients are now predicted to have a median survival of approximately 5 years (Bergsagel *et al.*, 2013). The hallmark of myeloma is the osteolytic bone disease that is present in 70–80% of patients (Kyle *et al.*, 2003). It is characterized by the presence of osteolytic lesions accompanied by the suppression of osteoblast differentiation and function (Christoulas *et al.*, 2009). Approximately 20% of patients with myeloma will present with a pathological fracture upon diagnosis, and almost 60% of patients will develop a pathological fracture over the course of their disease (Berenson *et al.*, 1996; Melton *et al.*, 2005). The most common site of fracture is in the spine (55–70% of patients). Other common sites of fracture include the femur, pelvis, ribs, and humerus (Lecouvet *et al.*, 1997).

Factors produced by tumour cells stimulate osteoclasts to resorb bone and inhibit osteoblastic activity. In turn, growth factors released by the increased bone resorption also promote the growth of myeloma cells, creating a vicious cycle of tumour expansion and bone destruction. The biological pathway of the receptor activator of NF-κB (RANK; also known as TNFRSF11A), its ligand (RANKL; also known as TNFSF11) and the soluble decoy receptor osteoprotegerin (OPG; also known as TNFRSF11B), is of major importance for the increased osteoclast activity observed in multiple myeloma (Terpos *et al.*, 2003). The relationship between the Wnt inhibitor Dickkopf 1 (DKK1) expression and osteoblast suppression has also emerged as a critical route to long-term osteoblast suppression in myeloma (Tian *et al.*, 2003).

Bisphosphonates are currently the standard approach for the management of the osteolytic bone disease associated with multiple myeloma (Kyle *et al.*, 2007). Bisphosphonates are pyrophosphate analogues that have high bone affinity and directly inhibit osteoclastic activity (Rogers *et al.*, 2011)., Furthermore, the potential exists for bisphosphonate treatment to reduce tumour burden, either via direct anti-tumour effects or indirect effects on the host microenvironment (Morgan *et al.*, 2011; 2012; Coleman *et al.*, 2012; Clezardin, 2013). Although bisphosphonates have been extremely effective for treating myeloma bone disease, they only reduce the severity of lytic lesions and do not repair existing bone loss (Levy and Roodman, 2009). The demand for additional novel therapeutic targets that prevent and/or repair bone destruction is increasing and would greatly improve treatment for myeloma patients. As bisphosphonates have been extensively reviewed elsewhere, the focus of this review will be novel and emerging targets that may represent the future for treatment of myeloma bone disease.

The RANK/RANKL signalling pathway

The RANK/RANKL/OPG signalling pathway has become known as one of the most important regulatory systems in maintaining the bone remodelling balance (Simonet *et al.*, 1997; Lacey *et al.*, 1998; Kong *et al.*, 1999). RANK is a transmembrane signalling receptor and is part of the TNF receptor family (see Alexander *et al.*, 2013a). It is expressed on the surface of osteoclast precursors. On binding to RANK, RANKL, expressed by bone marrow stromal cells and osteoblasts, induces a signalling cascade that induces osteoclastogenesis and activation of mature osteoclasts. OPG is a soluble member of the TNF superfamily that is secreted by osteoblasts and bone marrow stromal cells (BMSCs). It acts as a decoy receptor for RANKL, blocking the induction of osteoclasts. The ratio of RANKL and OPG has a major impact on the balance of bone resorption and deposition (Vega *et al.*, 2007; Boyce and Xing, 2008) and this signalling pathway is frequently deregulated in myeloma bone disease (Roodman and Dougall, 2008). In the myeloma bone marrow microenvironment, the interaction between BMSCs and myeloma cells results in increased RANKL expression and decreased OPG production, favouring bone resorption (Giuliani *et al.*, 2001; Pearse *et al.*, 2001). Furthermore, circulating levels of OPG and RANKL have been shown to correlate with increased lytic lesions and poor prognosis (Seidel *et al.*, 2001; Terpos *et al.*, 2003). Initial murine studies demonstrated the striking potential for targeting this system in myeloma bone disease (Croucher *et al.*, 2001). A human antibody targeting RANKL has been developed with high affinity and specificity for human RANKL. Upon binding to RANKL, denosumab inhibits RANKL from activating RANK on osteoclasts. Prevention of RANKL–RANK interaction inhibits osteoclast formation, function and survival, which decreases bone resorption and cancer-induced bone destruction (Narayanan, 2013). Denosumab has been successfully examined in phase II and phase III trials in patients with cancer-induced bone disease and those at risk of developing bone metastases (Coleman *et al.*, 2012). These studies found that denosumab increased bone mineral density (BMD) and decreased markers of bone resorption in several different cancers (Lipton *et al.*, 2007; 2008; Fizazi *et al.*, 2009). Several phase III trials comparing denosumab with zoledronic acid reported that denosumab was equal, if not superior, to zoledronic acid in preventing or delaying skeletal-related events in bone metastatic cancer or myeloma (Stopeck *et al.*, 2010; Henry *et al.*, 2011). These data indicate the potential for targeting RANKL for the treatment of myeloma bone disease.

Dickkopf-1

The Wnt signalling pathway plays a major role in myeloma and the associated bone disease. There has been much focus on targeting some of the key molecules in this pathway to treat myeloma bone disease. Dickkopf-1 (DKK1) is one of several inhibitors of the Wnt signalling pathway and acts by binding to LRP5/6, preventing Wnt signalling, and therefore, the translocation of β-catenin to the nucleus (Bafico *et al.*, 2001; Mao *et al.*, 2001). DKK1 has been shown to inhibit osteoblastogenesis in myeloma (Tian *et al.*, 2003; Gunn *et al.*, 2006). Inhibition of Wnt signalling is known to induce bone destruction by increasing osteoclast maturation and decreasing osteoblast function and differentiation (Tian *et al.*, 2003; Hideshima *et al.*, 2007). Myeloma patient sample analysis has also revealed that high serum and bone marrow DKK1 correlates with osteolytic lesions, and that patient serum can block bone morphogenetic protein (BMP)-2-induced osteoblast differentiation *in vitro* in a DKK1-dependent manner (Tian *et al.*, 2003). It has also been found that patients that are responsive to anti-myeloma treatment have reduced levels of DKK1 in their serum (Heider *et al.*, 2009). A study using a DKK1-DNA vaccine in the murine MOPC-21 myeloma model showed that active vaccination using the DKK1 vaccine protected mice from developing myeloma and also reduced tumour burden in mice with established myeloma (Qian *et al.*, 2012).

Fulciniti and colleagues evaluated a DKK1 neutralizing antibody (BHQ880) in myeloma. *In vitro* BHQ880 increased osteoblast differentiation and in co-culture with BMSCs, myeloma cell growth was also inhibited, suggesting an effect on the bone marrow micro-environment. An *in vivo* mouse model using BHQ880 led to a significant increase in osteoblast number, serum human osteocalcin level and trabecular bone and also inhibited myeloma cell growth (Fulciniti *et al.*, 2009). A study by Pozzi *et al.* also confirmed these results using a different neutralizing DKK1 antibody. However, they found that when using myeloma patient samples to study osteoclastogenesis, there was heterogeneity in the response to the DKK1 antibody (Pozzi *et al.*, 2013). A recent report regarding a phase II clinical trial of BHQ880 treatment of patients with smouldering myeloma likely to progress to full myeloma showed bone anabolic activity with treatment of BHQ880. The scale of the increase in bone strength observed was similar to that observed with other approved bone anabolic agents (Munshi *et al.*, 2012). Despite the conflicting roles of Wnt signalling in myeloma tumour growth (Qiang *et al.*, 2005; Edwards *et al.*, 2008), it is clear that blockade of DKK1 is highly effective in treating myeloma bone disease *in vivo*.

Sclerostin

Sclerostin, a well-characterized Wnt antagonist, is implicated in several bone-related pathological diseases such as sclerosteosis and van Buchem's disease (Balemans *et al.*, 2001; 2002) and now sclerostin in myeloma (Brunetti *et al.*, 2011; Colucci *et al.*, 2011; Terpos *et al.*, 2012a). Sclerostin has been shown to be secreted directly from myeloma cells and is elevated in myeloma patients with advanced bone disease (Brunetti *et al.*, 2011; Gkotzamanidou *et al.*, 2012; Terpos *et al.*, 2012a). Sclerostin binds to LRP5/6 to inhibit the Wnt signalling pathway during bone formation, leading to an increase in osteoclastogenesis via RANKL production and OPG inhibition (van Bezooijen *et al.*, 2005). Sclerostin also decreases the lifespan of osteoblasts by inducing apoptosis (Sutherland *et al.*, 2004). Sclerostin is also involved in BMP signalling. It competes with type I and type II BMP receptors for binding to BMPs. This results in a decrease of BMP signalling and subsequently, down-regulation of BMP-mediated mineralization in osteoblasts (Winkler *et al.*, 2003). Expression of this Wnt inhibitor suggests that myeloma cells can drive the inhibition of osteoblast differentiation and also promote osteoclastogenesis, which leads to progressive bone disease.

Sclerostin monoclonal antibodies have shown promising results in promoting bone formation after pathological and age-related bone loss in rodents (Li *et al.*, 2009; 2010; Agholme *et al.*, 2010; Ominsky *et al.*, 2011; Chen *et al.*, 2013) and in humans (McColm *et al.*, 2013) . Inhibiting sclerostin using such an approach may be a simple, non-invasive route to treat bone disease in myeloma patients. Using myeloma cell lines and patient samples, Colucci *et al.* showed that the addition of an anti-sclerostin mAb prevented the formation of the LRP5/6-sclerostin complex, and partially restored β-catenin translocation into osteoblast nuclei (Colucci *et al.*, 2011).

A human clinical trial in post-menopausal women was conducted using AMG 785 (romosozumab), a humanized mAb that inhibits the activity of sclerostin. AMG 785 resulted in a significant biochemical anabolic effect, which was also translated into statistically significant increases in BMD (Padhi *et al.*, 2011). A recent study also involving post-menopausal women with low bone mass reported that AMG 785 increased BMD and bone formation while decreasing markers of bone resorption (McClung *et al.*, 2014). To date, several studies reported on the effects of AMG 785 on bone disorders with low bone mass. However, studies involving patients with malignant disease have yet to be performed (Gkotzamanidou *et al.*, 2012).

Activin A

Activin A is a member of the TGFβ superfamily (Brosh *et al.*, 1995; Sternberg *et al.*, 1995). Activin binds to the type II receptor (ActRIIA or ActRIIB), which recruits and subsequently phosphorylates the type I receptor (ActRI is also known as the activin receptor-like kinase 4 (ALK4) receptor (Deli *et al.*, 2008). The phosphorylated type I receptor induces phosphorylation of Smads, which subsequently form a complex and are translocated to the nucleus to initiate transcription (Butler *et al.*, 2005). There is evidence that activin A is deregulated in primary bone tumours (Gobbi *et al.*, 2002; Masi *et al.*, 2002) and in bone metastatic tumours (Dowling and Risbridger, 2000; Risbridger *et al.*, 2001) and in myeloma (Vallet *et al.*, 2010). Activin A has been shown to act as a stimulator of osteoclastogenesis in tumour bone disease (Hashimoto *et al.*, 1992; Sugatani *et al.*, 2003; Futakuchi *et al.*, 2009). Furthermore, Galson *et al.* recently reviewed that IL-3, a potent osteoclast stimulator (Lee *et al.*, 2004), can induce the secretion of activin A from marrow macrophages in the myeloma microenvironment, providing a mechanism for the up-regulation of activin A in myeloma (Galson *et al.*, 2012). In myeloma, it has also been shown that activin A can inhibit osteoblast differentiation. Activin A levels are increased in the bone marrow plasma of myeloma patients with osteolytic disease (Terpos *et al.*, 2012b) and the interaction of myeloma and patient bone marrow stromal cells enhances activin A secretion. Adhesion mediated JNK activation was found to induce the secretion of activin A. This pathway was targeted by two groups using RAP-011, a soluble activin A receptor. In both studies, RAP-011 prevented the development of osteolytic lesions and inhibited tumour growth in myeloma-bearing mice (Chantry *et al.*, 2010; Vallet *et al.*, 2010).

The human version of RAP-011, ACE-011 (also known as sotatercept), is a receptor fusion protein consisting of the extracellular domain of human ActR11A. In myeloma patients a phase II study with ACE-011 reported that patients had reduced bone pain and cancer-induced anaemia (Adulkadyrov *et al.*, 2009). ACE-011 also effectively inhibited markers of bone resorption and stimulated bone formation parameters in post-menopausal women in a double-blind placebo-controlled study (Ruckle *et al.*, 2009). These data indicate that activin A is a promising new approach for reducing the debilitating bone disease that accompanies myeloma.

EphrinB2/EphB4 signalling

The Eph receptors are the largest subgroup of the receptor TK family (Hirai *et al.*, 1987). Bidirectional ephrin–Eph receptor signalling links the negative feedback loop in osteoclast differentiation to positive regulation of osteoblast differentiation, thereby maintaining bone homeostasis (Mundy and Elefteriou, 2006). Reverse signalling between the ligand ephrin B2 (EFNB2) in osteoclasts and its receptor EphB4 in BMSCs and osteoblasts has been found to negatively control osteoclast formation, whereas forward signalling was shown to promote osteoblast differentiation (Zhao *et al*., 2006). In addition, osteoblast-derived ephrin B2 can promote osteoblastic differentiation, suggesting a paracrine role for ephrin B2/EphB4 signalling in osteoblasts (Allan *et al*., 2008; Martin *et al*., 2010; Takyar *et al*., 2013). Myeloma cells have been shown to down-regulate EphB4 expression in osteoblasts (Bates *et al*., 2007). As EphB4 forward signalling enhances bone formation, reduction in EphB4 may account for impaired bone formation in myeloma bone disease. A study by Pennisi *et al*. (2009b) reported decreased ephrinB2 and EphB4 in mesenchymal stem cells (MSCs) from myeloma patients. The peptide ephrinB2-Fc activated EphB4 in MSCs and EphB4-Fc activated ephrinB4 in osteoclasts but not in MSCs. Myeloma bearing mice treated with both peptides demonstrated increased bone volume/tissue volume (BV/TV) and trabecular thickness in bones treated with ephrinB2-Fc or EphB4-Fc. EphB4-Fc and ephrinB2-Fc increased the numbers of osteoblasts, whereas only EphB4-Fc reduced osteoclast numbers. Exploiting this signalling pathway presents a potential mechanism for reducing osteolytic lesions and increasing bone formation in myeloma patients but may be complicated by the multiple receptor : ligand interactions and bidirectional signalling in this family.

Adiponectin

Adiponectin is an adipocyte-derived hormone with implications for the regulation of bone homeostasis. It is almost exclusively secreted by adipocytes, principally those in visceral adipose tissue, although peripheral fat and bone marrow adipocytes also contribute (DiMascio *et al*., 2007; Swarbrick and Havel, 2008). Adiponectin is also secreted from osteoblasts and BMSCs (Berner *et al*., 2004). The receptors for adiponectin, Adipo receptor 1 and Adipo receptor 2, have been identified on both osteoblasts and osteoclasts (Berner *et al*., 2004; Shinoda *et al*., 2006), suggesting a potential direct influence of this hormone on bone. Adiponectin has been shown to increase osteoblast proliferation and differentiation while inhibiting osteoclastogenesis *in vitro* (Oshima *et al*., 2005; Yamaguchi *et al*., 2007). Studies investigating adiponectin and its effect on bone have been contradictory in cell and murine studies (Oshima *et al*., 2005; Shinoda *et al*., 2006; Yamaguchi *et al*., 2007). In contrast to these contradictory findings in cell culture and animal studies, clinical studies demonstrate that circulating adiponectin concentrations are related to BMD (Lenchik *et al*., 2003; Jurimae *et al*., 2005; Richards *et al*., 2007). These data suggest that there is a strong connection between adiponectin and normal bone homeostasis in humans.

To date, there is little data on the impact that adiponectin could have on the bone disease induced by the presence of myeloma. Fowler *et al*. demonstrated that a reduction in host-derived adiponectin promoted myeloma development both in a murine model of myeloma and in patients with the non-malignant precursor monoclonal gammopathy of undetermined significance. Adiponectin was also found to be tumour suppressive, inducing myeloma cell apoptosis. The apolipoprotein peptide mimetic L-4F was used for pharmacological enhancement of host-derived adiponectin. L-4F reduced tumour burden, increased survival of myeloma-bearing mice and prevented myeloma bone disease (Fowler *et al*., 2011). These results indicate that decreased adiponectin is a novel mechanism that promotes myeloma growth and associated bone disease and represents a viable target for myeloma treatment.

Bruton's tyrosine kinase (BTK)

BTK plays a key role in the development and function of normal B-cells through activation of the B-cell antigen receptor signalling pathway on binding to antigens (de Weers *et al*., 1994). BTK has been implicated in bone resorption by regulating osteoclast differentiation and is expressed in osteoclasts but not in osteoblasts (Lee *et al*., 2008; Shinohara *et al*., 2008). BTK is highly expressed in both patients with multiple myeloma and human myeloma cell lines. (Chauhan *et al*., 2002; Tai *et al*., 2012; Rushworth *et al*., 2013). Liu *et al*. (2013) also identified increased BTK expression in myeloma and in the dexamethasone-resistant myeloma cell line MM1.R. In addition, a single nucleotide polymorphism (SNP) at cDNA position 2062 (T2062C) in the *BTK* gene was recorded in six out of eight (75%) patients and in U266 cells. This SNP in myeloma cells was not detected in other malignant haematopoietic cells of different lineages suggesting it is myeloma-specific and a potential prognostic indicator.

A potent BTK inhibitor, PCI-32765 (ibrutinib), has been reported to be cytotoxic to myeloma cells via inhibiting the NF-κB pathway and augments the activity of bortezomib and lenalidomide (Rushworth *et al*., 2013). Recently, Tai and colleagues (Tai *et al*., 2012) demonstrated PCI-32765 blocked BTK and PLC-γ2 in osteoclasts resulting in diminished osteoclast activity. PCI-32765 also inhibited the secretion of cytokines and chemokines from osteoclasts and BMSC cultures from normal and myeloma patient samples. PCI-32765 treatment significantly inhibits *in vivo* myeloma cell growth and myeloma cell-induced osteolysis of implanted human bone chips in SCID mice. These data suggest that BTK activation in myeloma mediates osteoclast differentiation and growth of myeloma cells and PCI-32765 merits further investigation as a novel therapeutic for myeloma cells and for myeloma-induced osteolytic bone disease.

Growth factor independence-1 (Gfi1)

Gfi1 is a zinc-finger transcriptional repressor that was originally identified in an *in vitro* screen for loci where the insertion of the Moloney murine leukaemia virus caused an IL-2-dependent T-cell leukaemia to progress to IL-2-independent growth (Gilks *et al*., 1993; Grimes *et al*., 1996; Zweidler-Mckay *et al*., 1996). A common characteristic of myeloma is the continued suppression of osteoblasts and their function through all stages of the disease. A study by D'Souza *et al*. (2011) showed that BMSCs from both myeloma-bearing mice and myeloma patients had increased expression of the transcriptional regulator Gfi1. Gfi1 was found to be a novel transcriptional regulator of the critical osteoblast differentiation factor Runx2. Gfi1 induction was blocked by anti-TNF-α and anti-IL-7 antibodies. BMSCs isolated from Gfi1$^{-/-}$ mice were resistant to myeloma-induced osteoblast suppression. In addition, knockdown of Gfi1 using siRNA in BMSCs from myeloma patients significantly restored expression of Runx2 and osteoblast differentiation markers (D'Souza *et al*., 2011). An abstract presented by Jin *et al*. demonstrated that Gfi1 binds directly to the Runx2 promoter and that there are multiple Gfi1 sites within the Runx2 promoter. Mutations in the Gfi1 binding site prevents TNF-α-mediated repression of Runx2 indicating that myeloma cell secretion of TNF-α could play a role in regulating Gfi1 expression in myeloma (Jin *et al*., 2011). These data indicate that Gfi1 could cause long-term suppression of osteoblasts in pathological disease and is a promising novel candidate for targeting myeloma bone disease.

Macrophage inflammatory protein-1α (MIP-1α)

MIP-1α/chemokine (C-C motif) ligand 3 (CCL3) is a chemokine that is highly expressed by myeloma cells and strongly associated with the development of the osteolytic bone disease. Myeloma cells, both those isolated from the bone marrow of patients with multiple myeloma and myeloma cell lines, express and secrete high concentrations of MIP-1α (Choi *et al*., 2000; Lentzsch *et al*., 2003). Indeed, levels of MIP-1α correlate with markers of bone resorption and osteolytic bone disease (Hashimoto *et al*., 2004). In addition, the primary receptor for MIP-1α, CCR1 (see Alexander *et al*., 2013b) is also expressed on both myeloma cells, and other cells from the host bone marrow microenvironment, including bone marrow stromal cells, osteoblasts and osteoclasts. MIP-1α is known to play a key role in myeloma cell survival, homing and in osteoclast formation and bone resorption (Choi *et al*., 2000; Han *et al*., 2001). A number of *in vivo* studies using murine models of multiple myeloma have taken different approaches to demonstrate the key role that MIP-1α plays in the pathogenesis of myeloma bone disease. Inhibition of MIP-1α expression in myeloma cells was found to significantly reduce tumour growth and osteoclast number (Oba *et al*., 2005). A neutralizing antibody to MIP-1α was also found to significantly reduce tumour burden and osteolytic bone disease (Oyayobi *et al*., 2001). The use of CCR1 antagonists was similarly found to reduce both tumour burden and bone disease (Menu *et al*., 2006) (Vallet *et al*., 2011). Taken together, these studies support the development of targeting the MIP-1α/CCR1 axis for the treatment of myeloma bone disease.

B-cell activating factor (BAFF)

BAFF (also know as TNFSF13B) is a member of the TNF superfamily that is expressed by myeloma cells, osteoclasts and bone marrow stromal cells, increased in the serum of patients with myeloma and acts to promote the growth and survival of myeloma cells within the bone marrow microenvironment (Novak *et al*., 2004; Abe *et al*., 2006; Tai *et al*., 2006). Targeting BAFF, using a neutralizing antibody, has proven effective in a murine model of myeloma, with a reduction in tumour burden, an increase in survival and a reduction in osteolytic bone disease (Neri *et al*., 2007). Initial phase 1 studies using the human anti-BAFF antibody tabalumab have been encouraging, with many patients with previously treated myeloma achieving a partial response or better following treatment with tabalumab (Raje *et al*., 2012) It will be of interest to see whether targeting BAFF in patients with myeloma reduces bone disease in addition to tumour burden, as suggested by *in vivo* preclinical models.

Proteasomes

The ubiquitin-proteasome pathway is responsible for the degradation of eukaryotic cellular proteins (Adams, 2002). The degradation of proteins by this pathway is critical for signal transduction, transcriptional regulation, response to stress and control of receptor function (Varshavsky, 1997). This pathway controls the activation of NF-κB (a major transcription factor) by regulating degradation of the NF-κB inhibitor (I-κB; Palombella *et al*., 1994; 1998). Bortezomib (N-acyl-pseudo dipeptidyl boronic acid) is a dipeptide that binds reversibly to the chymotrypsin-like b5 subunit of the catalytic chamber of the 20S proteasome inhibiting its function (Rajkumar *et al*., 2005). Myeloma cells secrete a large amount of different proteins, including immunoglobulins, leaving them vulnerable to killing by proteasome inhibition (Meister *et al*., 2007). Myeloma cells are exquisitely sensitive to proteasome inhibition, leading to tumour cell apoptosis and the fast-tracked approval for the use of proteasome inhibitors in the treatment of patients with multiple myeloma (Lawasut *et al*., 2012).

Proteasome inhibitors are also known to have direct effects on osteoblasts to promote osteoblast differentiation and bone formation (Garrett *et al*., 2003). In addition, recent studies have observed direct effects of proteasome inhibitors on osteoclasts, where decreased bone resorption has been shown to correlate with the extent of NF-κB binding (Zavrski *et al*., 2005). Bortezomib has also been shown to down-regulate TRAF 6, both at the protein and mRNA level (Hongming and Jian, 2009). TRAF 6 is a key signalling mediator between RANK and NF-κB (Darnay *et al*., 2007). In murine models, an increase in BMD, bone volume, trabecular thickness and bone formation was seen with treatment of bortezomib (Pennisi *et al*., 2009a) as well as an increase in osteoblast number in myeloma and non-myeloma mice (Deleu *et al*., 2009). Several studies have indicated that treatment with bortezomib can have bone anabolic effects in human myeloma patients. Clinical studies using bortezomib have demonstrated that levels of alkaline phosphatase and osteocalcin were enhanced and bone lesions were reduced in responders to bortezomib treatment (reviewed in (Zangari *et al*., 2012). Heider *et al*. also reported that bortezomib increased osteoblast activity markers, including alkaline phosphatase, in myeloma patients irrespective of level of response (Heider *et al*., 2006). Bortezomib stimulated osteoblast differentiation and bone formation in bone organ cultures in a BMP-dependent manner but this bone formation was blocked by DKK1, an osteoblast suppressor. However, bortezomib was found to inhibit DKK1 in bone and bone-derived cells (Oyajobi *et al*., 2007), and in myeloma patients in combination with lenalidomide and dexamethasone (Terpos *et al*., 2011) giving further weight to the potential bone anabolic capabilities of bortezomib. Although the retrospective analyses from these trials suggest promising results with the treatment of bortezomib, there is a need for prospective trials specifically designed to investigate the effect of bortezomib on myeloma bone disease.

The immune system

Immunomodulatory drugs (IMiDs) are a group of therapeutic agents consisting of thalidomide and its second generation derivatives lenalidomide and the newest member pomalidomide. IMiDs are known to have direct anti-tumour effects via several different mechanisms. In myeloma, IMiDs cause cell cycle arrest (Hideshima *et al*., 2000), prevent NF-κB activation leading to decreased expression of anti-apoptotic proteins and directly induce caspase-8 activation. It has recently been shown that the anti-myeloma activity of Thalidomide and its derivatives require the protein cereblon to produce the teratogenic effect seen with the use of IMiDs in myeloma cell lines (Ito *et al*., 2010). As seen in Figure 1, the interaction of myeloma cells with the BM microenvironment enhances myeloma cell growth and survival. IMiDs have been reported to prevent the adhesion of myeloma cells to non-myeloma cells in the BM microenvironment including BMSCs, osteoclasts and immune cells (reviewed in Chang *et al*., 2013). This would interfere with the vicious cycle of myeloma, reducing myeloma-induced osteoclastogenesis and the resultant growth factors released from bone destruction. A study by Breitkreutz *et al*. showed that lenalidomide inhibits osteoclast formation and activation by inhibiting key factors, such as PU.1 and pERK, during osteoclastogenesis and also by reducing myeloma burden (Breitkreutz *et al*., 2008). Lenalidomide decreased RANKL secreted from BMSCs from myeloma patients and in serum RANKL was decreased and OPG increased. In addition, a down-regulation of capthepsin K (which is secreted by osteoclasts and induces matrix degradation during bone resorption) was also observed upon treatment with lenalidomide (Breitkreutz *et al*., 2008). Pomalidomide (CC-4047) has also been shown to inhibit PU.1 and therefore, osteoclastogenesis (Anderson *et al*., 2006), further demonstrating the potential for IMiDs to target myeloma bone disease. Another study reported that at a dose that induced apoptosis in myeloma cells, IMiDs also showed an anti-osteoclast effect without affecting osteoblasts (Munemasa *et al*., 2008). It is increasingly being realized that IMiDs not only kill myeloma cells but have effects on the related bone disease. The results indicate the potential for IMiDs to increase osteoblastogenesis and inhibit osteoclastogenesis which would significantly improve the lytic lesions caused by myeloma.

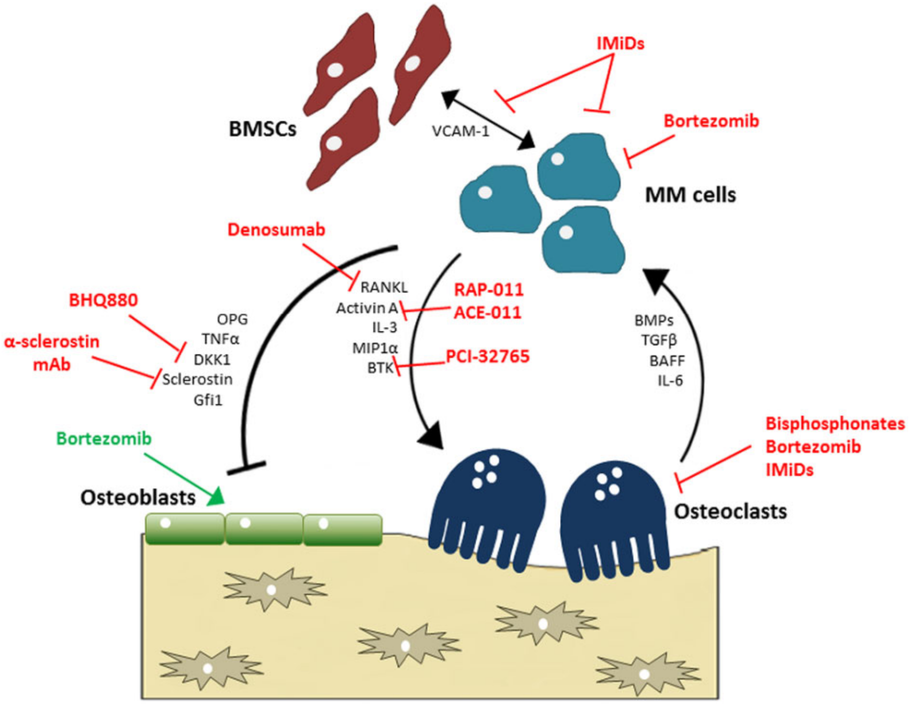

Figure 1

The vicious cycle of myeloma bone disease. The interactions between myeloma cells and cells of the bone marrow microenvironment, including stromal cells, osteoblasts and osteoclasts, promote both tumour growth and osteolytic bone disease. As our understanding of the mechanisms involved in disease pathogenesis increases, novel targets are revealed, which act on distinct components of the cycle to reduce tumour growth and/or bone disease.

Conclusions

The debilitating bone destruction that accompanies myeloma is the result of multiple factors that together contribute to the characteristic bone lesions. The severity of these lesions seen in the majority of patients with myeloma is explained by the vicious cycle of myeloma cell promotion and osteolysis via interaction with multiple cells in the bone marrow microenvironment. The development of novel bone anabolic drugs is essential. Patients with multiple myeloma are now surviving longer due to improved treatments for myeloma tumour burden. They are, however, left with multiple and often incapacitating bone lesions that still require treatment. Bisphosphonates remain the mainstay treatment of myeloma bone disease but come with limitations of their own. A study examining the effects of zoledronic acid and doxorubicin treatment in a breast cancer model highlighted the potential for increased drug potency in combination (Ottewell *et al*., 2008). It is likely that in order to ultimately eradicate myeloma and the associated bone disease, a combination of agents targeting tumour growth, osteoclastic bone resorption and osteoblastic bone formation are required. It is encouraging that a number of novel pathways and approaches have been identified that appear promising in preclinical studies. The development of novel bone disease targeting drugs that can be used as single treatments or in combination with bisphosphonates and other myeloma drugs will improve the quality of life, and possibly length of life, in myeloma patients.

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Conflict of interest

The authors declare no conflict of interest.

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