MEETING REPORTS

Novel Targets for and Mechanisms of Bone Metastasis: Meeting Report from Skeletal Complications of Malignancy V

October 25-27, 2007 in Philadelphia, Pennsylvania, USA

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Novel Targets for Bone Metastasis II (Session 5)

Future myeloma treatment strategies will likely involve standard and novel therapies based on targets identified by genomic and proteomic approaches (1). Recent additions to the myeloma arsenal include thalidomide, lenalidomide and bortezomib, which potentiate conventional melphalan/dexamethasone treatment (2-4). Bortezomib in combination with the hsp90 inhibitor doxil and lenalidomide may have added benefit. Also in development are combination lenalidomide and the Akt inhibitor perifosine and mTOR inhibitors (1). Medications that target adhesion of myeloma to bone marrow stromal cells, a process critical to myeloma progression, are in development (1).

Novel therapies are also being studied to treat prostate cancer bone metastasis. Although bone lesions associated with this type of tumor are osteoblastic, bone turnover is markedly increased and agents that inhibit osteoclast activity reduce skeletal-related events (SREs). The bisphosphonate zoledronic acid reduces SREs in men with advanced disease (5), and a recent trial is now evaluating whether this bisphosphonate benefits patients with early androgen-dependent disease in preventing bone metastases (6). Other trials are examining whether the RANKL monoclonal antibody denosumab is as effective as zoledronic acid in reducing SREs and whether denosumab alone prevents bone metastasis in men with advanced disease (6). Androgen ablation therapy reduces bone density and contributes to a high fracture rate in men with early and advanced disease. The selective estrogen receptor modulator (SERM) toremifene reduces treatment bone loss in men with prostate cancer (7).

The value of zoledronic acid in preventing SREs in patients with breast cancer bone metastasis has been reported (8). The ongoing AZURE trial will determine whether zoledronic acid prevents bone metastasis and disease progression in women with stage II/III disease. Subjects receive 19 treatments of zoledronic acid or placebo for 5 years. Preliminary data demonstrate that zoledronic acid does not increase adverse events (9). Denosumab is also currently being evaluated for the treatment of breast cancer bone metastasis. In a phase 2 trial, denosumab was compared to zoledronic acid in patients receiving standard therapy for advanced breast cancer. After 25 weeks, urinary NTX was reduced in both groups, and 12% of those receiving denosumab, and 16% of those receiving zoledronic acid, experienced an SRE (10). Another trial examined whether denosumab reduced bone turnover in patients who did not have a decrease in urinary NTX of less then 50 mM BCE/mmol creatinine after receiving zoledronic acid. At the conclusion of this study, 38% of patients randomized to zoledronic acid and 76% of patients randomized to denosumab had a NTX less than 50 mM BCE/mmol creatinine (10), indicating that denosumab may have benefit in patients who do not respond to zoledronic acid. Like RANKL inhibition, TGF-β blockade may also reduce breast cancer osteolysis. TGF-β is released from bone during osteoclast-mediated bone resorption

and stimulates breast cancer cells to proliferate. In an animal model of breast cancer bone metastasis, the TGF-β receptor I kinase inhibitor SD-208 had an additive effect with zoledronic acid in reducing breast cancer cell osteolysis (11).

Mechanisms of Bone Metastasis I (Session 6)

This session explored two signaling pathways with demonstrated roles in bone metastasis: Wnt and hypoxia, and important regulators of these pathways. Wnt signaling is critical for normal osteoblast development. Recent evidence now demonstrates that secreted factors from cancer cells manipulate osteoblastic Wnt signaling and steer bone metastasis to either an osteoblastic or osteolytic phenotype. Dickkopf-1 (DKK1), the extracellular Wnt signaling inhibitor, is increased in the bone marrow plasma of patients with myeloma osteolytic bone disease and is likely causal in osteoblast suppression characteristic of this disease (12). Similarly, DKK1 overexpression in the mixed osteoblastic/osteolytic prostate cancer cell line C4-2B resulted in the production of osteolytic lesions in an animal model (13). These studies show a clear inverse relationship between DKK1 and osteoblast activity.

Recent evidence now suggests that a reduced DKK1 concentration in the bone microenvironment may be required to support osteoblastic bone metastasis. In animal models of osteoblastic bone metastasis and prostate cancer clinical studies, endothelin-1 (ET-1) secreted by cancer cells stimulates osteoblast proliferation and new bone formation (14). The anabolic actions of ET-1 are dependent on reduced autocrine DKK1 secretion from the osteoblast (15;16). The osteoblastic response to prostate cancer bone metastasis may therefore be dually dependent on downregulation of microenvironment DKK1 secretion from osteoblasts via tumor-produced ET-1 and prostate cancer cells themselves. Furthermore, Wnts secreted by prostate cancer cells may further stimulate osteoblastogenesis and pathologic bone formation during prostate cancer bone metastasis (17).

Hypoxia signaling, mediated by the transcription factor hypoxia inducible factor (HIF), also promotes bone metastasis through regulation of cellular responses to low oxygen, including angiogenic, proliferative, prometastatic, and anti-apoptotic responses. Data recently published and presented at this meeting demonstrated that bone metastases are hypoxic (18) and that HIF may be an important mediator of bone metastasis by regulating prometastatic gene expression (19). One study showed that hypoxia regulates expression of heparan binding growth factors (HBGFs) in prostate cancer (20). C4-2B prostate cancer cell expression of vascular endothelial growth factor (VEGF)-A is increased 2-fold under hypoxic conditions. Bone marrow endothelial (BME) cells highly express the VEGF-A receptor, VEGF2R/flk-1. Culture of BMEs in the presence of conditioned media from C4-2B cells induced formation of angiogenic-like structures. These data suggest that inhibitors of heparan sulfate signaling may be a potential therapy for bone metastasis.

In addition, hypoxia signaling through HIF-1 may crosstalk with other signaling pathways that have demonstrated roles in bone metastasis, including the TGF-β pathway (19). TGF-β and hypoxia increased VEGF and CXCR4 promoter activity and mRNA expression *in vitro* and hypoxia may increase TGF-β signaling in bone by blocking mRNA expression of the TGF-β repressors Smad7 and SnoN. These studies suggest that combined inhibition of the hypoxia and TGF-β signaling pathways may be a useful therapeutic approach to treating bone metastases. Inhibitors of TGF-β have been shown to decrease tumor burden and promote survival in mouse models of breast cancer bone metastasis (11), while increasing bone mass (21).

Hypoxia inhibitors, of which numerous agents are currently being tested, may also have negative effects on tumor growth and positive effects on bone. Though the

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mechanism of action of these agents has not been entirely defined, their antitumor effect may result from numerous activities, including inhibition of HSP90 and prevention of stabilization of HIF-1, through agents such as geldanamycin and its derivatives, or depolymerization of microtubules, through agents such as 2-methoxyestradiol (2ME2). 2ME2 is a non-estrogenic, estrogen metabolite that has been tested in models of breast cancer and glioma (22-24). In models of osteosarcoma, 2ME2 induced growth arrest and tumor cell death. This may be due in part to increased OPG mRNA and protein expression by osteosarcoma cells treated with 2ME2 (25). In addition, data presented at this meeting show that, in combination with other prostate cancer therapies, such as zoledronic acid, 2ME2 may have positive effects to increase bone mass (26).

Transcriptional targets of HIF-1α may serve as additional therapeutic targets for treating bone metastasis. One study investigated the effect of bevacizumab, a humanized monoclonal antibody to VEGF, on breast cancer bone metastasis (27). Rats with MDA-MB-231 breast cancer bone metastases, treated with weekly injections of bevacizumab and monitored for lesion development by MRI and volume CT, developed decreased osteolytic lesions and soft tissue tumors.

These studies demonstrate that hypoxia contributes to bone metastasis. Further research may help to elucidate the effects of HIF-1 within the bone microenvironment and to identify and characterize antihypoxic agents that may be useful for treating bone metastasis.

Conflict of Interest: None reported.

Peer Review: This article has been peer-reviewed.

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