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PERSPECTIVES

RANKL: Targeting Bone and Cancer to Treat Skeletal Complications of Malignancy

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Abstract

The RANKL signaling pathway is a key mediator of osteoclastic bone resorption in response to stimuli from cancer cells. Bone resorption releases growth factors that then further stimulate cancer growth in a "vicious cycle". RANKL inhibition effectively decreases solid tumor bone metastases, myeloma and hypercalcemia of malignancy in animal studies. In clinical trials, humanized RANKL antibody therapy is effective in all of these settings and in cancer treatment-related bone loss as well. Although there are theoretical concerns that inhibition of RANKL signaling could impact the immune system or increase the incidence of neoplasms, neither of these adverse outcomes have been realized clinically to date. Emerging research suggests that RANKL signaling plays additional roles in cancer cells independent of osteoclasts by increasing homing of cancer cells to bone and invasive potential. Osteoclast eradication caused by RANKL inhibition may also offer advantages in suppression of bone turnover compared to bisphosphonates. Thus, targeting RANKL in skeletal complications of malignancy potently suppresses bone turnover to improve skeletal mortality in those conditions, and may have direct antitumor effects as well. *IBMS BoneKEy*. 2009 September;6(9):323-338.

Background

Bone is a fertile environment for cancer growth as initially described by the seed and soil hypothesis proposed by Stephen Paget in 1889. Over 100 years later, this theory is unchanged but has evolved to include a greater understanding of the interaction between cancer cells and the cells of the bone microenvironment that results in a "vicious cycle" of cancer growth. Cancer cells hijack osteoblasts and osteoclasts to disrupt normal bone remodeling. Increased bone resorption releases growth factors from the bone matrix that further stimulate cancer cell growth. The balance of resorption and formation determines the radiographic phenotype of osteolytic and osteoblastic bone metastases. Accumulating evidence, clinical and experimental, supports a pivotal role of osteoclasts in the pathogenesis and sequelae of bone metastases regardless of the radiographic phenotype. Bone resorption markers are increased in both osteolytic and osteoblastic solid tumor bone metastases and are higher in the latter (1). Bisphosphonates decrease skeletal morbidity from bone metastases from solid tumors or myeloma whether osteolytic or osteoblastic (2;3). Finally, the bone resorption marker, N-telopeptide, predicts death and skeletal morbidity from solid tumors and this relationship is stronger in osteoblastic disease due to prostate cancer (4;5). Thus osteoclasts play a pivotal role in bone metastases and their sequelae, and the rationale to target this bone resorbing cell is strong.

Cancer affects the skeleton in several ways; bone metastases, hypercalcemia and osteoporosis due to cancer treatment. Skeletal-related clinical events (SREs) are a direct result of bone metastasis and include bone pain, pathologic fractures and spinal cord compression. SREs contribute significantly to patient morbidity as up to 75% of advanced prostate and breast cancer and 30-40% of lung cancer patients have bone metastases (6). Systemic effects of tumor-derived PTHrP, which promotes calcium mobilization from bone and renal tubular reabsorbtion of calcium, is a major

causal factor in hypercalcemia of malignancy. Multiple myeloma also causes significant osteoclastogenesis and purely lytic lesions resulting in both hypercalcemia and SREs. Finally, cancer treatments such as estrogen or androgen blockade, chemotherapy and radiation therapy result in bone loss. Increased bone resorption by osteoclasts during cancer treatment releases a rich store of growth factors from the mineralized bone matrix and can enhance cancer growth in bone. Inhibiting osteoclast function to reduce bone resorption is paramount in each of these scenarios to reduce these related complications and possibly tumor growth in bone.

Bisphosphonates currently represent the only bone-targeted and approved therapy to treat skeletal complications of cancer and cancer treatment. They effectively inhibit osteoclast function, and decrease the risks of SREs in patients with solid tumor bone metastases. Bisphosphonates are also effective for treatment of hypercalcemia of malignancy, multiple myeloma-related bone resorption and bone loss during cancer treatment. Despite this effectiveness, bisphosphonates do not cause regression of bone metastases, so new therapies are needed. There are several limitations to the use of bisphosphonates: 1) association with osteonecrosis of the jaw, recognized recently with potent IV bisphosphonates, especially in cancer patients; 2) long half-life in bone and long-term potential to suppress bone turnover; 3) possible renal toxicity and contraindication in renal failure; 4) poor oral bioavailability; 5) failure to suppress markers of bone resorption in some patients with myeloma or solid tumor bone metastases (7;8). Thus, better options to target the osteoclast are needed.

An attractive target is the RANKL signaling triad that plays a dominant role in osteoclastogenesis. RANKL, when bound to RANK on the surface of osteoclast precursors, promotes osteoclastogenesis, whereas OPG, a soluble decoy receptor for RANKL, inhibits osteoclast formation. OPG was the first to be identified in this signaling pathway. It caused osteopetrosis in mice when overexpressed and protected mice from ovariectomy-induced bone loss (9). At the same time, another group purified OPG from fibroblasts and used it to significantly inhibit osteoclastogenesis (10). RANKL was then identified as the ligand for OPG and treated mice developed hypercalcemia from increased osteoclastogenesis (11). Further demonstrating a requirement of RANKL signaling for osteoclast development, both RANK and RANKL null transgenic mice lack osteoclasts (12;13).

OPG and RANKL are both expressed by osteoblasts and bone marrow stromal cells and the ratio of these determine the degree of osteoclastogenesis, effectively balancing new bone formation and resorption in healthy bone. In cancer the RANKL/OPG ratio is perturbed by signals from cancer cells and excessive bone resorption or formation occurs. PTHrP, IL-6 and IL-11 are secreted from cancer cells and increase RANKL expression in osteoblasts (14-16). IL-8 increases osteoclastogenesis in both a RANKL-dependent and -independent manner (17). Recent data in osteoarthritis models demonstrate increased RANKL expression in osteoblasts treated with IL-1β, TNFα, PGE2 and IL-17 as well and these factors could potentially play a role in cancer and bone interactions (18). In addition to enhancing bone resorption in response to cancer cell signals, RANKL, RANK and OPG may also play a role in cancer cell proliferation, migration to and invasion of bone.

Clinical and Experimental Evidence in Cancer

There is accumulating evidence that cancer cells express all components of the RANKL signaling pathway, but the exact function of this pathway may be different in different types of cancers. The relative role of this pathway in cancer cells and the relationship to bone cells is under intense investigation.

Evidence for RANKL: Osteoclast-dependent effects

Cancer cells produce many factors that stimulate osteoblast production of RANKL and subsequent osteoclastic bone

resorption. These effects are the most recognized role for RANKL in cancer.

Breast Cancer

Metastatic bone lesions are predominately osteolytic in breast cancer. Tumor induction of osteoblast and stromal cell RANKL expression plays a predominant role in osteoclastogenesis and bone destruction. It is possible that breast cancer cells activate osteoclasts directly, but this is likely a small contribution. Only 14% of breast cancers express RANKL (19), and most *in vitro* systems require osteoblasts to be cultured with breast cancer cells to induce osteoclastogenesis. When MDA-MB-231 breast cancer cells were co-cultured with calvarial osteoblasts, they induced bone resorption and RANKL mRNA expression in osteoblasts whereas MDA-MB-231 cells alone did not express RANKL (20;21). Consistent with this, MDA-MB-231, MCF-7 and T47D breast cancer cells did not express RANKL, but notably did express RANK and OPG. When these cells were co-cultured with murine osteoblasts and hematopoietic cells, they induced osteoclast formation. This process was PTHrP-dependent, and bone destruction and RANKL expression by osteoblasts was amplified in PTHrP-overexpressing breast cancer (14). This demonstrates the ability of tumor cells to induce RANKL expression in osteoblasts and stromal cells to indirectly increase osteoclast formation. Since many breast cancers express RANK (14), it also introduces the possibility that RANK expression by cancer cells may play a role in cancer growth in bone.

Myeloma

The vast majority of multiple myeloma cases are characterized by marked osteolysis with suppressed bone formation. Significant bone destruction occurs through alterations in the RANKL signaling pathway. Osteolytic lesions are identified adjacent to nests of myeloma cells and these can result in hypercalcemia and SREs. An increased RANKL/OPG ratio correlated with increased tumor burden in myeloma (22). OPG concentrations were lower in patients with myeloma compared to healthy controls (23). High RANKL/OPG ratios also predicted poor survival in patients with myeloma (24). Both myeloma cells isolated from patients and established myeloma cell lines express RANKL and can induce osteoclastogenesis directly (25-30). This is evidenced by data showing that myeloma cells and conditioned media are capable of inducing osteoclastogenesis in bone marrow mononuclear populations, bypassing a requirement for osteoblasts or stromal cells (29). Myeloma cells also upregulate RANKL expression in bone marrow stromal cells and osteoblasts (30;31) and in T lymphocytes (32). RANKL expressed by myeloma cells or induced in other cells in the bone microenvironment plays an important role in the pathogenesis of myeloma bone destruction. Since myeloma also suppresses osteoblastogenesis, myeloma expression of RANKL provides an additional route to stimulate osteoclastogenesis and may explain why it remains the malignancy so commonly associated with profound osteolysis.

Prostate cancer

Prostate cancer bone metastases are predominately osteoblastic, but osteoclastic bone resorption is also a prominent feature in osteoblastic disease and likely contributes to tumor growth in bone. Whereas the RANKL/OPG ratio is increased in predominately osteolytic bone lesions of breast cancer and myeloma, OPG is relatively increased in human prostate cancers. The contribution of this increased OPG to osteoclastic bone resorption or stimulation of bone formation is unclear. Human prostate cancers express all three components of the RANKL signaling triad, RANKL, OPG and RANK, and the expression of these correlated with advanced cancer stage and cancer metastasis (33). Furthermore, the highest concentration of OPG was observed in prostate cancer bone lesions. Consistent with these findings, both OPG and RANKL were significantly increased by immunohistochemistry in bone metastases compared to paired primary prostate cancers (34). Finally, RANKL expression on the surface of prostate cancer cells predicted recurrence of disease with a

hazard ratio of 11.6 (35). Different components of the RANKL signaling pathway are expressed in breast cancer, myeloma and prostate cancer and may have tumor-type specific roles. These tumor-specific roles have yet to be defined.

Hypercalcemia of malignancy

There are no published data on the direct alteration of RANKL signaling in hypercalcemia of malignancy, but there is abundant evidence that tumor products such as PTHrP that mediate hypercalcemia stimulate RANKL. Inhibition of RANKL effectively treats hypercalcemia of malignancy, reviewed later, and provides indirect evidence for a role of the RANKL pathway in the pathogenesis of hypercalcemia associated with malignancy.

Evidence for RANKL: Osteoclast-independent effects

Recent data suggest that the RANKL pathway may have important roles in various aspects of skeletal complications in cancer that do not involve the osteoclast.

Homing of cancer cells to bone

RANKL is clearly important for bone resorption and fueling cancer growth. However, there are data to suggest that bone-derived RANKL may also serve as "soil" or a chemoattractant to bone for RANK-expressing cancer cells independent of bone resorption and osteoclast activity. In normal mammary epithelium, melanoma and breast cancer cells, RANKL induced invasion and migration *in vitro*. These effects were blocked by OPG, and were not observed in a RANK-negative colon cancer cell line. In a melanoma metastases model that was previously shown not to activate osteoclasts and bone resorption, OPG did decrease bone metastases. Because OPG was effective in a system thought to be devoid of osteoclast activation, the authors concluded that the decrease in bone metastases was caused by inhibition of RANKL-mediated homing of cancer cells to bone and not by increased bone turnover (36). This conclusion is controversial as this mouse melanoma model may have some degree of osteoclast activation and increased bone turnover, not recognized by the authors. An intratibial inoculation model of these melanoma cells may shed some light on the role of RANKL in cancer cell migration to bone. In this model, there would be no cancer cell migration and OPG efficacy would implicate targets of osteoclast-induced bone resorption or direct anti-tumor effects.

Epithelial to mesenchymal transition (EMT) and invasion

EMT and invasion are important determinants of cancer growth in healthy tissue. Functional RANKL expression by prostate cancer cells is correlated with EMT and a threefold increase in bone metastases (37), supporting another tumor-specific role for RANKL signaling in cancer progression. In PC3 prostate cancer cells, treatment with RANKL increased invasion of collagen matrix and increased expression of MMP-9 and interleukin 6 and these effects were blocked with OPG (38). Consistent with these results, RANKL induced invasion of breast cancer cells in a matrigel invasion assay system (39). The role of RANKL as a chemoattractant or transformant independent of osteoclasts has yet to be established *in vivo*, but *in vitro* studies are suggestive.

Apoptosis

Components of the RANKL signaling pathway may have additional effects on tumor cells. There is evidence that blocking RANKL in certain settings with OPG could actually stimulate tumor growth by binding the pro-apoptotic factor TRAIL. Expression of OPG by MDA-MB-231 cells increased tumor inhibition of TRAIL and improved tumor survival (40). OPG over-expression in MCF-7 breast cancer cells also enhanced tumor growth in bone (41) and OPG expression by breast cancer cells correlated with bone homing and colonization potential (42). Recent data also suggest that interaction between TRAIL and OPG could increase RANKL expression in MDA-MB-231 cells (43). These data are in contrast to the inhibition of breast cancer tumor growth in bone by systemic treatment with OPG in

mice (44;45) and may be due to the anti-apoptotic or perhaps autocrine effects of OPG when produced directly by tumor cells. Although there are concerns that systemic OPG therapy could enhance tumor cell survival, OPG treatment consistently decreases bone metastasis in murine models of breast cancer metastasis.

Angiogenesis

OPG expression in endothelium of malignant breast tumors correlated with higher tumor grade and appears to have proangiogenic effects *in vitro*. OPG was expressed in the endothelium of 59% of malignant breast cancers, but not expressed in endothelium of nonmalignant tissue. OPG also supported endothelial cell survival *in vitro* and promoted cord structures in a matrigel tubule formation assay (46). OPG may decrease apoptosis of both tumor cells and endothelium, simultaneously increasing tumor growth and necessary blood supply.

Inhibition of RANKL: Animal Studies

Inhibition of RANKL *in vivo* is very effective in reducing bone destruction in murine models of breast cancer, myeloma and prostate cancer. In mice, OPG-Fc and RANK-Fc, recombinant chimeric human proteins, have been developed to study this. The fully humanized anti-RANKL antibody (huRANKL MAb), denosumab, does not recognize murine RANKL and is ineffective in mice. Therefore, these *in vivo* studies are a close approximation of huRANKL MAb treatment in humans (Table 1).

Breast cancer

In murine models of breast cancer, OPG-Fc prevented the development of osteolytic bone lesions (44;47) and treatment with OPG-Fc after establishment of osteolytic bone metastases resulted in a significant decrease in bone destruction and lesion growth (44;45;48). However, *in vitro* treatment with OPG did not affect tumor cells suggesting that the antiresorptive effects of OPG *in vivo* are necessary for inhibition of tumor growth (45). Both preventative and treatment models have resulted in improved survival in mice, though there was no difference in soft tissue tumor burden. OPG caused tumor cell apoptosis *in vivo*, but not *in vitro*, once again supporting osteoclast-mediated effects (44). There was increased tumor cell apoptosis in mice treated with OPG-Fc compared to those treated with zoledronic acid. This may be due to the eradication of osteoclasts by OPG-Fc but not by zoledronic acid (49). A study comparing ibandronate and OPG-Fc in mice with breast cancer bone metastases showed similar inhibition of bone destruction, and tumor growth in bone, and once again OPG-Fc treatment resulted in a total absence of osteoclasts (45).

Myeloma

Inhibition of RANKL in murine models of myeloma yields similar results to breast cancer studies. Although OPG and RANK do not have direct effects on multiple myeloma cells *in vitro*, both OPG-Fc and RANK-Fc treatment decrease melanoma burden and bone destruction (31;50-52), with comparable efficacy to zoledronic acid (53).

Prostate cancer

In prostate cancer, both osteoblastic and osteolytic lesions respond to RANKL inhibition. RANK-Fc and OPG-Fc treatment reduced both lesion area and tumor establishment in bone of osteolytic predominate prostate cancer cell lines (54-56). In contrast, osteoblastic lesions became established despite preventative RANKL inhibition, but treatment with RANKL inhibitors decreased growth of established lesions. (55;57). These findings were specific to tumor growth in bone as there was no impact on subcutaneous tumors. These data suggest that establishment of osteoblastic lesions is osteoclast-independent, but that ongoing growth is dependent on bone destruction. Consistent with these findings, OPG-Fc did not inhibit establishment of osteoblastic bone lesions, but did decrease tumor cell growth and PSA (58). As prostate cancer cells often express RANKL, it is important to determine the role of bone-derived and tumor-expressed RANKL. In C4-2B human prostate cancer cells, huRANKL MAb did not inhibit growth

Table 1. Animal studies: RANKL inhibition in solid tumor bone metastases, myeloma and SREs.

Solid tumor metastases	Agent used	Results	Reference
Breast	OPG-Fc	↓ tumor burden, absent OC with IC MDA-MB-231	(48)
	OPG-Fc	↓ tumor burden and bone destruction of IC MDA-MB-231	(47)
	OPG-Fc	↓ tumor burden and bone destruction of IT MDA-MB-231, comparable to IBN. OC eradicated with OPG only	(45)
	OPG-Fc	↓ tumor burden and bone destruction, ↑ survival of IC MDA-MB-231	(44)
	Adenoviral OPG-Fc	↓ tumor burden of IC MDA-MB-435, ↔ visceral mets or survival	(73)
Prostate	RANK-Fc	↓ tumor growth of IT, ↔ tumor of SC C4-2B	(57)
	OPG-Fc	↓ tumor growth ↔ tumor establishment of IT LuCaP 23.1	(58)
	OPG-Fc	↓ tumor burden and bone destruction of IT C4-2, comparable to ZA	(56)
	RANK-Fc	↓ tumor growth ↔ tumor establishment of IT LAPC-9	(55)
	Hu RANKL MAb	↔ tumor or bone destruction of IT C4-2	(59)
	OPG-Fc	↓ tumor burden of IT PC3	(38)
	RANK-Fc +/- Docetaxel	↓ tumor burden of IT PC3 ↑ BV/TV	(61)
	OPG-Fc +/- Docetaxel	↓ tumor burden of IC PC3 ↑ survival	(60)
Colon	OPG-Fc	↓ tumor burden of IC colon-26	(48)
Lung	RANK-Fc	↓ tumor burden and bone destruction of IT A549	(62)
Melanoma	OPG-Fc	↓ tumor burden, ↔ visceral mets, ↑ survival with IC B16F10	(36)
Myeloma	RANK-Fc	↓ myeloma burden and bone destruction with huMM, ARH-77	(31)
	OPG-Fc	↓ osteolysis with IV 5T2MM	(52)
	RANK-Fc	↓ myeloma burden and bone destruction with huMM, comparable to BP	(53)
	OPG-Fc	↓ myeloma burden, M protein ↑ survival with IV 5T33MM	(50)
HHM	muRANK-Fc	↓ bone destruction and serum calcium	(64)
	OPG-Fc	↓ bone destruction and serum calcium, greater effect than IV BP	(63)
Bone pain	OPG-Fc	↓ bone destruction and movement-evoked pain of established osteosarcoma	(65)

Breast cancer: MDA-MB-231, MDA-MB-245; Prostate cancer: C4-2, LuCaP, LAPC, PC3; Myeloma: huMM, ARH-77, 5T2MM, 5T33MM. ↔ no change, ↑ increased, ↓ decreased. IT: intratibial; IC: intracardiac; OC: osteoclasts; SC: subcutaneous; IV: intravenous; ZA: zoledronic acid; IBN: ibandronate; BP: bisphosphonate; HHM: humoral hypercalcemia of malignancy.

of tumors in a murine tibial injection model suggesting that the host or bone derived RANKL was critical for tumor growth (59). RANKL inhibition in combination with chemotherapy also improves outcomes. When given with docetaxel, RANK-Fc decreased bone destruction in mice and OPG-Fc improved survival in mice after intracardiac inoculation of PC3 cells (60;61). Inhibition of RANKL decreases tumor

burden in bone and bone destruction in both blastic and lytic bone metastases and may improve survival in mice with solid tumor metastases.

Lung cancer

Mixed blastic and lytic solid tumor bone metastases from a lung cancer cell line, A549, were significantly reduced with RANK-Fc treatment (62). Treatment with RANK-Fc reduced bone metastases and not subcutaneous tumors. These data are consistent with results for both breast and prostate bone metastases and highlight the importance of osteoclastic bone resorption in not only lytic bone metastases, but in blastic bone metastases as well.

Hypercalcemia of malignancy

RANKL inhibition is also effective in models of hypercalcemia of malignancy. In mice with subcutaneous syngeneic colon cancer C-26, a high-PTHrP-expressing cancer cell line, OPG-Fc treatment reduced serum calcium levels significantly more than either zoledronic acid or pamidronate (63). Although there was complete eradication of osteoclasts, there was a gradual increase of serum calcium at the end of the study period that the authors attributed to host immune responses to the subcutaneous delivery of a human protein and a waning of OPG-Fc efficacy. However, murine RANK-Fc treatment showed a similar rise of serum calcium when tested in mice inoculated with lung cancer that was attributed to unabated subcutaneous tumor growth and increasing PTHrP serum concentrations (64). The increasing PTHrP may lead to decreased renal clearance of calcium or increased 1,25 OH_3 vitamin D and intestinal calcium absorption. Consistent with the previous study, osteoclasts were significantly reduced, and the treatment was effective even when started after hypercalcemia had already developed.

Bone pain

Experimental evidence in mice indicates that OPG is also effective in the management of advanced cancer bone pain. In an osteolytic sarcoma model, OPG treatment decreased movement-evoked pain and other pain behaviors when given after significant bone destruction had already occurred (65). There was also decreased tumor growth and neurochemical change at the spinal cord with OPG treatment. RANKL inhibition, due to potent anti-osteoclast activity, is effective for management of both bone pain and hypercalcemia of malignancy.

Clinical Trials

Solid tumor bone metastases and myeloma

Clinical trials with denosumab mirror the data gleaned from *in vivo* studies with RANK-Fc and OPG-Fc (Table 2). RANKL MAb effectively reduced bone resorption in patients with solid tumor bone metastasis and myeloma in phase II clinical trials. A dosing regimen of 120 mg subcutaneously every four weeks in patients with myeloma or bone metastases from breast cancer rapidly suppressed urine N-telopeptide (uNTx), an effect that was sustained for 13 weeks. In these patients, 74% of patients receiving RANKL MAb had > 65% suppression of uNTx compared to 63% of the bisphosphonate-treated patients (66). SREs were similar between the two groups, affecting 12% of RANKL MAb-treated patients compared to 16% of bisphosphonate-treated patients at 25 weeks (67). However, a wide range of RANKL MAb dosing regimens were used and this may have diluted the effect of the higher dose. The dosing regimen of 120 mg every 4 weeks was the most effective and is now being evaluated in phase III trials. Superiority of denosumab to zoledronic acid was announced recently. Denosumab delayed time to first SRE compared to zoledronic acid with a hazard ratio of 0.82 (95% CI: 0.71-0.95) in 2,049 patients with advanced breast cancer.

RANKL MAb eliminated osteoclast activity in patients who continued to have elevated markers of bone resorption despite intravenous bisphosphonates. In 111 patients with myeloma or bone metastases from breast and prostate cancer fitting these criteria, RANKL MAb suppressed uNTx < 50 nM BCE/mM creatinine in 71% of patients

Table 2. Clinical trials with huRANKL MAb (denosumab)

Cancer studied	N receiving agent	Results	Reference
Myeloma Breast	N = 25 N = 29	↓ markers of bone resorption	(78)
Breast	N = 211	↓ markers of bone resorption and SREs	(67)
Myeloma	N = 85	↓ serum M-protein	(68)
Prostate Breast Myeloma/Other	N = 50 N = 46 N = 15	↓ markers of bone resorption and SREs	(8)
Giant cell tumor	N = 24	↓ tumor size	(69)
Treatment-induced bone loss: Aromatase inhibitor for nonmetastatic breast cancer	N = 127	↑ BMD at lumbar spine, total hip and distal radius	(74)

compared to 29% of patients receiving zoledronic acid (8). In this study, SREs were significantly reduced with RANKL MAb therapy with an incidence of 8% in the RANKL MAb group compared to 17% in the zoledronic acid group. In a separate evaluation of prostate cancer patients in this cohort, 69% had suppression of uNTx < 50 nM BCE/mM creatinine with RANKL MAb treatment compared to 38% of patients receiving zoledronic acid, suggesting that RANKL MAb is effective for both lytic and blastic bone metastases (7). RANKL MAb also stabilized myeloma as measured by serum M protein levels and decreased markers of bone resorption (68). The stable M protein suggests an inhibitory effect of RANKL MAb on myeloma progression. In several studies of a murine model of solid tumor bone metastases or myeloma, RANKL inhibition increased survival (36;44;50;60). Future studies with adequate power and duration will determine whether denosumab improves survival in humans with solid tumor bone metastases.

Other tumors: Giant cell tumors

Giant cell tumors of bone have also been treated effectively with RANKL MAb. RANKL inhibition decreased tumor volume or improved histologic grade in 87% of patients. Dramatic changes could be seen histologically and a tumor response required elimination of greater than 90% of giant cells. This resulted in substantial clinical benefit as measured by decreased pain, improved movement or return to work (69).

Hypercalcemia of Malignancy

Treatment of hypercalcemia of malignancy with RANKL MAb has not been evaluated in a clinical trial at this point, but *in vivo* studies suggest that it would be effective for this as well.

RANKL Inhibition for Cancer Treatment-Related Bone Loss

Estrogen and androgen blockade, common therapies for both breast and prostate cancer, increase bone turnover, cause bone loss and increase fracture risk. This makes bone a more attractive environment for cancer cell growth. In postmenopausal woman, RANKL inhibition increased bone mineral density at the lumbar spine, total hip and distal radius significantly more than placebo. These improvements were similar to or greater than changes observed in the alendronate arm (70). RANKL inhibition has also been studied in ovariectomy and orchiectomy models of bone loss and is very effective in suppressing bone resorption. Compared to alendronate, OPG treatment caused a greater increase in femur mechanical strength, femur bone mineral density and vertebral trabecular bone volume in ovariectomized mice. There was also a greater decrease in osteoclast number with OPG therapy compared to alendronate (71). OPG in orchiectomy models also preserves bone mass (72). In addition to preserving bone in these models, OPG prevented trabecular bone loss in tumor-bearing mice (59;73), and in mice receiving docetaxel (61). In clinical trials

RANKL MAb increased bone mineral density at the lumbar spine by 5.5% and 7.6% at 12 and 24 months, respectively, compared to placebo in women with nonmetastatic breast cancer treated with aromatase inhibitors (74). Inhibition of RANKL in settings of low estrogen or during cancer treatment effectively inhibits bone resorption, making bone a less fertile environment for cancer cell growth.

Adverse Effects

Determining adverse effects of RANKL MAb has relied on clinical studies. In mice, intact RANKL signaling is required for normal immune system development and absence of either RANKL or RANK causes lymph node agenesis and lymphocyte dysfunction, which was recently reviewed (75). RANK is expressed on the surface of T-cells and dendritic cells and inhibition of RANKL theoretically poses a risk of immune dysregulation. However, inhibition of RANKL after embryogenesis does not disrupt the immune system. Mice treated with OPG-Fc and RANK-Fc had a significant reduction of osteoclasts, but were still able to mount a normal immune response to influenza (76). Clinical trials do not show clinically significant immune disruption with denosumab. In 49 patients treated with a single dose of RANKL MAb, there were no decreases in lymphocyte number (77). After 2 years of RANKL MAb treatment in 319 patients, there was a small but significant increase in urinary tract infections and six cases of community-acquired infections that did not reach statistical significance and were easily treated with standard antibiotics. Acute phase reactions with myalgias and pyrexia were similar in the RANKL MAb and placebo groups. Other adverse effects may include hypertension that was more frequent in patients receiving either RANKL MAb or alendronate compared to placebo (70). RANKL MAb did not increase the incidence of neoplasm in this study, making potential interaction between RANKL inhibition and TRAIL less concerning. To date, no cases of osteonecrosis of the jaw have been reported with RANKL MAb therapy, but more frequent dosing schedules have been evaluated in only a limited number of patients. In 197 patients, there were transient and asymptomatic decreases in serum calcium at 2 weeks in 8% of RANKL MAb patients compared to 5% of patients receiving bisphosphonates. Finally several studies have documented absence of anti-denosumab antibodies with therapy (66;78). RANKL MAb is well-tolerated by patients and has not resulted in serious adverse events.

Summary

RANKL plays a major role in solid tumor bone metastases, myeloma, cancer treatment-induced osteoporosis and hypercalcemia of malignancy and the rationale to target RANKL in these disorders is strong. Fig. 1 summarizes potential mechanistic roles of RANKL in these skeletal complications of malignancy. Myeloma and solid tumor bone metastases are incurable and result in significant patient morbidity. In early clinical trials evaluating solid tumor bone metastases and multiple myeloma, RANKL MAb reduces bone resorption and SREs more effectively than bisphosphonates. Recent results from a large phase III trial also show superiority of RANKL MAb to zoledronic acid. *In vivo* studies show powerful reduction of calcium with RANKL inhibition in models of hypercalcemia of malignancy. RANKL inhibition also arrests bone loss in the setting of estrogen depletion with aromatase inhibitors. Thus RANKL inhibition effectively inhibits osteoclast-dependent skeletal complications of malignancy. *In vivo* studies suggest that RANKL inhibition completely eradicates osteoclasts from the bone environment, in contrast to bisphosphonates. Is it possible that osteoclasts provide stimulus to cancer cells independent of bone resorption? Are osteoclasts a source of important tumor growth factors? In addition to the known osteoclast-dependent effects, bone-derived RANKL may enhance homing of RANK-positive cancer cells independent of osteoclast activity or bone resorption. Tumor-derived RANKL may also play a role in EMT, providing another target for RANKL inhibition. Despite concerns about potential interference with TRAIL and the immune

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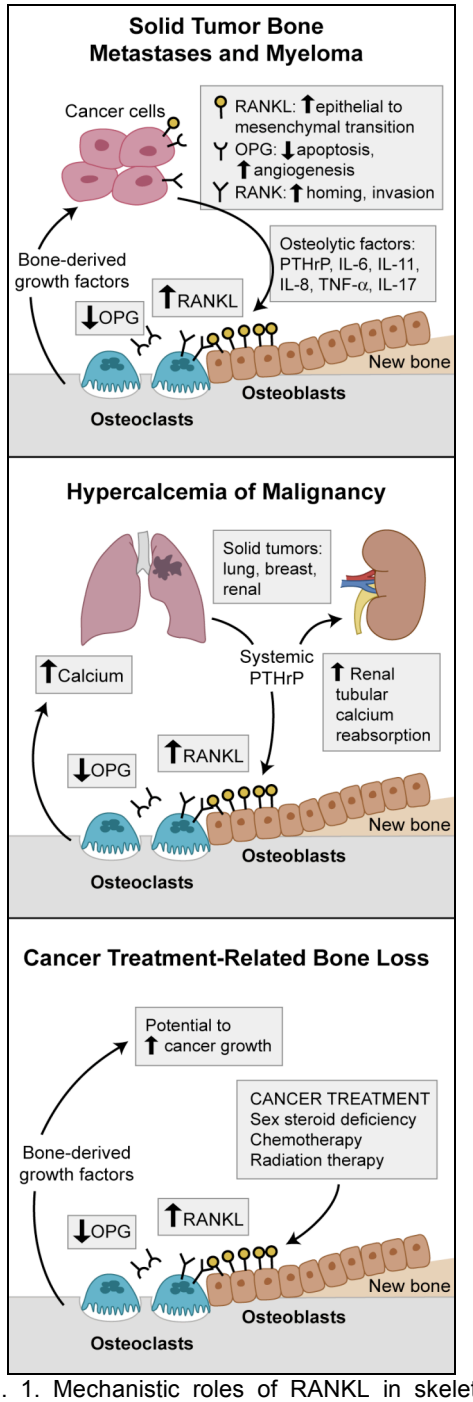

Fig. 1. Mechanistic roles of RANKL in skeletal complications of malignancy. Role of RANKL in solid tumor bone metastases and myeloma (top panel), hypercalcemia of malignancy (middle panel) and treatment-induced bone loss (bottom panel). Targeting RANKL effectively reduces bone resorption and associated complications in cancer and may also have direct anti-tumor effects.

system, RANKL inhibition does not appear to increase the incidence of neoplasm or interfere with lymphocytes. RANKL inhibitors are not stored in bone like bisphosphonates, and suppression of bone turnover is reversible. It is not apparent whether RANKL inhibitors will be associated with osteonecrosis of the jaw, but perhaps the reversibility will be advantageous and reduce this risk. There does appear to be increased risk of urinary tract infections with the use of RANKL MAb, and a higher frequency of hypertension that was comparable to alendronate. Longer and larger studies will need to be completed to further evaluate these potential concerns, and to determine whether RANKL inhibition has anti-tumor effects or impacts survival of patients with multiple myeloma or solid tumor bone metastases.

Conflict of Interest: Dr. Guise reports that she is on the advisory board, and consults, for Amgen and Novartis, and that she owns stock in Amgen. Dr. Crook reports no conflicts of interest.

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