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## VI Meeting on Cancer-Induced Bone Disease

December 10–14, 2006, San Antonio, TX, USA

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*Expert Rev. Anticancer Ther.* 7(4), 461–464 (2007)

This latest international conference on cancer-induced bone diseases, organized by the Cancer and Bone Society, attracted over 300 participants, who considered basic and clinical aspects of the many ways in which cancers affect the skeleton. Previous meetings have developed the theme of the importance of the bone microenvironment in influencing tumor behavior. This was a major topic, with many examples throughout the meeting from basic, translational and clinical aspects of both cancer and bone biology. Cancers of the breast and prostate, and the bone disease multiple myeloma were under the spotlight the most. In all cases there have been major advances in our understanding of the pathogenetic mechanisms, clinical application and appraisal of bone-targeted drugs and the identification of new drug targets.

### Stem cells & cancer cells in the bone marrow

Concepts of intercellular communication among cells in bone have informed recent discoveries of the microenvironment occupied by stem cells in marrow, as reviewed by David Scadden (Boston, MA, USA). The stem cell niche is crucial for adult stem cells in the marrow, protecting them from premature exhaustion and enabling their reproduction, as well as protecting the host from excess activity of stem cells. Osteoblast lineage cells are vital in enabling expansion of stem cells, with osteopontin limiting numbers and the calcium-sensing receptor affecting

localization. The role of the osteoblast is illustrated by the effect of parathyroid hormone (PTH) as an osteoblast stimulator, through an indirect action improving the production, preservation and efficiency of stem cells. Strikingly, early results were reported that demonstrate the ability of PTH treatment to increase the engraftment efficiency of hematopoietic stem cells transplanted into irradiated mice. Among the fascinating aspects and questions in niche biology is the observation that more mature, dependent cells can revert to a stem cell phenotype if they engage in a vacant niche, increasing their proliferation and decreasing differentiation. Relevant to this, in an intriguing presentation of the application of changes in the *Drosophila* eye to screening for anticancer drug discovery, R Cagan (St Louis, IL, USA) indicated that ectopic cell occupancy of the niche can lead to proliferation of the occupying cells and suggested that PTH treatment could be used to amplify the therapeutic application of, for example, umbilical vein stem cells. There are clear indications that the niche compartment might lend itself to drug development.

The niche was taken further into cancer biology by David Lyden (New York, NY, USA), describing how the bone marrow, as 'soil', is primed to receive the cancer cell 'seed'. Vascular endothelial growth factor receptor (VEGFR)-1<sup>+</sup> hematopoietic progenitor cells in the marrow form clusters before cancer cell arrival, but specify the subsequent location of metastases. The role

of placental growth factor in this process was outlined, acting through VEGFR-1 to promote matrix metalloproteinase (MMP)9 production at the osteoblastic zone in the endosteal surface, and mobilizing stem cells to the inner vascular zone. With the arrival of tumor cells, VEGF promotes vessel formation through action upon VEGFR-2<sup>+</sup> endothelial progenitors. Evidence was presented that tumor-conditioned medium could drive bone marrow cells in tumor-specific ways, contributing to the development of a key concept of circulating cells and/or secreted factors that prepare the metastatic niche for subsequent cancer growth. This extends the seed and soil hypothesis of Paget while maintaining its integrity, made all the more exciting by the demonstration that by blocking VEGFR-1 with antibody to target bone marrow cell clusters, metastasis could be blocked.

Another aspect of host-tumor interaction in the marrow discussed by Lynne Matrisian (Nashville, TN, USA) was the role of MMPs, a large group of enzymes that has many substrates. MMPs degrade the extracellular matrix and influence growth, apoptosis, migration and chemotaxis, and their actions on cell surface proteins clearly have effects on processes important in tumor progression. Low molecular weight MMP inhibitor drugs have hitherto been disappointing in trials, even when greater specificity has been achieved. Better understanding of stroma properties may improve prospects of anti-MMP therapy and resolution of exactly which MMPs are pro- and which are anti-tumorigenic. In Matrisian's experiments, loss of stromal MMP2 and 9 reduced tumor growth in bone, and their hypothesis proposed that since the stroma itself is a more universal and stable target, changing the stroma by antistromal therapy might provide a new approach. The new biology provided in the stem cell niche research will be a valuable resource, along with the evidence for tumor programming of the marrow niche in preparation for metastasis establishment. Much of this

also points to the likelihood that anti-stromal or anti-MMP therapy needs to be applied early.

These basic studies of metastasis mechanisms are at an early stage, but seem certain to provide ideas that will help in addressing the questions raised by Klaus Pantel (Hamburg, Germany), who described studies in which disseminated tumor cells (DTCs), often referred to as micrometastases, in bone marrow aspirates taken from patients at surgery for primary cancers were localized. A surprisingly high number of patients with operable breast cancer were positive for DTCs (199 out of 552 subjects), compared with only two out of 191 in subjects with no known malignancy. With other primary cancers, a high frequency of DTCs in bone marrow illustrated what a favored site the bone marrow is for cancer spread. For example, in patients with apparently early prostate cancer or lung cancer, 20–30 and 40–60% of bone marrow samples, respectively, were positive. Similarly, high rates of marrow positivity were seen in colorectal and stomach cancer patients, tumors not normally associated with bone metastases.

In a meta-analysis of 4703 patients with breast cancer included in studies evaluating bone marrow positivity, detection of DTC in bone marrow at the time of surgery was an independent predictor of poorer survival. Prognosis became even more unfavorable among a subset of patients in whom the DTC deposits survived chemotherapy administered after surgery and patients who remained DTC positive were subject to a higher relapse rate, indicating that the cells can escape dormancy. Crucial questions include: how is such dormancy regulated? Does the marrow/bone microenvironment dictate wholly or in part when and to what extent escape from dormancy occurs? Is there anything in the primary tumor that determines the establishment of DTCs? The latter question is being addressed by DNA/RNA profiling of primary lung cancers and marrow DTCs. Some stem cell characteristics of cells in the DTCs were noted: perhaps related to their survival after chemotherapy, long-term persistence and low proliferative activity.

### Translational research on tumor osteolysis & drug development

A number of invited and submitted papers addressed these topics. Constant themes were the central role of active osteoclasts in the process of establishment and growth of cells from solid cancers and myeloma in bone, and the many ways in which the bone microenvironment contributes to the survival and growth of malignancy in bone.

Transforming growth factor (TGF) $\beta$  has complex and multiple roles in cancer initiation, progression and metastasis. It is a tumor suppressor, but in some circumstances may act as an enhancer and thereby promote tumor initiation, progression and metastasis. In cancer cells, there are frequently inactivating mutations in the TGF $\beta$  receptor, in the signal transduction pathway, in the intracellular signaling proteins called SMADs and facilitators of SMAD signaling. Any of these may lead to cancer progression, since the consequence of inhibition of the suppressive effects of TGF $\beta$  is tumor promotion. H Moses (Nashville, TN, USA) presented studies using epithelial cells with impairment in TGF $\beta$  signaling and showed that in the presence of introduced oncogenes, these cells underwent enhanced tumor progression and metastasis. He also showed that mutations in the TGF $\beta$  signaling pathway in stromal cells enhanced tumor progression through mechanisms that involve hepatocyte growth factor.

There was great interest throughout all sessions of the meeting in the role of TGF $\beta$  in bone metastasis, since TGF $\beta$  is enriched in the bone matrix and released in its active form when bone resorbs. Several different approaches were used to examine the role of TGF $\beta$  in bone metastasis, including the use of inactivating mutations in tumor cells to interfere with TGF $\beta$  signaling and the use of TGF $\beta$  receptor kinase inhibitors. All workers agreed that bone metastasis is reduced by inhibition of TGF $\beta$  signaling by whatever means.

The mechanisms responsible for TGF $\beta$  enhancement of bone metastasis are not entirely clear. It may be due in part to increased PTH-related protein expression

or expression of other bone-resorbing cytokines, but may also be due to loss of more direct effects of TGF $\beta$  on osteoclastic bone resorption, or possibly other mechanisms. One interesting possibility that several people suggested was that TGF $\beta$  may affect tumor growth in bone indirectly by its inhibitory effects on osteoblast differentiation. Removal of this TGF $\beta$  effect leads to enhanced osteoblast differentiation and bone formation, and creates (in a manner undefined) an inhibitory effect on tumor growth in bone. This finding is of particular interest since breast cancer and myeloma bone disease cause a decrease in bone formation through mechanisms that are still unexplained. It suggests that an approach to enhance osteoblast differentiation may reduce tumor growth in bone.

Other factors in the bone microenvironment are probably involved in bone metastasis but their roles are not yet defined clearly and they have received less attention than TGF $\beta$  (e.g., MMP9, VEGF and stromal-derived growth factor (SDF)). Future meetings will probably clarify the means by which these molecules participate in the metastatic process.

The first morning of the meeting was devoted to presentations on the bone disease associated with prostate cancer. L Cheung (Atlanta, GA, USA) reviewed his studies on  $\beta$ -2-microglobulin, a factor he believes important in potentiating metastasis and enhancing the behavior of tumor cells so that they mimic patterns of gene expression in osteoblasts, a phenomenon called osteomimicry. In the session on prostate cancer, R Vessella (Seattle, WA, USA) reviewed the rapid autopsy program at the University of Washington and indicated the importance of a heterogeneous population of DTCs in the bone marrow of patients with not only advanced prostate cancer, but also in patients after apparent curative therapy and without other evidence of disease. G Stein (Birmingham, AL, USA) also addressed the issue of osteomimicry, particularly with respect to RUNX2 expression, and discussed the intranuclear trafficking mechanisms that direct transcription factors to specific locations in the nucleus. C Logothetis

(Houston, TX, USA) and Y De Clerck (Los Angeles, CA, USA) focused on the importance of the microenvironment in prostate cancer and neuroblastoma, respectively, and specifically the potential of Sr-89 as a bone-directed therapy in prostate cancer, and the pathophysiologic role of stromal production of interleukin (IL)-6 in neuroblastoma.

The myeloma session presented a number of themes. The different preclinical models for studying myeloma disease were in evidence in the presentations by P Croucher (Sheffield, UK), J Epstein (Little Rock, USA), D Roodman (Pittsburgh, PA, USA) and B Oyajobi (San Antonio, TX, USA). One of the most interesting aspects to come out of the myeloma session was further confirmation of the importance of the bone marrow microenvironment in influencing myeloma cell behavior in bone. M Abe (Tokushima, Japan) showed the effects of osteoblast differentiation on myeloma cell growth, and the potential role of TGF $\beta$  in influencing this process. C Edwards (Nashville, TN, USA) focused on Wnt signaling, its importance in the microenvironment and specifically the differential effects on bone formation and on tumor growth, as well as the potential it has for influencing myeloma growth. Croucher and P Epstein both indicated the importance of bisphosphonates and receptor activator of nuclear factor (NF) $\kappa$ B (RANK) ligand antibodies, which inhibit osteoclastic bone resorption and also influence myeloma growth, again reinforcing the notion of the link between tumor burden and local bone disease. Roodman pointed to a number of molecular targets with the potential for inhibiting osteoclastic activity and focused on recent work from his group on p62, a molecule in the NF $\kappa$ B pathway.

In the section on new drug discovery and development, M Rogers (Aberdeen, UK) updated information on current mechanisms or action of the bisphosphonates. Oyajobi indicated that proteasome inhibitors may influence DKK1 expression by their effects on histone deacetylase. W Dougall (Seattle, WA, USA) and A Lipton (Hershey, PA, USA) updated information on the potential for neutralizing antibodies to RANK ligand

as powerful inhibitors of bone resorption in both preclinical and clinical models. B Boyce (Rochester, MA, USA) discussed the potential for Src inhibitors in the treatment of bone metastasis.

In the section on breast cancer, there was continued interest in the role of integrin inhibition and cell–cell attachment with the use of new agents described by P Clezardin (Lyon, France) and K Weilbacher (St Louis, IL, USA). T Guise (Charlottesville, USA) indicated the potential for inhibition of TGF $\beta$  signaling and its multiple roles in promoting bone metastasis and the important influence of receptor kinase inhibitors in promoting bone formation. G Van der Pluijm (Leiden, Netherlands) emphasized the importance of the epithelial–mesenchymal transition and the reciprocal roles of TGF $\beta$  and bone morphogenic proteins in mediating these phenotypic changes. L Suva (Birmingham, AL, USA) discussed the potential of specific cytokines, such as IL-8, in promoting bone resorption. M Gillespie (Melbourne, Australia) emphasized roles of osteoprotegerin, in addition to its inhibitory effects on osteoclastic bone resorption, in regulating tumor cell growth. K Weilbacher gave a presentation on platelet activation in breast cancer metastasis and strategies to counteract this.

In the symposium on pain, D Clohisy (Minneapolis, MN, USA) and T Yoneda (Osaka, Japan) indicated the importance of understanding the responsible molecular and cellular mechanisms in bone metastasis, and the potential for osteoclast inhibition to reduce bone pain. Yoneda summarized current molecular mechanisms that may be responsible for pain, focusing on proton production by osteoclasts and tumor cells and mediated through specific acid-sensing nociceptors. Interestingly, he indicated that this process may be enhanced by insulin-like growth factor-1.

#### Clinical developments

J-J Body (Brussels, Belgium) updated information on the most recent clinical trial results with the powerful bisphosphonates zoledronic acid and ibandronate for the treatment of bone metastases. The

clinical value of these compounds is undisputed but uncertainties remain regarding timing, duration and choice of bisphosphonate. Many of these were alluded to in a panel discussion on the pros and cons of intravenous as opposed to oral bisphosphonates and in presentations on the association of bisphosphonates with osteonecrosis of the jaw, an uncommon but potentially severe complication observed in recent years in advanced cancer patients who have typically received long-term monthly intravenous bisphosphonate treatments.

More rational use of bisphosphonates and the development of new therapeutic agents might be facilitated by the measurement of specific biochemical markers of bone metabolism. M Siebel (Sydney, Australia) reviewed recent experience with bone marker measurements in oncology. Although most markers of bone turnover are increased in patients with established bone metastases, they are not useful for reliable and accurate early diagnosis of bone involvement. However, raised bone-resorption markers provide prognostic information and suppression of bone resorption appears to be associated with a favorable clinical outcome. Recent evidence suggests that the aim of bisphosphonate therapy should be to normalize increased rates of bone remodeling. They are useful in drug development for assessing cohorts of patients but use in individual patients cannot be recommended until the results of ongoing clinical trials are available.

Inhibition of the RANK ligand pathway presents the most exciting current therapeutic development. R Dansey (Thousand Oaks, CA, USA) reviewed the therapeutic rationale and development to date of denosumab, a highly potent, humanized monoclonal antibody to RANK ligand. Denosumab effectively suppresses bone resorption and is administered by subcutaneous injection and presents a serious therapeutic challenge to the bisphosphonates. Dose-finding studies were presented by Lipton and have defined a dose and schedule for Phase III trials in advanced malignancy and cancer treatment-induced bone loss. A broad portfolio of

Phase III trials across the range of cancers associated with bone metastases are in progress.

B Boyce reviewed a number of other new therapeutic approaches for cancer-induced bone disease. These included inhibitors of the Src tyrosine kinase, which is involved in many cellular processes including the formation of the osteoclast ruffled border and inhibitors of cathepsin K, which mediates the secretion of protons and chloride for the dissolution of the mineral and degradation of the matrix of bone. A number of small molecules interacting with these targets have passed through early clinical development, where they have been shown to effectively and safely inhibit bone resorption, and are now entering specific trials in cancer patients.

Bone metastases cause considerable morbidity and, alongside developments in drug therapy, therapeutic advances in interventional radiology and bone targeted radioisotopes have occurred. J Lane

(New York, NY, USA) described the therapeutic value of kyphoplasty in the management of vertebral collapse while C Perez (St Louis, IL, USA) reviewed our current knowledge on the role of radiopharmaceuticals.

Finally, cancer treatments themselves can have profound effects on bone health and may, usually through endocrine effects, increase the rate of bone loss and predispose patients to the development of osteoporosis. The effects of breast cancer treatments on bone, notably aromatase inhibitors, and strategies for management were reviewed by E McCloskey (Sheffield, UK), while the similar deleterious effects on bone health associated with androgen-deprivation therapy in the management of prostate cancer were considered by M Smith (Boston, MA, USA). Algorithms for monitoring individual patients and strategies for therapeutic intervention with bisphosphonates and denosumab are under development.

The pace of change is rapid in both our understanding of the biology underlying the metastatic process in bone and therapeutic options. The Cancer and Bone Society will meet again in June 2008 in Edinburgh, Scotland and yearly thereafter, with the aim of once again bringing together international experts in this important area of cancer biology and therapeutics.

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