EXPERT OPINION

Treatment of multiple myeloma bone disease: experimental and clinical data

Yvonne Mary Papamerkouriou, Eustathios Kenanidis, Zakareya Gamie, Kyriakos Papavasiliou, Thomas Kostakos, Michael Potoupnis, Ioannis Sarris, Eleftherios Tsiridis† & John Kyrkos

†*Aristotle University Medical School, "PapaGeorgiou" General Hospital, Academic Orthopaedic Unit, Thessaloniki, Greece*

Introduction: Bone disease is present in the majority of patients with multiple myeloma and can seriously affect quality of life and survival rate. In addition to suppression of osteoclastogenesis, there have been developments made in terms of the therapeutic agents available, such as novel immunomodulating agents, proteasome and receptor activator of nuclear factor κB ligand inhibitors.

Areas covered: Areas covered include *in vitro, in vivo* and clinical evidence was collected using MEDLINE® (1950 – May 2014), EMBASE (1980 – May 2014) and Google Scholar (1980 – May 2014) databases.

Expert opinion: Bisphosphonates are the mainstay of myeloma bone disease treatment. Oral clodronate and intravenous pamidronate and zoledronic acid are currently used drugs and seem to have comparable results in preventing skeletal-related events of the disease. Zoledronate can also have survival benefits and based on the available evidence is the superior bisphosphonate; however, its side effects have to be monitored. Denosumab had comparable results with zoledronate on myeloma bone disease treatment; its use has not been completely proven yet. There is an expanding set of drugs, proteasome inhibitors, under investigation with great potential to reduce the negative effects of myeloma cells on bone. Future clinical studies should compare both the catabolic and anabolic effects of these agents on bone.

Keywords: bisphosphonates, bone, immunomodulation, multiple, myeloma, osteoclast, proteasome

Expert Opin. Biol. Ther. [Early Online]

1. Introduction

Multiple myeloma (MM) is a malignant hematological disease that results from clonal multiplication of malignant plasma cells. The median age of patients at the time of diagnosis of MM is 65 years [1]. The incidence of the disease is about 6 patients per 100,000 of the population in the EU, and 27,500 new cases are reported every year [2]. It is more common in men rather than women and accounts for about 10% of all hemopoietic tumors in the Caucasian race. Over 50% of patients are over 65 and only 1% of the cases involve patients under 40 years old. The annual death rate of the disease is 4.1 per 100,000 and the 5-year survival rate is 28% [3,4].

Clonal multiplication of malignant plasma cells in MM leads to elevated immunoglobulin levels, anemia, immune system deficiency, renal failure and osteolytic bone lesions [5,6] The treatment of MM remains highly individualized [7]. When selecting the proper regimen, the following factors are to be considered: age of onset, whether the patient was symptomatic or not at the time of diagnosis and

informa
healthcare

Article highlights.

- Bisphosphonates (BPs) are the mainstay of myeloma bone disease treatment.
- Oral clodronate and i.v. pamidronate and zoledronic acid are currently used drugs.
- Among the drugs currently in use, zoledronate appears to be more effective in the treatment of myeloma bone disease.
- Denosumab is in Phase III study for myeloma patients with bone disease.

This box summarizes key points contained in the article

any detected high-risk cytogenic abnormalities [8]. Treatment can be divided into initial therapy, consolidation or maintenance therapy and stem cell transplantation for eligible patients [9]. There have been developments made in terms of the therapeutic agents available, such as novel immunomodulatory drugs and proteasome inhibitors, which have been associated with both tumor reduction and suppression [10,11]. There is the important issue of continuous therapy to maintain remission [12,13]. Recently, outcomes in MM have been remarkably improved due to the use of combination therapies [14,15].

Bone disease is present in 80% of patients with MM, causing pain, hypercalcemia and pathologic fractures that may warrant surgical treatment and/or radiotherapy. It may also lead to spinal cord compression following pathological vertebral fracture [5].

2. MM-induced bone disease

Bone disease is present in the majority of patients with MM and can seriously affect the quality of life and survival rate of MM patients [16]. This renders the understanding of the pathophysiology of bone disease in MM crucial in order to develop pharmacological agents that can slow down bone destruction. The underlying cause of bone disease in MM is the uncoupling of bone resorption from bone production [17]. MM is characterized by an elevated level of activated osteoclasts (OCs) parallel to reduced osteoblastic activity [18].

The binding of myeloma cells to bone marrow stromal cells (BMSCs) through vascular cell adhesion molecule-1 (VCAM-1) that binds to $\alpha4\beta1$ integrin on myeloma cells leads to differentiation and activation of OCs [19]. Factors that favor osteoclastogenesis include cytokines IL-6, IL-1α, IL-1β, IL11, macrophage-colony stimulating factor (M-CSF), TNF-α, TNF-β, macrophage inflammatory protein (MIP-1α), MIP-β, parathyroid hormone-related peptide and VEGF [20-22]. In addition, stromal cell derived factor-1α, IL-3 and hepatocyte growth factor [23-25] also act as osteoclast stimulants. The cytokines that favor the differentiation of OCs and increase their ability to absorb bone are produced either by myeloma cells or by stromal cells (BMSCs) that have been activated by myeloma cells. Overexpressed genes in OCs displayed additional mechanisms involved in osteoclast/MM cells interactions, suggesting targeting strategies to block this interaction and prevent drug resistance [26]. In addition to these factors, another important path of osteoclast activation is through the receptor activator of nuclear factor κB ligand (RANKL) – osteoprotegerin (OPG) system [19]. If left without treatment, patients with bone disease are expected to experience > 2 skeletal-related events (SREs) per year, defined as pathologic fractures, vertebral body compression fractures, hypercalcemia, pain and need for radiation or surgery [27]. As a result, patients suffering from MM-induced bone disease will probably need either anabolic or (more often) antiosteoclastic treatment [27].

For MM patients suffering from bone disease, antiosteoclast treatment has been widely used to prevent bone loss [28]. Although bisphosphonates (BPs) are the mainstay for the treatment of MM disease, several novel drugs for myeloma-related bone disease or novel antimyeloma drugs are very promising and have entered clinical trials [29]. Agents that have the ability to suppress osteoclastogenesis include BPs, immunomodulatory drugs such as thalidomide and lenalidomide, proteasome inhibitor bortezomib and agents with the ability to suppress RANKL. In addition, other agents that suppress bone absorption are being researched either in clinical trials or in experimental *in vitro* or *in vivo* models.

This article reviews the currently available literature on existing or developing antiosteoclast treatment regimens in patients suffering from MM bone disease.

3. Bisphosphonates

Currently, BPs are the cornerstone for the treatment of MM bone disease [29]. They are analogs of inorganic pyrophosphate and consist of two phosphonate groups linked to a carbon atom. The carbon substitution renders them resistant to breakdown by enzymatic hydrolysis. Also, it allows two additional chains of variable structure, the modification of which has led to the biosynthesis of different BPs [30]. Their high affinity for calcium allows them to bind to bone hydroxyapatite, something that is enhanced in areas of high bone turnover [31].

BPs are potent inhibitors of the activation and differentiation of OCs and, at the same time, also contribute to their apoptosis [32,33]. They have similar properties but differ with regard to their ability to prevent bone resorption. Their activity is drastically increased when an amino group is entered into the aliphatic carbon chain. Second and third generation BPs containing nitrogen, a group consisting of risedronate, alendronate, pamidronate, ibandronate and zoledronate, are far more potent than first generation non-nitrogen containing BPs (non-N-BPs), to which clodronate and etidronate belong [31].

During the bone remodeling process, OCs release BPs from the bone matrix and internalize them through fluid-based endocytosis [34] binding to the site of geranyl diphosphate and inhibiting farnesyl diphosphate synthase in the mevalonate pathway. This leads to the inhibition of protein prenylation, key regulators of cell signaling [35,36] and ultimately production of Appi, an ATP analog, and cell apoptosis [37]. The non-N-BPs are incorporated into analogs of ATP,

leading to accumulation of metabolites within OCs and finally cell apoptosis [38]. Other possible pathways of action of BPs include the reduction of IL-6 production by BMSCs and the increase of γ/δ Tα-cells with direct effect against MM cells [39]. The effects of BPs on osteoblasts and osteocytes are represented by a wide range of proanabolic or toxic effects depending most likely on the time and concentration of the BP tested, the time of exposure and the experimental methodology.

Oral clodronate, intravenous (i.v.) pamidronate and i.v. zoledronic acid have been found effective and licensed for the management of myeloma bone disease [40]. However, etidronate and ibandronate were found to be ineffective [41-43]. Until now, no data have demonstrated the ability of BPs to heal osteolytic lesions [44]. Although several bisphosphonate, placebo-controlled studies have been published (**Table 1**) concerning MM bone disease treatment, only three randomized comparative studies between two different BPs exist in the literature (**Table 2**).

3.1 BPs with low effectiveness in MM-related bone disease

3.1.1 *Etidronate*

Etidronate is considered ineffective in the treatment of myeloma bone disease. Etidronate, administered at 5 mg/kg/day per os (p.o.) in a randomized placebo controlled *in vivo* study showed no statistically significant difference between the etidronate and placebo group as far as reduction of pain, osteolytic lesions and SRE are concerned (**Table 1**) [41]. Similarly, another placebo controlled *in vivo* trial where etidronate was administered at 10 mgr/kg/day p.o., failed to show statistically significant difference between groups in all the previous mentioned categories [42].

3.1.2 *Ibandronate*

Ibandronate also proved to be ineffective. Mensen *et al.* reported no significant reduction of pain and development of new osteolytic lesions, as well as vertebral and nonvertebral fractures in MM patients who received 2 mg/month i.v. for 1 – 2 years [43]. However serum osteocalcin and C-terminal telopeptides were significantly reduced in ibandronate group (**Table 1**). This *in vivo* study alone provides only scarce evidence for the effectiveness of ibandronate on MM-related bone disease.

3.2 BPs with higher effectiveness in MM-related bone disease

3.2.1 *Clodronate*

Clodronate was the first non-N-BP that demonstrated usefulness in the clinical management of myeloma bone. Clodronate administered at 2.4 mg/day p.o. for 2 years in a randomized placebo controlled *in vivo* trial showed significant reduction of pain and osteolytic lesions. Progression of osteolytic bone lesions was twice as high in the placebo group ($p < 0.05$). Although the incidence of vertebral fractures was lower in the clodronate group, this difference was not statistically significant [45].

In another randomized placebo controlled *in vivo* study by Mc Closkey *et al.*, clodronate administered at 1.6 mg/day p.o. in 530 myeloma patients was found to significantly reduce vertebral and nonvertebral fractures at 1 year of follow-up. At 2 years follow-up, the patients who received clodronate had less myeloma-related pain than patients treated with placebo. However, the clodronate group failed to show a benefit in survival [46].

3.2.2 *Pamidronate*

Pamidronate is a nitrogen-containing BP drug that is effective on myeloma bone disease; it demonstrates, however, differential outcomes related to the route of administration. *In an in vivo study*, oral pamidronate failed to reduce SREs at 300 mg/day p.o. in 300 myeloma patients. Pamidronate was effective only in reducing pain [47]. Although there was reduction in the number and size of osteolytic lesions, this difference was not statistically significant ($p = 0.28$). In contrast, the IV administration of pamidronate was more effective. At 90 mg i.v. every 4 weeks, pamidronate significantly reduced osteolytic lesions, pain and SREs but had no effect on overall survival [48]. Additionally, patients treated with i.v. pamidronate experienced significantly fewer episodes of hypecalcemia after 3 cycles of therapy ($p = 0.007$).The overall positive result of this study was attributed to the better biocompatibility of i.v. administered BPs [48].

3.2.3 *Zoledronate*

There are three major comparative randomized studies between the most effective BPs on MM bone disease therapy. In a randomized Phase II *in vivo* trial reported by Berenson *et al.* (2001), escalating i.v. doses (0.4 – 4.0 mg) of zoledronate were compared with 90 mg of i.v. pamidronate on 280 MM patients or metastatic breast cancer [49]. Zoledronate administered at either 2 or 4 mg/month, proved to be as effective as 90 mg/month pamidronate in the reduction of both pain and SRE, whereas when it was administered at 0.4 mg/month, it was less effective [49]. Greater doses of zoledronate significantly reduced the need for radiation ($p = 0.05$), N-telopeptide marker levels and episodes of hypercalcemia, demonstrating at least equal efficacy to pamidronate.

In another *in vivo* study Rosen *et al.* (2001) studied 1648 patients with either Durie–Salmon stage III MM or advanced breast cancer and at least one bone lesion. Patients were randomly assigned to i.v. 4 or 8 mg of zoledronic acid versus 90 mg of pamidronate every 3 – 4 weeks for 1 year. Zoledronate administered at 4 mg proved to be equally as effective as pamidronate in reducing pain and skeletal related events [50]. Although skeletal morbidity was similar in patients treated with zoledronate, the incidence of radiation therapy was significantly reduced and the N-terminal cross-linking

Table 1. Major clinical trials of bisphosphonates versus placebo therapy on patients suffering from multiple myeloma bone disease.

Author (year)	Drug type/ dose/duration	Study/trial type	Number of patients	Concomitant medication	Bone markers/ electrolyte evaluation	Vertebral/ nonvertebral fractures reduction	Osteolytic lesions reduction	Pain reduction
Belch *et al.* (1991) [41]	Etidronate/5mg/kg/ day p.o.	Randomized, double-blind, placebo-controlled	173	Melphalan/ prednisone	No S.S.D. in hypercalcemia incidence between groups	No S.S.D. between groups	Not assessed	No S.S.D. between groups
Daragon *et al.* (1993) [42]	Etidronate 10mg/ kg/day p.o. 4 months	Multicenter controlled	94	Chemotherapy		No S.S.D. between groups		No difference between groups
Mensen *et al.* (2002) [43]	Ibandronate 2mg/ month i.v. (1 – 2 years)	Randomized, double-blinded, placebo-controlled	198	Conventional chemotherapy (+/- interferon)	>30% of serum osteocalcin/ > 50% urinary C-terminal telopeptides reduction in ibandronate group	No S.S.D between groups	No S.S.D. between groups	No S.S.D. between groups
Lahtinen *et al.* (1992) [45]	Clodronate 2.4g/ day p.o. 24 months	Multicenter, randomized, double-blind, placebo-controlled	350	Melphalan/ prednisone	Greater serum calcium reduction in clodronate group	Less progress of vertebral fractures in clodronate group (No S.S.D.)/ similar progress of non-vertebral fractures	Half reduction of lesions in Clodronate group ($p = 0.028$)	No S.S.D. reduction favoring clodronate group
McCloskey *et al.* (1998) [46]	Clodronate 1.6g/ day p.o	Randomized, double-blind, placebo-controlled	530	Allocated chemotherapy	50% reduction of severe hypercalcemia in clodronate group ($p = 0.064$)	Less vertebral ($p = 0.012$) and non-vertebral fractures ($p<0.025$) in clodronate group	Not assessed	Pain reduction in clodronate group ($p<0.05$)
Brincker *et al.* (1998) [47]	Pamidronate 300mg/day p.o	Randomized,, placebo-controlled	300	Melphalan/ prednisolone/ α- interferon	Less hypercalcemia events in pamidronate group ($p = 0.10$)	No S.S.D. between groups	Reduction in the number and size in pamidronate group ($p = 0.28$)	Less episodes of severe pain in pamidronate group ($p = 0.04$)
Berenson *et al.* (1996) [48]	Pamidronate 90mg iv/month for 21 cycles	Multi-center, randomized, placebo-controlled	392	Melphalan/ prednisone/ alkylating drugs/ doxorubicin	Lower incidence of hypecalcaemia after 3 cycles of therapy ($p = 0.007$) in pamidronate group	Less pathologic fractures in pamidronate group after 9 cycles of therapy ($p = 0.004$)	No S.S.D. between groups	S.S.D. favoring pamidronate Group

i.v.: Intravenously; p.o: Per os; S.S.D.: Statistical significant difference.

telopeptide of collagen type I (NTX) was more frequently normalized compared with pamidronate treated patients.

More recently, Morgan *et al.* (2011) [51] reported the results of the MRC Myeloma IX trial, a multi-center randomized, open-label, *in vivo* trial. This study was designed to compare the effect of oral clodronate 1600 mg/day versus i.v. zoledronate 4 mg every 21 – 28 days for patients with newly diagnosed symptomatic MM. Zoledronate was found superior in reducing SRE. There was statistically significant reduction of vertebral fractures ($p = 0.008$) and fewer new osteolytic lesions in the zoledronic acid group ($p < 0.0001$). Most importantly, zoledronate was associated with significant reduction in the risk of death (HR 0.84; $p = 0.0118$) and a significant improvement in progression-free survival (0.88, $p = 0.0179$) [51]. Extension of survival by 10 months was more pronounced for myeloma patients with osteolytic disease at diagnosis. Myeloma patients without bone disease at diagnosis had no survival advantage with zoledronic acid [52]. Based on the latter observation, the authors raised the hypothesis that zoledronate may exhibit an indirect or direct antimyeloma effect [53]. Pamidronate and zoledronic acid i.v. and oral clodronate are currently used in Europe in the treatment of MM. The International Myeloma Working Group suggested that BPs should be considered in all patients with MM receiving first-line antimyeloma therapy, regardless of the presence of osteolytic bone lesions on conventional radiography [40]. Treatment should be resumed in cases of relapse. As regard to the side effects of BPs and their tolerability, the European Myeloma Network suggests symptomatic treatment and continuation of therapy, monitoring of creatinine clearance and adjustment of dosage in cases of renal failure and tactic dentist surveillance in order to prevent osteonecrosis of the jaw (ONJ), one of the most serious side effects of BPs. BPs should be stopped before an invasive dental procedure. In the event of ONJ, BPs should also be discontinued [54].

4. Novel antimyeloma agents

BPs' side effects coupled with hesitance in administering them on a long term basis, channeled research toward other antiosteoclast agents. Immunomodulatory drugs such as thalidomide, lenalidomide and pomalidomide, and proteasome inhibitors, such as bortezomib and carfilzomib, are highly active agents for the treatment of both newly diagnosed and relapsed/refractory MM [29] Although they have established activity against myeloma, all share in common but to a different extent, the ability to reduce bone resorption, either by direct action against OCs or by modulating the influence of myeloma cells on OCs [29].

Although more sophisticated imaging modalities like MRI can be used for the evaluation of skeletal involvement in MM [47], plain radiographs remain the main diagnostic tool. However, normal radiographs cannot exclude significant bone turnover in patients suffering from MM [55]. Biochemical markers of bone turnover are mainly used in preclinical or even clinical practice in order to find out antiosteoclastic action of certain antimyeloma agents [49,50].

4.1 Thalidomide

Thalidomide has the ability to inhibit TNF-α, a cytokine, produced by macrophages of the immune system and also a mediator of inflammatory response. Thalidomide also alters the production of IL-1, IL-6, IL-12 inflammatory cytokines and IL-10, anti-inflammatory cytokine [56]. TNF-α is believed to be inhibited through enhanced TNF-α RNA degradation, resulting in diminished amounts of it secreted [57]. Thalidomide also favors myeloma cells apoptosis through upregulation of the activity of caspase-8. This causes apoptotic activity between caspase-8 and caspase-9. Secretion of IL-6 and the secretion of adhesion molecules are induced in the presence of TNF-α. The reduction of adhesion molecules ultimately affects OCs.

Thalidomide is considered to be an effective treatment of both newly diagnosed and refractory MM disease [58-63]. However, recent studies highlighted this novel antimyeloma agent as a promising drug against myeloma bone disease (**Table 3**) [60]. In a prospective nonrandomized *in vivo* study, Terpos *et al.* [60] reported that the combination of an intermediate dose of thalidomide (200 mg/day) with dexamethasone was very effective for patients suffering from refractory/relapsed MM. Although this combination had no effect on bone formation markers and no healing of the observed lytic lesions, demonstrated significant reduction of C-terminal type 1 collagen telopeptide (CTX) and tartrate-resistant acid phosphatase 5b (TRACP-5b) at 3 and 6 month and sRANKL and sRANKL/OPG at 6 months post-treatment [60]. It is possible that the combination of thalidomide and dexamethasone may have a positive effect on myeloma microenvironment, thus reducing the pathogenesis of myeloma bone disease.

Similar results were reported by Tosi *et al.* [64] who used thalidomide, dexamethasone and zoledronate for the treatment of patients with newly diagnosed MM. Following 4 months of treatment, there was statistically significant reduction in urinary NTX and serum crosslaps for those patients who partially responded to therapy. Although the relative contribution of each drug could not be determined, the combination of thalidomide, dexamethasone and zoledronate seemed to have a positive effect on myeloma bone disease.

4.2 Lenalidomide

Lenalidomide is a synthetic compound derived by modifying the chemical structure of thalidomide [65]. Lenalidomide is more potent than thalidomide with regard to its immunosuppressant and antitumor abilities. Particularly, in MM, it is able to alter the microenvironment. It directly decreases the formation of tartrate-resistant acid phosphatase (TRAP)-positive cells that form OCs [66]. Lenalidomide also decreases αVβ3-integrin, an adhesion molecule involved in osteoclast activation, and downregulates cathepsin K, a cysteine protease

Table 2. Comparative clinical trials between bisphosphonates' therapy on patients suffering from multiple myeloma bone disease.

Author (year)	Drug type/ dose/ duration	Study/trial type	Number of patients	Bone markers/ electrolyte evaluation	Vertebral/non vertebral fractures reduction	Osteolytic lesions reduction	Pain reduction/ other
Berenson *et al.* (2001) [49]	Zolendronic acid 0.4, 2.0, or 4.0 mg i.v. Pamidronate 90 mg i.v. per month for up to 10 months	Randomized, double-blind, double-dummy, multi-center study	108 multiple myeloma 172 metastatic breast cancer	Reduction in N-telopeptide marker levels and hypercalcemia episodes for all groups except 0.4 mg zoledronate group	Similar proportions (21 – 22%) of pathologic fractures for all groups/28% in the 0.4 mg zoledronate group	Not assessed/ incomplete response of patients	Pain reduction favoring the two higher dose zoledronate groups.
Rosen *et al.* (2001) [50]	Zolendronic acid 4 or 8mg i.v/month/ Pamidronate 90 mg i.v. for 12 months	Phase III, randomized, double-blind, study	1,648 Durie/ Salmon stage III multiple myeloma or advanced breast cancer	More frequent normalization of N-terminal cross-linking telopeptide of collagen type I (NTX) levels, in the zoledronic acid arm	Proportion of patients with at least one SRE similar in all treatment groups No difference of the time to first SRE between groups		Pain reduction in both groups
Morgan *et al.* (2011) [51]	Clodronate p.o. (1600 mg/day)/ Zoledronate i.v. (4 mg/month)	Multi-center randomized, open-label, clinical trial	1970 newly diagnosed, symptomatic myeloma patients		Reduction of vertebral fractures in the zoledronate group ($p = 0.0008$)	Reduction of new osteolytic lesions in the zoledronate group ($p < 0.0001$)	Benefit

i.v.: Intravenously; p.o.: Per os; SRE: Skeletal related events.

expressed in OCs that is involved in matrix degradation during resorption [66]. Furthermore, it reduces transcription factorPU.1 and MAP kinase (ERK [extracellular signal-regulated kinase]), all important mediators of osteoclastogenesis, and suppresses RANKL. Lenalidomide can reduce adhesion molecules (ICAM-1,VCAM-1 and E-selectin) that interfere in the adhesion of MM cells to BMSC [67].

Lenalidomide is an effective treatment for both the newly diagnosed and refractory MM as thalidomide [68-76] Lenalidomide is another novel drug targeting MM and its microenvironment that has shown promising clinical results on myeloma bone disease (**Table 3**) [66]. *In vitro* studies revealed that this novel drug can effectively inhibit osteoclast formation as well as reduce the serum levels of RANKL in BMSCs derived from MM patients [66].

In a randomized *in vivo* trial, Breikrutz *et al.* (2008) comparing the effect of two different doses of lenalidomide on osteolytic bone disease in patients with relapsed or refractory MM at 2 months of treatment recorded significant reduction of RANKL and increase of OPG [66]. In a similar retrospective study, Terpos *et al.* confirmed the same effect of lenalidomide on bone resorption markers for responders to therapy, although no effect on bone formation markers was recorded [77].

Lenalidomide effect seems to be enhanced by the action of bortezomib. In a prospective comparative *in vivo* study, the effect of lenalidomide, dexamethazone (RD) and bortezomib on 99 patients suffering from refractory or relapsed MM was studied. Patients suffering from peripheral neuropathy grade > 2 received RD, whereas those with grade < 2 neuropathy were treated with RD plus bortezomib; RD treatment significantly reduced only markers of bone resorption (CTX, Dkk-1). Although the addition of bortezomib has the same effect on CTX and Dkk-1, it also significantly increased bone alkaline phosphatase (bALP) and osteocalcin after six cycles of therapy, irrespective of treatment response [77].

4.3 Pomalidomide

Pomalidomide has been newly approved and may be used for relapsed and refractory MM. It has synergistic effects when combined with dexamethasone and has currently undergone Phase III trials [13,78]. Its combination with low-dose dexamethasone is effective and well tolerated in those with relapsed or refractory MM who have exhausted treatment with lenalidomide or bortezomib [79,80].

4.4 Bortezomib/proteasome inhibitors

Bortezomib is a proteasome inhibitor that specifically inhibits the threonine residue of the 26S proteasome, an enzyme complex that plays a key role in the cell by regulating protein degradation in a controlled fashion. Proteins that are no longer needed, including those involved in cell cycle control, apoptosis and cell signaling, are tagged with ubiquitin, which directs them to the proteasome to be degraded. This process maintains the balance of inhibitory and stimulatory proteins

involved in the cell cycle. The inhibition of the proteasome leads to a deregulation of the process, resulting in a build-up of cell cycle and regulatory proteins leading to cell death [81,82]. It is also possible that bortezomib dysregulates intracellular calcium metabolism, resulting in caspase activation and apoptosis [83].

Bortezomib is the first proteasome inhibitor with established *in vitro* and *in vivo* activity against myeloma (**Table 4**) [84-89]. This antimyeloma agent seems to downregulate the osteoclastic activity, leading to reduced bone resorption [29].

In an *in vitro* study by von Metzler *et al.* (2007), bortezomib inhibited early and late osteoclast differentiation, activation and resorptional activity, creating prerequisites for the clinical use of bortezomib in cancer-induced lytic bone disease. The early and late inhibition was mediated through p38 MAPK-triggered and inhibition of AP-1 and NF-B activation, respectively [90].

Terpos *et al.* [91] demonstrated for the first time that the administration of bortezomib in myeloma patients produced a significant reduction of serum levels of bone-related cytokines. These cytokines were responsible for both development of lytic bone disease and myeloma cell growth (DKK-1 and RANKL), osteoclast function (assessed by TRACP-5b) and bone resorption (assessed by CTX). Changes in markers were irrespective of response to therapy of the relapsed myeloma patients [91]. In the same *in vivo* study, bortezomib also produced a dramatic increase in both markers of bone formation, bALP and osteocalcin. Zangari *et al.* (2005) supported the same effect of bortezomib treatment on bone formation markers (bALP) concerning myeloma treated patients [92]. *In vitro* and *in vivo* observations supported the hypothesis that bortezomib treatment may have a direct stimulatory effect on bone formation process [93].

In a prospective case control study, Heider *et al.* tried to compare the effects of bortezomib monotherapy with other treatments on newly diagnosed or refractory myeloma patients [94]. There was statistically significant enhancement of markers of osteoblast activity (bALP) in myeloma patients treated with bortezomib in comparison with other treatment group, suggesting a possible direct effect on osteoblasts, unique to this proteasome inhibitor [94].

When bortezomib was administered with dexamethasone and zoledronate in 27 relapsed myeloma patients, significant decrease in serum NTX and increase in bALP and osteocalcin was reported. Additionally, bone mineral density (BMD) improvement in lumbar spine was observed very early at 6 months post-initiation of therapy in a subset of relapsed MM patients with low BMD and nonextensive lytic disease [95].

In a subanalysis of a Phase III *in vivo* study in newly diagnosed patients, the combination of bortezomib with melphalan and prednisone reduced substantially DKK-1 but significantly increased the serum levels of bALP in responding patients, in comparison with control group receiving only melphalan/prednisolone [96]. Bortezomib had also a bone formation effect evident in conventional radiography, providing sufficient evidence that bortezomib has an osteoblastic capacity.

Despite its efficiency, a high proportion of patients do not achieve sufficient clinical response with bortezomib. This has prompted the development of second generation proteasome inhibitors such as carfilzomib, marizomib, ixazomib [97] and oprozomib. Carfilzomib has a potential to overcome drug resistance and has less side effects compared to bortezomib. Furthermore, carfilzomib can promote the osteogenic differentiation of MSCs *in vitro and in vivo* by upregulating β-catenin/transcription factor [98]. Bortezomib is reported to induce osteoclast apoptosis and carfilzomib increased mineralization and reduced osteoclast formation, shifting bone microenvironment from catabolic to anabolic state [99]. As BPs, these novel agents, however, can cause serious side effects. Besides being dangerous due to its ability to cause congenital malformations when exposed to fetuses, thalidomide can also be responsible for sensory peripheral neuropathy and venous thromboembolism [100]. Neuropathy is less frequent with lenalidomide, although cytopenia and venous thromboembolism are serious side effects of the drug [100]. Bortezomib may cause both neuropathy and thrombocytopenia [100-102].

5. OPG and RANKL inhibitors

Osteoclastogenesis requires 'communication' between osteoclast precursors and stromal cells or osteoblasts [103,104]. BMSCs express two molecules that are essential for osteoclastogenesis, M-CSF and RANKL, which is a member of the TNF receptor family [105]. M-CSF expands the pool osteoclast precursors and RANKL stimulates it to commit to osteoclast phenotype. RANKL is expressed by activated T-cells, MSCs and osteoblasts and binds to its receptor RANK, which is expressed by osteoclast precursors, chondrocytes and mature OCs [106]. The binding of RANK to its receptor RANKL leads to the maturing of OCs through the metabolic pathway of NF-κB, c-fos and Jun N-terminal kinase. Also, the binding of RANK to RANKL leads to increased activity of mature OCs and inhibits their apoptosis [106].

Parathyroid hormone, vitamin D3, glucocorticoids, IL-1β, TNF-α, IL-11 and prostaglandin-E2 enhance the expression of RANKL, whereas TGF-β reduces it [107]. OPG, which belongs to the family of TNF receptors, binds to RANKL preventing it from binding to RANK, which in turn inhibits osteoclast differentiation and activation [108]. The expression of OPG is triggered by IL-1β, TNF-α, TGF-β and 17b estradiol, whereas glucocorticoids, vitamin-D3 and parathyroid hormone reduce it [109].

The imbalance of bone resorption and bone production in MM-induced bone disease is largely due to the disruption of the RANKL/RANK/OPG pathway [110]. Myeloma cells increase the expression of RANKL and reduce the expression of OPG [111]. According to some researchers, this could well be a result of an interaction between myeloma cells and

Table 3. Major studies of thalidomide or lenalidomide on bone metabolism of patients suffering from newly diagnosed or relapsed/refractory multiple myeloma.

Author/date	Drug/dose	Myeloma population	Study plan	Number of patients	Bone resorption markers	Bone formation markers	Lytic lesions/pain reduction
Terpos *et al.* 2005 [60]	Thalidomide 200 mg/day Dexamethasone 40 mg/day Zoledronic acid 4mg/per month	Refractory or relapsed myeloma patients	Prospective non-randomised study	35	Significant reduction of CTX and TRACP-5b at 3 and 6 month and sRANKL and sRANKL/OPG at 6 months	No change in markers of bone formation bALP, OPG, OPN	No healing of the lytic lesions of responders at 6 months post-treatment
Tosi *et al.* 2006 [64]	Thalidomide 100-200 mg/day Dexamethasone 40 mg/day Zoledronate i.v. 4 mg/28 day	Newly diagnosed symptomatic MM patients	Prospective non-randomised study	40	Significant decrease of urinary N-terminal cross-linking telopeptide of collagen type I ($p = 0.000$) and serum crosslaps ($p = 0.000$) in partially responsed patients vs non responsed at 4 months	Significant reduction of osteocalcin ($p = 0.000$) for sensitive and refractory ($p = 0.04$) and BAP for sensitive ($p = 0.007$) and for refractory ($p = 0.006$) patients.	Statistical significant reduction in bone pain in sensitive patients
Terpos *et al.* (2014) [77]	Lenalidomide 25 mg p.o/day Dexamethasone	Refractory or relapsed myeloma	Retrospective study	106	Significant reduction of DKK-1 ($p = 0.035$) and a mild reduction of CTX to responders on therapy after 6 cycles Significant increase in DKK-1 and CTX ($p = 0.04$) and in TRACP-5b values ($p = 0.046$) to non-responders on therapy after 6 cycles	No significant change in Osteocalcin and bALP between responders and nonresponders	
Terpos *et al.* (2014) [77]	RD for patients with prior neuropathy > Grade 2 VRD for patients with prior neuropathy < Grade 2 Bortezomib 1 mg/m^2 iv Lenalidomide 15 mg/p.o/day Dexamethasone 40 mg/p.o	Refractory or relapsed myeloma	Prospective comparative study	99	Borderline increase of Dkk-1 ($p = 0.01$) and significant reduction in CTX (p,0.01) between responding and nonresponding patients after 6 months of therapy with RD Significant reduction of sRANKL/OPG, DKK-1 and CTX after three and six cycles of VRD irrespective of treatment response	No change of bone formation markers for RD Significant increase of bALP and osteoclasts after three and six cycles of VRD irrespective of treatment response	

bALP: Bone alkaline phosphatase; CTX: C-terminal type 1 collagen telopeptide; i.v.: Intravenously; p.o.: Per os; RD: Dexamethazone + lenalidomide; VRD: RD + bortzomid.

Table 4. Major studies of bortezomib on bone metabolism of patients suffering from newly diagnosed or relapsed/refractory multiple myeloma.

Author (year)	Drug/dose	Myeloma population	Study plan	Number of patients	Bone resorption marker	Bone formation markers	BMD
Zangari *et al.* (2005) [92]	Bortezomib Thalidomide Dexamethasone	Myeloma patients	Retrospective analysis of three previous trials	523		Statistically significant elevation of ALP for responding patients within three cycles of therapy	
Terpos *et al.* (2006) [91]	Bortezomib 1.3 mg/m^2 (4 cycles) Zoledronate (26 patients)	Relapsed myeloma patients	Prospective non-randomized case control study	34	Significant reduction of serum DKK-1, sRANKL, CTX, and TRACP-5b after four cycles therapy	Dramatically increase bone-alkaline phosphatase and osteocalcin irrespectively of treatment response.	
Giuliani *et al.* (2007) [93]	Bortezomib (1.3 mg/m^2) i.v. monotherapy up to 8 cycles	Relapsed or refractory myeloma patients	Prospective case control study	21		Significant increase in the number of osteoblastic cells/mm^2 of bone tissue in MM responding patients but not in nonresponders	
Heider *et al.* (2006) [94]	Bortezomib monotherapy (1.3 mg/m^2) / +/-dexamethasone (25 patients) Control group other than bortzomib therapy (58 patients) (20 adriamycin/ dexamethasone, 22 melphalan/prednisone and 16 thalidomide)	Newly diagnosed or refractory myeloma patients	Prospective case control study	83		Significant increase of Osteocalcin levels ($p = 0.024$) and bALP ($p < 0.0005$) in bortezomib group Significant increase in BAP both in responders and non-responders.	
Terpos *et al.* (2010) [95]	Bortezomib Dexamethasone Zoledronic acid	Relapsed myeloma patients	Prospective case series	27	Significant reduction in N-terminal cross-linking telopeptide of collagen type I levels after eight cycles of therapy ($p = 0.013$)	Significant increase in bALP ($p < 0.01$) and osteocalcin levels ($p < 0.01$)	Significant increase in L1–L4 BMD after eight cycles of therapy ($p < 0.01$)
Delforge *et al.* (2011) [96]	VMP (bortezomib 1.3 mg/m^2 plus melphalan 9 mg/m^2 and prednisone 60 mg/m^2) or MP alone	Previously untreated myeloma patients	Prospective randomized comparative study	682	Significant greater DKK-1 decrease with VMP vs. MP ($p = 0.0069$)	Significant greater ALP increase with VMP vs. MP ($p = 0.029$)	

BMD: Bone mineral density.

BMSCs and not direct production of RANKL by myeloma cells [110]. In myeloma patients, RANKL/OPG ratio is increased, thus enhancing osteoclast differentiation and function, leading to bone lytic disease [29].

OPG reduction is a result of the interaction between myeloma cells and BMSCs. Furthermore, myeloma cells decrease OPG by internalizing and degrading it. They also produce Dkk-1, which blocks the production of OPG through the Wnt metabolic pathway. By blocking the Wnt pathway, myeloma cells interfere with the maturation of osteoblasts, resulting in the increase of RANKL production by immature osteoblasts and decrease of OPG production by mature osteoblasts [111].

Preclinical studies have demonstrated a strong interdependence between myeloma cells and OCs. Myeloma cells enhance the formation of OCs, whose activity or products, in turn, are essential for the survival and growth of the myeloma cells [29]. Yaccoby *et al.* (2002) reported that the treatment of myelomatous hosts with pamidronate, zoledronate or with a specific inhibitor of RANKL halted myeloma-induced bone resorption and inhibited myeloma cell growth and survival [112].

6. Treatment with recombinant OPG & anti-RANKL-Abs

Recombinant-OPG and anti-RANKL antibodies have already been studied in myeloma patients [113-117]. **Table 5** summarizes the clinical studies available for the latter treatment. In a randomized, double-blind Phase I *in vivo* study, patients with MM and with breast cancer were randomized into 2 groups so as to receive either recombinant OPG or pamidronate. The drop in urine NTX was found to be similar in both groups, indicating that recombinant OPG is at least as effective with standard i.v. pamidronate treatment in reducing bone resorption marker levels [113]. In another Phase I *in vivo* study, patients with MM and with breast cancer were randomized to receive either a subcutaneous dose of denosumab (four escalating dosages were tested) or an i.v. dose of pamidronate. There was a drop in urine NTX in all groups that lasted longer, however, in the group of patients who received the higher dosage of denosumab. The decrease of NTX lasted longer (84 days) compared to pamidronate group. No effect, however, was detected on bone formation markers between the two groups. [114].. In a Phase II *in vivo* study, patients with MM, prostate cancer or breast cancer who had been previously treated with BPs were randomized either to continue BPs or to receive denosumab every 4 or 12 weeks. In the denosumab group, fewer SREs occurred compared with i.v. BP group (8 versus 17%, respectively). Denosumab also decreased serum CTx, P1NP, TRAP-5b, bone specific alkaline phosphatase and osteocalcin levels and suppressed urinary NTX up until the 25th week of treatment [115]. Vij *et al.* (2007) reported that denosumab when administered monthly at a subcutaneous dose of 120 mg was more effective in plateau patients compared to relapsed cases. This denosumab effect was irrespective of the previous exposure of patients to BPs [116]. Finally, in a Phase III *in vivo* study, 5723 MM or patients suffering from other neoplasia were randomized to receive either 120 mg subcutaneous denosumab or 4 mg i.v. zoledronic acid [117]. Denosumab was superior to zoledronic acid in reducing the risk ($p < 0.001$) of the first on study SRE ($p = 0.0007$) as well as the risk for multiple events ($p = 0.001$). There was no difference between the two drugs regarding disease progression [117].

7. Future agents with possible use in MM disease

Continuous better understanding of the complexity of myeloma bone disease has revealed several other pathophysiogical pathways, leading to the development of new drugs. These new category of drugs have now entered into clinical trials in myeloma patients and may be used in the future in patients with bone disease [29].

7.1 Activin A inhibitors

Activin A is a TGF-β family member cytokine enriched in bone matrix, secreted by both osteoblasts and OCs and has been found to induce osteoclast development and differentiation through stimulation of RANK and other bone marrow precursors [118,119] It also inhibits osteoblasts both *in vitro* and *in vivo* via SMAD2-mediated DLX5 signaling downregulation [120].

Vallet *et al.* [120] in an *in vitro* and *in vivo* study demonstrated that Activin A correlates with osteolytic disease in MM patients. During broad cytokine profiling of bone marrow plasma derived from MM patients with and without osteolytic bone disease, Activin A showed a significantly higher expression in patients with more than one osteolytic lesion versus patients with one or no lesions ($p = 0.03$) [120].

A possible role of Activin in bone disease has been highlighted in other studies too [121-124]. In a preclinical *in vitro* study [121], Chantry *et al.* (2010) reported that a soluble Activin receptor type IIA fusion protein (ActRIIA.muFc) treatment in mice bearing murine myeloma cells stimulates osteoblastogenesis ($p < 0.01$), prevents myeloma-induced suppression of bone formation ($p < 0.05$), blocks the development of osteolytic bone lesions ($p < 0.05$) and increases survival ($p < 0.05$). The effect of sotatercept (ACE-011), an inhibitor of Activin A was evaluated in a randomized, double-blind, placebo-controlled *in vivo* study in 48 healthy, postmenopausal women. ACE-011 caused a rapid and sustained dose-dependent increase in serum levels of bALP and a dose-dependent decrease in CTX and TRACP-5b levels [122].

The effects of sotatercept have been also evaluated in a Phase II *in vivo* study, where the drug was administered in newly diagnosed and relapsed MM patients [123]. Thirty patients randomized to receive sotatercept or a combination

Table 5. Major studies of either recombinant osteoprotegerin or denosumab on bone metabolism of patients suffering from multiple myeloma or other neoplasia.

Author (year)	Drug type/dose	Study/trial type	Number of patients	Bone resorption Markers
Body *et al.* (2003) [113]	AMGN-0007(genetically engineered osteoprotegerin) Pamidronate 90 mg i.v.	Randomized, double-blind, double-dummy, single-dose study	26 women with breast carcinoma 28 patients with multiple myeloma and radiologic evidence of bone disease	Similar drop in urinary NTX levels between pamidronate and AMGN-0007 group
Body *et al.* (2006) [114]	Denosumab 0,1-0.3-1,0-3,0 mg/kg subcutaneously Pamidronate 90 mg IV	Phase I randomized, double-blind, double-dummy, multi-center study	25 patients with multiple myeloma and lytic bone disease 29 patients with breast cancer with bone metastases	Decrease in the levels of urinary and serum N-telopeptide lasted for longer period (84 days) at the higher denosumab doses
Fizazi *et al.* (2008) [115]	Zoledronic acid or pamidronate i.v./ 4weeks Denosumab 180 mg/month or 180 mg/12 weeks subcutaneously	Phase II randomized, open-label, multi-center study	111 patients (prostate cancer 50, breast cancer 47, multiple myeloma 15, solid tumors 6)	Reduction in sCTx, P1NP, TRAP-5b, BSAP, and Osteocalcin levels in denosumab group until week 25 Significant lower time for NTX reduction and greater odds of achieving urinary NTx lower than 50 with denosumab group at week 13 ($p = 0.01$)
Vij *et al.* (2007) [116]	Denosumab 120mg subcutaneously/ month for 4–6 cycles	Phase II single-arm trial	96 relapsed or plateau phase myeloma patients	Substantial reduction of serum CTX in both relapsed and plateau-phase myeloma patients
Henry *et al.* (2011) [117]	Denosumab 120 mg subcutaneously vs zoledronic acid iv 4 mg/ month	Three identically designed, randomized double-blind, double-dummy phase 3 trials	5723 patients (breast cancer, prostate cancer, other solid tumors or multiple myeloma)	Denosumab superior to zoledronate in reducing the risk ($p < 0.001$) and the median time of the first on-study SRE ($p = 0,0007$) and the risk of multiple SRE ($p < 0.001$)

BSAP: Bone specific alkaline phosphatase; NTX: N-terminal cross-linking telopeptide of collagen type I; SRE: Skeletal related events;TRAP: Tartrate-resistant acid-phosphatase.

of oral melphalan, prednisolone and thalidomide. Although Grade 3 and 4 adverse events, especially hematologic events, were more frequent in sotatercept group, the drug demonstrated clinically significant increases in biomarkers of bone formation, and decrease in bone pain.

Activin A seems to be a promising therapeutic target for myeloma bone disease. Its efficacy is evaluated also in conjunction with other antimyeloma drugs as lenalidomide on ongoing trials [124].

8. Dkk-1 antagonists

The secreted glycoprotein Dickkopf-1(Dkk-1), a soluble inhibitor of the Wnt signaling pathway, is broadly expressed in myeloma cells but highly restricted in normal tissues [125,126]. It is produced by myeloma cells, suppresses the osteoblast differentiation and its serum level correlates with focal bone lesions in MM [126].

In vitro and in vivo murine models [127] demonstrated that (BHQ880), a Dkk-1 neutralizing antibody increases osteoblast differentiation and inhibits myeloma cell growth and development of osteolytic lesions in MM. Statistically significant uniform increases in vertebral strength have been found using quantitive computerized tomography, after the administration of this agent [128]. Ongoing research has been targeting Dkk-1 to find novel therapeutic strategies for myeloma bone disease, such as Dkk-1 neutralizing antibodies.

8.1 Mitogen-activated protein kinase 1/2 inhibitors

The MAPK/ERK pathway is important in the pathogenesis of MM. Activation of this pathway mediates myeloma cell growth and survival and contributes to angiogenesis, as well as the development of drug resistance within the bone marrow microenvironment [89,129].

AZD6244 is a MEK1/2 inhibitor. It has been found to have specific antiosteoclast activity, blocking the M-CSF and RANKL related osteoclast differentiation and thus decreasing bone resorption [90,130]. There is an ongoing Phase II clinical trial of AZD6244 given to MM patients with relapsed refractory myeloma (www.clinicaltrials.gov).

8.2 Histone deacetylase inhibitors

PDX101 (belinostat) is a histone deacetylase inhibitor that has been found to inhibit osteoclastogenesis both *in vivo* and *in vitro* [131]. PDX101 has been given to patients with relapsed/refractory myeloma in combination with bortezomib

in a Phase II clinical trial that was discontinued due to toxicity resulting from the dosage administered. Another Phase II trial has been completed involving patients with advanced MM who were given PDX101, the results of which have not yet been reported (www.clinicaltrials.gov)

Vorinostat is also a histone deacetylase inhibitor. Results from a Phase I *in vivo* clinical trial concluded in finding the dosage at which vorinostat is well tolerated in patients with MM [132]. A Phase II clinical study involving patients with relapsed/refractory MM to which a combination of vorinostat and bortezomib was administered has been completed but its results have yet to be reported (www.clinicaltrials.gov).

There are also several ongoing trials concerning panobinostat, another histone deacetylase inhibitor, none of which have been completed (www.clinicaltrials.gov).

8.3 Anti-IL-6 antibody

IL-6 is produced by BMSCs and contributes to the development of myeloma cells and preventing their apoptosis as well. It also activates osteoclast precursors.

CNTO328 is a chimeric monoclonic antibody with high affinity for human IL-6. In a Phase II *in vivo* clinical study involving patients with relapsed/refractory myeloma, CNTO328 in combination with dexamethasone administration has shown this combination to have promising preliminary activity [133]. There are several ongoing clinical studies concerning the administration of CNTO328 in combination with other agents to patients with MM (www.clinicaltrials.gov).

8.4 Fenretinide

Fenretinide is an analog of all-trans retinoid acid that has been investigated in prostate and breast cancer. In an *in vitro study* investigating the effect of fenretinide on myeloma cells it was found to prevent their development. It also was found to prevent the differentiation of OCs and reduce their viability [134]. There is an ongoing clinical trial where fenretinide is administered to patients with hematological cancer, including MM (www.clinicaltrials.gov).

8.5 Other possible therapeutic agents in MM

TGF-β is a multifunctional cytokine that suppresses osteoclastogenesis [135]. It is a potent regulator of terminal osteoblast differentiation and mineralization [136-138] and is produced by osteoblasts and osteocytes [139]. It is released from bone matrices through bone resorption [140]. Two TGF-β inhibitors, SB431542 and Ki26894 have been shown both *in vitro* and *in vivo*, to favor osteoblast differentiation as well as preventing myeloma cell proliferation [141].

Valproic acid is used in the treatment of epilepsy but also has been found to be a histone deacetylase inhibitor. In an *in vitro* and *in vivo* study of valproic acid in MM, it was found to have a cytotoxic effect on myeloma cells and also an inhibitory effect on osteoclastogenesis [142].

Recently, brain derived neurotrophic factor (BDNF), which is essential to the development, function and survival of brain cells, has been identified as a factor that promotes osteoclastogenesis. The potential osteoclastogenic effects of BDNF may mediate stromal-MM cell interactions to upregulate RANKL secretion in myeloma bone diseases [143]. An antibody against BDNF that is produced by myeloma cells may reduce the production of OCs [144].

Additionally, the use of heparanase inhibitors in the treatment of MM was strongly suggested. Newer experimental *in vitro* and *in vivo* data provide important new insights into the role of heparanase in all aspects of myeloma bone disease [145]. It was demonstrated that heparanase significantly inhibited osteoblast differentiation and mineralization mainly by shifting the differentiation potential of osteoblast progenitors from osteoblastogenesis to adipogenesis. So the use of heparanase inhibitors would be promising in myeloma bone disease treatment.

Other promising agents are monoclonal antibodies (anti-CS1 and anti-CD38) [146,147] to surface glycoproteins expressed on myeloma cells, resulting in cell death through mechanisms such as apoptosis, complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity. Both appear to enhance treatment efficacy when combined with lenalidomide compared to lenalidomide alone.

9. Discussion

Bone disease is present in 80 – 90% of patients with MM and is responsible for most of the clinical features of this disease. It is primarily the result of an increase of osteoclast production and activation coupled with a decrease in osteoblast activity [18]. The binding of BMSCs to myeloma cells results in the production of factors that favor osteoclastogenesis [19]. Therefore, agents with either antiosteoclast action or the ability to suppress osteoclastogenesis are able to improve living conditions for MM patients.

BPs are the cornerstone of myeloma bone disease treatment.. To date, clodronate p.o. and i.v. pamidronate and zoledronic acid have shown positive results when given to patients with MM. Pamidronate has shown similar results to zoledronic acid when administered to patients with advanced bone disease, although both agents have to be given with adjusted dosage to patients with kidney failure [50]. Zoledronic acid has been found to be superior to clodronate with regard to preventing skeletal events and overall survival but also has a higher incidence of ONJ, one of the more serious side effects caused by BPs [51]. Administration of BPs is not recommended for patients with asymptomatic disease [54].

Certain immunomodulatory drugs, as thalidomide, lenalidomide and proteasome inhibitors, as bortezomib are potent antimyeloma drugs. They have been primarily administered to two categories of patients, those who are about to be treated with autologous stem cell transplantation and those who have relapsed/refractory myeloma, in combination with other

drugs. In addition to their antimyeloma effect, they have different levels of effect on abnormal bone metabolism, either by reducing bone resorption or by enhancing bone formation. Although there are no comparative studies between these antimyeloma agents concerning their efficacy on the treatment of bone myeloma disease, the dual effect of proteasome inhibitor as bortezomib on both resorption and formation seems to be favorable [95].

Anti-RANKl antibodies and especially denosumab has been used also *in vitro* and *in vivo* for myeloma bone disease treatment. It seems that denosumab can suppress biochemical markers of bone resorption and prevent to some extent SRE in myeloma patients. Denosumab, has currently been compared to zoledronic acid in a Phase III clinical study and found to be noninferior. Time to first SRE as well as disease progression was comparable between groups. As far as side effects are concerned, denosumab is capable of causing ONJ as well [129]. Denosumab has not yet been approved for use in MM patients.

There are a number of other agents currently being used in clinical studies or with established anti-OC action either *in vitro* or *in vivo* that may be used in the future in the treatment of MM. The discovery and eventual use of new agents may provide solutions to the serious side effects arising from the agents currently used and provide patients with MM new and more effective ways for coping with MM bone disease.

10. Expert opinion

BPs are the mainstay of myeloma bone disease treatment. Oral clodronate and i.v. pamidronate and zoledronic acid are currently used drugs. Zoledronate was found to be superior to clodronate as far as the prevention of SREs was concerned. Pamidronate and zoledronate seem to have comparable results in preventing SREs of the disease; however, current studies proved that zoledronate can also have survival benefits for the patients. This parameter makes zoledronate more preferable in the treatment of myeloma bone disease. All attention should be paid to avoid complications of BP treatment.

Although denosumab had comparable results with zoledronate on myeloma bone disease treatment, its use has not been proven yet. This drug is in Phase III study for myeloma patients with bone disease. Immunomodulatory drugs that are not currently in clinical use for MM bone disease as thalidomide and lenalidomide, as well as proteasome inhibitors as bortezomib, are potent antimyeloma drugs, having also the ability to alter bone metabolism. Out of all the latter, bortezomib appears to have beneficial effects on bone disease, inhibiting OCs and inducing osteoblast activity, possibly superior to all others. However, immunomodulatory drugs alone are not capable of reversing SREs and especially healing of bone lesions. There is an expanding set of drugs under investigation with great potential to reduce the negative effects of myeloma cells on bone, improve survival and quality of life.

Among the drugs currently in use, Zoledronate appears to be more effective in the treatment of myeloma bone disease. It has comparable results to pamidronate in preventing SREs and also has survival benefits for the patients. Although not yet licensed for myeloma bone disease, denosumab is under investigation as a potential new drug.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Bibliography

1. Haematology; UK myeloma forum. British Committee for Standards in Haematology. Diagnosis and management of multiple myeloma. Br J Haematol 2001;115:522-40
2. Ferlay JBF, Sankila R, Parkin DM. EUCAN: cancer incidence, mortality and prevalence in the European Union 1998, version 5.0. IARC CancerBase No 4. IARCPress; Lyon, France: 1999
3. Parker S.L, Davis KJ, Wingo PA, et al. Cancer statistics by race and ethnicity. CA Cancer J Clin 1998;48:31-48
4. Ries L, Krapcho M. editors. SEER Cancer Statistics Review, 1975-2004. National Cancer Institute; Bethesda, MD: 2007. based on November 2006 SEER data submission, posted to the SEER web site
5. Kyle RA, Gertz MA, Witzig TE, et al. Review of 1027 patients with newly diagnosed multiple myeloma. Mayo Clin Proc 2003;78:21-33
6. Gridelli C. The use of bisphosphonates in elderly cancer patients. Oncologist 2007;12:62-71
7. Richardson PG, Blade J. The comprehensive clinical management of multiple myeloma and related-plasma cell disorders. Expert Rev Hematol 2014;7:1-3
8. Mohundro MM, Mohundro BL. On the horizon for multiple myeloma. Am J Manag Care 2012;18:SP140-3
9. Reece D. Update on the initial therapy of multiple myeloma. Am Soc Clin Oncol Educ Book. 2013 doi:10.1200/EdBook_AM.2013.33.e307
10. Genadieva-Stavric S, Cavallo F, Palumbo A. New Approaches to Management of Multiple Myeloma. Curr Treat Options Oncol 2014;15(2):157-70
11. Laubach JP, Tai YT, Richardson PG, Anderson KC. Daratumumab granted breakthrough drug status. Expert Opin Investig Drugs 2014;23:445-52

12. Brioli A, Tacchetti P, Zamagni E, Cavo M. Maintenance therapy in newly diagnosed multiple myeloma: current recommendations. Expert Rev Anticancer Ther 2014;14:581-94

13. Palumbo A, Magarotto V. Novel treatment paradigm for elderly patients with multiple myeloma. Am J Blood Res 2011;1:190-204

14. Lopuch S, Kawalec P, Wisniewska N. Effectiveness of targeted therapy as monotherapy or combined therapy in patients with relapsed or refractory multiple myeloma: a systematic review and meta-analysis. Hematology (Am Soc Hematol Educ Program) 2014. [Epub ahead of print]

15. Anderson KC. New insights into therapeutic targets in myeloma. Hematology Am Soc Hematol Educ Program 2011;2011:184-90

16. Cocks K, Cohen D, Wisloff F, et al. An international field study of the reliability and validity of a disease-specific questionnaire module (the QLQ-MY20) in assessing the quality of life of patients with multiple myeloma. Eur J Cancer 2007;43:1670-8

17. Bataille R, Chappard D, Marcelli C, et al. Recruitment of new osteoblasts and osteoclasts is the earliest critical event in the pathogenesis of human multiple myeloma. J Clin Invest 1991;88:62-6

18. Terpos E, Dimopoulos MA. Myeloma bone disease: pathophysiology and management. Ann Oncol 2005;16:1223-31

19. Christoulas D, Terpos E, Dimopoulos MA. Pathogenesis and management of myeloma bone disease. Expert Rev Hematol 2009;2:385-98

20. Sati HI, Greaves M, Apperley JF, et al. Expression of interleukin-1beta and tumour necrosis factor-alpha in plasma cells from patients with multiple myeloma. Br J Haematol 1999;104:350-7

21. Oyajobi BO, Franchin G, Williams PJ, et al. Dual effects of macrophage inflammatory protein-1alpha on osteolysis and tumor burden in the murine 5TGM1 model of myeloma bone disease. Blood 2003;102:311-19

22. Mitsiades CS, Mitsiades NS, Munshi NC, et al. The role of the bone microenvironment in the pathophysiology and therapeutic management of multiple myeloma: interplay of growth factors, their receptors and stromal interactions. Eur J Cancer 2006;42:1564-73

23. Zannettino AC, Farrugia AN, Kortesidis A, et al. Elevated serum levels of stromal-derived factor-1alpha are associated with increased osteoclast activity and osteolytic bone disease in multiple myeloma patients. Cancer Res 2005;65:1700-9

24. Borset M, Hjorth-Hansen H, Seidel C, et al. Hepatocyte growth factor and its receptor c-met in multiple myeloma. Blood 1996;88:3998-4004

25. Lee JW, Chung HY, Ehrlich LA, et al. IL-3 expression by myeloma cells increases both osteoclast formation and growth of myeloma cells. Blood 2004;103:2308-15

26. Moreaux J, Hose D, Kassambara A, et al. Osteoclast-gene expression profiling reveals osteoclast-derived CCR2 chemokines promoting myeloma cell migration. Blood 2011;117:1280-90

27. Vallet S, Raje N. Bone anabolic agents for the treatment of multiple myeloma. Cancer Microenviron 2011;4:339-49

28. Modi ND, Lentzsch S. Bisphosphonates as antimyeloma drugs. Leukemia 2012;26:589-94

29. Terpos E, Kastritis E, Dimopoulos MA. Prevention and treatment of myeloma bone disease. Curr Hematol Malig Rep 2012;7:249-57

30. Terpos E, Rahemtulla A. Bisphosphonate treatment for multiple myeloma. Drugs Today (Barc) 2004;40:29-40

31. Lin JH. Bisphosphonates: a review of their pharmacokinetic properties. Bone 1996;18:75-85

32. Fleisch H. Bisphosphonates: mechanisms of action. Endocr Rev 1998;19:80-100

33. Suda T, Nakamura I, Jimi E, Takahashi N. Regulation of osteoclast function. J Bone Miner Res 1997;12:869-79

34. Thompson K, Rogers MJ, Coxon FP, Crockett JC. Cytosolic entry of bisphosphonate drugs requires acidification of vesicles after fluid-phase endocytosis. Mol Pharmacol 2006;69:1624-32

35. Rogers MJ. New insights into the molecular mechanisms of action of bisphosphonates. Curr Pharm Des 2003;9:2643-58

36. Roelofs AJ, Thompson K, Gordon S, Rogers MJ. Molecular mechanisms of action of bisphosphonates: current status. Clin Cancer Res 2006;12:6222s-30s

37. Raikkonen J, Crockett JC, Rogers MJ, et al. Zoledronic acid induces formation of a pro-apoptotic ATP analogue and isopentenyl pyrophosphate in osteoclasts in vivo and in MCF-7 cells in vitro. Br J Pharmacol 2009;157:427-35

38. Russell RG, Xia Z, Dunford JE, et al. Bisphosphonates: an update on mechanisms of action and how these relate to clinical efficacy. Ann N Y Acad Sci 2007;1117:209-57

39. Terpos E, Dimopoulos MA, Sezer O. The effect of novel anti-myeloma agents on bone metabolism of patients with multiple myeloma. Leukemia 2007;21:1875-84

40. Terpos E, Morgan G, Dimopoulos MA, et al. International myeloma working group recommendations for the treatment of multiple myeloma-related bone disease. J Clin Oncol 2013;31:2347-57

41. Belch AR, Bergsagel DE, Wilson K, et al. Effect of daily etidronate on the osteolysis of multiple myeloma. J Clin Oncol 1991;9:1397-402

42. Daragon A, Humez C, Michot C, et al. Treatment of multiple myeloma with etidronate: results of a multicentre double-blind study. Groupe d'Etudes et de Recherches sur le Myelome (GERM). Eur J Med 1993;2:449-52

43. Menssen HD, Sakalova A, Fontana A, et al. Effects of long-term intravenous ibandronate therapy on skeletal-related events, survival, and bone resorption markers in patients with advanced multiple myeloma. J Clin Oncol 2002;20:2353-9

44. Pozzi S, Raje N. The role of bisphosphonates in multiple myeloma: mechanisms, side effects, and the future. Oncologist 2011;16:651-62

45. Lahtinen R, Laakso M, Palva I, et al. Randomised, placebo-controlled multicentre trial of clodronate in multiple myeloma. Finnish Leukaemia Group. Lancet 1992;340:1049-52

46. McCloskey EV, MacLennan IC, Drayson MT, et al. A randomized trial of the effect of clodronate on skeletal

morbidity in multiple myeloma. MRC Working Party on Leukaemia in Adults. Br J Haematol 1998;100:317-25

47. Brincker H, Westin J, Abildgaard N, et al. Failure of oral pamidronate to reduce skeletal morbidity in multiple myeloma: a double-blind placebo-controlled trial. Danish-Swedish co-operative study group. Br J Haematol 1998;101:280-6

48. Berenson JR, Lichtenstein A, Porter L, et al. Efficacy of pamidronate in reducing skeletal events in patients with advanced multiple myeloma. Myeloma Aredia Study Group. N Engl J Med 1996;334:488-93

49. Berenson JR, Rosen LS, Howell A, et al. Zoledronic acid reduces skeletal-related events in patients with osteolytic metastases. Cancer 2001;91:1191-200

50. Rosen LS, Gordon D, Kaminski M, et al. Zoledronic acid versus pamidronate in the treatment of skeletal metastases in patients with breast cancer or osteolytic lesions of multiple myeloma: a phase III, double-blind, comparative trial. Cancer J 2001;7:377-87

51. Morgan GJ, Child JA, Gregory WM, et al. National Cancer Research Institute Haematological Oncology Clinical Studies, G, Effects of zoledronic acid versus clodronic acid on skeletal morbidity in patients with newly diagnosed multiple myeloma (MRC Myeloma IX): secondary outcomes from a randomised controlled trial. Lancet Oncol 2011;12:743-52

52. Morgan GJ, Davies FE, Gregory WM, et al. National cancer research institute haematological oncology clinical studies, g, effects of induction and maintenance plus long-term bisphosphonates on bone disease in patients with multiple myeloma: the medical research council myeloma ix trial. Blood 2012;119:5374-83

53. Croucher PI, De Hendrik R, Perry MJ, et al. Zoledronic acid treatment of 5T2MM-bearing mice inhibits the development of myeloma bone disease: evidence for decreased osteolysis, tumor burden and angiogenesis, and increased survival. J Bone Miner Res 2003;18:482-92

54. Terpos E, Sezer O, Croucher PI, et al. The use of bisphosphonates in multiple myeloma: recommendations of an expert panel on behalf of the European Myeloma Network. Ann Oncol 2009;20:1303-17

55. Berenson JR, Lichtenstein A, Porter L, et al. Long-term pamidronate treatment of advanced multiple myeloma patients reduces skeletal events. Myeloma Aredia Study Group. J Clin Oncol 1998;16:593-602

56. Martiniani R, Di Loreto V, Di Sano C, et al. Biological activity of lenalidomide and its underlying therapeutic effects in multiple myeloma. Adv Hematol 2012;2012:842945

57. Melchert M, List A. The thalidomide saga. Int J Biochem Cell Biol 2007;39:1489-99

58. Kropff MH, Lang N, Bisping G, et al. Hyperfractionated cyclophosphamide in combination with pulsed dexamethasone and thalidomide (HyperCDT) in primary refractory or relapsed multiple myeloma. Br J Haematol 2003;122:607-16

59. Hussein MA, Baz R, Srkalovic G, et al. Phase 2 study of pegylated liposomal doxorubicin, vincristine, decreased-frequency dexamethasone, and thalidomide in newly diagnosed and relapsed-refractory multiple myeloma. Mayo Clin Proc 2006;81:889-95

60. Terpos E, Mihou D, Szydlo R, et al. The combination of intermediate doses of thalidomide with dexamethasone is an effective treatment for patients with refractory/relapsed multiple myeloma and normalizes abnormal bone remodeling, through the reduction of sRANKL/osteoprotegerin ratio. Leukemia 2005;19:1969-76

61. Offidani M, Corvatta L, Marconi M, et al. Low-dose thalidomide with pegylated liposomal doxorubicin and high-dose dexamethasone for relapsed/refractory multiple myeloma: a prospective, multicenter, phase II study. Haematologica 2006;91:133-6

62. Palumbo A, Avonto I, Bruno B, et al. Intravenous melphalan, thalidomide and prednisone in refractory and relapsed multiple myeloma. Eur J Haematol 2006;76:273-7

63. Kropff M, Baylon HG, Hillengass J, et al. Thalidomide versus dexamethasone for the treatment of relapsed and/or refractory multiple myeloma: results from OPTIMUM, a randomized trial. Haematologica 2012;97:784-91

64. Tosi P, Zamagni E, Cellini C, et al. First-line therapy with thalidomide, dexamethasone and zoledronic acid decreases bone resorption markers in patients with multiple myeloma. Eur J Haematol 2006;76:399-404

65. Corral LG, Kaplan G. Immunomodulation by thalidomide and thalidomide analogues. Ann Rheum Dis 1999;58(Suppl 1):I107-13

66. Breitkreutz I, Raab MS, Vallet S, et al. Lenalidomide inhibits osteoclastogenesis, survival factors and bone-remodeling markers in multiple myeloma. Leukemia 2008;22:1925-32

67. Geitz H, Handt S, Zwingenberger K. Thalidomide selectively modulates the density of cell surface molecules involved in the adhesion cascade. Immunopharmacology 1996;31:213-21

68. Baz R, Walker E, Karam MA, et al. Lenalidomide and pegylated liposomal doxorubicin-based chemotherapy for relapsed or refractory multiple myeloma: safety and efficacy. Ann Oncol 2006;17:1766-71

69. Knop S, Gerecke C, Liebisch P, et al. Lenalidomide, adriamycin, and dexamethasone (RAD) in patients with relapsed and refractory multiple myeloma: a report from the German Myeloma Study Group DSMM (Deutsche Studiengruppe Multiples Myelom). Blood 2009;113:4137-43

70. Reece DE M-KE, Khan A. Phase I-II trial of oral cyclophosphamide, prednisone and lenalidomide (Revlimid)(CPR) for the treatment of patients with relapsed and refractory multiple myeloma. BLOOD 2009;116(21):1260-1260

71. Anderson KJS, Jakubowiak A. Lenalidomide, bortezomib, and dexamethasone in relapsed/refractory multiple myeloma (MM): encouraging outcomes and tolerability in a phase II study. J Clin Oncol 2009;27(15):8536

72. Schey SA, Morgan GJ, Ramasamy K, et al. The addition of cyclophosphamide to lenalidomide and dexamethasone in multiply relapsed/refractory myeloma patients; a phase I/II study. Br J Haematol 2010;150:326-33

73. Dimopoulos M, Spencer A, Attal M, et al. Multiple myeloma study, I, lenalidomide plus dexamethasone for relapsed or refractory multiple myeloma. N Engl J Med 2007;357:2123-32

74. Weber DKR, Chen C. Prolonged overall survival with lenalidomide plus dexamethasone compared with dexamethazone alone in patients with relapsed or refractory multiple myeloma. Blood 2007;110:412

75. Dimopoulos MA, Chen C, Spencer A, et al. Long-term follow-up on overall survival from the MM-009 and MM-010 phase III trials of lenalidomide plus dexamethasone in patients with relapsed or refractory multiple myeloma. Leukemia 2009;23:2147-52

76. Gay F, Hayman SR, Lacy MQ, et al. Lenalidomide plus dexamethasone versus thalidomide plus dexamethasone in newly diagnosed multiple myeloma: a comparative analysis of 411 patients. Blood 2010;115:1343-50

77. Terpos E, Christoulas D, Kastritis E, et al. Greek Myeloma Study G. The combination of lenalidomide and dexamethasone reduces bone resorption in responding patients with relapsed/refractory multiple myeloma but has no effect on bone formation: final results on 205 patients of the Greek myeloma study group. Am J Hematol 2014;89:34-40

78. San Miguel J, Weisel K, Moreau P, et al. Pomalidomide plus low-dose dexamethasone versus high-dose dexamethasone alone for patients with relapsed and refractory multiple myeloma (MM-003): a randomised, open-label, phase 3 trial. Lancet Oncol 2013;14:1055-66

79. Dimopoulos MA, Leleu X, Palumbo A, et al. Expert panel consensus statement on the optimal use of pomalidomide in relapsed and refractory multiple myeloma. Leukemia 2014;28(8):1573-85

80. Scott LJ. Pomalidomide: a review of its use in patients with recurrent multiple myeloma. Drugs 2014;74:549-62

81. Adams J, Palombella VJ, Sausville EA, et al. Proteasome inhibitors: a novel class of potent and effective antitumor agents. Cancer Res 1999;59:2615-22

82. Adams J. The proteasome: a suitable antineoplastic target. Nat Rev Cancer 2004;4:349-60

83. Landowski TH, Megli CJ, Nullmeyer KD, et al. Mitochondrial-mediated disregulation of Ca2+ is a critical determinant of Velcade (PS-341/bortezomib) cytotoxicity in myeloma cell lines. Cancer Res 2005;65:3828-36

84. Biehn SE, Moore DT, Voorhees PM, et al. Extended follow-up of outcome measures in multiple myeloma patients treated on a phase I study with bortezomib and pegylated liposomal doxorubicin. Ann Hematol 2007;86:211-16

85. Palumbo A, Ambrosini MT, Benevolo G, et al. Gruppo Italiano Malattie Ematologicche d.A. Bortezomib, melphalan, prednisone, and thalidomide for relapsed multiple myeloma. Blood 2007;109:2767-72

86. Pineda-Roman M, Zangari M, van Rhee F, et al. VTD combination therapy with bortezomib-thalidomide-dexamethasone is highly effective in advanced and refractory multiple myeloma. Leukemia 2008;22:1419-27

87. Reece DE, Rodriguez GP, Chen C, et al. Phase I-II trial of bortezomib plus oral cyclophosphamide and prednisone in relapsed and refractory multiple myeloma. J Clin Oncol 2008;26:4777-83

88. Popat R, Oakervee H, Williams C, et al. Bortezomib, low-dose intravenous melphalan, and dexamethasone for patients with relapsed multiple myeloma. Br J Haematol 2009;144:887-94

89. Khan ML, Reeder CB, Kumar SK, et al. A comparison of lenalidomide/dexamethasone versus cyclophosphamide/lenalidomide/dexamethasone versus cyclophosphamide/bortezomib/dexamethasone in newly diagnosed multiple myeloma. Br J Haematol 2012;156:326-33

90. von Metzler I, Krebbel H, Hecht M, et al. Bortezomib inhibits human osteoclastogenesis. Leukemia 2007;21:2025-34

91. Terpos E, Heath DJ, Rahemtulla A, et al. Bortezomib reduces serum dickkopf-1 and receptor activator of nuclear factor-kappaB ligand concentrations and normalises indices of bone remodelling in patients with relapsed multiple myeloma. Br J Haematol 2006;135:688-92

92. Zangari M, Esseltine D, Lee CK, et al. Response to bortezomib is associated to osteoblastic activation in patients with multiple myeloma. Br J Haematol 2005;131:71-3

93. Giuliani N, Morandi F, Tagliaferri S, et al. The proteasome inhibitor bortezomib affects osteoblast differentiation in vitro and in vivo in multiple myeloma patients. Blood 2007;110:334-8

94. Heider U, Kaiser M, Muller C, et al. Bortezomib increases osteoblast activity in myeloma patients irrespective of response to treatment. Eur J Haematol 2006;77:233-8

95. Terpos E, Christoulas D, Kokkoris P, et al. Increased bone mineral density in a subset of patients with relapsed multiple myeloma who received the combination of bortezomib, dexamethasone and zoledronic acid. Ann Oncol 2010;21:1561-2

96. Delforge M, Terpos E, Richardson PG, et al. Fewer bone disease events, improvement in bone remodeling, and evidence of bone healing with bortezomib plus melphalan-prednisone vs. melphalan-prednisone in the phase III VISTA trial in multiple myeloma. Eur J Haematol 2011;86:372-84

97. Kubiczkova L, Pour L, Sedlarikova L, et al. Proteasome inhibitors - molecular basis and current perspectives in multiple myeloma. J Cell Mol Med 2014;18(6):947-61

98. Hurchla MA, Garcia-Gomez A, Hornick MC, et al. The epoxyketone-based proteasome inhibitors carfilzomib and orally bioavailable oprozomib have anti-resorptive and bone-anabolic activity in addition to anti-myeloma effects. Leukemia 2013;27:430-40

99. Kupisiewicz K. Biological aspects of altered bone remodeling in multiple myeloma and possibilities of pharmacological intervention. Dan Med Bull 2011;58:B4277

100. Bird JM, Owen RG, D'Sa S, et al. On behalf of the Haemato-oncology Task Force of the British committee for Standards in Haematology (BCSH) and UK Myeloma Forum, Guidelines for the diagnosis and management of multiple myeloma. Br J Haematol 2011;154(1):32-75

101. Rosinol L, Blade J. Bortezomib and lenalidomide as front-line therapy for multiple myeloma. Leuk Lymphoma 2014;55(9):2024-31

102. Lok A, Mocquard J, Bourcier J, et al. Subcutaneous bortezomib incorporated into the bortezomib-thalidomide-dexamethasone regimen as part of front-line therapy in the context of autologous stem cell

transplantation for multiple myeloma. Haematologica 2014;99:e33-4

103. Roodman GD. Advances in bone biology: the osteoclast. Endocr Rev 1996;17:308-32

104. Voskaridou E, Terpos E. New insights into the pathophysiology and management of osteoporosis in patients with beta thalassaemia. Br J Haematol 2004;127:127-39

105. Itoh K, Udagawa N, Matsuzaki K, et al. Importance of membrane- or matrix-associated forms of M-CSF and RANKL/ODF in osteoclastogenesis supported by SaOS-4/3 cells expressing recombinant PTH/PTHrP receptors. J Bone Miner Res 2000;15:1766-75

106. Kong YY, Yoshida H, Sarosi I, et al. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature 1999;397:315-23

107. Hofbauer LC, Neubauer A, Heufelder AE. Receptor activator of nuclear factor-kappaB ligand and osteoprotegerin: potential implications for the pathogenesis and treatment of malignant bone diseases. Cancer 2001;92:460-70

108. Duplomb L, Baud'huin M, Charrier C, et al. Interleukin-6 inhibits receptor activator of nuclear factor kappaB ligand-induced osteoclastogenesis by diverting cells into the macrophage lineage: key role of Serine727 phosphorylation of signal transducer and activator of transcription 3. Endocrinology 2008;149:3688-97

109. Pearse RN, Sordillo EM, Yaccoby S, et al. Multiple myeloma disrupts the TRANCE/osteoprotegerin cytokine axis to trigger bone destruction and promote tumor progression. Proc Natl Acad Sci USA 2001;98:11581-6

110. Giuliani N, Colla S, Morandi F, et al. Lack of receptor activator of nuclear factor-kB ligand (RANKL) expression and functional production by human multiple myeloma cells. Haematologica 2005;90:275-8

111. Qiang YW, Chen Y, Stephens O, et al. Myeloma-derived Dickkopf-1 disrupts Wnt-regulated osteoprotegerin and RANKL production by osteoblasts: a potential mechanism underlying osteolytic bone lesions in multiple myeloma. Blood 2008;112:196-207

112. Yaccoby S, Pearse RN, Johnson CL, et al. Myeloma interacts with the bone marrow microenvironment to induce osteoclastogenesis and is dependent on osteoclast activity. Br J Haematol 2002;116:278-90

113. Body JJ, Greipp P, Coleman RE, et al. A phase I study of AMGN-0007, a recombinant osteoprotegerin construct, in patients with multiple myeloma or breast carcinoma related bone metastases. Cancer 2003;97:887-92

114. Body JJ, Facon T, Coleman RE, et al. A study of the biological receptor activator of nuclear factor-kappaB ligand inhibitor, denosumab, in patients with multiple myeloma or bone metastases from breast cancer. Clin Cancer Res 2006;12:1221-8

115. Fizazi KLA, Mariette X. Denosumab in patients with bone metastases from prostate, breast, and other cancers and elevated urinary N-telopeptide (uNTx) during intravenous bisphopshonate (IV BP) therapy: final results of a randomized. Phase II study.J Clin Oncol 2008;182:509-16

116. Vij RHN, Spencer A. An open-label, Phase II trial of denosumab in the treatment of relapsed (R) or plateau-phase (PP) multiple myeloma (MM). Blood 2007;110(Suppl 1):1054A

117. Henry DH, Costa L, Goldwasser F, et al. Randomized, double-blind study of denosumab versus zoledronic acid in the treatment of bone metastases in patients with advanced cancer (excluding breast and prostate cancer) or multiple myeloma. J Clin Oncol 2011;29:1125-32

118. Fuller K, Bayley KE, Chambers TJ. Activin A is an essential cofactor for osteoclast induction. Biochem Biophys Res Commun 2000;268:2-7

119. Sugatani T, Alvarez UM, Hruska KA. Activin A stimulates IkappaB-alpha/NFkappaB and RANK expression for osteoclast differentiation, but not AKT survival pathway in osteoclast precursors. J Cell Biochem 2003;90:59-67

120. Vallet S, Mukherjee S, Vaghela N, et al. Activin A promotes multiple myeloma-induced osteolysis and is a promising target for myeloma bone disease. Proc Natl Acad Sci USA 2010;107:5124-9

121. Chantry AD, Heath D, Mulivor AW, et al. Inhibiting activin-A signaling stimulates bone formation and prevents cancer-induced bone destruction in vivo. J Bone Miner Res 2010;25:2633-46

122. Ruckle J, Jacobs M, Kramer W, et al. Single-dose, randomized, double-blind, placebo-controlled study of ACE-011 (ActRIIA-IgG1) in postmenopausal women. J Bone Miner Res 2009;24:744-52

123. Abdulkadyrov KM, Salogub GN, Khuazheva NK, et al. Sotatercept in patients with osteolytic lesions of multiple myeloma. Br J Haematol 2014;165:814-23

124. Scullen T, Santo L, Vallet S, et al. Lenalidomide in combination with an activin A-neutralizing antibody: preclinical rationale for a novel anti-myeloma strategy. Leukemia 2013;27:1715-21

125. Zhou F, Meng S, Song H, Claret FX. Dickkopf-1 is a key regulator of myeloma bone disease: opportunities and challenges for therapeutic intervention. Blood Rev 2013;27:261-7

126. Politou MC, Heath DJ, Rahemtulla A, et al. Serum concentrations of Dickkopf-1 protein are increased in patients with multiple myeloma and reduced after autologous stem cell transplantation. Int J Cancer 2006;119:1728-31

127. Fulciniti M, Tassone P, Hideshima T, et al. Anti-DKK1 mAb (BHQ880) as a potential therapeutic agent for multiple myeloma. Blood 2009;114:371-9

128. Munshi NC AR, Beck JT. Early evidence of anabolic bone activity of BHQ880, a fully human anti-DKK1 neutralizing antibody: results of a Phase 2 study in previously untreated patients with smoldering multiple myeloma at risk for progression. Blood abstract meeting book ASH 2012;120(21):331

129. Hideshima T, Mitsiades C, Tonon G, et al. Understanding multiple myeloma pathogenesis in the bone marrow to identify new therapeutic targets. Nat Rev Cancer 2007;7:585-98

130. Breitkreutz IVS, Raab MS. AZD6244 (ARRY-142886), a potent and selactive MEK1/2 inhibitor blocks the ERK1/2 signaling pathway, inhibits osteoclast differentiation and activation in multiple myeloma: clinical implications. Blood 2006;108:989a

131. Feng R, Oton AB, Patrene K, et al. Combination of the proteasome inhibitor bortezomib and a histone deacetylase

inhibitor PXD101 results in synergistic inhibition of osteoclastogenesis and significantly stronger inhibition of multiple myeloma growth in vitro and in vivo. Blood 2006;108:153a-4a

132. Richardson PG, Mitsiades CS, Colson K, et al. Final results of a phase I trial of oral vorinostat in patients with advanced multiple myeloma. Blood 2007;110:11):1179

133. Voorhees PM, Manges RF, Somlo G, et al. A phase II multicenter study of CNTO 328,an anti-Il-6 monoclonal antibody, in patients with relapsed or refractory multiple myeloma (MM). J Clin Oncol 2009; 27 (15S): 8527

134. Li X, Ling W, Pennisi A, et al. Fenretinide inhibits myeloma cell growth, osteoclastogenesis and osteoclast viability. Cancer Lett 2009;284:175-81

135. Quinn JM, Itoh K, Udagawa N, et al. Transforming growth factor beta affects osteoclast differentiation via direct and indirect actions. J Bone Miner Res 2001;16:1787-94

136. Maeda S, Hayashi M, Komiya S, et al. Endogenous TGF-beta signaling suppresses maturation of osteoblastic mesenchymal cells. EMBO J 2004;23:552-63

137. Spinella-Jaegle S, Roman-Roman S, Faucheu C, et al. Opposite effects of bone morphogenetic protein-2 and transforming growth factor-beta1 on osteoblast differentiation. Bone 2001;29:323-30

138. Alliston T, Choy L, Ducy P, et al. TGF-beta-induced repression of CBFA1 by Smad3 decreases cbfa1 and osteocalcin expression and inhibits osteoblast differentiation. EMBO J 2001;20:2254-72

139. Pfeilschifter J, Bonewald L, Mundy GR. Characterization of the latent transforming growth factor beta complex in bone. J Bone Miner Res 1990;5:49-58

140. Guise TA, Chirgwin JM. Transforming growth factor-beta in osteolytic breast cancer bone metastases. Clin Orthop Relat Res 2003;415:S32-8

141. Takeuchi K, Abe M, Hiasa M, et al. TGF-β inhibition restores terminal osteoblast differentiation to supress myeloma growth. PLoS One 2010;5:9870

142. Kitazoe K, Abe M, Hiasa M, et al. Valproic acid exerts anti-tumor as well as anti-angiogenic effects on myeloma. Int J Hematol 2009;89:45-57

143. Ai LS, Sun CY, Zhang L, et al. Inhibition of BDNF in multiple myeloma blocks osteoclastogenesis via down-regulated stroma-derived RANKL expression both in vitro and in vivo. PLoS One 2012;7:e46287

144. Sun CY, Chu ZB, She XM, et al. Brain-derived neurotrophic factor is a potential osteoclast stimulating factor in multiple myeloma. Int J Cancer 2012;130:827-36

145. Ruan J, Trotter TN, Nan L, et al. Heparanase inhibits osteoblastogenesis and shifts bone marrow progenitor cell fate in myeloma bone disease. Bone 2013;57:10-17

146. Lonial S, Vij R, Harousseau JL, et al. Elotuzumab in combination with lenalidomide and low-dose dexamethasone in relapsed or refractory multiple myeloma. J Clin Oncol 2012;30:1953-9

147. van der Veer MS, de Weers M, van Kessel B, et al. Towards effective immunotherapy of myeloma: enhanced elimination of myeloma cells by combination of lenalidomide with the human CD38 monoclonal antibody daratumumab. Haematologica 2011;96:284-90

Affiliation

Yvonne Mary Papamerkouriou[1], Eustathios Kenanidis[1], Zakareya Gamie[2], Kyriakos Papavasiliou[1], Thomas Kostakos[1], Michael Potoupnis[1], Ioannis Sarris[1], Eleftherios Tsiridis[†1] & John Kyrkos[1]
[†]Author for correspondence
[1]Aristotle University Medical School, "PapaGeorgiou" General Hospital, Academic Orthopaedic Unit, Thessaloniki, Greece
E-mail: etsiridis@doctors.org.uk
[2]University of Newcastle upon Tyne, Institute of Cellular Medicine, Framlington Place Newcastle upon Tyne, Tyne and Wear, NE2 4HH, UK