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Bone Disease in Multiple Myeloma

Maja Hinge, Thomas Lund, Jean-Marie Delaisse and
Torben Plesner

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1. Introduction

Osteolytic bone disease in multiple myeloma (MM) is a common event. Already at diagnosis, approximately eighty percent of patients present with abnormal bone structure [1;2]. During disease progression a large proportion of patients will develop osteolytic lesions [3]. MM bone disease not only results in a reduced quality of life due to pain, pathological fractures, or symptomatic hypercalcaemia [4]; but may also be *the* deciding factor that determines if a patient requires anti-myeloma treatment or if a watch and wait strategy can be applied [5]. In this chapter we will discuss the normal bone remodelling process, and how it is affected in MM. During the last decades, increased knowledge about bone pathophysiology in general has led to an improved understanding of MM bone disease. The description of the receptor activator of nuclear factor kappa B (RANK) and its ligand in the nineties was one of the most significant steps. We will also address how biochemical markers may be used to monitor the velocity of the different processes in bone remodelling. The next part of the chapter will be dedicated to the treatment of MM bone disease. For many years, bisphosphonates have been a cornerstone in the treatment of MM bone disease and despite the occurrence of osteonecrosis of the jaw that was first reported as a result of the of bisphosphonate treatment in the early part of this century, these agents remain the most important components of treatment for MM bone disease. Lastly, we will discuss how various anti-myeloma treatments may influence bone turnover. During the last decade a number of novel drugs have been approved for the treatment of MM and especially proteasome inhibitors seems to have a positive effect on MM bone disease besides their anti-myeloma effect.

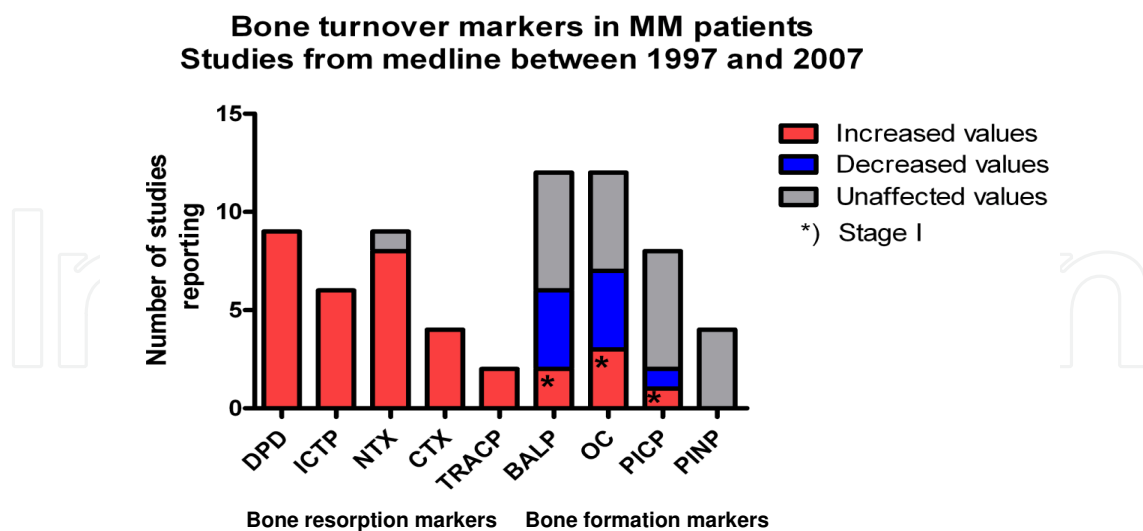
2. Pathogenesis of multiple myeloma bone disease

2.1. Introduction

The reason for the excessive loss of bone mass observed in MM is multi factorial. For many years attention was primarily focused on the increase in bone degradation which is observed in the majority of MM patients.

Over the last decade however, it has become increasingly evident that impaired bone formation also plays an important role in MM bone disease. In monoclonal gammopathy of unknown significance (MGUS) and early stage MM with preserved bone structure, normal or even increased bone formation may be observed. With disease progression and development of osteolytic lesions bone formation becomes impaired, and this may be an important contributing factor for the development of osteolytic lesions (see figure 1).

The interaction between the bone marrow microenvironment and the myeloma cells is also considered to be crucial. A large number of cytokines and chemokines, that regulate the activity of bone resorbing osteoclasts and bone forming osteoblasts, have been identified and studied in MM. Recently, a structure consisting of a flat layer of osteoblast lineage cells, that separates the bone surface from the bone marrow during bone remodelling, has been described. Disruption of this cell layer, called the bone remodelling compartment (BRC) canopy, allows direct contact between myeloma cells and the active bone remodelling cells, and this may affect both cell types. Osteocytes have been sparsely investigated in MM. However, a recent article illustrates that also this type of cell may be important for a better understanding of MM bone disease [6].



With permission from the author; Søndergaard T. The effect of simvastatin on bone markers in multiple myeloma and a description of the bone remodeling compartment. University of Southern Denmark, 2008.

Figure 1. Number of studies evaluating biochemical markers of bone turnover in MM patients in a ten year period. Bone resorption markers are uniformly elevated, while the bone formation markers are more divergent, with increased levels observed in early stages of MM.

3. Normal bone remodelling

Osteoclasts are the cells responsible for bone resorption. They originate from the monocyte-macrophage cell line. Differentiation of hematopoietic precursor cells into mature osteoclasts requires different environmental factors of which macrophage-colony stimulating factor (M-CSF) and receptor activator for NF- κ B ligand (RANKL) play an essential role. The early step in osteoclastogenesis seems to be influenced by M-CSF [7], whereas RANKL initiates differentiation, cell fusion, and activation of mature osteoclasts [8]. During osteoclast development the cell replaces the nonspecific esterase activity with tartrate-resistant acid phosphatase isotype 5b (TRACP 5b), which is believed to be specific for osteoclasts. Osteoclastogenesis results in the formation of large multinucleated cells located on the bone surface where bone degradation takes place. Bone degradation is achieved by an active secretion of protons from the osteoclasts into the resorption pits. The protons decrease the pH and cause decalcification of the bone matrix [9]. After decalcification the collagen fibres are degraded mainly by the proteolytic enzymes cathepsin K and various matrix metalloproteinases [10].

Osteoblasts are responsible for the formation of new bone following osteoclast-mediated bone resorption. Osteoblasts originate from differentiated mesenchymal stem cells under the influence of Runt-related transcription factor (Runx2) and the wingless type signalling (Wnt) factors. Runx2 is required for the differentiation of mesenchymal cells into osteoblasts [11]. The Wnt-pathway mediates the formation of a complex, which in turn inhibits the proteasomal degradation of β -catenin. The increasing level of β -catenin has a stimulating effect on osteoblast differentiation and maturation [12]. The Wnt-pathway can be inhibited by Dickkopf 1 (DKK1), resulting in decreased bone formation.

Mature osteoblasts are lined in groups located along the newly resorbed bone. Placed on the resorption site, the osteoblasts secrete the components needed to generate bone matrix, mainly collagen type 1 [13]. The bone formation ends with calcification of the newly synthesized bone. During bone formation some osteoblasts are incorporated into the bone matrix and become osteocytes. Bone lining cells and the canopy cells are also of osteoblast lineage.

Activation of bone remodelling is not yet clearly understood. However, it is thought that osteocytes may, at least partly, be of importance for the activation of bone remodelling. Osteocytes in the bone matrix may respond to mechanical stimulation and via communication through their networks of canaliculi initiate bone resorption. Osteocyte death probably also plays a role in the recruitment of osteoclasts.

Bone remodelling takes place on bone surface where the osteoclasts and osteoblasts are covered by a canopy of flattened cells of osteoblast lineage [14;15]. The space between the canopy and the bone surface undergoing remodelling is named the bone remodelling compartment (BRC). Disruption of the BRC canopy may impair bone remodelling [16]. Several factors of importance for the regulation of bone remodelling have been identified during the last decades. Within this chapter, we will only review some of the most important. The RANKL, RANK, and the decoy receptor osteoprotegerin (OPG) are probably the most significant factors in the regulation of normal physiological bone remodelling. RANK is expressed on the surface

of osteoclast precursor cells, and as mentioned above, stimulation with RANKL is essential for osteoclastogenesis [17]. RANKL is expressed by osteoblasts and bone marrow stromal cells. OPG has a high affinity for RANKL and functions as the physiological inhibitor of RANKL [18]. Since osteoblasts can stimulate osteoclast activity through the expression of RANKL and inhibit it through the secretion of OPG, osteoblasts hold a key position in the coupling between bone formation and bone degradation. Another interesting regulator of bone degradation is macrophage inflammatory protein 1- α (MIP-1 α). MIP-1 α has been shown to be a potent activator of osteoclasts [19]. MIP-1 α stimulates the activity and formation of osteoclasts indirectly by increasing the stromal cell expression of RANKL on the one hand [20; 21], but it also stimulates osteoclast formation independently of the RANKL system, though binding to the CCR1 or the CCR5 osteoclast receptor [21].

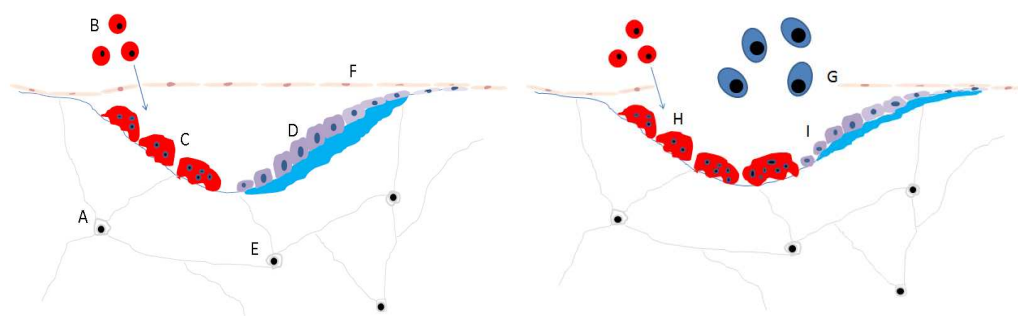


Figure 2. Normal bone remodelling and bone remodelling in multiple myeloma. A: Osteocytes sense mechanical stress and activate bone remodelling. B: Osteoclast precursors differentiate into mature multinucleated osteoclasts. C: The osteoclasts resorb bone matrix. D: Following bone resorption mononucleated osteoblasts lay down new bone in the resorbed area. E: During bone formation of new bone, osteoblasts are imbedded in the new bone matrix and differentiate into osteocytes. F: The bone remodelling takes place beneath a canopy of cells belonging to the osteoblast lineage G: Malignant plasma cells disrupt the bone remodelling compartment canopy and H: Increase osteoclastogenesis and I: Decrease osteoblastogenesis.

4. Abnormal bone remodelling in multiple myeloma

Increased bone degradation is an early event in MM. Retrospective studies using bone histomorphometry on bone marrow biopsies from patients diagnosed with MGUS harvested three to twelve months before these patients developed MM, were found to have increased bone degradation compared with MGUS patients who did not progress to MM during the first year after MGUS was diagnosed [22]. In MM both the number and the activity of the osteoclasts are found to be increased, and this may result in either focal or more diffuse loss of bone matrix when not compensated for by an equal increase in bone formation [23;24].

Several factors of importance for the development of MM bone disease have been identified during the last decades. The RANK/RANKL/OPG system is one of the most significant. In normal bone remodelling the RANKL/OPG ratio is tightly balanced. In MM the RANKL/OPG ratio is increased, both due to an elevated level of RANKL and as a result of a decrease in the

level of OPG, thus resulting in increased bone resorption [25]. The increased soluble RANKL/OPG ratio has been shown to correlate with the extent of bone disease and even with overall survival [25;26]. In addition, myeloma cells stimulate bone degradation by the secretion of MIP-1 α . In approximately 70% of MM patients, bone marrow serum levels of MIP-1 α are elevated [27] and peripheral blood levels of MIP-1 α have been found to correlate with bone disease and overall survival [28;29].

Vascular endothelial growth factor (VEGF) is known to be important for neovascularisation, but it probably also plays a role in the activation of osteoclasts in MM. VEGF has been demonstrated, *in vitro*, to act like macrophage colony-stimulating factor (M-CSF), thus inducing osteoclast differentiation [30]. Furthermore, a simultaneous blockade of VEGF and osteopontin has been shown to inhibit angiogenesis and bone resorption in co-cultures of myeloma cells and osteoclasts [31]. Taken together, these results indicate that VEGF could be of importance in bone resorption, and since the majority of myeloma cells can secrete VEGF it has been suggested that VEGF may support osteoclastic bone resorption in MM [32]. Interleukin-6 (IL-6), stromal-derived factor-1 α , tumor necrosis factor- α , and interleukin-11 are other examples of cytokines known to stimulate osteoclasts, which are suggested to be of importance in the development of MM bone disease [33;34].

The myeloma cells do not only affect the osteoclasts indirectly through the secretion of cytokines into the bone marrow microenvironment, but a direct contact between myeloma cells and bone marrow stromal cells or osteoclasts also seems to be an important factor in the development of MM bone disease.

Disruption of the BRC canopy is a frequent finding in MM. This breakdown of the BRC canopy allows a direct contact between the myeloma cells and the osteoclasts and osteoblasts involved in bone remodelling. This event probably contributes to impaired bone formation and enhanced bone resorption [16]. The extent of BRC canopy disruption in a histomorphometric study of iliac crest biopsies was found to correlate with the magnitude of osteolytic lesions in patients with MM [16]. Direct contact between human myeloma cells and bone marrow stromal cells or pre-osteoblasts tested in a co-culture system resulted in a marked decrease in the production of OPG, and thereby an imbalance in the RANKL/OPG ratio resulting in increased bone degradation [35]. Cell to cell contact between myeloma cells and bone marrow stromal cells has also been demonstrated to induce the secretion of IL-6 by bone marrow stromal cells [31]. IL-6 stimulates osteoclast formation and also has a promoting effect on myeloma cell proliferation [36]. It has also been suggested that myeloma cells can fuse with osteoclasts to create myeloma-osteoclast hybrid cells that may more aggressively erode bone than non-hybrid osteoclasts [16;37].

Co-cultures of myeloma cells and osteoclasts have demonstrated an increased viability of the myeloma cells caused by the direct cell to cell contact with osteoclasts [38]. Osteoclasts also produce factors capable of promoting myeloma cell growth, including IL-6 [39] and insulin-like-growth factor-1 (IGF-1) [40]. Osteoclasts can also support myeloma cell growth through the production of angiogenic factors, and the direct contact between myeloma cells and osteoclasts in co-cultures has been shown to enhance vascular tubule formation [41]. In animal models the inhibition of osteoclast activity with recombinant OPG or bisphosphonates has

resulted in an increased survival of mice inoculated with myeloma cells [42;43] but the clinical data from myeloma patients treated with bisphosphonates have been less consistent [44-48]. Nevertheless, the existence of a vicious cycle of bone resorption and tumour growth in patients with MM seems plausible and may be supported by the demonstration of a survival advantage in patients treated with zoledronic acid in the MRC IX trial [49].

Bone disease in MM is not only caused by an increased bone resorption, but the formation of new bone may also be affected. A reduced recruitment of osteoblasts, as well as reduced mineral deposition has been observed using histological methods in patients with MM [22]. In early stage of MM the number and activity of the osteoblasts can be increased but a marked decrease occurs as the plasma cell infiltration progresses [50]. Disruption of the BRC canopy in MM may be an important cause of the uncoupling of bone resorption and bone formation, with the result that bone resorption is not followed by bone formation or that the bone formation process is delayed or abolished [16]. Human plasma cells purified from bone marrow biopsies of MM patients have been found to express the gene for DKK1, and immunohistochemical analysis of bone marrow biopsies have shown that myeloma cells contain DKK1 [51]. In addition, blood and bone marrow serum levels of DKK1 have been demonstrated to be elevated in patients with MM bone disease [51]. Since DKK1 is believed to inhibit the stimulation of osteoblastogenesis via the Wnt-pathway this might cause impaired bone formation. Runx2 may also be affected by myeloma cells. Runx2 is required for osteoblast differentiation. The expression of Runx2 by mesenchymal cells has been found to decrease after direct cell to cell contact with myeloma cells in co-cultures [52].

Osteocytes have not been widely investigated, and their involvement in MM bone disease is unknown. Histological examination of compact bone from MM patients shows a significant change in the morphology of osteocytes and their lacunae [53]. Likewise, a major change in the gene expression profile of osteocytes in MM has also been observed. This indicates that osteocytes are markedly affected in MM. A recently published study showed that MM patients had significantly smaller numbers of viable osteocytes compared to healthy individuals [6]. Likewise MM patients with bone lesions were found to have a smaller number of viable osteocytes compared with MM patients without bone lesions. The amount of viable osteocytes was found to be negatively correlated with the number of osteoclasts and the authors suggest an involvement of the osteocytes in MM-induced osteoclast formation [6].

Furthermore, healing of bone lesions in MM bone disease does not occur frequently, even in patients who respond well to anti-myeloma treatment. It remains unclear why bone remodelling does not normalise when the influence from myeloma cells disappears after successful treatment. It may be due to irreversible damage of key elements in the bone formation process (i.e. the BRC).

5. Biochemical markers of bone turnover

Conventional radiography has for many years been the standard method for the diagnosis of myeloma bone disease. This modality, however, suffers from a low sensitivity, since 30% of the

trabecular bone mass must be absent for a lesion to become detectable. Computed tomography can increase the sensitivity at the cost of higher radiation exposure. Both modalities, however, only provide static information concerning the accumulated bone disease. Biochemical markers of bone turnover can provide dynamic information concerning the velocity of bone turn-over at any given time point, and can be measured from either blood or urine samples. Furthermore, bone formation and bone resorption can be evaluated separately. Bone markers can be divided into two categories: they are either collagen fragments released during the formation or destruction of the collagen triple helix structure of which bone consists, or they are enzymes released from either osteoblasts or the osteoclasts (see figure 3). Bone resorption markers from the first group include the cross-linked telopeptides of type-1 collagen NTX, CTX, ICTP and DPD (Table 1). They are products of osteoclast-mediated degradation of collagen and therefore reflect bone resorption. Bone formation markers from this group include PINP and PINC (Table 1). These markers are products of the cleavage process of procollagen into collagen and therefore the measured levels will reflect the amount of newly formed bone matrix. The second group of bone markers include TRACP-5b, bALP and OC (Table 1). TRACP-5b is secreted by osteoclasts and used as a marker of osteoclast number and activity, whereas bALP and osteocalcin are produced by osteoblasts and used as markers of osteoblast number and activity. The levels of bone markers have been shown to correlate with the degree of bone resorption or bone formation using classical bone histomorphometry [54;55]. Furthermore, bone resorption markers decrease when treatment with anti-resorptive drugs is initiated [56]. Conversely, the discontinuation of anti-resorptive drugs leads to a rise in bone resorption markers [57]. However, when using biochemical markers it is important to be aware of the fact that the level of markers may be influenced by a number of factors, such as age, gender, drugs, renal- and liver function or diet. Especially the collagen-mediated markers are sensitive to food intake. Despite the interest in bone markers, there is still no consensus on how they should be used to monitor disease activity and response to treatment in MM [58].

Bone marker	Abbreviation	Type	Analytical specimen
C-terminal cross-linking telopeptide of type-1 collagen	CTX	Bone resorption marker	Serum, Urine
N-terminal cross-linking telopeptide of type-1 collagen	NTX	Bone resorption marker	Serum, Urine
C-terminal cross-linking telopeptide of type-1 collagen generated by metalloproteinase	ICTP	Bone resorption marker	Serum
Deoxypyridinoline	DPD	Bone resorption marker	Serum, Urine
Tartrate-resistant acid phosphatase isotype 5b	TRACP-5b	Bone resorption marker osteoclast activity	Serum
Bone-specific alkaline phosphatase	bALP	Bone formation marker	Serum
Osteocalcin	OC	Bone formation marker	Serum
Procollagen type-1 N-propeptide	PINP	Bone formation marker	Serum
Procollagen type-1 C-propeptide	PICP	Bone formation marker	Serum

Table 1. Biochemical markers of bone turnover

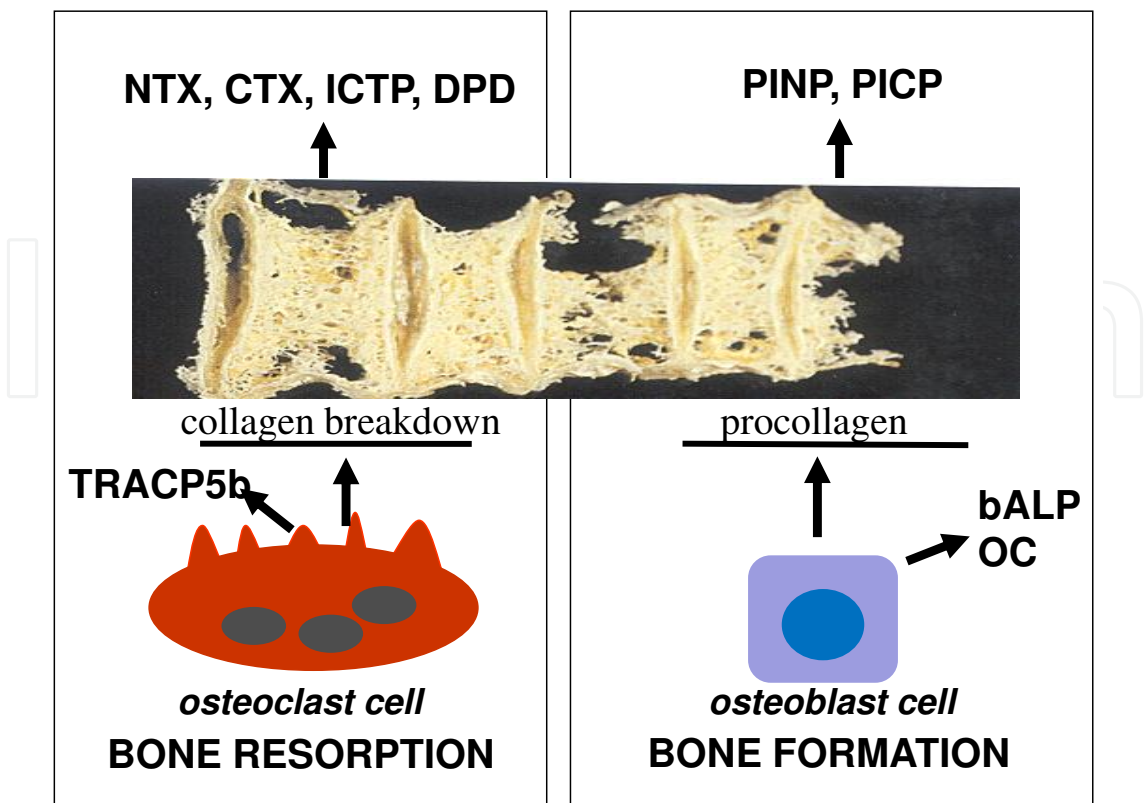


Figure 3. Biochemical markers of bone remodelling can be divided into markers reflecting bone resorption (left) and marker reflecting bone formation (right). They can also be divided in markers reflecting a change in the collagen matrix (upper part) or markers reflecting the activity of bone resorbing or bone forming cells (lower part).

6. Treatment of multiple myeloma bone disease

6.1. Anti-resorptive treatments:

Until now, bisphosphonates remain the only registered agents for the treatment of osteolytic bone disease in MM. Bisphosphonates are synthetic analogues of pyrophosphate with a high affinity for the hydroxyapatite in the bone. After administration, bisphosphonates are rapidly cleared from the blood and incorporated into the bone matrix or excreted through the kidneys. If imbedded in the bone matrix they remain incorporated for many years, or until the bone is degraded by the osteoclasts [59]. Three generations of bisphosphonates exist, and each is many fold more potent than the previous [60]. The different bisphosphonates can be distinguished by the absence or presences of a nitrogen atom in the R² position of the bisphosphonate, with the amino-bisphosphonates being the most potent. When the osteoclast degrades bone, the bisphosphonate is taken up through endocytosis and causes apoptosis either through the incorporation into non-functional adenosine triphosphate (non-nitrogen containing bisphosphonates), or through the inhibition of farnesyl pyrophosphate synthase (nitrogen containing bisphosphonates)[61]. Early studies, using the least potent bisphosphonate, etidronate, showed no clinical benefit on MM bone disease [62], whereas the slightly more potent clodronate could diminish progression of osteolysis, but had no effect on bone pain or

pathological fractures [63]. In 1996 and 1998, Berenson *et al.* published two studies, in which patients were randomised to placebo or the amino-bisphosphonate pamidronate. A significant effect was observed with regard to reduced pain, fewer skeletal related events, and improved quality of life [64;65]. Initially, no effect could be observed in overall survival, however using a Cox multivariable regression analysis a slight increase in overall survival was observed for a subgroup of patients. A subsequent phase III trial, comparing the more potent bisphosphonate zoledronic acid with pamidronate in breast cancer patients with bone metastases and MM patients, demonstrated a superiority of zoledronic acid over pamidronate in reducing skeletal events in the breast cancer group but not in the MM sub-population. No difference was observed in overall survival [66]. Later publications indicated that there could be an effect on overall survival but only with the most potent bisphosphonates [67-70]. In 2010 a large meta-analysis concluded that there was no effect on overall survival in MM provided by bisphosphonates in general [71]. However, later the same year the large MRC IX trial, reported that zoledronic acid was superior to the non-nitrogen containing bisphosphonate clodronate, not only with regard to the control of bone disease, but zoledronic acid also increased overall survival by 5.5 months [49]. Because of the MRC IX data, an updated version of the meta-analysis was published in 2012. Still, no significant effect on overall survival was observed for bisphosphonates in general, but “meta regression analysis indicated that the beneficial effect of bisphosphonates on mortality in patients with MM may be a function of drug potency, with zoledronate being the most potent” [72].

Bisphosphonates are potential nephrotoxic compounds and dosage adjustment according to creatinine clearance are required [73].

In 2003, it was reported for the first time, that exposure to bisphosphonates could also cause osteonecrotic lesions, especially in the oral cavity. This complication was termed bisphosphonate-associated osteonecrosis of the jaw (BON) [74]. BON is commonly observed after surgical dental procedures, e.g. tooth extractions, but spontaneous cases do occur [75]. The incidence of BON increases with treatment duration [76], as well as with the potency of the bisphosphonate used [77]. The aetiology of BON remains controversial. One possible explanation could be that the profound suppression of osteoclast activity results in the accumulation of microfractures in the bone. This explanation is in accordance with the fact that BON incidence increases with treatment duration and potency of bisphosphonate type and that BON is also observed after treatment with denosumab, a monoclonal antibody that inhibits osteoclast activity by binding to RANKL. It has also been suggested that BON may occur because of the anti-angiogenic effects of bisphosphonates [78]. Indeed, BON seems to be more commonly observed in patients receiving other anti-angiogenic compounds such as thalidomide [77]. Thirdly, it has been speculated that the frequent findings of actinomycosis in the lesions may be part of the pathogenesis and not only a secondary event, especially since prophylactic antibiotics during dental procedures seem to reduce the incidence of BON [79]. Recently, osteomalacia, which in adults is often a consequence of vitamin D deficiency, has been suggested as a risk factor for BON [80]. Once established BON is difficult to cure, and surgical treatment may worsen the situation [75]. Case-reports suggest several treatment modalities, including low-level laser therapy [81;82], hyperbaric oxygen treatment [83], long-term

administration of antibiotics [84], autologous bone marrow transplantation [85], and ozone therapy [86]. Because of the difficulties in treating BON, focus has mainly been on preventing the occurrence in the first place. This has been done partly by implementing preventive dental procedures prior to the initiation of therapy with bisphosphonates, but probably more importantly by reducing the exposure time to bisphosphonates. The oral microflora also seems to play a role in the development of BON and antibiotic prophylaxis before dental procedure may reduce the risk of developing BON [79]. Concerning the preventive procedures, there are data indicating a positive effect [87;88]. Concerning the reduced exposure time there are few supportive data, but recommendations based on expert opinion do exist [89-92]. Corso *et al.* demonstrated that monthly infusions for one year followed by four infusions the following year offered equal bone protection but reduced BON incidence compared to the monthly infusions for two years [93]. Lund *et al.* have provided evidence that one year of monthly infusions offers inferior anti-resorptive protection after discontinuation compared with two years of monthly infusions based on consecutive measurement of markers of bone turnover [57]. A more rational approach to reduce the bisphosphonate load without increasing the risk of osteolysis, could be to monitor the patient's ongoing bone remodelling using biochemical markers of bone turnover in order to provide individualized treatment. Data now exist which indicate that bone remodelling markers may predict osteolysis before it becomes manifest by X-ray or CT-scan [94].

Denosumab is a humanized antibody with high affinity for RANKL. By targeting RANKL, denosumab mimics physiological OPG and thus blocks the stimulation of the osteoclasts through the NF- κ B receptor. Denosumab could be expected to have a favourable impact on MM bone disease due to its effect on the increased RANKL/OPG ratio observed in MM patients. In 2006 Body *et al.* published a study investigating the effect of a single dose of subcutaneous denosumab compared with a single dose of intravenous pamidronate on the urinary and serum levels of the bone resorption marker NTX. The study population consisted of 54 patients with bone lesions and either MM (n=25) or breast cancer (n=29). The study reported that the compounds were well-tolerated and to a similar extent decreased the investigated bone resorption marker NTX [95]. A phase II study including 96 MM patients, in either relapse or plateau phase, where denosumab was administered every fourth week also demonstrated a decrease in bone resorption markers, even in patients previously treated with bisphosphonates, with an acceptable safety profile [96]. In a phase III trial patients (n=1776) with cancer bone metastases (excluding breast and prostate cancer) or MM (10% of the study population) were randomized to treatment with either zoledronic acid or denosumab. Denosumab was found to be equivalent to zoledronic acid in delaying time to first on-study skeletal-related event. Noteworthy, in a subgroup analysis of the MM patients (n=180), mortality appeared to be increased in those treated with denosumab with a hazard ratio of 2.26 (95% CI: 1.13-4.50) [97]. Recently, new data from this trial has been published. Results of patient-reported outcomes of pain and health-related quality of life were reported to be equal in the two treatment arms [98]. The frequency of osteonecrosis of the jaw seemed to be equal for treatment with denosumab or zoledronic acid [97;99]. Denosumab is currently not registered for the treatment of MM bone disease by US Food and Drug Administration or the European Medicines Agency. [100;101], but it could perhaps in the future be used for the

treatment of bone disease in patients with renal failure who are not suitable for treatment with bisphosphonates due to the risk of aggravation of renal function.

6.2. Possible future anti-resorptive drug treatments

Several drugs targeting MM bone disease are under development e.g. the CCR1-inhibitor (MLN3897) that blocks the CCR1 receptor on osteoclasts and thereby prevents stimulation by MIP-1 α [102]. Another candidate for the treatment of MM bone disease is the anti-DKK1 human antibody BHQ880. The agent has been shown to increase osteoblast differentiation in vivo and in animal models to significantly increase the number of osteoblasts and trabecular thickness [103]. Whether this bone anabolic effect will be found in humans will be of interest because it raises the possibility for not only preventing bone loss, but also supporting new bone formation. Clinical trials with BHQ880 are ongoing [104].

6.3. Anti-myeloma treatments

Treatment of MM using conventional chemotherapy usually does not induce healing of osteolytic lesions even if patients respond well to the anti-myeloma treatment and obtain long progression free periods [105-107]. Although markers of bone resorption may decrease [55] serum levels of bone formation markers remain suppressed as a sign of continuously impaired bone formation even in patients who have obtained complete response after treatment with conventional chemotherapy [56;108].

Proteasome inhibitors have a well-documented anti-myeloma effect and they may also have an impact on MM bone disease through the inhibition of osteoclasts and stimulation of osteoblasts.

In vitro studies have demonstrated that proteasome inhibitors inhibit osteoclast differentiation and resorptive activity by reducing the activity of NF- κ B [109;110]. In vivo studies of the effect of bortezomib on bone resorption markers show a rapid and significant decrease in CTX and urinary NTX, but it has also been observed that the levels begin to increase again already 2-3 days after the intravenous injection of bortezomib [111]. The levels of the bone resorption markers CTX and TRACP-5b and the RANKL/OPG ratio were also found to decrease after four cycles of treatment with bortezomib in a clinical study including 34 myeloma patients [112]. The ubiquitin-proteolytic pathway is a regulator of bone formation [113] and by blocking this pathway proteasome inhibitors can stimulate osteoblast differentiation. Suggestions of the underlying mechanism have been that proteasome inhibitors may increase the level of bone morphogenetic protein 2 [114] and prevent the proteolytic degradation of RUNX-2 [115]. In an in vitro study, it has been suggested the bortezomib may enhance bone formation through the inhibition of DKK1 expression in osteogenic cells [116]. More studies have provided evidence that proteasome inhibitors stimulate osteoblasts and bone formation in vitro as well as in animal models [114;116-118], and histological investigations have demonstrated increased numbers of osteoblasts in bone marrow sections from MM patient treated with bortezomib [115]. Clinical studies have demonstrated that anti-myeloma treatment with bortezomib induces an increased level of biochemical markers of bone formation both with

regard to markers of osteoblast activation and also bone matrix deposition [118;119]. Alkaline phosphatase was found to be significantly increased in patients who responded to bortezomib treatment [119]. In another clinical study bone-specific alkaline phosphatase (bALP) and osteocalcin were found to be increased not only in responding patients, but also in patients who did not achieve an anti-myeloma response to treatment with bortezomib [120]. This result supports the assumption that bortezomib may have a bone anabolic effect independent of its anti-myeloma effect. Enhancement of bone matrix deposition after mono-therapy with bortezomib, has also been shown by the demonstration of increased serum levels of PINP (Procollagen Type-I N-terminal propeptide) [118]. Both bALP and osteocalcin were found to be increased after treatment with bortezomib in a clinical study of 34 relapsed myeloma patients in non-responders and responders but the increase was highest in responding patients. However no radiographic signs of healing of the baseline osteolytic lesions were observed six month post-treatment [112]. Radiologic evidence of healing of lytic lesions was observed in six out of 11 patients who responded to combination treatment with bortezomib, melphalan, and prednisone while none of the evaluated patients who had achieved a response to treatment with melphalan and prednisone without bortezomib showed radiological signs of healing [121].

Pomalidomide (originally CC-4047), is a derivative of thalidomide that is anti-angiogenic and acts as an immunomodulator. Pomalidomide is now tested in Phase III clinical trials and will hopefully soon become available treatment of patients with relapsed or refractory MM. The drug has been granted orphan status for the treatment of MM by the European Medicines Agency [122]. Pomalidomide has been shown to inhibit osteoclasts differentiation in bone marrow cultures which leads to a strong inhibition of bone resorption [123]. The inhibition of osteoclast formation seems to occur through a reduction of the PU.1 expression. PU.1 is a critical transcription factor in the development of mature osteoclasts. Lenalidomide, another thalidomide derivative, has been shown to inhibit both an early step in osteoclastogenesis through reduction of PU.1 expression and to reduce secretion of RANKL from bone marrow stroma cells derived from patients with MM [124]. In a clinical study including 20 MM patients with bone disease Breitkreutz *et al.* found a significant decrease in the serum levels of the RANKL/OPG ratio after two cycles of treatment with lenalidomide [124]. Likewise, treatment with thalidomide in combination with dexamethasone has a favourable effect on the RANKL/OPG ratio [125]. Treatment with thalidomide in combination with dexamethasone can also decrease the levels of the bone resorption markers CTX, NTX and TRACP-5b, however the treatment does not increase the bone formation marker bALP or osteocalcin [126]. The failure to increase bone formations markers in serum, correlates with the observation that none of the responding patients in a clinical study of patients treated with a thalidomide/dexamethasone combination, showed any radiological signs of healing of osteolytic lesions [125].

7. Conclusion

The pathophysiology in multiple myeloma bone disease is complex. There is evidence that not only osteoclast activity but also other cells and structures responsible for normal bone

metabolism are affected in different ways, suggesting that different targets for treatment may be identified. The notion that myeloma-induced stimulation of osteoclast may promote growth of myeloma cells and thus create a vicious circle emphasise the importance of improved understanding as well as development of more efficient treatment of myeloma-induced bone disease. Bisphosphonates remain so far the only registered drugs for treatment of multiple myeloma bone disease. Due to risk of renal damage and bisphosphonate-associated osteonecrosis of the jaw after treatment with the potent amino-bisphosphonates, alternatives are wanted and several new drugs are under investigation. Furthermore, the optimal duration of treatment with bisphosphonates remains unknown.

Treatment with conventional chemotherapy does not induce healing of osteolytic lesion even in patients who have obtained complete response. However, novel drugs used for treatment of multiple myeloma seem to affect bone metabolism besides their anti-myeloma effect and cases with radiological signs of healing following treatment with bortezomib have been reported.

The last decade has brought the understanding of multiple myeloma bone disease to a higher level, new anti-myeloma drugs with positive effect on bone disease have been registered and more are undergoing investigation. Still many questions regarding the pathophysiology and treatment of multiple myeloma bone disease remain to be answered.

Author details

Maja Hinge^{1*}, Thomas Lund², Jean-Marie Delaisse¹ and Torben Plesner³

*Address all correspondence to: maja.hinge@slb.regionsyddanmark.dk

1 Department of Clinical Cell Biology, Vejle/Lillebælt Hospital, University of Southern Denmark, Vejle, Denmark

2 Department of Haematology, Odense University Hospital, Odense, Denmark

3 Department of Internal Medicine, Division of Haematology, Vejle/Lillebælt Hospital, University of Southern Denmark, Denmark

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