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Biology and management of myeloma-related bone disease

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ABSTRACT

Bone disease is one of the most common complications of multiple myeloma. It is the result of increased osteoclast activity which is not compensated by osteoblast activity and leads to osteolytic lesions characterized by bone pain and increased risk for pathological fracture, spinal cord compression and need for radiotherapy or surgery to the bone. Recent studies have revealed novel pathways and molecules that are involved in the biology of myeloma bone disease including the receptor activator of nuclear factor-kappa B ligand/osteoprotegerin pathway, the Wnt signaling inhibitors dickkopf-1 and sclerostin, macrophage inflammatory proteins, activin A, and others. A thorough study of these pathways have provided novel agents that may play a critical role in the management of myeloma related bone disease in the near future, such as denosumab (anti-RANKL), sotatercept (activin A antagonist), romosozumab (anti-sclerostin) or BHQ-880 (anti-dickkopf 1). Currently, bisphosphonates are the cornerstone in the treatment of myeloma related bone disease. Zoledronic acid and pamidronate are used in this setting with very good results in reducing skeletal-related events, but they cannot be used in patients with severe renal impairment. Furthermore, they have some rare but serious adverse events including osteonecrosis of the jaw and acute renal insufficiency. This review paper focuses on the latest advances in the pathophysiology of myeloma bone disease and in the current and future treatment options for its management.

Introduction

Multiple myeloma (MM) is a plasma cell malignancy which is characterized by the presence of bone destruction due to an elevated function of osteoclasts that is not balanced by a comparable elevation of osteoblast function. This bone destruction develops lytic lesions that lead to bone pain, hypercalcemia and skeletal-related events (SREs) such as pathological fractures, requirement for surgery and/or radiation

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to the bone and spinal cord compression (SCC) [1]. At diagnosis 70% of the patients present with bone pain, while during the course of the disease 50% of the patients develop at least one SRE if they do not receive a bone-targeted agent [2]. In two studies, Melton et al. has shown that MM patients have generalized bone loss and osteoporosis that make them vulnerable to osteoporotic fractures [3]. They also showed that even patients with monoclonal gammopathy of undetermined significance (MGUS) have a >2-fold increase in fracture rate of the axial skeleton [4]. Bone disease has a serious impact on the quality of life and survival of MM patients [5] and affects both clinical and economic aspects of their life [6]. The risk of death in MM patients who develop a pathologic fracture increases by 20% in comparison with MM patients without pathologic fractures [7]. Thus, it is important to diagnose early and treat properly bone disease and its complications. This paper reviews the latest available details of pathophysiology and treatment of myeloma related bone disease.

Biology of multiple myeloma bone disease

In the adult skeleton, skeletal integrity is coordinated by the synchronized activity of three cell types. Osteoblasts create new bone matrix; osteoclasts are responsible for bone resorption and osteocytes regulate bone turnover. In multiple myeloma patients, bone disease is the result of an uncoupling in bone remodeling. It consists of an increase in the osteoclast-mediated bone resorption, which is combined with suppression in the osteoblast, mediated bone mineralization and defects in osteocyte functions [8]. Until today, several direct and indirect interactions between myeloma and stromal cells in the bone marrow microenvironment have been recognized. The fact that osteolytic lesions occur close to MM cells suggests that factors secreted by tumor cells lead to direct stimulation of osteoclast mediated bone resorption and inhibition of osteoblast mediated bone formation [9]. In addition, the increased bone resorptive progress leads to the release of growth factors that increase the growth of MM cells, leading to a vicious cycle of tumor expansion and bone destruction. Apart from this, interactions via adhesion between MM cells and bone marrow cells result in the production of factors that promote angiogenesis and make the myeloma cells resistant to chemotherapy [10, 11]. One example is that of T-regulatory and T-helper cells. In MM patients the stimulated T-regulatory cells by myeloma cells up-regulate pro-osteoclastic molecules and have been implicated with disease progression, whereas T-helper cells secrete IL-17 which promotes osteoclast formation [12–14]. On the other hand, Yaccoby et al. showed that osteoblasts inhibit MM cell growth in most of the patients [15].

Increased osteoclast activity

The main regulator of the osteoclast stimulation and activation is the system of the receptor activator of nuclear factor-kappa B (RANK), its ligand (RANKL) and its decoy receptor, osteoprotegerin (OPG). An important step in the osteoclast stimulation is the binding of myeloma cells to the bone marrow stromal cells (BMSCs). This adhesion is mediated by interactions between α4β1 on myeloma cells and vascular cell adhesion molecule 1 (VCAM-1) on BMSCs, and leads to the up-regulation of a variety of pro-osteoclastic cytokines and chemokines which directly or indirectly stimulate osteoclast formation differentiation and activity. These factors include interleukin-6 (IL-6), IL-1α, IL-1β, IL-11, macrophage-colony stimulating factor (M-CSF), tumor necrosis factor alpha and beta (TNF-α and TNF-β), macrophage inflammatory proteins-1 alpha and beta (MIP-1α and β), parathyroid hormone-related peptide (PTHrP), vascular endothelial growth factor (VEGF) and others [16–18]. These factors are excreted by MM cells directly, or indirectly after stimulation of bone marrow cells by the MM cells.

TNF Superfamily members – the RANK/RANKL signaling pathway

RANK is a transmembrane signaling receptor. It is located on the surface of osteoclast precursors [19, 20]. RANKL is expressed by a range of cell types, including marrow stromal cells and osteoclasts. Its expression is stimulated by cytokines that stimulate bone resorption [21] such as parathyroid hormone (PTH), 1,25-dihydroxy vitamin D3 and prostaglandins [22, 23]. RANKL binds to its receptor on osteoclast precursors and stimulates osteoclast differentiation formation and survival. These functions are mediated through the nuclear factor kappa-B (NFκB) and p38 MAP-kinase pathways. Apart from this, RANKL has direct enhancement effects on mature osteoclasts that inhibit their apoptosis. The importance of the role of RANKL in osteoclastogenesis has been shown in RANKL or RANK gene knockout mice. These animals lack osteoclasts and as a result they develop osteopetrosis [24–27]. In the absence of RANKL almost no chemokine with osteoclast activity can act.

OPG, another member of the TNF receptor superfamily, is a soluble decoy receptor for RANKL [28]. It is produced by several cells, including osteoblasts, and interacts with RANKL, causing inhibition of its action, thereby reducing osteoclastogenesis. The important role of OPG has been shown in studies with knock-out mice. OPG deficient mice develop severe osteopenia and osteoporosis [29–31]. An abnormal RANKL/OPG ratio is found in the majority of malignant bone disorders [32].

Myeloma cells turn the balance of the RANKL/OPG ratio in favor of RANKL. In the bone marrow microenvironment, MM cells play a double role: they induce the expression of RANKL from stromal cells, while they directly express RANKL, although in low amounts [33–37]. Apart from this they decrease the OPG availability within the bone marrow microenvironment. This is maintained in two different ways .The MM cells reduce OPG secretion from osteoblasts and stromal cells. In addition, they remove the remaining OPG by lysosomal degradation [38, 39]. The up-regulation of RANKL, in combination with down-regulation of OPG, leads to the formation and activation of osteoclasts. Levels of RANKL and OPG have been shown to correlate with clinical activity of MM, severity of bone disease and poor prognosis[40]. In individuals with MGUS, the RANKL/OPG is also increased when compared to that in control subjects but remains significantly lower than that in patients with myeloma [41],

partly explaining the higher incidence of osteoporosis in these patients.

Macrophage inflammatory proteins-1 alpha and beta (MIP-1α, -β)

These two cytokines play an important role in the biology of myeloma bone disease. Both are produced and secreted by MM cells. MIP-1α, also known as chemokine (C-C motif) ligand 3 (CCL-3), is a low molecular weight chemokine, which belongs to the RANTES (regulated on activation, normal T cell expressed and secreted) family of chemokines, primarily associated with cell adhesion and migration. MIP-1α is chemotactic for monocytes and monocyte-like cells, including osteoclast precursors. MIP-1α induces late stage differentiation in human osteoclast progenitors and promotes osteoclast formation in a dose-dependent way in bone marrow cultures [17, 42–44]. MIP-1α enhances the effects of RANKL and IL-6 on osteoclast formation [45]. However, it has also been shown to enhance osteoclast formation independent of RANKL. MIP-1α and MIP-1β enhance the RANKL expression in stromal cells [27]. MIP-1β is a highly homologous chemokine of MIP-1α that similarly to MIP-1α induces the development of osteolytic bone lesions [46].

Both MIP-1α and MIP-1β are produced and secreted by myeloma cells. MIP-1α m-RNA has been detected in MM cells, while MIP-1α protein was found elevated in the microenvironment of MM patients in whom it correlated with stage and disease activity. MIP-1α was also elevated in the blood of myeloma patients with severe bone disorders, but not in MGUS patients with increased bone resorption [41, 47]. Gene expression profiling showed that MIP-1α is one of the genes that is highly correlated with bone destruction in MM [48].

Furthermore, MIP-1α has direct action on myeloma cells, since they express the receptor CCR5, promoting growth, survival and migration of myeloma cells [49]. MIP-1α up-regulates the expression of β1 integrin on MM cells, increasing adhesive interactions between MM cells and marrow stromal cells. This results in increased production of RANKL, IL-6, VEGF and TNF-α by marrow stromal cells, which further enhances MM cell growth, angiogenesis and bone destruction [27].

Interleukin-3

IL-3 mRNA levels were found to be increased in myeloma cells and IL-3 protein levels were found to be increased in bone marrow plasma from MM patients. IL-3 in combination with MIP-1α or RANKL significantly enhances human osteoclast formation and bone resorption compared with MIP-1α or RANKL alone. IL-3 also stimulates the growth of myeloma cells independently of the presence of IL-6. These data suggest that increased IL-3 levels are present in the marrow microenvironment of myeloma patients, increasing bone destruction and tumor cell growth [50, 51].

Interleukin-6

IL-6 is a growth factor for both osteoclasts and myeloma cells, promoting their survival and preventing their apoptosis. IL-6 causes an increase in the osteoclast precursors, which leads to the increase in the number of mature osteoclasts. The levels of circulating IL-6 and its receptor (IL-6R) are increased in MM and correlate with stage, advanced myeloma features and disease-free survival [52]. Levels of IL-6 are elevated in MM patients with osteolytic bone disease when compared with MM patients without bone disease, as well as in patients with MGUS [53].

Interleukin-1β

IL-1β has potent osteoclastogenesis activity: it enhances the expression of adhesion molecules and induces paracrine IL-6 production, resulting in osteolytic disease. IL-β has been found to be increased in myeloma cell cultures [50]. Elevated IL-1β m-RNA levels were also detected in MM patients, while anti-IL-1β antibodies failed completely to abolish osteoclastogenesis activity of myeloma bone marrow [54].

Tumor necrosis factor alpha (TNF-α)

High plasma levels of TNF-α have been found in patients with MM [55]. TNF-α causes proteolytic breakdown of I-kappa B (the inhibitor of NF-κB), leading to NF-κB activation and enhancement of gene transcription, including IL-6 and adhesion molecules, which are involved in promoting bone resorption [56].

Hepatocyte growth factor (HGF)

Myeloma cells can transform HGF to its active form. HGF plays an important role in osteoclast activation and angiogenesis. HGF can up-regulate the osteoclast-like cell-mediated IL-11 expression [57].

Vascular endothelial growth factor (VEGF)

VEGF plays a major role in tumor neovascularization and has been recently implicated in osteoclastogenesis in MM. It is excreted by myeloma cells and binds to VEGFR-1 receptor that is mainly expressed by osteoclasts. It has a direct role in enhancing osteoclast function and survival [11]. VEGF stimulates the IL-6 production by stromal cells, while IL-6 enhances VEGF secretion by myeloma cells, suggesting the existence of paracrine interactions among stromal and MM cells [58].

Osteopontin

Osteopontin is a non-collagenous matrix protein which is produced by different cells including osteoblasts and myeloma cells. It is involved in tumor metastasis, adhesion, apoptosis and angiogenesis. Marrow cells from myeloma patients with advanced disease produced increased levels of osteopontin compared with that from asymptomatic MM or MGUS patients. Furthermore, plasma osteopontin levels of MM patients were significantly higher than those of MGUS and controls, and correlated with both disease progression and bone destruction. These observations suggest that myeloma cells actively produce osteopontin, which contributes to osteoclastic bone resorption [59].

Stromal-derived factor-1α (SDF-1α)

SDF-1α is another chemokine which is expressed by both stromal and myeloma cells. MM patients have elevated plasma levels of SDF-1α when compared with normal, age-matched subjects. The SDF-1α levels have been correlated with multiple radiological osteolytic lesions in MM patients.

SDF-1α binds to its receptor CXCR4, which is widely expressed on leukocytes, mature dendritic cells, osteoclast precursors, and myeloma cells, and up-regulates the expression of the matrix degrading enzyme, matrix metalloproteinase 9 (MMP-9), promoting the recruitment, migration and activation of the osteoclasts [27].

Parathyroid hormone-related protein (PTHrP)

PTHrP is produced by a number of tumors that grow in bone and mediates the development of bone metastases, particularly of breast and lung cancer [60–62]. It is possible that PTHrP stimulates bone resorption and mediates its effect by up-regulating RANKL in osteoblasts via the PTH-R1 [62]. PTHrP has been shown to be expressed by myeloma cells and PTHrP signaling, via the PTH-RI, increases expression of RANKL in myeloma cells [63, 64].

Osteoblast suppression

The inhibition of osteoblasts is another crucial step in the pathogenesis of myeloma bone disease. As myeloma burden increases, osteoblast-driven bone formation is suppressed which further results in the development of osteolytic bone lesions. Osteoblast suppression is maintained even in patients in long-term remission. Osteoblast inhibition is maintained through the secretion of cytokines, which is the result of interactions between MM cells and osteoblasts or osteocytes [55].

Wnt signaling pathway

The osteoblast function is maintained by several pathways, including the canonical Wingless-type (Wnt) pathway. Wnt proteins bind to the Wnt receptor and its co-receptors LRP5/LRP6 and lead to a stabilization of β-catenin. This results in the increase of cytoplasmic levels of β-catenin, leading to translocation into the nucleus. This event stimulates the expression of osteoblastic target genes [65]. When the Wnt signal is absent, β-catenin is phosphorylated and degraded by the proteasome. Wnt antagonists prevent the binding of Wnt glycoproteins to their receptors and include the following molecules [66]. Members of the dickkopf (DKK) family and sclerostin bind to the LRP5/LRP6 component, while secreted frizzled-related proteins (sFRP), for example sFRP-2 and sFRP-3, bind to Wnt proteins. Both result in a suppression of Wnt signaling and a reduced osteoblast function.

DKK-1 is secreted by myeloma cells and has been shown to inhibit differentiation of osteoblast precursor cells in vitro. In MM patients with lytic lesions, immunohistochemical analysis of bone marrow biopsies showed that myeloma cells overexpress DKK-1. In fact, bone marrow plasma from newly diagnosed MM patients contains nearly 3 times more DKK-1 protein compared to that from control subjects: marrow plasma from patients with MM that contained >12 ng/ml of DKK-1 inhibited osteoblast differentiation. Furthermore, gene expression levels of DKK-1 correlated with extensive bone disease [67]. DKK-1 is increased in the serum of MM patients [68] and correlates with the extent of bone disease [69]. Serum DKK-1 decreases in myeloma patients who respond to therapy, but not in those who did not respond [70]. DKK-1 is secreted in vivo mainly by myeloma cells. Furthermore, since Wnt signaling in osteoblasts increases the expression of OPG and downregulates the expression of RANKL [71, 72], inhibition of Wnt signaling promotes osteoclastogenesis. Taken together, DKK-1 seems to be a key regulator of bone metabolism in myeloma.

Soluble FRP-2 is secreted from MM cells and inhibits mineralized nodule formation and osteoblast differentiation induced by bone morphogenetic protein 2 (BMP-2) [73]. It inhibits osteoblastic differentiation at multiple steps, not only early osteoblastic differentiation to express alkaline phosphatase (ALP), but also terminal differentiation to acquire mineralizing properties. It is thought to be a decoy receptor that interferes with Wnt binding to its receptor, Frizzled. MM patients with advanced bone disease had elevated expression of sFRP-2 in their myeloma cells [73].

Finally, sclerostin is a cysteine-knot-containing protein, which is produced by osteocytes, inhibits canonical Wnt pathway and thus inhibits osteoblast function [74]. Circulating sclerostin reflect bone marrow plasma sclerostin levels [75]. In patients with multiple myeloma, sclerostin is overproduced in the marrow microenvironment either by the myeloma cells [76] or the osteocytes, and its circulating levels correlate with advanced bone disease and abnormal bone remodeling [77].

Activin-A

Activin is a member of the transforming growth factor-beta (TGF-β) superfamily with complex effects on the bones. Activin-A has been shown to inhibit bone formation and in some studies promote osteoclastic bone resorption, although this may prove to be context specific. Activin-A is increased in the bone marrow of patients with myeloma and serum level is increased in patients with newly diagnosed myeloma and associated with elevated bone resorption [78, 79]. Activin-A signaling occurs through the Activin A type IIA receptor to inhibit osteoblastic bone formation. Blocking activin-A signaling using a soluble ActRIIA murine Fc fusion protein (ActRIImuRc) has been shown to prevent activin A mediated osteoblast suppression, but has no effect on osteoclast formation in vitro [80].

Diagnosis and monitoring of myeloma bone disease

The diagnostic procedures that are used widely today in the diagnosis and monitoring of myeloma related bone disease include conventional radiography, computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography/CT (PET/CT).

Conventional radiography

It is the standard diagnostic procedure for the detection of skeletal involvement, although it lacks sensitivity as it requires a 30–50% of the trabecular bone loss to reveal a detectable lytic lesion [81]. The skeletal survey should include anteroposterior and lateral views of the skull, posteroanterior view of the chest, anteroposterior and lateral views of the thoracic, lumbar and cervical spine (including an open

mouth view), humeri and femora, and anteroposterior view of the pelvis. In addition, symptomatic areas should also be specifically visualized. Approximately 75% of patients with multiple myeloma have abnormal skeletal radiographs. The most common sites involved include the central skeleton, the skull, and the femur, whereas involvement of distal bones is not very frequent [27]. The osteolytic lesions of myeloma are well circumscribed, and sclerosis of surrounding bone is usually absent. In approximately 15% of patients, generalized osteopenia is the only bone manifestation of myeloma [82]. In cases of disease progression, skeletal survey should be performed again [83].

Computed tomography

It is a sensitive tool for the detection of the bone-destructive effects in MM as it can detect small osteolytic lesions, unseen with plain radiographs. CT is not necessary for the initial staging of patients with MM, but is useful for directing needle biopsy for histological diagnosis. Urgent CT may be used when SCC is suspected and MRI is contraindicated (intraorbital metallic foreign bodies, cardiac pacemakers) due to patient intolerance or unavailability [27, 84]. A new CT technique for the whole body with low dose radiation (LDWBCT) has now been recognized as a simple and very sensitive method for the depiction of lytic lesions in myeloma patients, but its value is still under investigation.

Magnetic resonance imaging

It can sample a large volume of bone marrow and depict bone marrow abnormalities in MM with greater sensitivity than conventional radiography and CT. MRI should be performed in all MM patients with negative skeletal survey [83]. Focal lesions are identified in more than one half of patients lacking osteolysis in plain radiography. The converse, detection of focal lesions on plain radiography without corresponding MRI abnormalities, was seen in 20% of patients [27]. MRI is a useful tool in the detection and staging of nonsecretory and macrofocal myeloma or relapse. Whole spine MRI is a staging tool in patients with solitary plasmacytoma of bone, irrespective of the site of the index lesion. MRI plays a role in determining the infiltration of the bone marrow and the adjacent soft tissue structures. This can lead to detection of bone marrow alterations, before bone destruction is detected in conventional radiography or in CT scans [84–86]. Myelomatous lesions of bone marrow can be classified into three patterns: focal, diffuse and variegated. MRI pattern of bone marrow involvement correlates with prognosis in MM. Dimopoulos et al. found that patients with diffuse pattern had a median survival of 24 months; patients with variegated pattern had 52 months, patients with focal pattern had 51 months while those with normal pattern had 56 months ($p = 0.001$) [87]. MRI images accurately reflect response to treatment by showing a decrease or resolution of focal lesions seen on initial studies, whereas lytic lesions are seen on CT even if a patient has complete remission (CR) on MRI. Resolution of diffuse disease can also be identified [87]. Complete response to therapy, as accessed by MRI, favors prolonged survival, especially among patients with a higher number of focal lesions [87]. These data justify the wider application of MRI in MM, as the appropriate imaging tool that permits early detection of eventually devastating focal lesions and as an independent staging tool with prognostic implications [83].

Positron emission tomography/computed tomography (PET/CT)

PET/CT is a technique that combines both anatomical and functional characteristics. It consists of the injection of labeled radiopharmaceuticals such as FDG, followed by tomographic imaging. It combines a high resolution contrast of pet along with a high resolution of CT. Focal lesions show high glucose utilization, due to their high metabolic rate [88]. Lammeren-Veneva et al. showed that, in comparison with conventional radiography, PET-CT revealed more lytic lesions with the exception of those in the skull [89]. In another study, PET-CT was found to have 92% specificity and 85% sensitivity in the detection of myelomatous involvement. This study demonstrated the superiority of PET-CT in the detection of extra medullary disease in comparison with MRI and radiographic bone survey. PET-CT can also play an important role in the assessment of response to treatment and mainly in the most accurate definition of stringent complete response in MM [90].

Bone markers

With the exception of MRI, imaging modalities do not provide information about the rate of the bone turnover. Bone remodeling in MM patients has been tried to be monitored through biochemical markers. The assessment of bone resorption has been made mainly through the urinary and serum products of bone collagen degradation. These are the C- and N-terminal cross-linking telopeptide of collagen type-I (NTX, and CTX or ICTP, respectively) and the serum levels of tartrate-resistant acid phosphatase type-5b (TRACP-5b), an enzyme produced by activated osteoclasts. For the evaluation of bone formation we can evaluate two enzymes that are produced by the osteoblasts: the bone-specific ALP (bALP) and the osteocalcin (OC) [84]. Coleman et al. have shown that high levels of NTX correlated with increased risk of disease progression and skeletal complications in comparison with low NTX levels ($p < 0.001$) [85]. The levels of bALP correlated with risk of negative clinical outcomes. Urinary NTX and serum ICTP are sensitive markers for the identification of patients with increased risk of early bone disease progression [86].

Treatment of myeloma bone disease

Radiation therapy

Radiotherapy is mainly used for the management of solitary plasmacytoma, and less frequently when there is evidence of symptomatic SCC, extensive and symptomatic lytic lesions, and for the prevention of pathologic fractures. Approximately 20% of patients with MM required radiation therapy in the

past, but since current novel agents work rapidly the need for palliative radiotherapy has been decreased [91]. It should be clear that radiotherapy can lead to delays in other treatments including drugs that may be effective anti-MM agents but quite radiosensitizing such as anthracyclines and proteasome inhibitors. The International Myeloma Working group guidelines suggest that a low-dose radiation therapy (up to 30 Gy) is useful as palliative treatment for uncontrolled pain, for impending pathologic fracture, or impending SCC [92]. Upfront external beam radiation therapy is useful for patients with plasmacytoma, extra medullary masses and SCC. However, radiotherapy for palliation and local disease control should be used with caution taking into consideration the prior history of treatment, response and the need for urgent response. It should be limited, in order to spare the patient's marrow function. Novel agents have decreased the need for palliative radiotherapy.

Kyphoplasty and vertebroplasty

Vertebroplasty consists of percutaneous injection of polymethylmethacrylate (PMMA) into the vertebral body under fluoroscopy guidance, and is used in the treatment of painful vertebral compression fractures (VCFs). Approximately 80% of patients with pain unresponsive to medical treatment experience pain relief [93]. The role of vertebroplasty has not been studied in MM patients. Kyphoplasty represents a modification of vertebroplasty, where a balloon is inflated prior to PMMA injection. This can stabilize the fractured vertebral body, reduce kyphotic deformity and restore vertebral height [94, 95]. According to the latest IMWG guidelines, balloon kyphoplasty (BKP) should be considered for symptomatic VCFs and is the procedure of choice to improve QoL in patients with painful VCFs [92]. However risk for subsequent fracture significantly increases in patients undergoing vertebroplasty or kyphoplasty compared with that in patients with previous VCFs who were not treated with either procedures [96].

Surgery

There should be a close cooperation and continuous Orthopedic consultation regarding long-bone fractures, bony compression of the spinal cord, or vertebral column instability (grade D). Consideration and indications for surgery should be done in consultation with the treating oncologist/hematologist and the orthopedic and neurosurgeon to determine when MM treatment can be safely restarted [92].

Bisphosphonates

Bisphosphonates are artificial analogs of pyrophosphates. In comparison with natural pyrophosphates, bisphosphonates are resistant to phosphatase induced hydrolysis [97]. Bisphosphonates cause osteoclast suppression. They bind to calcium containing molecules such as hydroxyapatite [98]. Osteoclast-induced bone resorption causes exposure of hydroxyapatite. Bisphosphonates bind to the exposed molecules of hydroxyapatite. This fact leads to increased concentration of bisphosphonates within the lytic lesions [98–100]. There are two main groups of bisphosphonates, each with a differently proposed mechanism of action [98]. Non-nitrogen containing bisphosphonates induce osteoclast apoptosis via their cytotoxic ATP analogs. On the other hand, nitrogen containing bisphosphonates downregulate osteoclast activity by inhibiting the HMG-CoA reductase pathway. Etidronate and clodronate are non-nitrogen containing bisphosphonates. Zoledronic acid, ibandronate, pamidronate and risedronate are nitrogen-containing bisphosphonates. All bisphosphonates have similar physicochemical properties; however, their anti-resorbing activity is different. Their activity is drastically increased when an amino group is entered into the aliphatic carbon chain. Thus, pamidronate is 100- and 700-fold more potent than etidronate, while zoledronic acid and ibandronate have 10 000- to 100 000-fold higher potency than etidronate, both in vitro and in vivo [101]. Bisphosphonates also appear to affect the microenvironment in which tumor cells grow and may have direct anti-tumor activity [102–107]. Possible mechanisms include the reduction of IL-6 secretion by BMSCs or the expansion of gamma/delta T-cells with possible anti-MM activity. The aim of bisphosphonates use is the reduction of SREs in patients with myeloma bone disease [27].

Etidronate

Etidronate was found to be ineffective in two placebo-controlled studies in myeloma patients [108, 109].

Clodronate

Two major, placebo-controlled, randomized trials have been performed to date in MM. Lahtinen et al. reported the reduction of new osteolytic lesions by approximately 50% in myeloma patients who received oral clodronate for two years. The benefits of clodronate were independent of the presence of lytic lesions at baseline [110, 111]. McCloskey et al. showed a survival advantage in patients who received clodronate and who did not have vertebral fractures at diagnosis (59 vs. 37 months), even though there was no difference regarding overall survival in the two groups. After one year of follow-up, both vertebral and non-vertebral fractures as well as the time to first non-vertebral fracture and severe hypercalcemia were reduced in the clodronate group. At two years, the patients who received clodronate had better performance status and less myeloma-related pain than patients treated with placebo [112, 113].

Pamidronate

Pamidronate is an aminobisphosphonate which has been administered either orally or intravenously. In one trial, patients with at least one lytic lesion and advanced disease were randomized to placebo or intravenous pamidronate [114, 115]. In the pamidronate group, there was a reduction in time to the first skeletal event and in the total number of SREs per year. At nine months, the incidence of SREs was nearly 50% lower in MM patients treated with pamidronate compared with placebo (24% vs. 41%, respectively; $p < 0.001$), and at 21 months the difference remained significant. Pain scores and quality of life were also significantly improved in the pamidronate group. In another trial patients were randomized to receive either placebo or oral pamidronate, in

addition to conventional therapy. There were no reduction in SREs although there was reduction in severe pain. The overall negative result of the study was possibly due to the low absorption of orally administered bisphosphonates [116].

Zoledronic acid

In a randomized trial Berenson et al. compared the effects of zoledronic acid and pamidronate. Both pamidronate at a dose of 90 mg and zoledronic acid at doses of 2 mg and 4 mg in comparison with zoledronic acid 0.4 mg significantly reduced SREs [117]. This trial did not show any superiority of zoledronic acid in comparison with pamidronate, in terms of SREs in myeloma population. In a large randomized phase III, double-blind, study the effects of zoledronic acid and pamidronate were compared [118]. Regarding time to first SRE there were no differences between the study groups. Patients treated with zoledronic acid (4 mg) showed slightly lower skeletal morbidity rate. However, the use of radiation to bone was significantly lower in patients treated with 4 mg zoledronic acid compared with pamidronate. In patients treated with zoledronic acid in comparison with those treated with pamidronate, the levels of NTX showed better normalization. A subsequent analysis of data from a long-term (25-month) extension phase of this study confirmed the equivocal findings that zoledronic acid and pamidronate had similar efficacy in reducing the risk of SREs in MM patients [119]. There is a relatively recent study exploring the role of zoledronic acid in patients with asymptomatic myeloma. No difference was observed regarding the time to progression to symptomatic disease requiring chemotherapy between patients receiving zoledronic acid and the patients who were only observed. However, SREs were reduced in the zoledronic acid group at progression (55.5%) vs. the observation group (78.3%; $p = 0.041$) [120]. In MRC-IX study, there was a comparison between intravenous zoledronic acid (4 mg every 3–4 weeks or at doses according to creatinine clearance rates) and oral clodronate (1600 mg orally daily) in newly diagnosed patients with symptomatic MM ($n = 1960$ evaluable for efficacy). Zoledronic acid reduced the incidence of SREs in both myeloma patients with and without bone lesions as assessed using conventional radiography, compared to clodronate [121, 123]. The median number of SREs after a median period of 3.7 years was 35% for patients receiving clodronate versus 27% of patients receiving zoledronic acid ($p = 0.004$). More importantly, zoledronic acid reduced mortality and extended median survival. Further subset analysis showed that this treatment extended survival by 10 months over clodronate for patients with osteolytic disease at diagnosis, whereas myeloma patients without bone disease at diagnosis as assessed using conventional radiography had no survival advantage with zoledronic acid [122]. These results confirm preclinical studies suggesting indirect and direct anti-myeloma effects of zoledronic acid [123].

Bisphosphonates adverse events

Even though bisphosphonate therapy is well tolerated in patients with MM, clinicians should be alert for symptoms and signs suggesting adverse events (AEs) and patients and healthcare professionals should be instructed on how to prevent and recognize AEs. Potential AEs associated with bisphosphonate administration include hypocalcemia and hypophosphatemia, gastrointestinal events after oral administration, inflammatory reactions at the injection site, and acute-phase reactions after IV administration of aminobisphosphonates. Renal impairment and ONJ represent infrequent but potentially serious AEs with bisphosphonate use.

Hypocalcemia

Hypocalcemia is usually relatively mild and asymptomatic with bisphosphonate use in most MM patients. The incidence of symptomatic hypocalcemia is much lower in MM patients compared to that in patients with solid tumors. Although severe hypocalcemia has been observed in some patients [124] these events are usually preventable via the administration of oral calcium and vitamin D3. Patients should routinely receive calcium (600 mg/day) and vitamin D3 (400 IU/day) supplementation since 60% of MM patients have vitamin D deficiency or insufficiency [125, 126]. In vitamin D deficient patients there is an increase in bone remodeling. This fact shows that MM patients should be calcium and vitamin D sufficient [127]. Calcium supplementation should be used with caution in patients with renal insufficiency.

Renal impairment

Bisphosphonate infusions are associated with both dose- and infusion rate-dependent effects on renal function. The potential for renal damage is dependent on the concentration of bisphosphonate in the bloodstream, and the highest risk is observed after administration of high dosages or rapid infusion. Both zoledronic acid and pamidronate have produced acute renal damage or increases in serum creatinine [115, 119]. Patients should be closely monitored for compromised renal function by measuring CrCl before administration of each IV bisphosphonate infusion. Current guideline recommendations [92] state that the dosages of zoledronic acid and clodronate, when administered intravenously, should be reduced for patients who have preexisting renal impairment (CrCl 30–60 mL/min) but there are no clinical studies demonstrating the efficacy of this approach. For patients with CrCl between 30 and 60 mL/min, zoledronic acid dose should be adjusted. The effect of zoledronic acid has not been studied in patients presented with severe renal impairment (CrCl <30 mL/min), and it is not recommended for these patients. We suggest that pamidronate may be given at a dose of 90 mg infused over 4–6 h for myeloma patients with osteolytic disease and renal insufficiency. Furthermore, serum creatinine and CrCl should be measured before each infusion of pamidronate or zoledronic acid, while BPs should not be administered in short infusion times (<2 h for pamidronate and less than 15 min for zoledronic acid). Bisphosphonate therapy can be resumed after withholding zoledronic acid or pamidronate for patients who develop renal deterioration during therapy, when serum creatinine returns to within 10% of baseline [92].

Osteonecrosis of the Jaw

It is an uncommon complication of intravenous bisphosphonates. It is potentially serious and its main characteristic is the

development of exposed bone in the mouth. Incidence may vary from 2 to 10% [128–130]. Risk factors include the invasive dental procedures, poor oral hygiene, older age, increased duration and number of bisphosphonate infusions, and zoledronic acid use [129, 130]. In approximately one half of patients, ONJ lesions will heal [131], but in the other 50% of patients who restart bisphosphonate after having stopped it, recurrence of ONJ will develop. According to recent IMWG guidelines preventive strategies should be adopted to avoid ONJ [85]. A dental examination is necessary before beginning the bisphosphonate course. Patients should also be alerted regarding dental hygiene. All existing dental condition should be treated before initiation of bisphosphonate therapy. After bisphosphonate treatment initiation, unnecessary invasive dental procedures should be avoided, and dental health status should be monitored on an annual basis. Patients' dental health status should be monitored by a physician and a dentist. Dental problems should be managed conservatively if possible. If invasive dental procedures are necessary there should be temporary suspension of bisphosphonate treatment. The panel consensus suggests the interruption of bisphosphonates before and after dental procedures for a total of 180 days (90 days before and 90 days after procedures such as tooth extraction, dental implants and surgery to the jaw). Bisphosphonates do not need to be discontinued for routine dental procedures including root canal. Initial treatment of ONJ should include discontinuation of bisphosphonates until healing occurs. The physician should consider the advantages and disadvantages of continued treatment with bisphosphonates, especially in the relapsed/refractory MM setting. Preventive measures during bisphosphonate treatment have the potential to reduce the incidence of ONJ about 75% [132]. Prophylactic antibiotic treatment may prevent ONJ occurrence after dental procedures [133]. Management of patients depends on ONJ stage. Stage I (asymptomatic exposed bone; no soft tissue infection) can be managed conservatively with oral antimicrobial rinses. Stage II (exposed bone and associated pain/swelling and/or soft tissue infection) requires culture-directed long-term and maintenance antimicrobial therapy, analgesic management and occasionally, minor bony debridement. Stage III disease (pathological fracture and exposed bone or soft tissue infection not manageable with antibiotics) requires surgical resection in order to reduce the volume of necrotic bone in addition to the measures described in stage II [134].When ONJ occurs initial therapy should include discontinuation of bisphosphonates until healing occurs [98].

The administration of medical ozone (O3) as an oil suspension directly to the ONJ lesions that are below $\leq$2.5 cm may be another possible therapeutic strategy for those patients who fail to respond to conservative treatment. In such patients, there are reports suggesting that ONJ lesions resolved with complete reconstitution of oral and jaw tissue, with 3–10 applications [135, 136]. In addition, treatment with hyperbaric oxygen has been reported to be helpful.

Future treatment options

RANKL antagonists

Preclinical models of MM demonstrated that RANKL inhibition can prevent bone destruction from MM. RANKL inhibition with recombinant RANK-Fc protein not only reduced MM-induced osteolysis, but also caused a marked decline in tumor burden [35, 137]. Similar results were obtained using recombinant OPG for the treatment of MM-bearing animals [138]. These data gave the rationale for using RANKL inhibition in the clinical setting.

Denosumab, a fully human monoclonal antibody, has showed high affinity and specificity in binding RANKL and inhibits RANKL-RANK interaction, mimicking the endogenous effects of OPG. In knock-in mice with chimeric (murine/human) RANKL expression, denosumab showed inhibition of bone resorption [139].

In a phase I trial, 54 patients with breast cancer ($n = 29$) or MM ($n = 25$) with osteolytic lesions received a single dose of denosumab or pamidronate. Denosumab decreased bone resorption within 24 hours of administration, as reflected by levels of urinary and serum NTX. This was similar in magnitude but more sustained than with intravenous pamidronate [140]. These results were confirmed in another phase I trial, in which denosumab was given at multiple doses [141].

In a phase II trial, the ability of denosumab (120 mg given monthly as a subcutaneous injection) to affect bone resorption markers and monoclonal protein levels in MM patients, who relapsed after response to prior therapy, and in patients who responded to most recent therapy and had stable disease for 3 or more months was evaluated. No patient experienced complete or partial response ($\geq$50% reduction in M-protein) but seven patients had maximum reduction of $\geq$25% in serum M-protein. Bone resorption markers were reduced by more than 50% with denosumab [142].

In another phase II trial, Fizazi et al. evaluated the effect of denosumab in patients with bone metastases and elevated urinary NTX levels despite ongoing intravenous bisphosphonate therapy. Patients were stratified by tumor type (total 111 patients; nine patients with multiple myeloma, 50 patients with prostate cancer, 46 patients with breast cancer and six patients with another solid tumor) and screening NTX levels and randomly assigned to receive subcutaneous denosumab 180 mg every four or every 12 weeks or continue intravenous bisphosphonates every four weeks. Denosumab normalized urinary NTX levels more frequently than the continuation of intravenous bisphosphonate (64% vs. 37% respectively; $p = 0.01$) and reduced on-study SREs compared to intravenous bisphosphonate (8% vs. 17%) [143]. This study showed that denosumab inhibits bone resorption and prevents SREs even in patients who are refractory to bisphosphonate therapy.

A meta-analysis of major phase 3 studies comparing denosumab versus zoledronic acid that included mainly patients with bone metastases due to solid tumors showed that denosumab was superior in terms of delaying the time to first on-study SRE by 8 months and reducing the risk of the first SRE by 17%. No difference between the two drugs was reported regarding disease progression and overall survival. Hypocalcaemia was more common in denosumab arm, while ONJ was similar with the two drugs [144].

Denosumab appears to have little toxicity, mainly asthenia, and multiple phase III trials of denosumab in patients with bone metastasis are ongoing. However it is crucial to mention that RANKL is involved in dendritic cell survival and that the anti-RANKL strategy may have an effect on the

immune system and a possible increase in infection rate, especially in cancer patients who have already had severe immunodeficiency. For MM patients, while denosumab was comparable to zoledronic acid with respect to the occurrence of SREs, inferior survival occurred in denosumab compared to zoledronic acid-treated patients, but this was a subset analysis from a large phase III trial that involved mostly solid tumor patients with metastatic bone disease [145]. Interpretation is limited based on the small numbers of MM patients who were enrolled in the trial and imbalance in baseline disease characteristics.

Activin-A inhibitors

Sotatercept (ACE-011) is a novel fusion protein of the extracellular domain of the activin receptor IIA (ActRIIA) and human immunoglobulin G (IgG) Fc domain with potent inhibitory effect on activin, enhancing the deposition of new bone tissue and preventing bone loss. In the preclinical setting, RAP-011, a murine counterpart of sotatercept, prevented the formation of osteolytic lesions in a murine MM model by stimulating bone formation but with no effect on osteoclast activity [146].

In a phase 1 study, a single-dose of sotatercept decreased bone resorption and increased bone formation in healthy postmenopausal volunteers with no severe AEs [147]. In a multicenter phase IIa study we evaluated the safety and tolerability of sotatercept and its effects on bone metabolism and hematopoiesis in newly diagnosed and relapsed MM patients. Patients were randomized to receive four 28-day cycles of sotatercept (0.1, 0.3, or 0.5 mg/kg) or placebo. Patients also received six cycles of combination oral melphalan, prednisolone, and thalidomide (MPT). Thirty patients were enrolled; six received placebo and 24 received sotatercept. In patients without bisphosphonate use, anabolic improvements in bone mineral density and in bone formation relative to placebo occurred, whereas bone resorption was minimally affected. Increases in hemoglobin levels, versus baseline, and the duration of the increases were higher in the sotatercept-treated patients, with a trend suggesting a dose-related effect [148]. Further research is needed to support these findings.

Moreover, increased activin-A secretion was enhanced by lenalidomide and was inhibited by the addition of an activin A-neutralizing antibody. This effectively restored osteoblast function and subsequently inhibited myeloma-related osteolysis without abrogating the cytotoxic effects of lenalidomide on malignant cells [149] and thus supporting the combination of lenalidomide with an anti-activin-A molecule.

DKK-1 antagonists

DKK-1 plays an important role in the dysfunction of osteoblasts observed in MM. The production of this soluble Wnt inhibitor by MM cells inhibits osteoblast activity, and its serum level reflects the extension of focal bone lesions in MM [67, 150]. Serum DKK-1 is increased not only in symptomatic MM patients at diagnosis but also in relapsed MM, correlating with advanced disease features and the presence of lytic lesions, while serum DKK-1 levels of asymptomatic patients at diagnosis and plateau do not differ from control values [68, 151].

BHQ880, a phage-derived IgG antibody, the first-in-class, fully human anti-DKK-1 neutralizing antibody, seems to promote bone formation inhibiting tumor-induced osteolytic disease in preclinical studies [152]. Inhibiting DKK-1 with BHQ880 in the 5T2MM murine model of myeloma reduced the development of osteolytic bone lesions and in vivo growth of MM cells [153]. Finally in a recent study in humans BHQ880 managed to increase bone strength in the majority of myeloma patients with relapsed and/or refractory disease [154].

Sclerostin antagonists

Circulating sclerostin is elevated in patients with myeloma and extended bone disease [77]. Romosozumab is a humanized monoclonal antibody that targets sclerostin. In a phase II study in women with postmenopausal osteoporosis, romosozumab demonstrated increases in the bone mineral density of the lumbar spine after 12 months of therapy [155]. Studies in MM are going to start soon.

Effects of antimyeloma agents on bone metabolism

Bortezomib

Bortezomib is a first-in-class proteasome inhibitor with known activity against myeloma. Bortezomib plays an important role in osteoclast function and differentiation. It affects both late and early stages of osteoclast differentiation causing reduction of subsequent bone resorption [156–158]. Clinical trials with bortezomib indicated that it may also increase osteoblast activity and induce new bone formation. In mice bortezomib induces mesenchymal stem cells to differentiate into osteoblasts [159]. Bortezomib upregulates the transcription factor Runx2/Cbfa1 activity in human osteoblast progenitors and osteoblasts [160]. Bortezomib administration in relapsed/refractory patients resulted in a significant reduction of DKK-1, enhancement of bone formation and increase in bone mineral density [161–163]. Furthermore, bortezomib in combination with thalidomide and dexamethasone (VTD) as consolidation therapy post autologous transplantation produces no SREs in patients with no progressive disease, indicating that patients who respond to consolidation may not need concomitant bisphosphonate administration [164].

Immunomodulatory agents

Immunomodulatory drugs (IMiDs), such as thalidomide, lenalidomide and pomalidomide, are highly active agents in the treatment of both newly diagnosed and relapsed/refractory MM. These agents also alter interactions between bone marrow microenvironment and malignant plasma cells, and modify abnormal bone metabolism in MM [27].

Thalidomide almost completely blocks RANKL-induced osteoclast formation in vitro. In relapsed/refractory MM patients, intermediate dose of thalidomide (200 mg/d) in combination with dexamethasone produced a significant reduction of serum markers of bone resorption [C-telopeptide of collagen type-I (CTX) and TRACP-5b] and also of sRANKL/OPG ratio [165].

Lenalidomide also inhibited osteoclast formation, by targeting PU.1, a critical transcription factor for the development of osteoclasts, and downregulating cathepsin K. The downregulation of PU.1 in hematopoietic progenitor cells resulted in a complete shift of lineage development toward granulocytes. Lenalidomide also reduced the serum levels of sRANKL/OPG ratio in MM patients [166]. However, lenalidomide seems to have modest or no effect on bone formation of myeloma patients [167].

Pomalidomide, like thalidomide, blocks RANKL-induced osteoclastogenesis in vitro, even at concentrations of one μM, which is similar or even lower than that achieved in vivo after the therapeutic administration of this agent. Pomalidomide downregulates transcription factor PU.1, affecting the lineage commitment of osteoclast precursors toward granulocytes instead of mature osteoclasts [168].

IMWG recommendations for treatment of myeloma-related bone disease

The International Myeloma Working group recently produced recommendations regarding the management of myeloma-related bone disease. The IMWG experts recommended that all patients with MM, who are receiving antimyeloma therapy with or without osteolytic bone lesions, as well as patients with osteopenia or osteoporosis due to MM should receive bisphosphonates. Intravenous pamidronate and zoledronic acid are recommended for the prevention of SREs in patients with active disease due to their efficacy in SREs reduction. Intravenous zoledronic acid has shown better efficacy in SREs prevention compared with oral clodronate. Zoledronic acid is recommended for MM patients with bone disease at diagnosis rather than CLO. This is because ZOL has shown better survival benefit and has potential antimyeloma activity. MM patients who are ineligible for transplantation may benefit from the combination of antimyeloma treatment with zoledronic acid. In patients with asymptomatic MM of low and intermediate risk, bisphosphonates are recommended when dual-energy X-ray absorptiometry (DXA) scan reveals osteoporosis. For patients with high-risk asymptomatic MM, or if it is unclear whether bone loss is MM or age related, schedule and dose of bisphosphonates should follow those of symptomatic MM, especially in patients with abnormal MRI pattern. Intravenous bisphosphonates should be administered at 3- to 4-week intervals to all patients with active MM. Zoledronic acid improves OS and reduces SREs over clodronate in patients who have been treated for more than two years; thus it should be given until disease progression in patients, not in CR or a very good partial remission (VGPR) and further continued at relapse. There is no similar evidence for pamidronate. Zoledronic acid and pamidronate should be discontinued after 1 or 2 years in patients in CR or VGPR because of the higher rates of ONJ [92].

Authors' contributions/Wkład autorów

According to order.

Conflict of interest/Konflikt interesu

Evangelos Terpos has received honoraria by Janssen-Cilag, Celgene, Novartis and Amgen. Nikolaos Kanellias has no conflicts to declare. Krzysztof Giannopoulos has no conflicts to declare.

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Ethics/Etyka

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; and Uniform Requirements for manuscripts submitted to Biomedical journals.

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