Bone 75 (2015) 161–169

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Review

Dynamic interplay between bone and multiple myeloma: Emerging roles of the osteoblast

Michaela R. Reagan [a,b], Lucy Liaw [b,c], Clifford J. Rosen [b,c,*], Irene M. Ghobrial [a,**]

[a] Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA
[b] Maine Medical Center Research Institute, Scarborough, ME, USA
[c] Tufts University School of Medicine, Boston, MA, USA

ARTICLE INFO

Article history:
Received 21 January 2015
Revised 15 February 2015
Accepted 18 February 2015
Available online 26 February 2015

Edited by: Sundeep Khosla

Keywords:
Multiple myeloma
Osteoblasts
Bone

ABSTRACT

Multiple myeloma is a B-cell malignancy characterized by the unrelenting proliferation of plasma cells. Multiple myeloma causes osteolytic lesions and fractures that do not heal due to decreased osteoblastic and increased osteoclastic activity. However, the exact relationship between osteoblasts and myeloma cells remains elusive. Understanding the interactions between these dynamic bone-forming cells and myeloma cells is crucial to understanding how osteolytic lesions form and persist and how tumors grow within the bone marrow. This review provides a comprehensive overview of basic and translational research focused on the role of osteoblasts in multiple myeloma progression and their relationship to osteolytic lesions. Importantly, current challenges for *in vitro* studies exploring direct osteoblastic effects on myeloma cells, and gaps in understanding the role of the osteoblast in myeloma progression are delineated. Finally, successes and challenges in myeloma treatment with osteoanabolic therapy (i.e., any treatment that induces increased osteoblastic number or activity) are enumerated. Our goal is to illuminate novel mechanisms by which osteoblasts may contribute to multiple myeloma disease progression and osteolysis to better direct research efforts. Ultimately, we hope this may provide a roadmap for new approaches to the pathogenesis and treatment of multiple myeloma with a particular focus on the osteoblast.

Contents

* Correspondence to: C.J. Rosen, Center for Molecular Medicine, Maine Medical Center Research Institute, Maine Medical Center, 81 Research Drive, Scarborough, ME 04074, USA. Fax: +1 207 396 8174.
** Correspondence to: I.M. Ghobrial, Medical Oncology, Dana-Farber Cancer Institute, 450 Brookline Av, Boston, MA 02115, USA. Fax: +1 617 632 4862.
E-mail addresses: rosenc@mmc.org (C.J. Rosen), Irene_Ghobrial@dfci.harvard.edu (I.M. Ghobrial).

http://dx.doi.org/10.1016/j.bone.2015.02.021
8756-3282/

Introduction

Multiple myeloma is an incurable plasma cell dyscrasia, a type of B-cell cancer that progresses through stages from monoclonal gammopathy of undetermined significance (MGUS), to asymptomatic smoldering myeloma, and lastly, to overt, symptomatic myeloma. This last stage is associated with significant morbidity, particularly in the form of fractures. Recent reports show that MGUS is also correlated with enhanced skeletal risks and osteopenia at this early stage of plasma cell transformation [1]. During multiple myeloma progression, osteolytic lesions are found throughout the skeleton with multiple tumors or "omas" packing the bone marrow. Osteolysis, a hallmark of multiple myeloma-induced bone disease, results from decreased osteoblastic activity and increased osteoclastic activity, releasing growth factors and cytokines embedded in bone matrix to form a "vicious cycle" [2–5]. The degree of osteolysis is an important parameter in the assessment of multiple myeloma patients. While the numbers of osteoblasts and bone formation rates are often increased in the early stages of tumor burden, due to increased osteoclast activity (which feeds back to activate increased osteoblast activity), these numbers become significantly lower when plasma cell infiltration occupies more than 50% of bone marrow [6]. Although bone-building (bone anabolic) treatments are currently being explored in early clinical trials to delay the time to first skeletal-related events (SREs) [7,8], much work remains to be done to validate if these are truly anti-myeloma strategies with long-term clinical benefits.

Preliminary research has demonstrated that osteoblast numbers can be decreased in hematologic malignancies, even in non-osteolytic tumors (a decrease of 55% was found in myelodysplasia and acute myeloid leukemia patients) and that osteoblasts can have an anti-tumor effect in blood cancers [9]. In support of this concept, the genetic depletion of osteoblasts in mouse models of acute leukemia led to increased circulating tumor cells and tumor marrow and spleen engraftment, higher tumor burden, and shorter survival [9]. Myelopoiesis increased and was coupled with a reduction in B-lymphopoiesis and compromised erythropoiesis, suggesting alterations in hematopoietic differentiation. When mice with acute myeloid or lymphoblastic leukemia were treated with a pharmacological inhibitor of duodenal serotonin, a hormone that suppresses osteoblast numbers, osteoblast numbers were increased, as expected. Remarkably, this treatment and subsequent maintenance of the osteoblast pool restored normal marrow function, reduced tumor burden and prolonged survival [9]. Therefore, osteoblasts may play a fundamental role in propagating leukemia in the marrow; pathways mediating this regulation still need identification.

One of the most pressing gaps in multiple myeloma biology is a basic biological understanding of the role of osteoblasts in disease progression (see Fig. 1). Recently, bone microstructural changes have been identified, along with elevated DKK1 and MIP-1α levels, in patients with MGUS [10]. Moreover, epidemiological data have demonstrated that low bone mineral density, increased fracture rate, and osteoporosis correlate with MGUS [1]. This provides more evidence that decreased osteoblast number/function, or weaker bones, could not only result from, but also cause or accelerate multiple myeloma [11]. As reviewed here, *in vitro* and *in vivo* studies to interrogate this hypothesis are crucial to elevate these correlations to mechanistically defined causal relationships. Studies are ongoing to identify underlying biological mechanisms by which osteoporosis could contribute to the development of multiple myeloma, and to gain insights into the roles of bone strength and bone-matrix forming cells in the etiology and pathogenesis of the disease. These studies are focused on several key questions: Do osteoblasts typically inhibit or stimulate the growth of myeloma cells? Would augmenting this specific cell type within the microenvironment decelerate or accelerate the progression of the disease, or affect its initial establishment? In which ways do osteoblasts directly or indirectly, through interactions with other bone marrow cells, affect the pathogenesis of multiple myeloma? Herein we review current concepts that begin to address these questions.

Ontogeny and developmental biology of the osteoblast

Osteoblasts are highly specific bone cells lining and formulating the mineralized matrix of the skeleton. They result from the osteogenic differentiation of mesenchymal stem cells (MSCs) and pass through a series of pre-osteoblastic stages as osteoprogenitor cells [12], until they become fully functional osteoblasts. When making bone, osteoblasts first deposit a dense organic extracellular matrix, primarily collagen I, and then harden this matrix by producing an inorganic calcium and phosphate-based mineral, hydroxyapatite. Different types of bone are formed by osteoblasts throughout the skeleton during skeletogenesis, remodeling, and fracture healing, including lamellar bone and woven bone [13]. During embryonic development, bone forms predominantly through a complex process termed endochondral ossification, a process including an intermediate cartilage stage [14]. A smaller fraction of human bones, such as the plates of the skull, are formed by intramembranous ossification, a process of direct differentiation of MSCs into mineralizing osteoblasts.

Osteoblasts in distinct anatomical locations respond uniquely to different stimuli and would likely respond differently to tumor cells, complicating studies aimed at using osteoblasts to inhibit multiple myeloma and other osteolytic cancers. What governs osteoblast phenotype and bone turnover in different bone compartments is largely unknown, but much work has been done to unravel the signaling mechanisms, pathways and relationships governing osteogenesis [15,16]. In 2009, Colnot [17] provided direct evidence that the major sources for skeletal stem cells are the periosteum, endosteum, and bone marrow and that while each give rise to osteoblasts, only the periosteum gives rise to chondrocytes, implicating different cellular populations within each distinct microenvironment. The periosteum also contributes to the growth and healing of long bones, demonstrating important differences in cell populations within various anatomical locations [18]. Recent evidence demonstrates that Wnt16 knockout mice have lower cortical bone mass, but no changes to their trabecular bone mass [19], whereas prior reports provide evidence that Wnt10a is necessary for trabecular bone formation, but not for cortical bone formation or maintenance [20,21]. These studies, and others using *Klotho*, *Src*, *and Sfrp4* null mice [22], demonstrate that osteoblasts and osteoclasts from different anatomical locations respond differently to ligands, trauma/disease, and treatments. This is also found clinically, where some therapeutics show different effects on long bones compared to vertebrae, or cortex versus trabeculae [23]. In sum, these studies suggest that osteoblast progenitors derived from these different locations may have disparate effects on bone remodeling and possibly cancer growth. This is a key nuance often ignored but which must be thoroughly understood before effective bone anabolic agents can be designed and targeted successfully.

Effects of osteoblasts on multiple myeloma

Unlike bone marrow MSCs, which support myeloma disease progression [24–26], evidence suggests that osteoblasts may suppress myeloma [27]. Osteoblast-derived growth factors play a large role in stimulating the growth of prostate cancers within the bone [28], raising the question of why this does not occur in myeloma. It is not clear if myeloma cells respond differently to these same osteoblast-derived factors, or if myeloma cells, like prostate cancer cells, actually benefit from osteoblasts, but proliferate even more strongly when they activate osteoclastic activity rather than osteoblastic activity. It is interesting that, although very rare, myeloma can also cause osteosclerostic lesions, without other symptoms of POEMS syndrome, suggesting again the possibility that osteoblastic activity may not necessarily be detrimental to plasma-cell-proliferative disorders [29–33].

As recently reviewed by Olechnowicz and Edwards [34], there are numerous other components of the host bone marrow that contribute to the pathogenesis of multiple myeloma, including fibroblasts, immune cells, adipocytes, endothelial cells, and osteoclasts. Contributions from

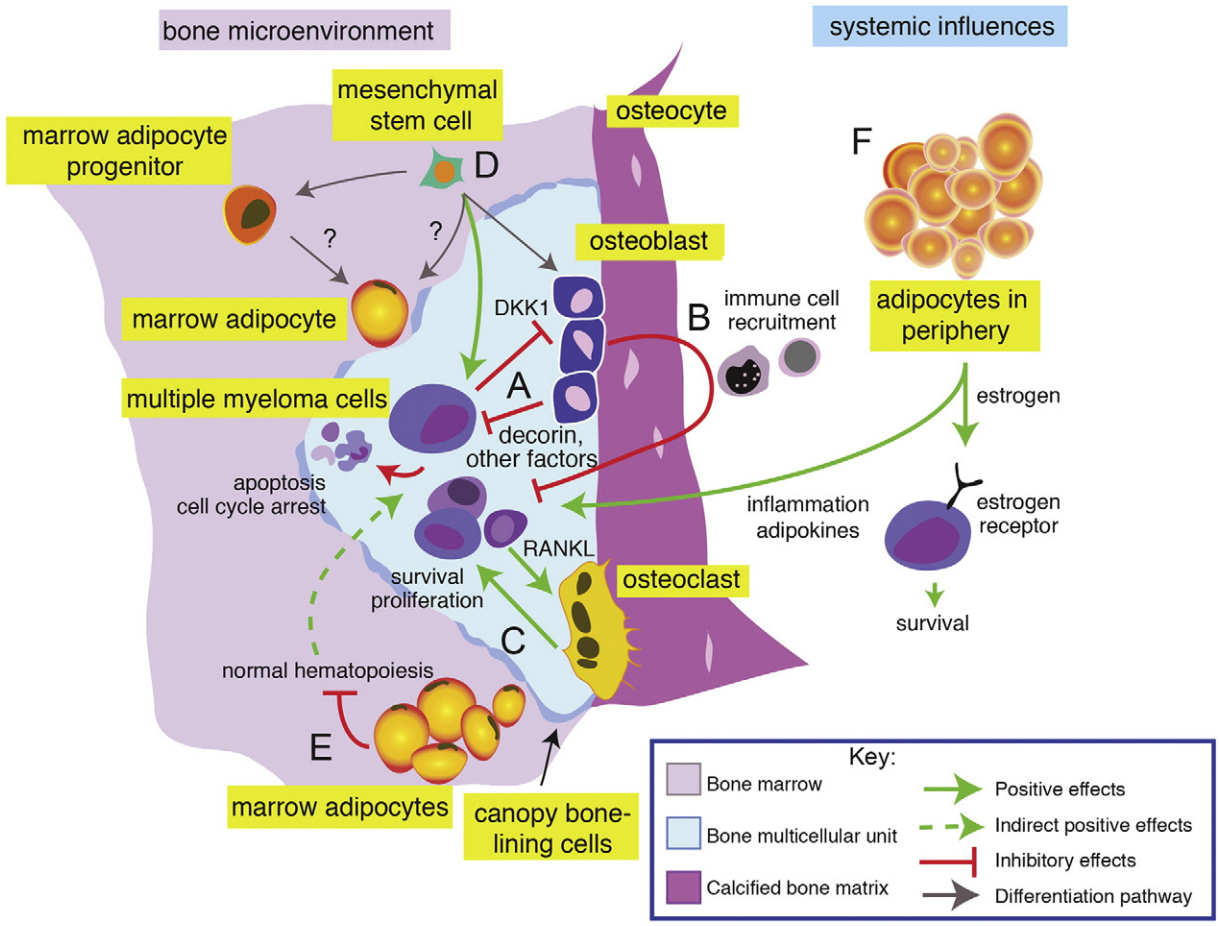

Fig. 1. The osteoblast as a central mediator of multiple myeloma growth. Multiple myeloma is a disease of the plasma cell. Multiple myeloma tumor cells grow within the bone microenvironment. Increasing evidence shows that osteoblasts play a central role in regulating the growth of multiple myeloma in the bone marrow through direct interactions or influences on other bone marrow niche cells. Within the bone microenvironment (left), osteoblasts secrete factors such as decorin (A) that directly lead to myeloma cell apoptosis and cell cycle arrest. In a reciprocal interaction, myeloma cells suppress osteoblast generation via DKK1. In addition, osteoblasts recruit immune cells to the bone marrow (B) where they can have anti-tumor effects, although recruitment of regulatory T cells and myeloid-derived suppressor cells can promote myeloma growth by inhibiting the anti-tumor immune response. (C) Increased osteoblastic activity leads to increased osteoclast activity, which can promote the survival and proliferation of myeloma cells. In turn, myeloma cells increase osteoclastic activity. (D) The mesenchymal stem cell within the marrow niche can have direct positive effects on myeloma cells and also determines the balance of resident osteoblasts and adipocytes. (E) The contribution of marrow adipocytes is still under active investigation, but marrow adipocytes may suppress normal hematopoiesis, leading to the development of myeloma cells. Other systemic influences (right) include adipose tissue, which, under conditions of excess adipocyte accumulation, induces systemic inflammation and release of adipokines and estrogen that may promote myeloma growth and survival.

the sympathetic nervous system and abnormalities in the myeloma-associated extracellular matrix also can support multiple myeloma progression. Perturbation of the osteoblast can lead directly, and spontaneously, to myelodysplasia or AML [35,36], demonstrating the critical influence of the bone microenvironment on hematological malignancies.

Direct effects of osteoblasts on myeloma growth

Biology of direct effects of osteoblasts on myeloma cells

Osteoblasts have been reported to directly inhibit multiple myeloma cells *in vitro*. One group demonstrated that some osteoblastic cells (MC3T3-E1 pre-osteoblastic cells and bone marrow-derived stromal cells), when differentiated into mineralized osteoblasts, induce apoptosis and cell cycle arrest in myeloma cells (i.e., cells such as RPMI8226, U266, KMS-12, INA6, 5TGM1, and primary patient samples) [27]. Decorin, the main small leucine-rich proteoglycan produced by osteoblasts, has also been identified as an endogenous, osteoblast-derived factor that suppresses multiple myeloma cell growth and survival [37]. In general, however, there is controversy about the net effect of osteoblasts on myeloma cells, as osteoblasts also produce factors that could support myeloma growth, such as osteocalcin, osteopontin, fibroblast growth factors, and transforming growth factor beta family members, although direct studies on this are lacking. One study demonstrated that quiescent myeloma cells prefer to reside in the endosteal/osteoblastic regions of the bone marrow compared with the vascular regions or spleen, indicating that osteoblasts may play a unique role in maintaining myeloma cells within a specific niche [38]. Another study showed that osteoblasts may be either supportive or inhibitory of multiple myeloma cells, and interestingly, these effects were dependent on the patient source of myeloma cells [39,40]. A better understanding of the direct anti-myeloma effects of osteoblasts is mandatory before bone anabolic treatments can be used successfully to inhibit multiple myeloma progression.

In vitro challenges of studying osteoblasts and myeloma interaction

There are several challenges that must be overcome to understand the role of osteoblasts in impeding myeloma growth. First, *in vitro* co-culture studies with plasma cells and osteoblasts are limited by the lack of relevant osteoblast cell lines. The human bone marrow stroma cell lines HS-5 and HS-27 do not differentiate into osteoblasts, and other cell lines that do mineralize, such as Saos2 [41] and MG-63 [42], are actually osteosarcoma rather than osteoblast cell lines. Certain cell lines, such as the human fetal osteoblastic cell line hFOB1.19 [43], which proliferate at 33.4 °C and differentiate at 39.4 °C or in osteogenic

medium, have been explored in multiple myeloma *in vitro* cultures [44] and could be exploited further. The two best options for osteoblast models may be primary human osteoblasts [45], or primary bone marrow-derived MSCs, which can be expanded and then induced to differentiate into mineralizing osteoblasts [24,46]. The challenge with using differentiated MSCs to model osteoblasts is delineating the moment when an MSC becomes a "pre-osteoblast," or has matured into a fully differentiated osteoblast, or has overshot the osteoblast stage to become an osteocyte. If the MSC is not differentiated far enough down the osteogenic pathway, it may still appear as a supportive stromal cell, accelerating the growth of myeloma cells rather than inhibiting them, as it is believed that osteoblasts may do *in vivo*. The mouse cell line MC3T3-E1 [47] is a well-accepted albeit unique pre-osteoblast cell line that undergoes linear osteogenic differentiation. Primary mouse calvarial osteoblasts are also widely used [48], but studies with mouse osteoblasts add some risk of missing human cell-specific signaling.

A second challenge for studying direct effects of osteoblasts on multiple myeloma is that, as with myeloma-derived MSCs [49], myeloma-derived osteoblasts differ substantially from their healthy-donor counterparts [50]. Specifically, their proliferation and osteogenic potential are significantly inhibited and their expression of the CCL3 receptor (CCR1) is significantly increased, which is one pathway contributing to their decreased osteogenic capacity [50]. By studying interactions between normal osteoblasts and myeloma cells, we may not observe the changes that occur in patients, which are between myeloma cells and myeloma-associated osteoblasts. Future studies may more accurately understand the relationships between these cells types if myeloma-patient-derived osteoblasts are utilized.

A third challenge to studying the direct effects of osteoblasts on myeloma cell growth relates to the *in vitro* conditions in which co-cultures are maintained. Most *in vitro* cultures are performed in two-dimensions on flat tissue-culture plates, but models to better mimic the physiologically relevant three-dimensional (3D) nature of the bone microenvironment are now become more established [24,51,52]. Tissue-engineered 3D bone built on silk scaffolds allows for highly reproducible, cost-effective replicates of cultures of osteoblasts and myeloma cells and can be used to model the process of differentiation of MSCs into a mineralized, porous artificial bone environment. These cultures are now being adapted to model the interactions between myeloma cells and any other cells, in the microenvironment, to better elucidate MM-bone stromal cell relationships (unpublished data).

Indirect effects of osteoblasts on myeloma cells through interaction with other cells

Osteoclasts

The most well-documented osteoblast relationship in the bone is the forward-feedback mechanism with osteoclasts known as remodeling. Increased osteoblastic activity leads to increased osteoclastic activity, which can then trigger recruitment of more osteoblasts and vice versa. This cycle is essential for maintaining bone mass and strength [53]. The pathophysiology of myeloma-induced bone disease progressing through the "vicious cycle" occurs when myeloma cells hijack the normal bone remodeling process and skew the balance towards increased osteolytic processes. This state of inhibited osteoblastic activity and increased osteoclastic activity, stimulated through molecules such as RANKL from myeloma cells and osteoblasts [54], leads to osteolytic lesions, weakened bone, pathological fracture, and a release of bone-embedded growth factors that further promote tumor cell growth [2,4,55].

Osteoclasts not only degrade bone matrix to release tumorigenic factors, but also directly promote the survival and proliferation of myeloma cells [40]. Hence, it is possible that use of bone anabolic treatments to increase osteoblastic activity would have a counter effect of also stimulating osteoclastic activity, thereby mitigating the tumor-suppressing effect of newly formed osteoblasts. Similarly, decreasing osteoclastic activities through agents such as bisphosphonates may have the opposite effect, due to the subsequent suppression of osteoblast function *in vivo*, hence diminishing any potential osteoblastic anti-multiple myeloma action. Therefore, understanding the regulation of the timing, location, and responses of osteoclasts to osteoblasts, and the reverse, is crucial for optimizing bone anabolic treatment regimens.

Adipocytes

There is growing interest in understanding interactions between bone and fat cells in normal physiology and disease, and the dynamic relationships between osteoblasts and marrow adipocytes are likely to affect multiple myeloma within the microenvironment in numerous ways. Previously, it was thought that obesity was associated with stronger bones, but more evidence has surfaced that obesity and osteoporosis share common genetic and environmental factors and that excessive fat and obesity may not protect against osteoporosis but could, in fact, accelerate it [56]. The interaction between adipocytes and osteoblasts has traditionally been considered as mutually exclusive such that the transcription factors that induce osteoblastogenesis inhibit adipogenesis and vice versa [56]. Interestingly, there is a significant degree of lineage plasticity between adipocytes and osteoblasts, which share a common progenitor, that further complicates dissecting the relationship between these two cell types in healthy and cancer-containing bone marrow [57,58]. Recent evidence suggests, however, that bone marrow adipocytes may derive from a progenitor cell distinct from the progenitor for osteoblasts, chondrocytes, and other bone marrow stromal cells [59,60].

There are also intriguing data that suggest adipocytes may regulate the pathogenesis and progression of multiple myeloma. A high body mass index (BMI) correlates with increased risk for multiple myeloma [61,62], possibly through increased conversion of androgens to estrogens that in turn stimulate estrogen receptor positive multiple myeloma cells [63–65]. High BMI may also lead to increased multiple myeloma development through increases in inflammatory mediators or CCL2- and COX-2-driven pathways that stimulate tumor growth in the bone marrow [66], but more mechanistic studies are needed to understand these signals. *In vitro* experiments have demonstrated a role for adipocytes in increasing the proliferation of multiple myeloma cells, but whether this is mediated by leptin or other adipokines has not been resolved [67]. Increased bone marrow adiposity in high BMI patients may also support multiple myeloma progression through the disruption of normal hematopoiesis and immune function [68]. In contrast, other reports have shown no difference, or even better overall survival or progression-free survival with certain treatments (e.g., melphalan and total body irradiation) in obese and extremely obese patients compared with normal and overweight patients [69]. Based on preliminary data, it appears that increasing osteoblastic differentiation and activity could decrease myeloma activity in part by decreasing the recruitment of adipocytes within the bone marrow niche, but this remains an open area of research.

Hematopoietic niche and immune cell interaction with myeloma cells

Because the osteoblastic niche is also a site for hematopoietic stem cell (HSC) and immune cell homing and homeostasis [70], osteoblasts may inhibit multiple myeloma growth partially by supporting anti-myeloma immune cell homing to the bone marrow. However, specific types of osteoblasts may play different immune supportive roles, as it appears that only a subtype of osteoblasts, those termed spindle-shaped N-cadherin+/CD45- Osteoblasts (SNOs), located next to the endosteal surface of bone, function to retain the so called Long-Term (LT)-HSCs in a quiescent status [12]. The relationship between osteoblasts and immune cells is complex. For example, although sclerostin null mice have high bone mineral density, they have increased B-cell apoptosis due to decreased osteoblast-derived CXCL12, resulting from increased Wnt signaling [71]. Moreover, although the immune system in general suppresses multiple myeloma [72], not all immune cells

mediate this role. Regulatory T-cells and immunosuppressive myeloid-derived suppressor cells [73] are now being identified as important new targets that inhibit the immune response in multiple myeloma [74]. Interestingly, cellular immunity was found to be decreased in myeloma patients, including decreased ratio of $CD4^+/CD8^+$, DC1/DC2, and Th1/Th2 cells, as well as an increased ratio of regulatory T cells, and some of these metrics of immune function ($CD4^+/CD8^+$ ratio and $CD4^+CD25^+/CD3^+$T ratio) were significantly positively correlated with the quantity of osteoblasts [75]. Hence, the potential effects of osteoblast loss on multiple myeloma via inhibition of the immune system require further investigation.

Other cells in the osteoprogenitor lineage

MSCs, the osteoblast progenitors, and myeloma progression. Bone marrow-derived MSCs are osteoprogenitor cells capable of differentiating into osteoblasts, adipocytes, and chondrocytes, among other cells, and much research has demonstrated their support of multiple myeloma adhesion, growth, and drug-resistance *in vitro* [24–26,76]. The expression of signaling cytokines, extracellular matrix factors, and adhesion molecules is the basis for their important role in myelomagenesis, bone marrow homing, and proliferation [24,77–80]. MSCs from myeloma patients are abnormal in terms of osteogenic differentiation, proliferation, gene expression, and other functions [24,49,81]. By inhibiting osteogenic differentiation of MSCs, multiple myeloma cells may be cleverly retaining a population of cells known to support their survival while inhibiting the maturation of osteoblasts, which generate bone matrix and have suppressive effects on myeloma cells.

In vitro, both osteoblasts and osteocytes can support MSC osteogenesis, in part due to soluble osteogenic cytokines [82]. However, osteoblasts seem to support an initial proliferation of MSCs and a delayed differentiation, while osteocytes promote an initial osteogenic differentiation [82]. However, contrasting the HSC niche roles of osteoblasts, it is less well understood how osteoblasts affect MSC homing, quiescence, and differentiation *in vivo* in healthy bone marrow, and even less so in myeloma-infiltrated bone marrow.

One study found that human placenta-derived adherent cells (PDACs), a type of MSC, inhibit H929 myeloma cell growth in a subcutaneous tumor model (tumor cells grown subcutaneously, later injected with PDACs). However, when the tumor was grown instead in a rabbit bone that was implanted subcutaneously into a SCID mouse (the SCID-rab model), the injected PDACs inhibited growth of H929 myeloma cells [82]. This may indicate that MSCs in multiple myeloma are dependent on the presence of the bone microenvironment to show anti-myeloma effects. Hence, osteoblasts may be essential regulators of the osteoprogenitor phenotype, and they may support a more anti-myeloma phenotype in MSCs.

Osteocytes, the osteoblast descendant, and myeloma progression. Upon becoming encased in osteon, osteoblasts become osteocytes and play a key regulatory role in bone homeostasis, osteoclast activity, and osteoblast regulation. Osteocytes are the mechanosensing cells that reside in lacuna and connect with each other through dendritic processes extending through lacunar-canalicular networks. They have been considered switchboard operators, as they direct a number of different signals that control cells behavior. For example, they extend processes into the vasculature within the bone, and out into the osteoblast-lined surfaces of the marrow and periosteum. With age, these lacunar-canalicular networks become compromised with large sections of bone lacking live osteocytes, suggesting one mechanism whereby diseases that have increasing incidence with age, such as myeloma, may have enhanced growth potential and progression. In several cancers, it has been shown that osteocytes affect tumor evolution through a number of local signaling and endocrine mechanisms [12,83,84].

The relationship between osteoblasts and osteocytes is complicated by the addition of myeloma cells. Since osteoblasts give rise to osteocytes as they become encased in bone matrix, myeloma inhibition of osteoblasts and osteoblastic activity may be a major cause of the decreased osteocytes observed in clinical samples [85]. However, since osteocytes are one of the major producers of sclerostin, a Wnt antagonist, a decrease in osteocytes for any reason typically decreases sclerostin levels, which then stimulates osteoblastic activity to produce a stable bone equilibrium. Unfortunately, the net balance in multiple myeloma patients is osteolysis and loss of osteoblasts/osteocytes; the attempt by the bone to normalize itself is futile and eventually toppled by the burden of osteoclastic activity. For more on the roles of osteocytes in multiple myeloma, refer to the elegant review by Roodman et al. [86].

Canopy-lining cells, the osteoblast cousin, and myeloma progression. Although similar in lineage to active, bone-matrix-secreting osteoblasts, canopy lining cells are quiescent, bone marrow protecting cells. These cells isolate areas of turnover to create a tightly connected, single-cell wide physical barrier to seal-off the osteoclast/osteoblast resorption pit from the marrow [87]. The relatively flat, elongated cells immunostain for osteoblast markers osteocalcin, osteonectin, pro-collagen type I (PINP), pro-collagen type III (PIIINP) and NCAM (CD56), demonstrating that the cell originates from the osteoblast lineage [88]. Importantly, they are Ki-67 negative (hence, non-proliferative) and negative for lymphocytic and monocytic markers. How these cells differ, if at all, from the more classically described quiescent bone lining cells remains to be delineated

Canopy lining cells may play an important role in the dysregulation of bone remodeling in general [89] and could be a novel target cell type in multiple myeloma. Osteoblasts seem to require these cells to properly lay down matrix, as multiple myeloma biopsies analyzed for the presence of these canopies over the bone remodeling compartment (BRC) demonstrated frequent disruptions in 66% of the biopsies. Importantly, frequent disruption (holes) in the canopies correlated with extensive resorption without matrix reconstruction, not observed in biopsies with normal, intact canopies over the BRCs [83,88,90]. Only in multiple myeloma bone surfaces with disrupted canopies did the researchers observe an absence of coupling between bone formation and resorption in patient biopsies [90]. It remains to be determined if BRC canopy destruction in multiple myeloma is a cause or result of deficient bone formation. The microanatomical structures may function through multiple unclear mechanisms (e.g., exerting physical constraints for cells or chemoattractants or acting as anchorage points for certain progenitors), but it is evident that their disruption results in direct physical contact between myeloma cells, osteoclasts, and osteoblasts, and coincides with the occurrence of osteolytic lesions.

Systemic effects

Osteoblasts, osteoclasts, and osteocytes contribute not only to local modifications of bone but also to systemic changes in whole body homeostasis through secretion of specific peptides and growth factors. Traditionally, the action of these cells define the bone as an endocrine organ, responding to hormones and soluble signaling molecules such as estrogen via estrogen receptor α (ERα) [91], calcium, PTH, 1, 25-Dihydroxycholecalciferol, and vitamin D, which communicate with other endocrine organs throughout the body, such as the thyroid, parathyroid, pituitary glands, adrenal glands, and pancreas, as reviewed elsewhere [92]. More recently, the secretion of metabolically active peptides such as osteocalcin has been shown to regulate insulin sensitivity and secretion. During states of high bone turnover, the release of matrix and cell-derived undercarboxylated osteocalcin impact adipose tissue sensitivity to insulin which in turn could release adipokines that further modulate myeloma progression [93]. On the other hand, bone metabolism may appear normal, as judged by biochemical measurements such as urinary excretion of calcium, hydroxyproline, and n-telopeptide, but significant bone destruction may be present. Hence, experiments to alter the local bone milieu, to dissect the roles of osteoblasts on

myeloma growth, must be performed in conjunction with monitoring other systemic changes resulting from alterations of osteoblasts. Measuring bioactive factors that are liberated during bone destruction may help quantify bone turnover, but cannot be used as a definite readout, due to a variety of confounding systemic effects resulting from osteoblast stimulation or inhibition [6].

In vivo models of osteoanabolism as a therapeutic approach to multiple myeloma

Osteoanabolic treatment is defined as any treatment that stimulates osteoblastic activity and bone formation. As a therapy for multiple myeloma, this strategy has yielded conflicting conclusions, with *in vivo* efficacy depending on the model system and treatments used. Some mouse models, such as the patient xenograft SCID-hu model, demonstrated that osteoanabolic treatments hold promise for inhibiting multiple myeloma, although results were highly variable and patient-specific [40]. There is mounting evidence that the anti-myeloma proteasome inhibitors carfilzomib [94] and bortezomib [95] have bone anabolic effects on bone and induce osteogenic differentiation of MSCs, which may contribute to their anti-myeloma effects [96], but concrete evidence remains elusive to demonstrate that these agents can produce anti-myeloma effects via changes in the bone microenvironment. The use of anti-resorptive agents, such as bisphosphonates, cathepsin K inhibitors, or RANKL inhibitors [54], in combination with osteoanabolic agents, may maximize the use of the bone microenvironment to inhibit myeloma. Collectively, these results highlight the need for better *in vivo* models and deeper understanding of exactly how we predict osteoanabolic treatments may function to inhibit multiple myeloma.

Anti-sclerostin and anti-DKK1 antibodies also have osteoanabolic effects in preclinical models and in clinical trials and are currently under investigation for the treatment of osteoporosis and osteolytic disease [97,98]. Anti-sclerostin treatments may prove useful for osteolytic cancers in general, but especially for myeloma, since myeloma cells secrete sclerostin that inhibits osteoblast activity [99]. Anti-DKK1 treatments may also be viable mechanisms for inhibiting myeloma bone disease, as DKK1, a canonical Wnt pathway inhibitor, is overexpressed in myeloma cells and patient serum [100], and DKK1 levels correlate with the extent of lytic bone disease [101]. Anti-DKK1 antibody therapy has also been shown to significantly increase osteoblast bone formation and bone mineral density in both murine and human healthy and multiple myeloma models [97,102,103]. Anti-DKK1 therapy in myeloma also inhibits osteolysis in multiple myeloma SCID-rab models (SCID mouse with rabbit bone subcutaneous implantation) [104]. However, the rates of success at lowering IgG levels or decreasing tumor growth rate, measured by tumor size, were only 36% (4/11) and were patient specific, suggesting that bone anabolic treatments may work only for a subset of myeloma patients [104]. A different myeloma model used to test anti-DKK1 antibodies is the SCID-hu model with fetal bone chips in a SCID mouse, injected with INA-6 myeloma cells. In this model, treatment with the Novartis antibody BHQ880, which neutralizes both human and murine DKK1, promoted osteoblastogenesis and decreased tumor burden, as measured by *in vivo* IL6 levels [102].

Other bone anabolic agents, including dasatinib, a multitargeted tyrosine kinase inhibitor [105], and soluble decoy receptors of activin A, a known osteoclast activating factor [106], also inhibit multiple myeloma, suggesting their clinical utility and supporting the hypothesis that increasing bone volume and osteoblast number is a practical method for inhibiting multiple myeloma [107,108]. Similarly, TGF-β, a potent inhibitor of terminal osteoblast differentiation abundant in the bone matrix, has also been identified as a novel target. Anti-TGF-β therapies are able to restore osteoblast differentiation suppressed in MM conditions *in vitro* and suppress myeloma cell growth within the bone marrow (using the SCID-rab/INA6 myeloma model) while preventing bone destruction in myeloma-bearing animal models [27]. This study demonstrated that osteoblasts, defined as mineralized MC3T3-E1 cells, were able to induce apoptosis and G1 cell cycle arrest in 5TGM1 myeloma cells, although the exact mechanisms by which osteoblasts potentiated these effects were not explored [27].

In vivo studies using daily administered parathyroid hormone (PTH) in SCID-rab and SCID-hu mouse models demonstrated that PTH treatment increased bone mineral density and reduced tumor burden [109]. PTH also increased the number of osteoblasts and other bone formation parameters and pre-treatment with PTH before injecting tumor cells also increased bone mineral density and delayed tumor progression. This research supports the hypothesis that an increase in bone mineral density and osteoblast number may provide a net anti-myeloma effect. Importantly, PTH can clinically lead to increased bone formation and osteoblast activity within the first 6 months of treatment. However, with longer-term PTH administration, osteoblast activation slows, and importantly, bone resorption increases significantly. Theoretically, this could compromise any positive effects of this approach for slowing myeloma progression [110]. Thus, osteoanabolic therapies for bone utilize a range of different approaches and target pathways, nicely summarized in a recent review [111]. However, it still remains controversial whether reported anti-tumor effects of bone-modulating therapies are clinically significant [112]. The current challenge in myeloma therapeutics thus becomes not only to develop biologic agents that have the desired effect of killing cancer cells but also to prevent any rebound or compensation that could make the skeletal changes worse.

Clinical studies have shown that the treatment of multiple myeloma patients with bisphosphonates significantly overall survival and progression-free survival [113,114]. Zoledronic acid and bortezomib both have anti-myeloma effects. Zoledronic acid is thought to directly impact myeloma cells, and bortezomib additionally may induce "pro-bone" mechanisms, including increasing osteogenic differentiation and inhibiting osteoclasts [95,115]. In fact, based on these studies, a phase II clinical trial recently completed in smoldering multiple myeloma patients treated with low dose bortezomib had a primary endpoint, "to evaluate the bone anabolic effect of bortezomib in patients with smoldering myeloma" and a secondary endpoint "to evaluate the effect of bortezomib on the natural history of smoldering myeloma" [116]. Another interesting phase II trial in the recruitment stage aims to test Sotatercept, an activin-A antagonist that interferes with the SMAD pathway. This signaling network, when activated, can lead to increased bone formation and anti-tumor activity in multiple myeloma [117] and bone anabolic improvements in bone mineral density and in bone formation [118]. The effects of Sotatercept on patient-specific outcomes such as skeletal-related events (i.e., fractures, impaired healing, bone pain) as well as delayed-progression, or progression-free or overall survival, remain to be elucidated. Results from these ongoing and future trials may open the door for similar treatments to be tested in patients with early stage or even overt myeloma.

Future directions and conclusions

One of the new directions in osteoblast-myeloma research, and in tumor-host interaction studies in general, is the use of CRISPR-Cas9 knockout technologies [119]. With this technology, researchers have already demonstrated an ability to more specifically target genes such as Ikaros family zinc finger proteins 1 and 3 (IKZF1 and IKZF3) in myeloma and hence dissociate the anti-tumor and teratogenic activities of thalidomide-like drugs [120]. The use of CRISPR technologies for modulating host osteoblasts and bone marrow cells would provide abundant information regarding the roles of different genes in bone cells and could suggest novel mechanisms for modulating the bone microenvironment to induce a less hospitable environment for the growth of cancer cells. Also, if investigators can overcome the obstacles and potential off–target effects of microRNA delivery, these may be a potential novel osteoanabolic treatment. "Osteogenic microRNAs" have been identified [121] and are currently under investigation for *in vivo* efficacy. Interestingly, some of these have been identified as differentially expressed in

multiple myeloma versus healthy MSCs and capable of functionally rescuing MSCs for their ability to produce bone matrix [24].

Multiple myeloma is considered by some a prototype for metastatic bone disease although there are clear phenotypic distinctions from other malignancies such as prostate cancer. Nevertheless, studies of osteoblastic function in myeloma could be extrapolated to other conditions that have classically been considered osteolytic, such as metastatic breast cancer [122–125]. This review described the multitude of ways in which osteoblasts may function to support or inhibit myeloma growth, and discussed new potential targets in the relationship between osteoblasts and myeloma cells to treat or prevent multiple myeloma. Osteoblasts act as an important hub of activity, affecting other cells within the bone marrow niche and mediating both direct and indirect effects on myeloma cells (Fig. 1). The future of bone anabolic treatments for anti-myeloma therapy is bright, but to optimize the use and design of such agents, it will be critical to view the osteoblast within a larger context and to visualize its interactions with other cells in the bone microenvironment and roles in whole body homeostasis.

Author contributions

MRR conceived and wrote the manuscript; LL designed and created the figure; LL, CJR, and IMG provided intellectual input and edited the manuscript.

Acknowledgments

This work was supported by the NIH R01CA154648 and by a Pilot Project Grant from NIH/NIGMS P30GM106391 at the Maine Medical Center Research Institute. This work was also supported by the Office of the Assistant Secretary of Defense for Health Affairs, through the Peer Reviewed Cancer Research Program, Visionary Postdoctoral Fellowship Award, under Award FY14 DoD Congressionally Directed Medical Research Program No. W81XWH-13-1-0390. Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the DoD.

Conflicts of interest

The authors declare no competing financial interest. Ghobrial: *BMS*: Advisory Board; *Celgene*: Advisory Board, Advisory Board Other; *Millennium*: Advisory Board, Advisory Board Other; *Onyx*: Advisory Board, Advisory Board Other.

References

[1] Drake MT. Unveiling skeletal fragility in patients diagnosed with MGUS: no longer a condition of undetermined significance? J Bone Miner Res 2014;29:2529–33. http://dx.doi.org/10.1002/jbmr.2387.
[2] Kingsley LA, Fournier PGJ, Chirgwin JM, Guise TA. Molecular biology of bone metastasis. Mol Cancer Ther 2007;6:2609–17. http://dx.doi.org/10.1158/1535-7163.MCT-07-0234.
[3] Guise TA, Mohammad KS, Clines G, Stebbins EG, Wong DH, Higgins LS, et al. Basic mechanisms responsible for osteolytic and osteoblastic bone metastases. Clin Cancer Res 2006;12:6213s–6s. http://dx.doi.org/10.1158/1078-0432.CCR-06-1007.
[4] Fowler JA, Edwards CM, Croucher PI. Tumor-host cell interactions in the bone disease of myeloma. Bone 2011;48:121–8. http://dx.doi.org/10.1016/j.bone.2010.06.029.
[5] Kovacic N, Croucher PI, McDonald MM. Signaling between tumor cells and the host bone marrow microenvironment. Calcif Tissue Int 2013;94:125–39. http://dx.doi.org/10.1007/s00223-013-9794-7.
[6] Taube T, Beneton MN, McCloskey EV, Rogers S, Greaves M, Kanis JA. Abnormal bone remodelling in patients with myelomatosis and normal biochemical indices of bone resorption. Eur J Haematol 1992;49:192–8.
[7] A Double-blind, Placebo-controlled, Randomized Phase 2 Study of BHQ880, an Anti-Dickkopf1 (DKK1) Monoclonal Antibody (mAb), in Patients With Untreated Multiple Myeloma and Renal Insufficiency n.d. https://clinicaltrials.gov/ct2/show/record/NCT01337752 (accessed December 18, 2015).
[8] A Phase Ib/II Multicenter Dose-determination Study, With an Adaptive, Randomized, Placebo-controlled, Double-blind Phase II, Using Various Repeated IV Doses of BHQ880 in Combination With Zoledronic Acid in Relapsed or Refractory Myeloma Patients With Prio n.d. https://clinicaltrials.gov/ct2/show/NCT00741377 (accessed January 18, 2015).
[9] Krevvata M, Silva BC, Manavalan JS, Galan-Diez M, Kode A, Matthews BG, et al. Inhibition of leukemia cell engraftment and disease progression in mice by osteoblasts. Blood 2014;124:2834–46. http://dx.doi.org/10.1182/blood-2013-07-517219.
[10] Ng AC, Khosla S, Charatcharoenwitthaya N, Kumar SK, Achenbach SJ, Holets MF, et al. Bone microstructural changes revealed by high-resolution peripheral quantitative computed tomography imaging and elevated DKK1 and MIP-1α levels in patients with MGUS. Blood 2011;118:6529–34. http://dx.doi.org/10.1182/blood-2011-04-351437.
[11] Berenson JR, Anderson KC, Audell RA, Boccia RV, Coleman M, Dimopoulos MA, et al. Monoclonal gammopathy of undetermined significance: a consensus statement. Br J Haematol 2010;150:28–38. http://dx.doi.org/10.1111/j.1365-2141.2010.08207.x.
[12] Capulli M, Paone R, Rucci N. Osteoblast and osteocyte: games without frontiers. Arch Biochem Biophys 2014;561:3–12. http://dx.doi.org/10.1016/j.abb.2014.05.003.
[13] Kronenberg HM, Kobayashi T. Skeletal Development and Repair. Methods and Protocols. London: Springer; 2014. http://dx.doi.org/10.1007/978-1-62703-989-5.
[14] Kronenberg HM. Developmental regulation of the growth plate. Nature 2003;423:332–6. http://dx.doi.org/10.1038/nature01657.
[15] Baron R, Rawadi G, Roman-Roman S. Wnt signaling: a key regulator of bone mass. Curr Top Dev Biol 2006;76:103–27. http://dx.doi.org/10.1016/S0070-2153(06)76004-5.
[16] Baron R, Kneissel M. WNT signaling in bone homeostasis and disease: from human mutations to treatments. Nat Med 2013;19:179–92. http://dx.doi.org/10.1038/nm.3074.
[17] Colnot C. Skeletal cell fate decisions within periosteum and bone marrow during bone regeneration. J Bone Miner Res 2009;24:274–82. http://dx.doi.org/10.1359/jbmr.081003.
[18] Colnot C, Zhang X, Knothe Tate ML. Current insights on the regenerative potential of the periosteum: molecular, cellular, and endogenous engineering approaches. J Orthop Res 2012;30:1869–78. http://dx.doi.org/10.1002/jor.22181.
[19] Movérare-Skrtic S, Henning P, Liu X, Nagano K, Saito H, Börjesson AE, et al. Osteoblast-derived WNT16 represses osteoclastogenesis and prevents cortical bone fragility fractures. Nat Med 2014;20:1279–88. http://dx.doi.org/10.1038/nm.3654.
[20] Stevens JR, Miranda-Carboni GA, Singer MA, Brugger SM, Lyons KM, Lane TF. Wnt10b deficiency results in age-dependent loss of bone mass and progressive reduction of mesenchymal progenitor cells. J Bone Miner Res 2010;25:2138–47. http://dx.doi.org/10.1002/jbmr.118.
[21] Bennett CN, Longo KA, Wright WS, Suva LJ, Lane TF, Hankenson KD, et al. Regulation of osteoblastogenesis and bone mass by Wnt10b. Proc Natl Acad Sci U S A 2005;102:3324–9. http://dx.doi.org/10.1073/pnas.0408742102.
[22] Brommage R, Liu J, Hansen GM, Kirkpatrick LL, Potter DG, Sands AT, et al. High-throughput screening of mouse gene knockouts identifies established and novel skeletal phenotypes. Bone Res 2014;2:14034. http://dx.doi.org/10.1038/boneres.2014.34.
[23] Pritchard JM, Giangregorio LM, Atkinson SA, Beattie KA, Inglis D, Ioannidis G, et al. Changes in trabecular bone microarchitecture in postmenopausal women with and without type 2 diabetes: a two year longitudinal study. BMC Musculoskelet Disord 2013;14:114. http://dx.doi.org/10.1186/1471-2474-14-114.
[24] Reagan MR, Mishima Y, Glavey SV, Zhang Y, Manier S, Lu ZN, et al. Investigating osteogenic differentiation in multiple myeloma using a novel 3D bone marrow niche model. Blood 2014;124:3250–9. http://dx.doi.org/10.1182/blood-2014-02-558007.
[25] Roccaro AM, Sacco A, Purschke WG, Moschetta M, Buchner K, Maasch C, et al. SDF-1 inhibition targets the bone marrow niche for cancer therapy. Cell Rep 2014;9:118–28. http://dx.doi.org/10.1016/j.celrep.2014.08.042.
[26] Azab AK, Runnels JM, Pitsillides C, Moreau A-S, Azab F, Leleu X, et al. CXCR4 inhibitor AMD3100 disrupts the interaction of multiple myeloma cells with the bone marrow microenvironment and enhances their sensitivity to therapy. Blood 2009;113:4341–51. http://dx.doi.org/10.1182/blood-2008-10-186668.
[27] Takeuchi K, Abe M, Hiasa M, Oda A, Amou H, Kido S, et al. Tgf-Beta inhibition restores terminal osteoblast differentiation to suppress myeloma growth. PLoS ONE 2010;5:e9870. http://dx.doi.org/10.1371/journal.pone.0009870.
[28] Logothetis CJ, Lin S-H. Osteoblasts in prostate cancer metastasis to bone. Nat Rev Cancer 2005;5:21–8. http://dx.doi.org/10.1038/nrc1528.
[29] Langley GR, Sabean HB, Sorger K. Sclerotic lesions of bone in myeloma. Can Med Assoc J 1966;94:940–6.
[30] Morán Blanco LM, Encinas Rodríguez C. Multiple myeloma with diffuse osteosclerosis: Distinct from POEMS syndrome. Radiologia 2013;56:e29–33. http://dx.doi.org/10.1016/j.rx.2013.04.003.
[31] Chen M, Green R. Circulating plasma cells with Russell bodies in osteosclerotic myeloma. Blood 2013;122:160.
[32] Kuo MC, Shih LY. Primary plasma cell leukemia with extensive dense osteosclerosis: complete remission following combination chemotherapy. Ann Hematol 1995;71:147–51.
[33] Mulleman D, Gaxatte C, Guillerm G, Leroy X, Cotten A, Duquesnoy B, et al. Multiple myeloma presenting with widespread osteosclerotic lesions. Joint Bone Spine 2004;71:79–83. http://dx.doi.org/10.1016/S1297-319X(03)00152-0.
[34] Olechnowicz SWZ, Edwards CM. Contributions of the host microenvironment to cancer-induced bone disease. Cancer Res 2014;74:1625–31. http://dx.doi.org/10.1158/0008-5472.CAN-13-2645.
[35] Park D, Spencer JA, Koh BI, Kobayashi T, Fujisaki J, Clemens TL, et al. Endogenous bone marrow MSCs are dynamic, fate-restricted participants in bone maintenance and regeneration. Cell Stem Cell 2012;10:259–72. http://dx.doi.org/10.1016/j.stem.2012.02.003.
[36] Kode A, Manavalan JS, Mosialou I, Bhagat G, Rathinam CV, Luo N, et al. Leukaemogenesis induced by an activating β-catenin mutation in osteoblasts. Nature 2014;506:240–4. http://dx.doi.org/10.1038/nature12883.

[37] Li X, Pennisi A, Yaccoby S. Role of decorin in the antimyeloma effects of osteoblasts. Blood 2008;112:159–68. http://dx.doi.org/10.1182/blood-2007-11-124164.

[38] Chen Z, Orlowski RZ, Wang M, Kwak L, McCarty N. Osteoblastic niche supports the growth of quiescent multiple myeloma cells. Blood 2014;123:2204–8. http://dx.doi.org/10.1182/blood-2013-07-517136.

[39] Yaccoby S. Osteoblastogenesis and tumor growth in myeloma. Leuk Lymphoma 2010;51:213–20. http://dx.doi.org/10.3109/10428190903503438.

[40] Yaccoby S, Wezeman MJ, Zangari M, Walker R, Cottler-Fox M, Gaddy D, et al. Inhibitory effects of osteoblasts and increased bone formation on myeloma in novel culture systems and a myelomatous mouse model. Haematologica 2006; 91:192–9.

[41] Nash LA, Sullivan PJ, Peters SJ, Ward WE. Rooibos flavonoids, orientin and luteolin, stimulate mineralization in human osteoblasts through the Wnt pathway. Mol Nutr Food Res 2014. http://dx.doi.org/10.1002/mnfr.201400592 [Epub ahead].

[42] Ishtiaq S, Edwards S, Sankaralingam A, Evans BAJ, Elford C, Frost ML, et al. The effect of nitrogen containing bisphosphonates, zoledronate and alendronate, on the production of pro-angiogenic factors by osteoblastic cells. Cytokine 2014;71:154–60. http://dx.doi.org/10.1016/j.cyto.2014.10.025.

[43] Guo D, Li Q, Lv Q, Wei Q, Cao S, Gu J. MiR-27a targets sFRP1 in hFOB cells to regulate proliferation, apoptosis and differentiation. PLoS ONE 2014;9:e91354. http://dx.doi.org/10.1371/journal.pone.0091354.

[44] Zhang W, Lee WY, Siegel DS, Tolias P, Zilberberg J. Patient-specific 3D microfluidic tissue model for multiple myeloma. Tissue Eng Part C Methods 2014;20:663–70. http://dx.doi.org/10.1089/ten.TEC.2013.0490.

[45] Tinhofer I, Biedermann R, Krismer M, Crazzolara R, Greil R. A role of TRAIL in killing osteoblasts by myeloma cells. FASEB J 2006;20:759–61. http://dx.doi.org/10.1096/fj.05-4329fje.

[46] Kassen D, Moore S, Percy L, Herledan G, Bounds D, Rodriguez-Justo M, et al. The bone marrow stromal compartment in multiple myeloma patients retains capability for osteogenic differentiation in vitro: defining the stromal defect in myeloma. Br J Haematol 2014;167:194–206. http://dx.doi.org/10.1111/bjh.13020.

[47] Rubin J, Fan X, Rahnert J, Sen B, Hsieh C-L, Murphy TC, et al. IGF-I secretion by prostate carcinoma cells does not alter tumor-bone cell interactions in vitro or in vivo. Prostate 2006;66:789–800. http://dx.doi.org/10.1002/pros.20379.

[48] Standal T, Johnson RW, McGregor NE, Poulton IJ, Ho PWM, Martin TJ, et al. gp130 in late osteoblasts and osteocytes is required for PTH-induced osteoblast differentiation. J Endocrinol 2014;223:181–90. http://dx.doi.org/10.1530/JOE-14-0424.

[49] Reagan MR, Ghobrial IM. Multiple myeloma-mesenchymal stem cells: characterization, origin, and tumor-promoting effects. Clin Cancer Res 2012;18:342–9. http://dx.doi.org/10.1158/1078-0432.CCR-11-2212.

[50] Fu R, Liu H, Zhao S, Wang Y, Li L, Gao S, et al. Osteoblast inhibition by chemokine cytokine ligand3 in myeloma-induced bone disease. Cancer Cell Int 2014;14:132. http://dx.doi.org/10.1186/s12935-014-0132-6.

[51] Ferrarini M, Steimberg N, Ponzoni M, Belloni D, Berenzi A, Girlanda S, et al. Ex-vivo dynamic 3-D culture of human tissues in the RCCS™ bioreactor allows the study of multiple myeloma biology and response to therapy. PLoS ONE 2013;8:e71613. http://dx.doi.org/10.1371/journal.pone.0071613.

[52] Narayanan NK, Duan B, Butcher JT, Mazumder A, Narayanan BA. Characterization of multiple myeloma clonal cell expansion and stromal Wnt/β-catenin signaling in hyaluronic acid-based 3D hydrogel. In Vivo (Brooklyn) 2014;28:67–73.

[53] Cappariello A, Maurizi A, Veeriah V, Teti A. The great beauty of the osteoclast. Arch Biochem Biophys 2014;558:70–8. http://dx.doi.org/10.1016/j.abb.2014.06.017.

[54] Schmiedel BJ, Scheible CA, Nuebling T, Kopp H-G, Wirths S, Azuma M, et al. RANKL expression, function, and therapeutic targeting in multiple myeloma and chronic lymphocytic leukemia. Cancer Res 2013;73:683–94. http://dx.doi.org/10.1158/0008-5472.CAN-12-2280.

[55] Clines GA, Guise TA. Hypercalcaemia of malignancy and basic research on mechanisms responsible for osteolytic and osteoblastic metastasis to bone. Endocr Relat Cancer 2005;12:549–83. http://dx.doi.org/10.1677/erc.1.00543.

[56] Colaianni G, Brunetti G, Faienza MF, Colucci S, Grano M. Osteoporosis and obesity: role of Wnt pathway in human and murine models. World J Orthop 2014;5:242–6. http://dx.doi.org/10.5312/wjo.v5.i3.242.

[57] Berendsen AD, Olsen BR. Osteoblast-adipocyte lineage plasticity in tissue development, maintenance and pathology. Cell Mol Life Sci 2014;71:493–7. http://dx.doi.org/10.1007/s00018-013-1440-z.

[58] Zhou BO, Yue R, Murphy MM, Peyer JG, Morrison SJ. Leptin-receptor-expressing mesenchymal stromal cells represent the main source of bone formed by adult bone marrow. Cell Stem Cell 2014;15:154–68. http://dx.doi.org/10.1016/j.stem.2014.06.008.

[59] Worthley DL, Churchill M, Compton JT, Tailor Y, Rao M, Si Y, et al. Gremlin 1 identifies a skeletal stem cell with bone, cartilage, and reticular stromal potential. Cell 2015;160:269–84. http://dx.doi.org/10.1016/j.cell.2014.11.042.

[60] Chan CKF, Seo EY, Chen JY, Lo D, McArdle A, Sinha R, et al. Identification and specification of the mouse skeletal stem cell. Cell 2015;160:285–98. http://dx.doi.org/10.1016/j.cell.2014.12.002.

[61] Pan SY, Johnson KC, Ugnat A-M, Wen SW, Mao Y. Association of obesity and cancer risk in Canada. Am J Epidemiol 2004;159:259–68.

[62] Wallin A, Larsson SC. Body mass index and risk of multiple myeloma: a meta-analysis of prospective studies. Eur J Cancer 2011;47:1606–15. http://dx.doi.org/10.1016/j.ejca.2011.01.020.

[63] Sola B, Poirot M, de Medina P, Bustany S, Marsaud V, Silvente-Poirot S, et al. Antiestrogen-binding site ligands induce autophagy in myeloma cells that proceeds through alteration of cholesterol metabolism. Oncotarget 2013;4:911–22.

[64] Wang X, Yan Z, Fulciniti M, Li Y, Gkotzamanidou M, Amin SB, et al. Transcription factor-pathway coexpression analysis reveals cooperation between SP1 and ESR1 on dysregulating cell cycle arrest in non-hyperdiploid multiple myeloma. Leukemia 2014;28:894–903. http://dx.doi.org/10.1038/leu.2013.233.

[65] Islam R, Altundag K, Kurt M, Altundag O, Turen S. Association between obesity and multiple myeloma in postmenopausal women may be attributed to increased aromatization of androgen in adipose tissue. Med Hypotheses 2005;65:1001–2. http://dx.doi.org/10.1016/j.mehy.2005.05.014.

[66] Hardaway AL, Herroon MK, Rajagurubandara E, Podgorski I. Bone marrow fat: linking adipocyte-induced inflammation with skeletal metastases. Cancer Metastasis Rev 2014;33:527–43. http://dx.doi.org/10.1007/s10555-013-9484-y.

[67] Caers J, Deleu S, Belaid Z, De Raeve H, Van Valckenborgh E, De Bruyne E, et al. Neighboring adipocytes participate in the bone marrow microenvironment of multiple myeloma cells. Leukemia 2007;21:1580–4. http://dx.doi.org/10.1038/sj.leu.2404658.

[68] Adler BJ, Kaushansky K, Rubin CT. Obesity-driven disruption of haematopoiesis and the bone marrow niche. Nat Rev Endocrinol 2014;10:737–48. http://dx.doi.org/10.1038/nrendo.2014.169.

[69] Vogl DT, Wang T, Pérez WS, Stadtmauer EA, Heitjan DF, Lazarus HM, et al. Effect of obesity on outcomes after autologous hematopoietic stem cell transplantation for multiple myeloma. Biol Blood Marrow Transplant 2011;17:1765–74. http://dx.doi.org/10.1016/j.bbmt.2011.05.005.

[70] Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, et al. Osteoblastic cells regulate the haematopoietic stem cell niche. Nature 2003;425:841–6. http://dx.doi.org/10.1038/nature02040.

[71] Panaroni C, Tzeng Y-S, Saeed H, Wu JY. Mesenchymal progenitors and the osteoblast lineage in bone marrow hematopoietic niches. Curr Osteoporos Rep 2014; 12:22–32. http://dx.doi.org/10.1007/s11914-014-0190-7.

[72] Wang L, Jin N, Schmitt A, Greiner J, Malcherek G, Hundemer M, et al. T cell-based targeted immunotherapies for patients with multiple myeloma. Int J Cancer 2014;136:1751–68. http://dx.doi.org/10.1002/ijc.29190.

[73] Ramachandran IR, Martner A, Pisklakova A, Condamine T, Chase T, Vogl T, et al. Myeloid-derived suppressor cells regulate growth of multiple myeloma by inhibiting T cells in bone marrow. J Immunol 2013;190:3815–23. http://dx.doi.org/10.4049/jimmunol.1203373.

[74] Braga WMT, Atanackovic D, Colleoni GWB. The role of regulatory T cells and TH17 cells in multiple myeloma. Clin Dev Immunol 2012;2012:293479. http://dx.doi.org/10.1155/2012/293479.

[75] Fu R, Gao S, Peng F, Li J, Liu H, Wang H, et al. Relationship between abnormal osteoblasts and cellular immunity in multiple myeloma. Cancer Cell Int 2014;14:62. http://dx.doi.org/10.1186/1475-2867-14-62.

[76] McMillin DW, Delmore J, Weisberg E, Negri JM, Geer DC, Klippel S, et al. Tumor cell-specific bioluminescence platform to identify stroma-induced changes to anticancer drug activity. Nat Med 2010;16:483–9. http://dx.doi.org/10.1038/nm.2112.

[77] Roccaro AM, Sacco A, Maiso P, Azab AK, Tai Y-T, Reagan M, et al. BM mesenchymal stromal cell-derived exosomes facilitate multiple myeloma progression. J Clin Invest 2013;123:1542–55. http://dx.doi.org/10.1172/JCI66517.

[78] Reagan MR, Kaplan DL. Concise review: mesenchymal stem cell tumor-homing: detection methods in disease model systems. Stem Cells 2011;29:920. http://dx.doi.org/10.1002/stem.645.

[79] Houthuijzen JM, Daenen LGM, Roodhart JML, Voest EE. The role of mesenchymal stem cells in anti-cancer drug resistance and tumour progression. Br J Cancer 2012;106:1901–6. http://dx.doi.org/10.1038/bjc.2012.201.

[80] Azab F, Vali S, Abraham J, Potter N, Muz B, de la Puente P, et al. PI3KCA plays a major role in multiple myeloma and its inhibition with BYL719 decreases proliferation, synergizes with other therapies and overcomes stroma-induced resistance. Br J Haematol 2014;165:89–101. http://dx.doi.org/10.1111/bjh.12734.

[81] Corre J, Mahtouk K, Attal M, Gadelorge M, Huynh A, Fleury-Cappellesso S, et al. Bone marrow mesenchymal stem cells are abnormal in multiple myeloma. Leukemia 2007;21:1079–88. http://dx.doi.org/10.1038/sj.leu.2404621.

[82] Birmingham E, Niebur GL, McHugh PE, Shaw G, Barry FP, McNamara LM. Osteogenic differentiation of mesenchymal stem cells is regulated by osteocyte and osteoblast cells in a simplified bone niche. Eur Cell Mater 2012;23:13–27.

[83] Sims NA, Vrahnas C. Regulation of cortical and trabecular bone mass by communication between osteoblasts, osteocytes and osteoclasts. Arch Biochem Biophys 2014;561C:22–8. http://dx.doi.org/10.1016/j.abb.2014.05.015.

[84] Compton JT, Lee FY. A review of osteocyte function and the emerging importance of sclerostin. J Bone Joint Surg Am 2014;96:1659–68. http://dx.doi.org/10.2106/JBJS.M.01096.

[85] Giuliani N, Ferretti M, Bolzoni M, Storti P, Lazzaretti M, Dalla Palma B, et al. Increased osteocyte death in multiple myeloma patients: role in myeloma-induced osteoclast formation. Leukemia 2012;26:1391–401. http://dx.doi.org/10.1038/leu.2011.381.

[86] Delgado-Calle J, Bellido T, Roodman GD. Role of osteocytes in multiple myeloma bone disease. Curr Opin Support Palliat Care 2014;8:407–13. http://dx.doi.org/10.1097/SPC.0000000000000090.

[87] Del Fattore A, Teti A. The tight relationship between osteoclasts and the immune system. Inflamm Allergy Drug Targets 2012;11:181–7.

[88] Andersen TL, Sondergaard TE, Skorzynska KE, Dagnaes-Hansen F, Plesner TL, Hauge EM, et al. A physical mechanism for coupling bone resorption and formation in adult human bone. Am J Pathol 2009;174:239–47. http://dx.doi.org/10.2353/ajpath.2009.080627.

[89] Jensen PR, Andersen TL, Hauge E-M, Bollerslev J, Delaissé J-M. A joined role of canopy and reversal cells in bone remodeling—lessons from glucocorticoid-induced osteoporosis. Bone 2014;73C:16–23. http://dx.doi.org/10.1016/j.bone.2014.12.004.

[90] Andersen TL, Søe K, Sondergaard TE, Plesner T, Delaisse J-M. Myeloma cell-induced disruption of bone remodelling compartments leads to osteolytic lesions and generation of osteoclast-myeloma hybrid cells. Br J Haematol 2010;148:551–61. http://dx.doi.org/10.1111/j.1365-2141.2009.07980.x.

[91] Kondoh S, Inoue K, Igarashi K, Sugizaki H, Shirode-Fukuda Y, Inoue E, et al. Estrogen receptor α in osteocytes regulates trabecular bone formation in female mice. Bone 2014;60:68–77. http://dx.doi.org/10.1016/j.bone.2013.12.005.
[92] Hofbauer LC, Rachner TD, Coleman RE, Jakob F. Endocrine aspects of bone metastases. Lancet Diabetes Endocrinol 2014;2:500–12. http://dx.doi.org/10.1016/S2213-8587(13)70203-1.
[93] Patti A, Gennari L, Merlotti D, Dotta F, Nuti R. Endocrine actions of osteocalcin. Int J Endocrinol 2013;2013:846480. http://dx.doi.org/10.1155/2013/846480.
[94] Hu B, Chen Y, Usmani SZ, Ye S, Qiang W, Papanikolaou X, et al. Characterization of the molecular mechanism of the bone-anabolic activity of carfilzomib in multiple myeloma. PLoS ONE 2013;8:e74191. http://dx.doi.org/10.1371/journal.pone.0074191.
[95] Swami A, Reagan MR, Basto P, Mishima Y, Kamaly N, Glavey S, et al. Engineered nanomedicine for myeloma and bone microenvironment targeting. Proc Natl Acad Sci U S A 2014;111:10287–92. http://dx.doi.org/10.1073/pnas.1401337111.
[96] Zangari M, Terpos E, Zhan F, Tricot G. Impact of bortezomib on bone health in myeloma: a review of current evidence. Cancer Treat Rev 2012;38:968–80. http://dx.doi.org/10.1016/j.ctrv.2011.12.007.
[97] Daoussis D, Andonopoulos AP. The emerging role of Dickkopf-1 in bone biology: is it the main switch controlling bone and joint remodeling? Semin Arthritis Rheum 2011;41:170–7. http://dx.doi.org/10.1016/j.semarthrit.2011.01.006.
[98] Clarke BL. Anti-sclerostin antibodies: utility in treatment of osteoporosis. Maturitas 2014;78:199–204. http://dx.doi.org/10.1016/j.maturitas.2014.04.016.
[99] Colucci S, Brunetti G, Oranger A, Mori G, Sardone F, Specchia G, et al. Myeloma cells suppress osteoblasts through sclerostin secretion. Blood Cancer J 2011;1:e27. http://dx.doi.org/10.1038/bcj.2011.22.
[100] Politou MC, Heath DJ, Rahemtulla A, Szydlo R, Anagnostopoulos A, Dimopoulos MA, 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. http://dx.doi.org/10.1002/ijc.22033.
[101] Kaiser M, Mieth M, Liebisch P, Oberländer R, Rademacher J, Jakob C, et al. Serum concentrations of DKK-1 correlate with the extent of bone disease in patients with multiple myeloma. Eur J Haematol 2008;80:490–4. http://dx.doi.org/10.1111/j.1600-0609.2008.01065.x.
[102] Fulciniti M, Tassone P, Hideshima T, Vallet S, Nanjappa P, Ettenberg SA, et al. Anti-DKK1 mAb (BHQ880) as a potential therapeutic agent for multiple myeloma. Blood 2009;114:371–9. http://dx.doi.org/10.1182/blood-2008-11-191577.
[103] 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. http://dx.doi.org/10.1016/j.blre.2013.08.002.
[104] Yaccoby S, Ling W, Zhan F, Walker R, Barlogie B, Shaughnessy JD. Antibody-based inhibition of DKK1 suppresses tumor-induced bone resorption and multiple myeloma growth in vivo. Blood 2007;109:2106–11. http://dx.doi.org/10.1182/blood-2006-09-047712.
[105] Garcia-Gomez A, Ocio EM, Crusoe E, Santamaria C, Hernández-Campo P, Blanco JF, et al. Dasatinib as a bone-modifying agent: anabolic and anti-resorptive effects. PLoS ONE 2012;7:e34914. http://dx.doi.org/10.1371/journal.pone.0034914.
[106] Silbermann R, Bolzoni M, Storti P, Guasco D, Bonomini S, Zhou D, et al. Bone marrow monocyte-/macrophage-derived activin A mediates the osteoclastogenic effect of IL-3 in multiple myeloma. Leukemia 2014;28:951–4. http://dx.doi.org/10.1038/leu.2013.385.
[107] Vallet S, Mukherjee S, Vaghela N, Hideshima T, Fulciniti M, Pozzi S, et al. Activin A promotes multiple myeloma-induced osteolysis and is a promising target for myeloma bone disease. Proc Natl Acad Sci U S A 2010;107:5124–9. http://dx.doi.org/10.1073/pnas.0911929107.
[108] Chantry AD, Heath D, Mulivor AW, Pearsall S, Baud'huin M, Coulton L, 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. http://dx.doi.org/10.1002/jbmr.142.
[109] Pennisi A, Ling W, Li X, Khan S, Wang Y, Barlogie B, et al. Consequences of daily administered parathyroid hormone on myeloma growth, bone disease, and molecular profiling of whole myelomatous bone. PLoS ONE 2010;5:e15233. http://dx.doi.org/10.1371/journal.pone.0015233.
[110] Jilka RL. Molecular and cellular mechanisms of the anabolic effect of intermittent PTH. Bone 2007;40:1434–46. http://dx.doi.org/10.1016/j.bone.2007.03.017.
[111] Martin TJ. Bone biology and anabolic therapies for bone: current status and future prospects. J Bone Metab 2014;21:8–20. http://dx.doi.org/10.11005/jbm.2014.21.1.8.
[112] Walker RE, Lawson MA, Buckle CH, Snowden JA, Chantry AD. Myeloma bone disease: pathogenesis, current treatments and future targets. Br Med Bull 2014;111:117–38. http://dx.doi.org/10.1093/bmb/ldu016.
[113] Coleman R, Gnant M, Morgan G, Clezardin P. Effects of bone-targeted agents on cancer progression and mortality. J Natl Cancer Inst 2012;104:1059–67. http://dx.doi.org/10.1093/jnci/djs263.
[114] Pozzi S, Raje N. The role of bisphosphonates in multiple myeloma: mechanisms, side effects, and the future. Oncologist 2011;16:651–62. http://dx.doi.org/10.1634/theoncologist. 2010-0225.
[115] Mohty M, Malard F, Mohty B, Savani B, Moreau P, Terpos E. The effects of bortezomib on bone disease in patients with multiple myeloma. Cancer 2014;120:618–23. http://dx.doi.org/10.1002/cncr.28481.
[116] Effect of Low Dose Bortezomib on Bone Formation in Smoldering Myeloma Patients n.d. https://clinicaltrials.gov/ct2/show/record/NCT00983346 (accessed January 04, 2015).
[117] A Phase IIa Study of Sotatercept on Bone Mass and Turnover in Patients With Multiple Myeloma - Tabular View - ClinicalTrials.gov n.d. https://clinicaltrials.gov/ct2/show/NCT02230917 (accessed January 04, 2015).
[118] Abdulkadyrov KM, Salogub GN, Khuazheva NK, Sherman ML, Laadem A, Barger R, et al. Sotatercept in patients with osteolytic lesions of multiple myeloma. Br J Haematol 2014;165:814–23. http://dx.doi.org/10.1111/bjh.12835.
[119] Platt RJ, Chen S, Zhou Y, Yim MJ, Swiech L, Kempton HR, et al. CRISPR-Cas9 knockin mice for genome editing and cancer modeling. Cell 2014;159:440–55. http://dx.doi.org/10.1016/j.cell.2014.09.014.
[120] Lu G, Middleton RE, Sun H, Naniong M, Ott CJ, Mitsiades CS, et al. The myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins. Science 2014;343:305–9. http://dx.doi.org/10.1126/science.1244917.
[121] Eskildsen T, Taipaleenmäki H, Stenvang J, Abdallah BM, Ditzel N, Nossent AY, et al. MicroRNA-138 regulates osteogenic differentiation of human stromal (mesenchymal) stem cells in vivo. Proc Natl Acad Sci U S A 2011;108:6139–44. http://dx.doi.org/10.1073/pnas.1016758108.
[122] Sung B, Oyajobi B, Aggarwal BB. Plumbagin inhibits osteoclastogenesis and reduces human breast cancer-induced osteolytic bone metastasis in mice through suppression of RANKL signaling. Mol Cancer Ther 2012;11:350–9. http://dx.doi.org/10.1158/1535-7163.MCT-11-0731.
[123] Roodman GD. Genes associate with abnormal bone cell activity in bone metastasis. Cancer Metastasis Rev 2012;31:569–78. http://dx.doi.org/10.1007/s10555-012-9372-x.
[124] Zhai Z, Qu X, Yan W, Li H, Liu G, Liu X, et al. Andrographolide prevents human breast cancer-induced osteoclastic bone loss via attenuated RANKL signaling. Breast Cancer Res Treat 2014;144:33–45. http://dx.doi.org/10.1007/s10549-014-2844-7.
[125] Zinonos I, Luo K-W, Labrinidis A, Liapis V, Hay S, Panagopoulos V, et al. Pharmacologic inhibition of bone resorption prevents cancer-induced osteolysis but enhances soft tissue metastasis in a mouse model of osteolytic breast cancer. Int J Oncol 2014;45:532–40. http://dx.doi.org/10.3892/ijo.2014.2468.