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REVIEW

Interaction between the skeletal and immune systems in cancer: mechanisms and clinical implications

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Abstract The skeletal and immune systems have a complex relationship. Both systems are intimately coupled, with osteoclastogenesis and hematopoiesis occurring in the bone marrow. Bone and immune cells also share common hematopoietic precursors. Furthermore, the skeletal and immune systems share various cytokines, receptors, and transcription factors that regulate signal transduction pathways involved in osteoclastogenesis and immune system activation, including the receptor activator of nuclear factor-κB ligand/receptor activator of nuclear factor-κB/osteoprotegerin (RANKL–RANK–OPG) pathway. Cancer cells can disrupt both the skeletal and immune systems. Interaction between cancer and bone cells results in a vicious cycle of bone destruction and cancer growth. Bone remodeling generates a growth-factor-rich environment that attracts cancer cells and promotes their proliferation. In turn, cancer cells stimulate osteoclast formation and activity, resulting in additional bone resorption that further stimulates cancer cell growth. Currently available bone-targeted therapies may also modulate the immune system. Bisphosphonates such as zoledronic acid exert stimulating effects on the immune system, resulting in possible anticancer activity against malignant cells. Denosumab, an anti-RANKL monoclonal antibody with proven antiosteoclast activity, may suppress immune responses. This may result in the reported association with an increased risk of neoplasms, as well as serious skin and other infections as reported in some studies, mainly in the postmenopausal setting. When assessing bone-targeted therapies, it is important to consider the shared signaling pathways between bone and the immune system, as well as the clinical risk:benefit ratio.

Keywords Anticancer · Bisphosphonates · Denosumab · Osteoimmunology · RANKL inhibition · Zoledronic acid

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Introduction

The skeletal and immune systems are interconnected in normal (physiologic) and pathologic conditions. Both systems are intimately coupled, as osteoclastogenesis and hematopoiesis occur in the bone marrow. Osteoclasts, macrophages, and dendritic cells also share common precursors. Furthermore, the skeletal and immune systems share various cytokines, receptors, adaptor proteins, signaling molecules, and transcription factors, thereby allowing crosstalk to occur between the various cells and their respective signal transduction pathways involved in osteoclastogenesis and hematopoiesis.

Osteoclastogenesis and hematopoiesis

Hematopoietic stem cells are maintained in the bone marrow. Adjacent osteoblast precursors produce signals that control hematopoietic stem cell replication and differentiation. Hematopoietic stem cells may either maintain their pluripotency or differentiate into multipotential progenitor cells, which have the capacity to form common lymphoid progenitor or common myeloid precursor cells. Common lymphoid progenitor cells undergo additional differentiation to form T lymphocytes, B lymphocytes, or natural killer cells, whereas common myeloid precursor cells form all other myeloid lineages and preosteoclasts.

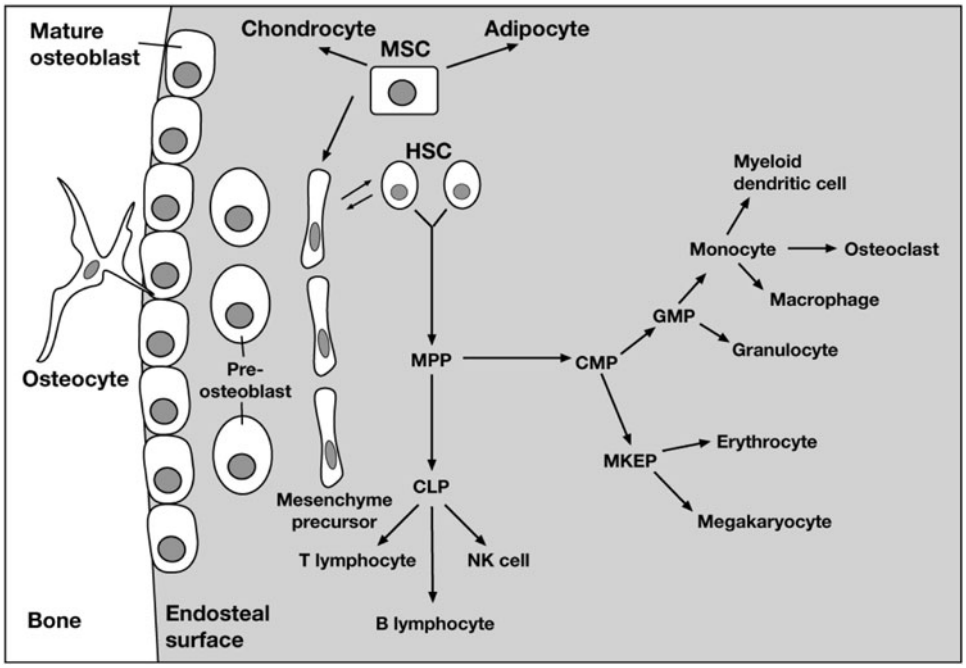

Fig. 1 Interaction between osteoblastic and hematopoietic cell lineages. Abbreviations: CLP, common lymphoid progenitor; CMP, common myeloid precursor; GMP, granulocyte–macrophage progenitor; HSC, hematopoietic stem cell; MKEP, megakaryocyte-erythroid progenitor; MPP, multipotential progenitor; MSC, mesenchymal stem cell; NK, natural killer. Reprinted from Lorenzo J, et al. (2008) Osteoimmunology: interactions of the bone and immune system. *Endocr Rev* 29:403–440. Copyright 2008 [1]

Activated osteoclasts are formed from the fusion of pre-osteoclasts and multinucleated osteoclasts, the regulation of which is complex and affected by multiple factors. Multipotential stem cells differentiate into chondrocytes, adipocytes, and mesenchyme precursors; the latter undergo differentiation to form preosteoblasts and, eventually, mature matrix-producing osteoblasts. Osteoblasts may remain on the bone surface as lining cells or undergo terminal differentiation to form osteocytes, which become encased in the mineralized bone matrix (Fig. 1) [1]. The shared lineages and paracrine signaling between osteoclasts and hematopoietic cells highlight the potential for bone-targeted agents to influence the immune system.

Crosstalk between skeletal and immune system components

The skeletal and immune systems share various signal transduction pathways, thereby allowing a complex interplay to occur between bone metabolism and immunology. Furthermore, immune system components, such as T cells, cytokines, and chemokines, can exert substantial effects on osteoclastogenesis.

Signal transduction in osteoclastogenesis

Osteoclastogenesis is primarily regulated via interactions between c-FMS and macrophage colony-stimulating factor, receptor activator of nuclear factor (NF)-κB (RANK) and RANK ligand (RANKL), and immunoglobulin (Ig)-like receptors and their ligands (Fig. 2) [2]. The role of RANK signaling in osteoclastogenesis has also been reviewed elsewhere [2–16]. Other key regulatory pathways are described below.

RANK signaling

Receptor activator of NF-κB ligand is a member of the tumor necrosis factor (TNF) cytokine superfamily that is expressed by osteoblasts, monocytes, neutrophils, dendritic cells, B lymphocytes, and T lymphocytes [3]. Secretion of RANKL by osteoclastogenesis-supporting cells (osteoblasts and synovial fibroblasts) occurs in response to osteoclastogenic factors such as 1,25-dihydroxyvitamin D3, prostaglandin E2, and parathyroid hormone [2]. T cells express RANKL as a type-2 membrane-bound protein and also release it in soluble form, although the function of the soluble form remains unknown [16]. Inflammatory cytokines, such as interleukin (IL)-1, IL-6, and TNF-α, also potently induce RANKL expression on osteoblasts and synovial fibroblasts, thereby stimulating RANKL signaling [2].

Receptor activator of NF-κB, the RANKL receptor, shares high homology with CD40, which is expressed on lymphocytes and, similar to RANKL, is reported to play a role in atherosclerosis and coronary artery disease [17–19]. Interaction of RANK with RANKL is inhibited by osteoprotegerin (OPG), a soluble competitor (decoy) receptor that binds to RANKL [12, 13]. Receptor activator of NF-κB lacks intrinsic enzymatic activity in its intracellular domain and transduces signals by recruiting adaptor molecules such as the TNF-receptor-associated factor (TRAF)

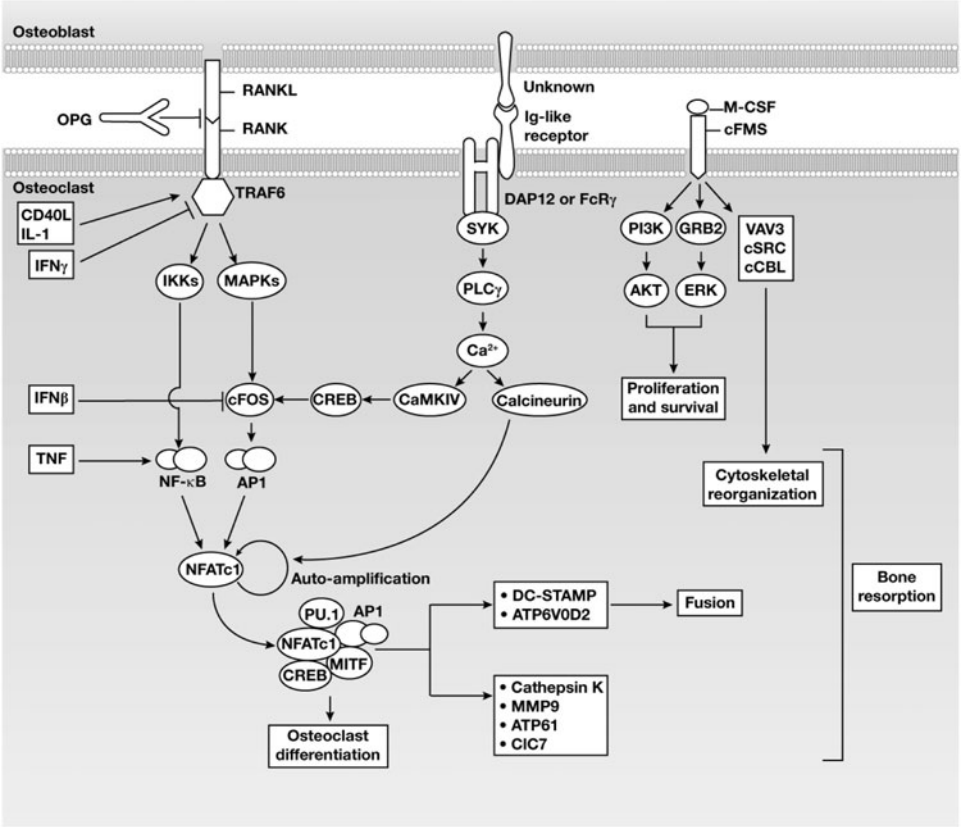

Fig. 2 Osteoimmunologic interactions between osteoblasts and osteoclasts. Abbreviations: AP1, activator protein 1; CaMKIV, calcium-/calmodulin-dependent protein kinase type IV; CD40L, CD40 ligand; ClC, chloride channel; CREB, cyclic AMP responsive-element-binding protein; DAP, DNAX-activating protein; DC-STAMP, dendritic-cell-specific transmembrane protein; FcRγ, Fc-receptor common γ-subunit; GRB2-ERK, growth-factor-receptor-bound protein 2–extracellular-signal-regulated kinase; Ig, immunoglobulin; IFN, interferon; IL, interleukin; IKK, inhibitor of NF-κB kinase; MAPKs, mitogen-activated protein kinases; M-CSF, macrophage colony-stimulating factor; MITF, microphthalmia-associated transcription factor; MMP, matrix metalloproteinase; NFATc1, nuclear factor of activated T-cell cytoplasmic 1; NF-κB, nuclear factor-κB; OPG, osteoprotegerin; PI3K, phosphatidylinositol 3-kinase; PLC, phospholipase C; RANK, receptor activator of nuclear factor-κB; RANKL, receptor activator of nuclear factor-κB ligand; SYK, spleen tyrosine kinase; TNF, tumor necrosis factor; TRAF6, tumor necrosis factor receptor-associated factor 6. Reprinted by permission by Macmillan Publishers Ltd: *Nature Reviews Immunology*. Takayanagi H (2007) Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems. *Nat Rev Immunol*. 7:292–304. Copyright 2007 [2]

family of proteins, especially TRAF6 [4, 5, 15]. By an unknown mechanism, RANKL binding to RANK induces trimerization of RANK and TRAF6, leading to activation of NF-κB and of mitogen-activated protein kinases such as Jun N-terminal kinase and p38 [6]. Activated RANK can also lead to stimulation of Ig-like receptor signaling.

Signaling through nuclear factor of activated T-cell cytoplasmic (NFATc)-1

Expression of NFATc-1, the master regulator of osteoclast differentiation, depends on induction of the TRAF6–NF-κB and c-FOS pathways, in addition to activation of calcium signaling [20]. Nuclear factor of activated T-cell cytoplasmic-1 is initially induced by TRAF6-activated NF-κB and NFATc-2. After translocation into the nucleus, NFATc-1 autoregulates its own expression by binding to the NFAT-binding site of its promoter, enabling robust induction of NFATc-1 expression [21]. Activator protein 1 and continuous activation of calcium signaling by calcineurin are crucial for NFATc-1 autoamplification [20]. Nuclear factor of activated T-cell cytoplasmic-1 cooperates with other transcription factors, such as AP1, PU.1, microphthalmia-associated transcription factor, and cyclic AMP responsive-element-binding protein, to regulate various osteoclast-specific genes, including tartrate-resistant acid phosphatase, cathepsin K, calcitonin receptor, osteoclast-associated receptor, and β3-integrin [2, 20, 22–24].

Effects of cytokines and chemokines on osteoclastogenesis

Immune cells produce a variety of proinflammatory cytokines that contribute to bone damage [25]. Tumor

Table 1 Cytokines involved in osteoclastogenesis

Cytokine	Main producer cells	Primary target in osteoclastogenesis	Effect on osteoclastogenesis	Role in osteoimmunology
RANKL	T-cells; Osteoblasts	Osteoclast precursor cells	Activation	Induction of osteoclast differentiation
TNF-α	Macrophages; Th1 cells	Osteoclast precursor cells; mesenchymal cells	Activation	RANKL induction on mesenchymal cells, RANKL synergy, inflammation
IL-6	Th2 cells; dendritic cells	Mesenchymal cells; T cells	Activation	RANKL induction on mesenchymal cells, Th17-cell differentiation, inflammation
IL-17	Th17 cells; memory T cells	Mesenchymal cells	Activation	RANKL induction on mesenchymal cells, inflammation
IFN-γ	Th1 cells; natural killer cells	Osteoclast precursor cells	Inhibition	RANKL signaling inhibition, cellular immunity
IL-4	Th2 cells; natural killer T cells	Osteoclast precursor cells	Inhibition	RANKL signaling inhibition, humoral immunity
IL-10	Th2 cells	Osteoclast precursor cells	Inhibition	RANKL signaling inhibition, antiinflammatory
IL-12	Macrophages; dendritic cells	T cells	Inhibition	Th1-cell differentiation, IFN-γ and GM-CSF induction
IL-18	Macrophages; dendritic cells	T cells	Inhibition	Th1-cell differentiation, IFN-γ induction
GM-CSF	Th1 cells	Osteoclast precursor cells	Inhibition	RANKL signaling inhibition, granulocyte differentiation

GM-CSF granulocyte–macrophage colony-stimulating factor, *IFN* interferon, *IL*, interleukin, *RANKL* receptor activator of nuclear factor-κB ligand, *Th* T-helper, *TNF* tumor necrosis factor

necrosis factor-alpha and IL-1, -3, -6, -7, -11, -15, and -17 potentiate bone loss by inducing RANKL expression on osteoblasts or by increasing osteoclast differentiation and activation. In contrast, IL-4, -5, -10, -12, -13, and -18 and interferon (IFN)-α, -β, and -γ inhibit osteoclastogenesis by directly or indirectly blocking RANKL signaling (Table 1). Interleukin-1 stimulates TRAF6 expression, thereby potentiating the RANKL–RANK signaling cascade and inducing mature osteoclasts to perform bone-resorbing activity. Interferon gamma down-regulates TRAF6 expression via proteosomal degradation, resulting in termination of osteoclast formation [26, 27]. Receptor activator of NF-κB induces expression of IFN-β in osteoclast precursor cells, and IFN-β functions as a negative feedback regulator of osteoclast differentiation by interfering with RANKL-induced c-FOS expression [28]. Tumor necrosis factor-alpha stimulates NF-κB activation primarily via interacting with TRAF2. Although TNF-α alone cannot induce osteoclastogenesis and TNF-α overexpression cannot rescue RANKL deficiency, TNF-α combined with transforming growth factor (TGF)-β induces osteoclastogenesis even in the absence of RANK or TRAF6 [29–31]. These results suggest that TNF-α plays a pivotal role in the pathologic activation of osteoclasts associated with inflammation (Fig. 2) [2]. Osteoblast-mediated bone formation is also affected by various soluble cytokines such as TNF-α, IL-1, and IL-4 [32]. The molecular mechanisms involved in osteoblast regulation by the immune system and the pathologic significance of such regulation are less understood than in osteoclasts.

Effects of T cells on osteoclastogenesis

In general, activated T cells exert an inhibitory effect on osteoclastogenesis. The $CD4^+$ T-helper (Th) cells have traditionally been divided into 2 main subtypes—Th1 and Th2—based on their associated cytokine profiles. The Th1 cells mainly produce IFN-γ and IL-2 and mediate cellular immunity. In contrast, Th2 cells mainly produce IL-4, IL-5, and IL-10 and mediate humoral immunity. Although T cells express RANKL, most Th1 cytokines, as well as certain Th2 cytokines (e.g., IL-4 and IL-10), exert an inhibitory effect on osteoclastogenesis. However, the Th-cell subset involved in producing IL-17 (Th17 cells) is considered to be the typical osteoclastogenic Th subset. The Th17 cells express RANKL at higher levels than Th1 or Th2 cells and, as a result, may directly participate in osteoclastogenesis. In addition, Th17 cells do not produce large amounts of IFN-γ, an inhibitor of osteoclastogenesis. Furthermore, Th17 cells activate local inflammation, triggering release of proinflammatory cytokines that potentiate RANKL expression on osteoclastogenesis-supporting cells and RANKL–RANK signal transduction in osteoclast precursor cells [33]. Interleukin-17, produced by Th17 cells, induces the synthesis of matrix-degrading enzymes,

such as matrix metalloproteinases, that mediate bone and cartilage degradation [34]. The effects of Th17 cells on osteoclastogenesis are balanced by regulatory T cells, which suppress osteoclast formation via a cytokine-dependent mechanism mediated by TGF-β, IFN-γ, IL-4, and IL-10 [35–37]. Therefore, the effects of T cells on osteoclastogenesis depend on the balance between positive and negative factors expressed by these cells under pathologic conditions.

Disruption of the skeletal and immune systems in cancer

Tumorigenesis can disrupt the skeletal and immune systems. Tumor growth and metastasis necessitate evasion of the immune system, especially phosphoantigen-targeted gamma delta T cells ($\gamma\delta$ T cells), which can detect and destroy cancer cells. Immune system components also play other key roles in tumor development and progression. For example, tumor-associated macrophages (TAMs) are abundant in the bone microenvironment and influence multiple steps in tumor development, including growth, survival, invasion, and metastasis, as well as angiogenesis and lymphangiogenesis [38, 39]. During early metastasis of solid tumors, disseminated tumor cells (DTCs) survive in the bone marrow of patients with various tumor types. Cancers for which DTCs have been detected in patients who have not developed overt metastases include breast, colon, gastric, lung, and prostate cancers [40–46]. The hematopoietic niche in the bone marrow also provides a "harbor" for DTCs to survive despite anticancer therapies. Whether this niche also harbors cancer cells against anticancer immune defenses is unknown. However, the shared signal transduction pathways among the bone remodeling and immune system machineries in this common microenvironment suggest that activation of this vicious cycle of tumor growth and osteolytic bone destruction could also lead to localized immunosuppression or recruitment of metastasis-supporting TAMs, an unfortunate juxtaposition of osteoimmunology effects. Later in the disease course, interactions between malignant cells and bone may result in a vicious cycle of bone destruction and cancer growth (the "seed and soil theory") [47]. The effects of cancer on bone can result in skeletal-related events (SREs) that include pathologic fracture, spinal cord compression, hypercalcemia of malignancy, and the need for radiotherapy. Furthermore, some cancers such as myeloma can exert additional deleterious effects on bone metabolism via inducing osteolysis, systemic bone loss, and suppression of new bone formation throughout the skeleton [48, 49].

Effects of osteoclastogenesis on cancer growth and metastases

Osteoclast-mediated osteolysis results in release of growth factors in the bone microenvironment that facilitate cancer growth and metastases. Bone-derived cytokines provide a chemotactic stimulus for directed tumor cell migration [50]. Recent studies established that RANKL is a chemoattractant that increases migration and invasion of RANK-positive cancer cells (bone tropism) [51, 52]. In preclinical models, bone resorption by bone cell cultures stimulated proliferation of various tumor cell types, including breast cancer that possessed bone-metastasizing properties [53]. In animal models, cancer cells located immediately adjacent to bone surfaces had significantly greater proliferation rates compared with those distant from bone, suggesting a mitogenic effect within the bone microenvironment [54]. Furthermore, in an animal model wherein bone resorption was stimulated by tumor cells, the proliferation rate of metastatic cancer cells was increased in bone but not in other tissues [55].

Effects of tumorigenesis on bone resorption

Cancer cells stimulate osteoclast-mediated osteolysis via several mechanisms. Cancer cells may express RANKL and RANK, up-regulate RANKL expression by other osteoimmune cell types, down-regulate OPG expression, and stimulate release of factors that activate RANKL–RANK signaling in osteoclasts (Fig. 3) [56]. Expression of RANKL has been detected in prostate cancer cells [57] and

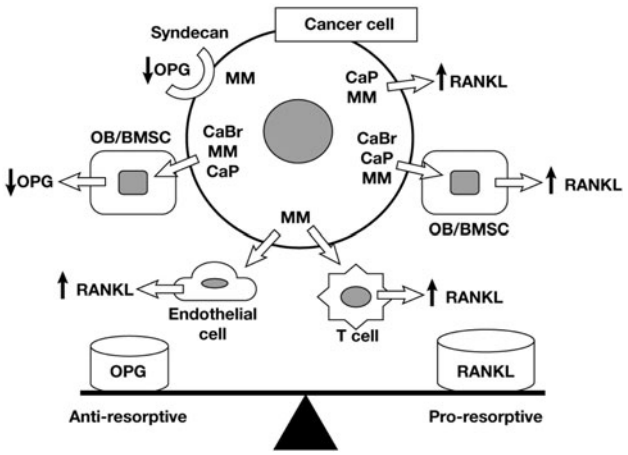

Fig. 3 Mechanisms by which cancer cells may promote bone resorption via regulation of RANKL and/or OPG. Abbreviations: BMSC, bone marrow stromal cells; CaBr, breast cancer; CaP, prostate cancer; MM, multiple myeloma; OB, osteoblasts; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor-κB ligand. Reprinted from Kearns AE, et al. Receptor activator of nuclear factor κB ligand and osteoprotegerin regulation of bone remodeling in health and disease. *Endocr Rev.* 29:155–192. Copyright 2008 [56]

multiple myeloma (MM) cells [58, 59], and RANKL expression by MM cells correlated with the propensity to cause bone destruction [58]. Although breast cancer cells do not typically express RANKL [60, 61], they can up-regulate RANKL expression by osteoblasts [60, 61] and bone marrow stromal cells [61, 62]. Prostate cancer cells can up-regulate RANKL expression in osteoblasts [63], and MM cells up-regulate RANKL expression in bone marrow stromal cells [64], endothelial cells [65], and T cells [66]. Several studies also reported expression of functional RANK by breast cancer, prostate, and melanoma cell lines [51, 52]. Breast cancer cells and MM cells down-regulate OPG production by osteoblasts and bone marrow stromal cells [60, 64]. Multiple myeloma cells express the heparin sulfate proteoglycan, syndecan, on their surface, which sequesters and degrades heparin-binding proteins including OPG [67]. Notably, the RANKL–OPG balance is disturbed in severe osteolytic pathologies in favor of RANKL, with large quantities of OPG being released within the tumor microenvironment to counterbalance high RANKL concentrations [64, 68].

Effects of cancer treatment on the skeletal and immune systems

Bone-targeted therapies

Early generation bisphosphonates

In general, early generation bisphosphonates do not appear to activate the immune system against cancer cells. However, clodronate combined with IL-2 stimulated proliferation of $\gamma\delta$ T cells in the absence of other cellular components in peripheral blood mononuclear cell (PBMC) cultures (wherein nitrogen-containing bisphosphonates have been tested), and clodronate-treated $\gamma\delta$ T cells exhibited higher cytotoxic activity against neuroblastoma cells compared with untreated control cells [69]. There are currently no data on whether these effects can result in meaningful anticancer activities in in vivo models. Clodronate has shown efficacy in preventing SREs in patients with bone metastases from MM [70–73] and breast cancer [74] and was recently reported to significantly prolong survival in men with bone metastases from prostate cancer [75] (Table 2) [70, 72, 74–82]. Results from trials in the adjuvant breast cancer setting were inconsistent and provided some evidence to suggest that clodronate can delay not only metastasis to bone but also to visceral sites.

Nitrogen-containing bisphosphonates

Nitrogen-containing bisphosphonates, such as zoledronic acid (ZOL) and pamidronate, cause immune system activation against cancer cells via activating $\gamma\delta$ T cells [83, 84]. By blocking G-protein signaling, these agents prevent differentiation of monocytes into osteoclasts, inhibit osteoclast recruitment and maturation, induce osteoclast apoptosis, and inhibit adhesion of osteoclasts to bone [85].

Pamidronate—Pamidronate therapy is associated with SRE reductions in patients with bone metastases from MM [76]. Although there was no overall difference in survival between pamidronate- and placebo-treated patients, pamidronate prolonged survival among patients who had

Table 2 Efficacy of bone-targeted agents in patients with bone metastases

Bisphosphonate	Cancer type	Patients, *N*	Reduction of SREs	Reduction of pain	Acute-phase reaction	Survival benefit
Clodronate [70]	Multiple myeloma	350	Yes	Yes	No	NE
Clodronate [72]	Multiple myeloma	536	Yes	Yes	No	±[a]
Clodronate [74]	Breast cancer	173	Yes	Yes	No	No
Clodronate [75]	Prostate cancer	819	NR	NR	No	Yes
Pamidronate [76]	Multiple myeloma	392	Yes	Yes	Yes	±[b]
Ibandronate [77]	Multiple myeloma	198	No	No	±	No
Zoledronic acid [78]	Multiple myeloma or breast cancer	1,648	Yes	Yes	Yes	Yes
Zoledronic acid [79]	Breast cancer	228	Yes	Yes	Yes	NE
Zoledronic acid [80]	Lung cancer and other solid tumors	773	Yes	NE	Yes	No
Zoledronic acid [81]	Hormone-refractory prostate cancer	122	Yes	Yes	Yes	NE
Denosumab [82]	Breast cancer	2,046	Yes	NE	Yes	NE

NE not evaluated, *NR* not reported, *SREs* skeletal-related events

[a] In a post hoc analysis, patients without vertebral fracture at study entry survived significantly longer on clodronate therapy (median survival was 23 months longer compared with patients receiving placebo)

[b] Survival of patients with more advanced disease was significantly increased in the pamidronate group (median survival of 21 vs. 14 months, $P = .041$)

received more than 1 previous antimyeloma regimen (14 vs. 21 months; $P = .041$; $N = 392$) [86].

Although evidence is limited, pamidronate has demonstrated effects on the immune system that may result in anticancer activity. Treatment with pamidronate induced expansion of $\gamma\delta$ T cells in PBMC cultures from healthy donors, and pamidronate-activated $\gamma\delta$ T cells produced immunostimulatory cytokines and exhibited specific cytotoxicity against lymphoma and myeloma cell lines. Furthermore, pamidronate-treated bone marrow cultures from patients with MM exhibited reduced plasma cell survival compared with untreated cultures, especially in pamidronate-treated cultures, in which activation of bone marrow $\gamma\delta$ T cells was evident (14 of 24 patients) [87].

Ibandronate—Administration of ibandronate to patients with advanced MM failed to reduce bone morbidity or prolong survival [77]. Ibandronate also produced a lesser reduction in markers of bone resorption and disease activity, including N-telopeptide of type I collagen (NTX), IL-6, and β_2-microglobulin, compared with pamidronate [88]. However, ibandronate has demonstrated efficacy in the reduction of skeletal complications in other tumor types such as breast cancer [89].

Zoledronic acid—Numerous studies established that ZOL exhibits consistent efficacy in delaying and preventing SREs in patients with malignant bone disease from MM [78, 90, 91] and various solid tumors including breast [79], lung [80, 92], and prostate cancers [81]. In a 25-month randomized trial comparing ZOL with pamidronate in patients with bone lesions from MM or breast cancer ($N = 1{,}648$), a 15-minute infusion of 4 mg ZOL was at least as effective as a 2-hour infusion of 90 mg pamidronate at reducing the risk of SRE complications in the overall population [78]. Similarly, treating patients with lung cancer and other solid tumors with ZOL resulted in fewer patients developing SREs (ZOL 8 mg reduced to 4 mg = 36%, placebo = 46%; $P = .023$; $N = 773$) [80]. Administration of ZOL to men with hormone-refractory metastatic prostate cancer also reduced the proportion of patients with SREs (38% vs. 49%; $P = .028$ vs. placebo; $N = 122$) [81].

A recent study also demonstrated that ZOL may elicit anticancer effects associated with immune system stimulation. Zoledronic acid activated $\gamma\delta$ T cells in vitro, and administration of ZOL to patients with prostate cancer resulted in the activation of $\gamma\delta$ T cells in peripheral blood after the first infusion. Moreover, after the first ZOL infusion, serum prostate-specific antigen (PSA) levels were reduced in 3 of 11 evaluable patients, and PSA velocity was reduced in 5 of 10 evaluable patients [93]. These results suggest that ZOL-activated $\gamma\delta$ T cells may be associated with the induction of an anticancer response in patients with prostate cancer.

Anticancer and antitumor activity of bisphosphonates

Numerous in vitro studies established that ZOL directly and indirectly inhibits multiple steps involved in the processes of cancer development and progression. In addition, ZOL stimulates cancer cell apoptosis and expansion of $\gamma\delta$ T cells, which play an important role in immune surveillance against neoplasia [94]. Preclinical studies reported that ZOL elicits anticancer activity in various cancer types and exhibits synergy with cytotoxic agents [95–100]. Four separate studies reported that ZOL reduced the persistence of DTCs in the bone marrow of patients with breast cancer [101–104]. In the clinical setting, adding ZOL to standard anticancer therapy improved clinical outcomes in early breast cancer. Administration of ZOL combined with adjuvant endocrine therapy to premenopausal women improved disease-free survival (hazard ratio [HR] = 0.64; $P = .01$) compared with endocrine therapy alone in the ABCSG-12 trial ($N = 1{,}803$) [105]. Similarly, ZOL plus neoadjuvant chemotherapy reduced residual invasive tumor size by 44% compared with chemotherapy in an exploratory subgroup from the AZURE trial ($P = .006$; $n = 205$) [106]. A multivariate analysis adjusted for potential prognostic factors in addition to neoadjuvant treatment group demonstrated that patients treated with ZOL plus neoadjuvant chemotherapy had a twofold greater complete pathologic response rate (breast and axilla) compared with patients treated with chemotherapy alone (odds ratio = 2.2; $P = .1457$). In the ZO-FAST ($N = 1{,}065$; median follow-up = 48 months; HR = 0.59; $P = .0176$) and Z-FAST ($N = 602$; median follow-up = 61 months; $P = .6283$) studies in postmenopausal women receiving adjuvant letrozole, immediate addition of ZOL reduced disease recurrence [107, 108]. In contrast with ABCSG-12, which had disease-free survival as a primary endpoint, ZO-FAST and Z-FAST were not designed or powered to evaluate disease recurrence (primary endpoints were bone loss); however, these studies demonstrated that upfront administration of ZOL resulted in improved disease-free survival among women with breast cancer. Subset analyses of the phase III clinical studies revealed that ZOL significantly prolonged survival compared with placebo among patients with high baseline NTX levels. Benefits were independent of SRE prevention, and multiple anticancer mechanisms, some of which involved immune system activation, may have contributed [109, 110]. Additionally, ZOL elicited anticancer responses in patients with MM, bladder cancer, lung cancer, or advanced solid tumors [111–114]. The Medical Research Council (MRC) Myeloma IX trial demonstrated that, after median follow-up of 3.7 years, ZOL significantly improved overall survival (by 5.5 months; 16% reduction in risk of death; $P = .0118$) and progression-free survival (by 2 months; 12% reduction

in risk of disease progression; $P = .0179$) versus clodronate in patients with newly diagnosed MM ($N = 1{,}960$ evaluable patients) [111]. The survival benefit associated with ZOL was maintained in analyses adjusting for the potential effects of SREs on survival ($P = .0178$ vs. clodronate), again supporting anticancer mechanisms for ZOL, which may involve positive effects on anticancer immune responses [111].

Denosumab

Denosumab is a fully human IgG2 monoclonal antibody that binds to RANKL with high affinity and specificity, thereby inhibiting osteoclastogenesis. The effects of denosumab on bone remodeling have been evaluated in patients with postmenopausal osteoporosis, rheumatoid arthritis, and various cancers [115–118]. Limited safety data from the advanced cancer setting have been released. However, results from phase III studies in bone-loss settings suggested that adverse immunologic effects might occur.

The FREEDOM trial, a phase III clinical study of 7,868 healthy postmenopausal women with osteoporosis, demonstrated that denosumab reduced the risk of new vertebral fractures by 68% compared with placebo ($P < .001$) [117]. A number of recent studies also demonstrated that denosumab can prevent SREs among patients with bone metastases from breast cancer, prostate cancer, other solid tumors, or MM. Denosumab was superior to ZOL in delaying time to first on-study SRE (HR = 0.82; $P = .01$ superiority), time to first and subsequent on-study SREs (rate ratio = 0.77; $P = .001$) in 2,046 patients with advanced breast cancer [82], and in delaying time to first on-study SRE in patients with advanced castration-resistant prostate cancer (CRPC) (HR = 0.82; $P = .008$ superiority; $N = 1{,}901$) [119]. Median time to first on-study SRE was 20.7 months for denosumab versus 17.1 months for ZOL [119]. However, a significantly greater proportion of denosumab-treated patients experienced increased PSA levels compared with ZOL-treated patients (3.8% vs. 2.0%, respectively; $P < .05$) [119]. Based on these results, it is possible that RANKL inhibition may impair immunosurveillance. Denosumab was noninferior to ZOL in delaying time to first SRE in 1,776 patients with other advanced solid tumors or MM (HR = 0.84; $P = .0007$) [115].

Anticancer and antitumor effects of denosumab therapy—Denosumab demonstrated antitumor activity in a phase II trial in 37 patients with benign giant-cell tumor (GCT) of bone, a tumor type that overexpresses RANKL and is associated with increased osteoclastic activity [120]. Given the low metastatic potential of GCT, the results observed in this patient population may not translate to patients with malignancies wherein the pathophysiology is distinct from that of GCT. Anticancer activity of blocking RANKL has been recently described in mouse models. RANKL inhibition was acting directly on hormone-induced mammary epithelium at early stages in tumorigenesis, and the permissive contribution of progesterone to increased mammary cancer incidence was due to RANKL-dependent proliferative changes in the mammary epithelium [121]. Based on these data, we assume that denosumab may have an anticancer activity; however, this has not yet been demonstrated in the clinical setting.

Risk of infections or new malignancies with denosumab therapy—Signaling via the RANKL–RANK pathway is involved in B-cell and T-cell differentiation and in survival of dendritic cells. As a result, concerns have been raised regarding possible immunosuppression with RANKL inhibitors. Recent clinical studies suggest that increased infection risk may be associated with denosumab therapy. The incidence of skin infections requiring hospitalization (cellulitis: 0.3 vs. <0.1% for placebo; $P = .002$) and endocarditis (3 patients vs. 0 for placebo) was increased among postmenopausal women with osteoporosis who received denosumab therapy (FREEDOM) [115, 117]. A meta-analysis of 10,329 patients with osteopenia or osteoporosis also reported an increased risk of serious infections (odds ratio = 4.54 for denosumab vs. placebo; $P = .03$) [122]. Serious infections were reported in 2.3% of denosumab-treated patients with early stage breast cancer compared with 0.8% of placebo-treated patients (P = not reported [NR]; $N = 249$; HALT-BC trial) [123]. Similarly, serious infections occurred at a higher incidence among denosumab-treated patients with androgen-dependent prostate cancer (5.9% vs. 4.6% for placebo; P = NR; $N = 1{,}468$; HALT-PC trial) [124]. Urinary tract infections also occurred more frequently among denosumab-treated patients with prostate cancer-related bone metastases (15% vs. 6% for bisphosphonates; P = NR; N = 49) [125].

Denosumab is specific for human and certain nonhuman primate RANKL and fails to inactivate rodent RANKL. Consequently, no carcinogenicity studies have been performed with denosumab because of the absence of an appropriate animal model. However, safety analyses from clinical trials of denosumab to prevent bone loss in patients receiving hormone ablation therapy (HALT) for early stage breast or prostate cancer suggest that the potential for cancer progression may be increased with denosumab therapy. Among 1,456 patients with androgen-dependent prostate cancer in HALT-PC, 8.2% ($n = 60$) of denosumab-treated patients and 5.5% ($n = 40$) of placebo-treated patients experienced metastatic events (P = NR) [115]. Similarly, metastatic events were reported in 7% ($n = 9$) of denosumab-treated patients compared with 4.2% ($n = 5$) of placebo-treated patients with breast cancer in HALT-BC (P = NR; $N = 249$) [115]. Indeed, given the significantly

increased rates of PSA progression in patients with CRPC and the significantly reduced survival in patients with MM treated with denosumab versus ZOL in the phase III clinical trials program (HR = 2.26) [126], further investigations on the potential effects of RANKL inhibition on cancer immunosurveillance and response are warranted.

Conclusions

The skeletal and immune systems have a complex relationship under normal (physiologic) and pathologic conditions. The RANKL–RANK–OPG signal transduction pathway plays a key role in regulating osteoclastogenesis. However, the effects of RANKL signaling are not limited to the skeletal system; RANKL is also expressed in other regulatory systems including the immune, cardiovascular, endocrine, and nervous systems. Expression of RANKL in the immune system regulates antigen-specific T-cell and B-cell responses, as well as the ability of T cells to interact with dendritic cells. Furthermore, RANKL directly affects the survival of antigen-presenting dendritic cells, which help other cells in the immune system to recognize and destroy abnormal cells and foreign antigens. Because of the systemic nature of RANKL expression, RANKL inhibition to prevent bone destruction may result in unintended consequences outside of the bone, including immune suppression with resulting possible increases in risk of infection or new malignancies. The long-term safety profiles of agents targeting this pathway are not yet known.

Currently available therapies designed to reduce pathologic osteolysis may also result in modulation of the immune system. Nitrogen-containing bisphosphonates such as ZOL exert beneficial effects on the immune system, resulting in activation of anticancer responses, as demonstrated in several clinical studies in various malignancies. Careful consideration should be paid to the shared pathways in bone immunology to maximize beneficial and minimize potentially negative effects in the clinical setting.

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