MEETING REPORTS

Meeting Report from Skeletal Complications of Malignancy IV

A symposium jointly sponsored by The Paget Foundation for Paget’s Disease of Bone and Related Disorders, the National Cancer Institute, and the University of Virginia School of Medicine

April 28-30, 2005 in Bethesda, Maryland, USA


- Prostate Cancer - Robert L. Vessella
- Biology and Anti-Vascular Therapy of Bone Metastases - Theresa A. Guise and Edward S. Susman
- Breast Cancer - Larry J. Suva
- Multiple Myeloma - Gregory A. Clines
- Primary Bone Tumors/Osteosarcoma - Scott L. Kominsky and Kristy L. Weber
- Cancer Bone Pain - John M. Chirgwin
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- Bone Complications of Cancer Therapy - Wende Kozlow
- Current and Future Directions in Treatment - Wende Kozlow and Theresa A. Guise

PROSTATE CANCER

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The session on prostate cancer opened with an overview of advanced disease, and focused on tumor cell interactions with the host, particularly in regard to the bone microenvironment, which facilitate metastases (1). Two long-standing rapid autopsy programs at the University of Washington and at the University of Michigan have enabled the study of metastatic prostate cancer, especially metastases to bone. Collectively, these investigations have revealed that nearly 100% of all patients have at least microscopic metastases in bone at the time of death from advanced disease, and that bone metastases are extraordinarily heterogeneous in histology and biomarker expression. These studies have also provided insight into the novel pathophysiology responsible for bone metastases. For example, over-expression of CD-55 by metastatic prostate cells enables the tumor cells to avoid the complement system of the immune response (1).

A common theme in this session was the vicious cycle between cancer and bone, and its complexity was illustrated by the diversity of factors implicated in the pathophysiology of bone metastases. The normal calcium-regulating hormone, parathyroid hormone (PTH), and the tumor-peptide PTH-related protein (PTHrP), may have an important role in prostate cancer bone metastases through their stimulation of osteoblasts and subsequently osteoclasts (2). Investigators have shown that PTH and PTHrP have both...
The molecular mechanisms by which ET-1 stimulates the osteoblast via ETA receptors were investigated using a molecular approach (9). Gene array analysis, and validation, of ET-1 treated osteoblasts showed suppression of Dickkopf homolog 1 (Dkk1), a tonic inhibitor of the Wnt signaling pathway, and increased production of CCN1 and CCN2 (Cyr61 and CTGF, respectively) and IL-6 (10). Overexpression of Dkk1 suppresses bone formation in multiple myeloma and blocks new bone induced by ET-1. The investigators hypothesized that ET-1 increases osteoblast activity in prostate cancer by suppressing Dkk1 (10); this was proposed independently by other investigators at the meeting (11;12). In addition, CCNs and IL-6 may mediate the effects of ET-1 on osteoblast activity. Since these factors may also affect the osteolytic side of the vicious cycle, the role of combined ETA receptor blockade and bone resorption inhibition was studied in the prostate cancer xenograft model, LuCaP 23.1, which stimulates an osteoblastic response when inoculated into bone (13). Treatment of tumor-bearing mice with either the ETA receptor antagonist, atrasentan, or the bisphosphonate zoledronic acid, slightly reduced tumor growth and osteoblast response. However, the combination was significantly better than either alone. Since this did not completely abrogate tumor growth, it is clear that other tumor osteoblastic factors have a role. LuCaP23.1 also produces adrenomedullin (AM), which stimulates osteoblasts, induces new bone formation and inhibits apoptosis. Expression of AM by the highly osteolytic PC-3 cell line induced new bone formation. Furthermore, an inhibitor of AM, a phenyl acetic acid derivative, blocked the effect of AM on new bone formation. Collectively, these data confirm that osteolysis has an important role in the pathophysiology of osteoblastic bone metastases, but also that tumors produce multiple factors which stimulate osteoblast activity.

The theme of osteoblast stimulatory factors continued with descriptions of studies on the role of bone morphogenetic protein 7 (BMP-7) in prostate cancer bone metastases (14).
BMP-7 inhibits proliferation of LNCaP, an androgen sensitive prostate cancer cell line, but not C4-2, an androgen independent prostate cancer cell line. Further study in these lines revealed that BMP-7 treatment increased androgen receptor dependent transcriptional activity in LNCaP cells more than in C4-2 cells. The transcriptional pathways were further explored and it was noted that BMP-7 treatment dramatically increased the association between the androgen receptor and beta-catenin. Thus, BMP signaling can interact with both the androgen receptor and Wnt signaling pathways. BMP-7 may enhance androgen receptor signaling or the Wnt pathways, depending upon the beta-catenin status of the cell. These studies provided further evidence for the role of the Wnt pathway in prostate cancer metastases, an emerging theme in tumor metastases to bone.

Receptor activator of NF-κB (RANK) and RANK ligand (RANKL) are critical mediators of osteoclastic differentiation and bone resorption. Osteoprotegerin (OPG) is the soluble decoy receptor which binds RANKL and neutralizes its effects. Emerging evidence indicates that the RANKL, RANK and OPG system may have important roles in cancer, as well as in normal bone remodeling. Investigators developed a fully humanized monoclonal antibody, designated AMG-162, that targets RANKL as a means of decreasing bone resorption (15). Using the PC-3 prostate cancer cell line that expresses RANK, they identified 40 genes that were upregulated, and 20 genes that were downregulated, upon exposure of PC-3 to RANKL. Furthermore, when PC-3 was treated with Fc-OPG or the bisphosphonate zoledronate (ZA), they noted a selective subset of genes downregulated by Fc-OPG but not ZA. Thus, RANKL inhibition may have indirect effects on tumor cell growth in addition to its inhibition of osteoclast activity.

Matrix metalloproteinases (MMPs) have long been implicated in cancer metastases. The MMP, Mt1-MMP, may have a role in metastases to bone. Investigators showed that Mt1-MMP is highly expressed in prostate cancer bone metastasis and proposed that Mt1-MMP degrades collagen and facilitates tumor cell engraftment in bone (16). In the LNCaP model, which expresses low levels of Mt1-MMP, transfection with Mt1-MMP resulted in an increase in osteolytic lesions when engrafted in bone. Alternatively, silencing Mt1-MMP in the DU-145 prostate line, which expresses high levels of Mt1-MMP, with siRNA reduced proliferation and collagen degradation while the xenografts revealed an increase in new bone formation. Mt1-MMP may play a key role in prostate cancer bone metastasis.

The above reports represent the exponential growth of our knowledge of specific factors responsible for prostate cancer metastases to bone. However, it has long been recognized that prostate cancer cells lie dormant in bone marrow, even at the time of diagnosis. What activates some of these dormant prostate cancer cells to yield clinically relevant bone metastases is unknown and a critical area of investigation. Detection, isolation and characterization of disseminated prostate cancer cells from the peripheral blood or bone marrow aspirates (BM) that then allows for isolation of single DTC (17). Using sets of 10-20 DTC, they have begun characterization studies involving quantitative RT-PCR, gene array profiling and array CGH. Their results suggest that a majority of, and perhaps all, patients at the time of initial diagnosis have DTC in their bone marrow. A significant proportion of patients who were considered free of disease for more than five years after a radical prostatectomy still harbored DTC, although presumed dormant at this point in time, in their BM. Results from the array CGH studies indicate that genomic alterations are observed among the DTC from patients early in their disease and that the frequency increases as the disease...
develops the ability to form aggregates with either lymphocytes or platelets. Platelets were designed to stop bleeding but also facilitate tumor metastases since some tumor cells express adhesion molecules that will bind platelets to form multi-cell clumps. Such cellular groups arrest in distant capillary beds by adhering to receptors on the surface of endothelial cells. Few of these cells can extrapolate into the organ parenchyma where the cells proliferate. Here, if the tumor is to grow larger than a millimeter at the new site, it must respond to the microenvironment and stimulate angiogenesis, since oxygen is an absolute requirement for tumor proliferation and survival. It is also necessary for the tumor to be within one hundred microns of a blood vessel.

Investigators hypothesized that induction of apoptosis in tumor endothelial cells will reduce oxygen delivery to tumors and induce secondary apoptosis in the surrounding tumor cell and normal cell (18). To test this idea, the targeted agent Gleevec and the cytotoxic agent paclitaxel were utilized in a mouse model of human metastatic prostate cancer. Gleevec targeted growth factors, such as PDGF, which prevented the tumor from developing an oxygen supply. While mice that were not treated with the combination all developed tumors, only one-fourth of the animals treated with Gleevec/paclitaxel had tumors (19). In a clinical trial that translated the laboratory work to the clinic, 21 patients were treated with Gleevec at 1600 mg per day and docetaxel. After months and months of treatment, 67 percent of the patients responded, with 50% being long-term responders. Combination treatment requires that clinicians accept three principles. First, targeted therapy requires a target. Gleevec should only be used if the tumors express the PDGF receptor. Second, heterogeneous disease cannot be treated by homogeneous therapy. Multimodality treatment is the name of the game. Third, chronic diseases require chronic treatment. Cancer metastases are a chronic disease, and should be treated as such, with long-term plans and goals in place.
Conflict of Interest: Dr. Guise reports that she has received a research grant and speaker’s fee from Novartis and that she is a Member of the Board of Directors of the Paget Foundation. Mr. Susman reports that no conflict of interest exists.

BREAST CANCER

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A clear distinction between this meeting and preceding Skeletal Complications of Malignancy meetings, in terms of the breast cancer research presented, was the relatively small number of breast cancer (and other predominantly lytic cancers) papers presented. The field as a whole seems to have become aware of the relative lack of primary data regarding the mechanism(s) of prostate cancer progression. Still, the studies of breast cancer presented were fascinating and novel.

Overall, the overriding conclusion of the breast cancer presentations was that the phenotype of metastatic breast cancers is complex and involves numerous factors. Several presentations identified important components of the metastatic phenotype that collectively may represent a better determinant of the phenotype of aggressive breast tumors than the individual factors alone. In fact, one presentation (20) discussed the multigene nature of osteolytic tumors, focusing on the importance of degradative enzymes, cytokines and the extracellular proteoglycan signature in defining the tumor phenotype.

Other data presented (21), based on microarray analyses, suggested an important role for Smad4 in the metastatic cascade. Similarly, data from two studies (22;23) suggested important roles for the αVβ3 integrin in tumor cell lines and the expression of a range of chemokines, including IL-8 and IL-1, in primary human breast cancer. In other studies (24), a laminin-α1 peptide was shown to increase tumor adhesion and enhance the development of lytic lesions in vivo, demonstrating the importance of adhesion and the extracellular matrix in tumor progression, as did the identification of a role for connexin-43 in modulating the invasive phenotype of tumor cells (25). Supporting these data, another presentation used an antibody to bone sialoprotein to successfully target breast cancer progression in a preclinical rat model (26).

There were several gene expression profiling papers presented (27-29) that collectively identified some unique factors, but confirmed the expression of many previously identified genes. Interesting, but not too surprising, is the growing body of evidence identifying similar patterns of gene expression between breast and prostate cancer cells in preclinical models. These data stress the importance of the interaction of the bone marrow microenvironment with metastatic tumor cells.

In terms of clinical papers, perhaps the most informative was the detailed presentation of the status of current clinical trials with emerging therapies for breast cancer (30). In addition, a presentation (31) described the status of anti-OPG and anti-RANKL therapy. Perhaps the most intriguing finding is the observation that the potency of OPG in a phase 1 study of cancer patients was seemingly less than in osteoporosis patients. The interpretation that elevated RANKL levels are present in cancer patients remains hypothetical, and alternate interpretations, such as the existence of other non-RANKL activities, inducing osteolysis in cancer patients, do exist. In support of this latter idea, a presentation (32) clearly demonstrated that the tumor-associated macrophages are a local source of osteoclast precursors responsive to both RANKL-dependent and -independent mechanisms in many primary human cancers.

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MULTIPLE MYELOMA

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Therapy for multiple myeloma is changing rapidly, based on expanding knowledge of pathophysiology. Standard initial therapy for multiple myeloma consists of an alkylating agent such as melphalan and prednisone. Hematopoietic stem cell transplantation is also considered in selected patients. The combination of vincristine, doxorubicin and dexamethasone is often used as an initial regimen in preparation for stem cell transplantation or in the treatment of relapsing or refractory disease. In the last five years, many new therapeutic agents have been introduced. Thalidomide and the proteosome inhibitor bortezomib have shown benefit in refractory or relapsing disease, but more recent evidence also shows benefit in the initial treatment of multiple myeloma. Investigators detailed the reactions and interactions of the critical ubiquitin-proteasome pathway within normal and cancer cells involved in the necessary protein breakdown and mechanism vital to cell growth (33). In cancer, disruption of the reactions should result in ways of preventing metastases and cancer cell growth. The knowledge gained through the laboratory bench discovery of these mechanisms resulted in new medicines, including the promising PS-341 (Velcade) now in clinical use. Additional innovative therapies that target multiple myeloma cells, the bone microenvironment, and interactions between myeloma cells and the microenvironment are under development or are in clinical trials (34).

The antitumor actions of thalidomide occur through induction of myeloma apoptosis, augmentation of host immunity and inhibition of angiogenesis. Used alone or in combination with high-dose dexamethasone in the treatment of refractory disease, this agent increases time to progression and survival (35;36). The addition of thalidomide to the standard initial therapy of melphalan and prednisone or of dexamethasone also shows benefit (34;37). The thalidomide analogue revlimid appears to have similar activity as thalidomide but with an improved side-effect profile (34).

The proteosome inhibitor bortezomib decreases myeloma cell proliferation, inhibits DNA repair and angiogenesis, and overcomes myeloma drug resistance. In a recently reported phase III trial comparing bortezomib to dexamethasone, treatment with bortezomib resulted in an increased time to progression in patients with relapsed/refractory disease (38). Another proteosome inhibitor, NPI-0052, triggered apoptosis in bortezomib-resistant patients (34). Clinical studies are now underway to evaluate the cytotoxic effects of the hsp90 inhibitor 17AAG and a p38 MAPK inhibitor. Preclinical studies are evaluating the effectiveness of inhibitors to VEGF, telomerase, histone deacetylase and IGF-1R.

Effective therapies will likely take a multi-targeted approach. The combination of a p38 MAPK inhibitor enhanced bortezomib-induced cytotoxicity, and bortezomib and 17AAG prolonged survival in an animal model of multiple myeloma (34). Whether these newer agents, alone or in combination, will replace autologous BMT for treatment of multiple myeloma is unclear.

Mechanisms of bone destruction and net bone loss in multiple myeloma are increasingly being understood. Unlike most other osteolytic processes, multiple myeloma bone disease is characterized by both increased osteoclast bone resorption and decreased osteoblast-mediated bone formation. The adhesion of myeloma cells to bone marrow stromal cells markedly increased the production of stromal cell IL-6 and RANKL, which increase osteoclastogenesis (39). The importance of RANKL was demonstrated in a myeloma animal model where administration of RANK-Fc in mice decreased tumor burden (40). MIP-1α, a myeloma cell product, is a potent osteoclast stimulatory factor that was increased in the marrow plasma of 70% of
patients with myeloma (39). Another osteoclast stimulatory factor, IL-3, was also increased in myeloma patients and inhibited osteoblast mineralization (39).

Recently, Dickkopf homolog 1 (Dkk1) was reported to be involved in the suppression of osteoblasts in multiple myeloma (41). Using gene microarray technology, Dkk1 transcripts were found at higher levels in plasma cells of patients with more advanced disease. Moreover, Dkk1 protein levels were elevated in the bone marrow plasma and peripheral blood of patients with myeloma bone disease compared to control plasma cells. Dkk1 is a secreted inhibitor of the Wnt signaling pathway and binds to the LDL-receptor-related proteins 5 and 6 (LRP5 and 6), preventing interaction of these coreceptors with the frizzled (Frz) receptor family (42).

The Wnt pathway is important in the differentiation of mesenchymal stem cells to mature osteoblasts. Dkk1 appeared to alter the bone microenvironment by suppressing osteoblast differentiation (43). In vitro experiments support this statement. The addition of recombinant Dkk1 to osteoblast cultures decreases the BMP-2-mediated increases in alkaline phosphatase, a marker of osteoblast differentiation (41). Dkk1 also blocked the osteoblast proliferative effects of endothelin-1, and decreased levels of this factor may also have a role in promoting osteoblastic disease of prostate cancer and some breast cancers (10). Dexamethasone increases the expression of osteoblast Dkk1 and may explain bone loss associated with chronic administration of glucocorticoids (44). Conversely, bortezomib decreases osteoblast Dkk1 production and increases new bone formation in calvarial organ cultures (45). A similar anabolic effect was observed with Dkk1 neutralizing antibodies (10). Preclinical studies with anti-Dkk1 antibodies show promise. In a mouse animal model of myeloma bone disease, anti-Dkk1 antibodies increased osteoblast activation and osteoclast inactivation, and decreased bone loss and tumor burden (43). Dkk1 may also indirectly promote osteoclastogenesis by stimulating IL-6 production from mesenchymal stem cells.

An additional role for Dkk1 in other osteolytic diseases is likely. Human prostate cancer PC-3 cells produce osteolytic lesions in a mouse model of bone metastases. PC-3 cells abundantly express Dkk1 compared to the osteoblastic prostate cancer cell lines C4-2B and LuCaP-35. PC-3 cells were stably transfected with a Dkk1 siRNA construct and tested in an in vitro mineralization assay. These clones failed to stimulate mineralization. Furthermore, an opposite response was observed in C4-2B cells that overexpress Dkk1 (12).

Conflict of Interest: The author reports that no conflict of interest exists.

PRIMARY BONE TUMORS/OSTEOSARCOMA

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Ewing's sarcoma (ES) is the second most common primary bone malignancy in childhood and adolescence (46). Clinical trials have yielded advances in the treatment of this disease, however, the low number of children eligible for phase I clinical trials (47) emphasizes the need for clinically relevant murine models. Investigators (48) described the development of a human xenograft model of ES in immature nude mice. Intrafemoral administration of TC-71 ES cells resulted in the development of osteolytic lesions in all animals within 6 weeks, as determined by radiography and micro-CT scans showing reduced trabecular bone volume. Correspondingly, multinucleated osteoclasts were observed in close proximity to ES cells, confirmed by positive staining for the Ewing's marker CD99. Radiation therapy, either primary, postsurgical, or presurgical, is used in approximately 20% of ES cases (49), however, the role of radiation therapy in the local treatment of ES remains to be
determined. Using the murine model described above, radiation treatment inhibited tumor growth in a dose-dependent fashion. However, as observed in previous studies using Sprague-Dawley rats (50), radiation treatment resulted in growth plate damage, as evidenced by limb length discrepancies.

Paget's disease of bone is characterized by an excessive and abnormal remodeling of bone (51). While infrequent, this condition can be complicated by sarcoma. Sarcomas occur in approximately 1% of cases and develop more frequently in men than in women, suggesting a potential hormonal influence. Osteosarcoma is the most common histological type, occurring in 80-90% of cases, followed by fibrosarcoma. While tumors commonly occur in the skull, humerus, pelvis, and femur, the spine is typically uninvolved despite the high incidence of Paget's disease in the vertebrae. Loss of heterozygosity studies suggest that the development of Paget's disease and osteosarcoma may be linked by a single gene or two tightly linked genes on chromosome 18, which may be associated with loss of a tumor suppressor gene (52,53). The specific identity of this gene remains unknown.

Fibrous dysplasia is a very uncommon disorder of the skeleton that causes expansion of one or more bones due to abnormal development of the fibrous, or connective, tissue within the bone. Sarcoma can be a complication of fibrous dysplasia occurring in 0.5% of patients with monostotic disease and 4% of patients with McCune-Albright syndrome. Incidence is equal in males and females and does not appear to be correlated with age. Similar to Paget's disease, osteosarcoma occurs most frequently followed by fibrosarcoma and chondrosarcoma. Factors linking sarcoma incidence with fibrous dysplasia have not been discovered, however, some evidence suggests that radiotherapy may be involved in sarcoma development (54).

While studies on cancer development and progression have placed much emphasis on the cellular microenvironment, recent work has emphasized the importance of the nuclear microenvironment. Intranuclear organization of transcriptional regulatory machinery has profound effects on normal cell biology as well as tumorigenesis. Evidence of this has been demonstrated in the Runx (Cbfa/AML) family of transcription factors (TF), which function as scaffolds for chromatin remodeling machinery and play a role in tissue differentiation during embryonic development (55). A unique 31-38 amino acid nuclear matrix targeting signal (NMTS) has been identified in Runx that directs the TF to distinct nuclear matrix-associated sites within the nucleus (56). When mice carrying a homozygous deletion of the NMTS in Runx2/CBFA1/AML3 were generated, they failed to develop bone due to maturational arrest of osteoblasts, despite successful entry of mutant Runx2/CBFA1/AML3 into the nucleus (57). This suggests that subnuclear targeting is essential for proper transcriptional regulation. Under cancerous conditions, expression of mutant Runx2/CBFA1/AML3 in the breast cancer cell line, MDA-MB-231, inhibited tumor growth and osteolysis in mice, and reduced invasiveness in vitro (58).

In acute myeloid leukemia (AML), the commonly occurring (8;21) chromosomal translocation alters the subnuclear targeting of the TF AML1. Interestingly, when a single amino acid substitution was used to disrupt the NMTS of AML1, differentiation of myeloid progenitors was blocked, cells continued to proliferate, and exhibited a transformed leukemia phenotype (59). Taken together, these studies suggest that the fidelity of nuclear organization plays an important role in both normal and disease processes, and may provide future targets for therapy.

Osteosarcoma is the most common primary bone tumor in children and adolescents (60). The standard treatment is neoadjuvant chemotherapy followed by wide surgical resection and continued systemic treatment. The current 5-year survival rate for patients with nonmetastatic extremity osteosarcoma is approximately 65-70%. No breakthroughs
in treatment or overall survival have been made in the last 10-15 years and further research is needed to better understand this disease and identify new options for treatment.

There is a lack of information at present regarding the pathogenesis of osteosarcoma and related genetic mutations, and this dearth of information was addressed in a subset of patients with osteosarcoma (61). A separate peak of incidence of osteosarcoma was noted in older patients. The majority of this subgroup is due to secondary osteosarcoma in patients with monostotic or polyostotic Paget's disease. There is a 1% chance of osteosarcoma developing in monostotic Paget's disease and a 5-10% chance in patients with polyostotic disease. The relationship of Paget's disease to osteosarcoma has been studied in light of a mutation in the SQSTM1 gene, which is part of the RANKL signaling pathway (62). This P392L mutation (CGC->CTC) is the most common mutation in Paget's disease, is located in the ubiquitin-binding domain, and is present in 46% of familial and 16% of sporadic cases of Paget's disease. It has been shown that blood from patients with Paget's disease is negative for the mutation. Bone cells captured and sequenced from pagetic bone are heterozygous for the P392L mutation, but cells from the Pagetoid osteosarcoma are homozygous for the mutation. There is no mutation in adolescent patients with osteosarcoma. The available clinical data suggests that mutations in the SQSTM1 gene are predisposing but not sufficient for pagetoid osteosarcoma.

The presence of RANK has been noted in proliferating mesenchymal stem cells (63). As cells differentiate along the osteoblast lineage, RANK is turned off and osteoprotegerin (OPG) begins to increase. Further investigation revealed that RANK is strongly expressed in both childhood osteosarcoma and in the secondary form of osteosarcoma associated with Paget's disease. Confocal microscopy has shown that RANK localizes to intracellular areas rather than on the cell membrane. The question of whether RANK is causative of, or just a marker for, osteosarcoma was addressed with small interference RNA (siRNA) techniques. Osteosarcoma cell lines transfected with siRNA for RANK died, presumably because of apoptosis. Those cells transfected with siRNA for RANKL were strongly inhibited in their ability to grow on soft agar.

A controversial topic involves the influence of estrogen on the development of osteosarcoma in children and adolescents. It has been suggested that estrogen is a strong modulator of normal bone physiology in both men and women. Published studies show that 40% of osteosarcoma samples (n=65) are estrogen receptor positive. Estrogen induces changes in cells that suggest a more osteoblast-like phenotype (64). The influence of this hormone on high-grade osteosarcoma was studied by analyzing the SEER database (65). By separating 2017 cases from 1973 to the present by gender into prepubertal (0-9 years), peripubertal (10-16 years), postpubertal (17-30 years) and mature (>30 years), a higher incidence of osteosarcoma was noted in postpubertal males compared to females (p<0.0003). Females in the peripubertal group had a significantly better prognosis (p<0.05) than prepubertal females, peripubertal males, and postpubertal males. The status of ERα and ERβ in untreated human osteosarcoma tissues was correlated to clinical outcome in 24 cases. The expression of ERα was associated with localized disease at presentation (p=0.04) and a significantly improved event-free survival (p=0.05). Evaluation of osteosarcoma cell lines revealed a dose-dependent growth inhibition with increasing doses of 17B estradiol in the line that was ERα+ compared to no inhibition in the lines that were ERα-(66). This work raises the question of whether estrogen can be used as a prognostic marker or potential therapeutic target in patients with osteosarcoma.

Future directions for research in osteosarcoma should be focused in the areas that will allow improved clinical outcome in the treatment of patients (67).
Work is needed in many areas, including diagnosis/prognosis; neoadjuvant systemic cytotoxic agents; methods to detect tumor response to therapy; safe surgical treatment to maximize function outcome; and treatment of systemic metastasis. Specific areas where breakthroughs are needed include new genetic and molecular tests to identify the etiology of the disease, an expression signature of osteosarcoma, and possible markers with prognostic significance (68). Immunotherapy and cardioprotective agents added to current multidrug cytotoxic chemotherapy regimens may decrease toxicity. Molecular or radiologic assays to assess the response of the tumor prior to surgical resection may allow better function-preserving resection and identify responders and nonresponders to front-line chemotherapy (69). Aerosolized drugs or targeted molecular therapy to prevent or treat pulmonary metastasis is necessary to improve the approximately 20% survival of patients with systemic disease (70;71). The main issues to consider in the surgical resection of osteosarcoma relate to achieving adequate margins and developing implants that are durable enough to last the extended lifespan of these patients (60). The options for limb reconstruction include the use of cadaveric allografts, prostheses, or biologic options using autologous bone. Metal endoprostheses have become the reconstruction of choice because they are durable and allow immediate weightbearing. Patients resume systemic chemotherapy regimen soon after surgery. Skeletally immature patients provide a major challenge for long term successful reconstruction as major growth plates are often removed with the tumor. Historically, there has been a high failure rate of expandable endoprostheses, but there are innovative noninvasive options currently available. In addition, vascularized epiphyseal plate transfer using the fibula is a novel way to provide a biologic growing reconstruction in a skeletally immature patient.

Neuroblastoma is a childhood cancer that primarily metastasizes to bone (72). The type I insulin-like growth factor receptor (IGF-RI) induces neuroblastoma cell motility, migration, invasion, and survival (73). It was shown that transfection of the IGF-RI into neuroblastoma cells not expressing the native gene increases neuroblastoma adhesion to endothelial cells, transendothelial migration, and attachment to bone stromal cells (74). Tumor cells expressing IGF-RI are able to grow in the bone after intratibial or intracardiac injection, in a mouse model.

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CANCER BONE PAIN

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Bone pain is a major source of morbidity for cancer patients with skeletal involvement, particularly for those with multiple myeloma or tumors of the breast or prostate. Three oral presentations clearly summarized the future directions that need to be taken in this understudied area.

Emphasis was placed on the importance of the adoption of uniform, objective instruments for pain evaluation so that results from clinical trials can be meaningfully evaluated (75). The treatment of cancer bone pain was summarized (76). Opioids are the standard treatment, and dose-escalation is usually needed as tumor burden increases. However, pain is often under-treated for fear of addiction or dependency, as well as to avoid side effects, and also because of concerns that the pain is psychogenic or idiopathic. In fact, all of these feared outcomes are rare, and patients need to be treated aggressively to improve quality of life.

Other investigators described the molecular mechanisms of bone pain and the animal models used to study them, including behavioral testing in mice and specific neurochemical staining of nerve endings in
bone and associated dorsal root ganglia (77). Pain responses were elicited by acid, ATP, and a series of peptides, such as nerve growth factor (NGF), TNF-α, IL-1, prostaglandin E2, and endothelin-1, released in the tumor/bone microenvironment. In addition to the standard clinical treatment of irradiation, bone pain can be alleviated by specific receptor antagonists against several of these factors, as well as osteoprotegerin, NSAIDs, and COX-2 inhibitors. Antibody neutralization of NGF appears to be particularly effective and is being entered into clinical trials. Treatments that target tumor, bone, or their interactions are all likely to ameliorate pain. There may also be specific neurotransmitters in the path from bone to the CNS that will be recognized in future work and could be selectively targeted to reduce tumor-induced bone pain.

The oral presentations were complemented by three posters presenting data on a rat model of prostate cancer bone pain (78), on the assessment of resource utilization by prostate cancer patients with bone pain (79), and on the reduction of metastatic bone pain in patients treated with the bisphosphonates pamidronate and ibandronate (80). Bone pain in cancer is an area in need of much more work at all levels, from better education of hospice care providers to closer collaborations between cancer metastasis researchers and neuroscientists.

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**NEW DIRECTIONS IN IMAGING**

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Tumor imaging technology has evolved exponentially and new techniques were described. Investigators (81) used micro-CT to evaluate the impact of TGF-β on breast cancer metastasis. The model system used was an orthotopic model where 4T1 cells were injected into the mammary fat pad and followed over time. At one week, a palpable tumor was detected; at 2 weeks, lung lesions were present; at 3 weeks, liver metastases were present; and at 4-5 weeks, bone metastases were detectable. Using an antibody to TGF-β that blocks TGF-β 1, 2 and 3, the tumor grade and number of lung lesions was significantly reduced.

In order to better quantify the extent of lesions, several strategies were used, each of which has advantages and disadvantages. Micro-CT at a resolution of 16 microns is effective at tumor detection and is more sensitive than histology because of the difficulty of evaluating the entire depth of tissue with single histologic sections. There are machines that can perform micro-CT *in vivo*, but the resolution is at the 40 micron level, and in this particular model system would be difficult due to the challenge of anesthesia with lung metastases over time. Micro-CT also provides volumetric analysis of the tumor, for example in the lung, and volume of bone loss in the osseous sites. Traditionally, for the lung, metastases have been scored on gross exam, but this can underestimate the impact of lesions below the surface that the micro-CT can detect. Micro-CT is also more sensitive than microradiographic analysis for osseous lesions. Micro-CT analysis of osseous lesions with anti-TGF-β antibody revealed an approximately 5% decrease in bone volume with antibody alone, a 28% decrease in bone volume with tumor, but a 14.3% increase in bone volume with antibody and tumor combined groups. For mouse melanoma studies, cleared scoring of lung lesions is more sensitive but misses the volumetric measures. In contrast, micro-CT misses some small tumors and thus underestimates the numbers of lesions, but provides volumetric measures. Finally, imaging of the vasculature can be performed using three separate color markers such as CD31 to mark endothelial cells, actin to measure late stage pericytes and collagen type IV to track the basement membrane.

Bioluminescence for animal imaging has rapidly emerged to study tumor metastases
Bioluminescent (BLI) techniques are non-invasive methods that permit longitudinal evaluation of cell/tumor growth where the amount of light is proportional to the numbers of living cells. The benefits are that the technique is non-invasive, can be used for functional applications (e.g. regulatory promoter constructs), is much more sensitive than radiology, can be used to follow animals longitudinally and thus reduces the numbers of animals necessary to obtain multiple time data points, and has extremely low background. The two main systems are firefly luciferase, which uses luciferin as a substrate, and renilla luciferase, which uses coelentrazine as a substrate. The basis of the reaction is: Luciferin (injected into the animal bearing luciferase cells or constructs) + ATP + O₂ yields oxyluciferin and light (photons). The wavelength of the light is in the near infrared spectrum >580-600 nanometers. Two commercial systems available are the IVIS system and a system developed by GE. Newer software programs are permitting 3-D reconstruction of multiple images and will be further developed to improve the ability to evaluate the volumetric analysis of tumors. One cautionary note is that the cell lines used are transfected with luciferase expressing constructs and it is important for investigators to verify that transfections have not altered the phenotypic behavior of the cells and that they retain their luciferase expression in vivo.

Numerous poster presentations included data obtained using imaging techniques. Of interest was a presentation (83) where bioluminescence was used to track tumor growth in vivo and then also to recover tumor cells from lesions for culture in vitro and reinoculation and relocation in vivo. The luciferase tag facilitates the identification of cell populations rederived from the metastatic sites. One of the poster presentations selected for an oral presentation described an innovative strategy of dual labeling with luciferase and GFP (21). Two constructs were transfected to breast cancer cells. The first contained a CMV promoter to drive expression of red fluorescent protein (RFP) and luciferase. The second construct had a TGF-β response element in front of a GFP and HSV-TK construct. Cells expressing red fluorescent protein were identified in vitro to confirm the transfected phenotype. Upon injection in vivo bioluminescence was utilized to identify tumor cells, e.g., in one mouse a metastatic lesion was identified in the adrenal gland and one was identified in the craniofacial bone. Micro-PET revealed that the craniofacial lesion was positive but the adrenal lesion was negative. These findings suggest that lesions in the bone are more sensitive to TGF-β activity and demonstrate an effective strategy to correlate tumor localization with specific response elements. Another poster (84) utilized GFP labeling to track tumors and also to image cellular trafficking and growth of dual color cancer cells in the skeletal vasculature of live mice. Cells expressing H2B-GFP to tag the nucleus, and retroviral RFP to highlight the cytoplasm, were injected into the carotid artery and tracked to demonstrate their localization in bone within 20 minutes.

Conflict of Interest: The author reports that no conflict of interest exists.

BONE COMPLICATIONS OF CANCER THERAPY

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Treatment-Related Osteoporosis in Prostate Cancer Survivors

There are 2 million men in the United States with osteoporosis, and an additional 8 million American men with osteopenia. These men have a 1 in 4 lifetime risk for fracture. Androgen-deprivation therapy (ADT) for the treatment of prostate cancer has increased over the past 15 years, making hypogonadism the leading cause of decreased bone mineral density (BMD) in men. ADT can be achieved with the use of gonadotropin-releasing hormone (GnRH)
agonists or orchiectomy. GnRH agonists have been used for neoadjuvant/adjuvant therapy, but now are increasingly used for localized prostate cancer and salvage therapy for PSA-only recurrence.

Men achieve higher peak bone mass than women, and do not experience a rapid rate in loss of BMD, as noted in women during the first few years of menopause. The rate of change in BMD in older men, however, approximates that of older women. Within one year of treatment with GnRH agonists, men lose 2-3% of BMD in the hip and lumbar spine. GnRH agonists also increase the risk of fractures by 25% for any site, by 46% for the hip and by 63% for the vertebral bodies (85;86).

Studies to determine if GnRH agonist-related bone loss can be prevented are promising. Forty-seven men with locally advanced or recurrent prostate cancer were randomized to receive the GnRH agonist leuprolide alone or leuprolide plus the bisphosphonate (BP) pamidronate for 48 weeks. Men who received leuprolide alone had an overall 2-3% loss of BMD in the lumbar spine and hip, and their markers of bone turnover rose progressively. The combination of leuprolide and pamidronate prevented bone loss at all sites, but there was no increase in BMD (87). More potent bisphosphonates increased BMD in a similar population. Men with localized prostate cancer beginning ADT who received 4 mg zoledronic acid intravenously every 3 months for one year had a 5.6% increase in lumbar spine BMD compared to a 2.2% decrease in the placebo group (p<0.001) (88).

Estradiol concentrations correlate positively with BMD and negatively with fracture risk in older women. Estrogens are also important for skeletal health in men. Interestingly, men with inactivating mutations in the aromatase gene have decreased BMD despite normal testosterone levels. In addition, medical castration with estrogen spares BMD in men. Elderly men have estradiol levels of 25-60 pmol/L; however, estradiol levels are not detectable in men taking GnRH agonists.Raloxifene, a selective estrogen receptor modulator (SERM) that prevents bone loss in postmenopausal women, is also effective in men on ADT. To study the effect of raloxifene in men, 48 men receiving a GnRH agonist were given raloxifene 60 mg/day or placebo. Total hip BMD increased by 1.1% in the men that received raloxifene, and decreased by 2.6% in the men that received placebo (p<0.001) (89). In a second study, 46 men on ADT for at least 12 months were randomized to 20 mg, 40 mg, or 60 mg/day of the SERM toremifene citrate, or to placebo, for six months. There was a dose dependent improvement in BMD after six months of toremifene citrate compared to placebo (p<0.01) (90).

Bicalutamide (Casodex) is a nonsteroidal antiandrogen that completely inhibits the action of androgens by binding to the androgen receptor. Bicalutamide is used as monotherapy for prostate cancer patients with stage M0 disease only. It increases testosterone and estradiol levels; estradiol levels approximate the low-normal levels of a premenopausal woman. Men on the bicalutamide have a higher rate of breast pain and enlargement, whereas medical/surgical castration results in a higher rate of hot flashes. Fifty-two men with prostate cancer and no bone metastases were randomized to bicalutamide 150 mg/day or leuprolide for 12 months. Men on the bicalutamide had two-fold increases in testosterone and estradiol levels, significant increases in BMD and no rise in urinary excretion of N-telopeptide. In contrast, men on leuprolide had decreased BMD, increased urinary excretion of N-telopeptide and increased risk of fracture (91).

These studies demonstrate that bisphosphonates, SERMs and bicalutamide monotherapy prevent loss of BMD and fractures in prostate cancer patients on ADT. A new study that looks at the effect of a monoclonal antibody to RANK ligand (AMG 162) on BMD in men on GnRH agonists is currently underway.

Bone Marrow Transplantation and Bone Loss
Bone marrow transplantation (BMT) is the most common transplant performed. The mean age of a patient who undergoes a BMT is 40 years old; 70-80% of these patients are expected to achieve long-term survival. Skeletal health is an increasing concern for BMT patients. Osteoporosis is more common after allogeneic BMT, as opposed to autologous BMT, which is attributed to immunosuppression for GVHD treatment. The average loss of femoral neck BMD after allogeneic BMT is 12% in the first year, but can be as great as 30%. Complete recovery of BMD does not occur at the femoral neck. Conversely, there is also loss of BMD at the lumbar spine, with a 4-16% risk of vertebral fracture within one year after BMT, but BMD can recover at this site (92).

The mechanism for loss of BMD after allogeneic BMT is multifactorial and complex. In addition to the underlying malignancy, post-transplant factors, including immunosuppressive therapy such as glucocorticoids, cyclosporine A and FK506, hypogonadism, renal impairment, decreased calcium absorption, chemotherapy and radiation therapy contribute to the loss of BMD after BMT. These factors increase cytokines, including IL-6 (from stromal cells) and IL-7 (from monocytes) that, in turn, increase RANK ligand and osteoprotegerin (OPG) (93). The net result is an increase in bone resorption after BMT. In a clinical study that prospectively followed thirty-six patients after BMT, serum OPG levels initially rose until they peaked at three weeks post-BMT, and then declined (94). The OPG levels had a positive, yet not significant, correlation with the bone resorption marker serum type I collagen carboxyterminal telopeptide (ICTP). In a second study, bone marrow IL-6 levels had a significant positive correlation to serum ICTP levels three weeks post-BMT, when significant changes in bone turnover are seen (95). In addition, the differentiation of bone marrow stromal cells into osteoblasts is impaired after BMT (96), which may be attributable to glucocorticoids, chemotherapy, and total body irradiation.

Hormone replacement therapy, calcium, and calcitonin have been ineffective at preventing loss of BMD after BMT. Vitamin D has been shown to improve BMD in BMT patients who are vitamin D deficient, which is common in those with GVHD or living at high latitudes. Clinical trials with bisphosphonates have also been promising. Treatment with oral risedronate after allogeneic BMT significantly improved lumbar spine BMD and prevented further loss of femoral neck BMD after BMT (97). In another clinical trial, intravenous pamidronate after BMT prevented loss of BMD from the spine and reduced loss of BMD from the total hip and femoral neck (92). Intravenous pamidronate was most beneficial for patients who required high-dose prednisolone in the first six months post-BMT (92). A clinical trial with zoledronic acid showed that this potent bisphosphonate increased BMD at both the lumbar spine and femoral neck one year post-BMT (98). The same study demonstrated that zoledronic acid significantly increased osteoblastic precursor cell growth in vitro (98).

The most devastating skeletal complication of allogeneic BMT is avascular necrosis (AVN), which occurs in 10-20% of patients, with a median onset of 12 months after BMT (92). AVN is less common after autologous BMT, occurring in 1.9% of patients. The principal risk factor for AVN post-BMT is the total dose of glucocorticoids required for treatment of GVHD (92). Older age, but not gender, is also important. AVN may develop secondary to deficient bone marrow stromal cells post-BMT (92).

Bisphosphonate Use and Osteonecrosis of the Jaw

There have been reports of osteonecrosis of the jaw (ONJ) occurring in cancer patients who have received bisphosphonates, however, the incidence of ONJ is unknown. In fact, the incidence of ONJ in the general population is unknown. The pathogenesis of ONJ is not well understood, but compromised bone vasculature leading to mechanical failure is likely. ONJ can be aseptic, ischemic or avascular. Potential risk
factors include glucocorticoids, anticoagulants, alcohol, tobacco, infection, inflammation, trauma and bisphosphonates. Bisphosphonates may be associated with site-specific osteonecrosis, perhaps by changing the architecture of the jaw.

ONJ was first described in the 1500s: “Phossy jaw” began with toothache and swelling of the gums and jaw. The lower jaw was more commonly affected, but sometimes the upper jaw also was attacked. Abscesses formed in the jaw bone(s), destroying them and draining a fetid discharge that offended those around the victim while gradually disfiguring him/her. If the patient was to survive, the only treatment was a disfiguring operation to remove the jaw bone (99). “Phossy jaw” most commonly affected factory workers using white phosphorus to make matches.

To determine the incidence of ONJ in cancer patients who received bisphosphonates, the M.D. Anderson Cancer Center pharmacy database was reviewed to identify all patients who received intravenous bisphosphonates between January 1, 1994 and December 31, 2003 (100). The use of intravenous bisphosphonates was identified in 4032 patients. ONJ was a clinical diagnosis defined as “exposed non-healing bone with/without pain of at least 3-6 months duration” (100). Although the review of medical and dental records has not been completed, 29 patients have been identified as having ONJ; estimated frequency is 1.1%. The first 11 patients were described (100). Four of the 11 patients had multiple myeloma; 7 patients had metastatic breast cancer. Potential predisposing factors included dental extraction prior to development of ONJ (7 patients), mandibular or maxillary exostosis (3 patients), denture trauma (2 patients), periodontal disease (5 patients) and radiotherapy to the maxilla (1 patient). The range of time between initiating bisphosphonate therapy and diagnosis of ONJ was 17-59 months. Nine patients were treated with pamidronate and zoledronic acid, 1 patient was treated with zoledronic acid alone, and 1 patient was treated with alendronate and zoledronic acid. Therapy included aggressive oral hygiene, salicept or sodium bicarbonate oral rinse, debridement of necrotic bone and antibiotics if infection was detected. At last follow-up, the ONJ lesion was stable in 4 patients, improved in 4 patients and progressive in 3 patients (100).

A second retrospective review used the medical, dental and pharmacy databases at the Memorial Sloan-Kettering Cancer Center (between January 1, 2000 and September 14, 2003) to determine incidence of and risk factors for ONJ after bisphosphonate treatment. The review identified 934 patients treated for metastatic breast cancer. These patients received a total of 13,143 doses of intravenous bisphosphonates. Dental records were available for 64 patients; 6 patients were diagnosed with ONJ (0.6%). ONJ was defined as exposed necrotic bone in the oral cavity. Invasive manipulation was associated with extension of the disease. Therefore, ONJ was a clinical diagnosis; neither imaging studies nor invasive manipulation/biopsy were required. All 6 patients were female, and median age was 53.5 years (range 27-66 years). ONJ was in the maxilla (2 patients) and mandible (4 patients); no patients had ONJ in both the maxilla and mandible. Four patients had a recent dental extraction. Oral hygiene was described as good (1 patient), fair (4 patients) or poor (1 patient). All 6 patients received cancer treatment. Four patients were taking glucocorticoids, 4 patients used tobacco and 4 patients had co-morbid diseases, such as diabetes mellitus. Management of ONJ included chlorhexidine rinses, oral antibiotics and conservative local debridement. ONJ either resolved in one patient or remained unchanged or progressed in four patients. One patient died before the outcome of ONJ could be assessed (101).

A member of the audience noted that chemotherapeutic agents may have a role in the development of ONJ. He remarked that in contrast to pamidronate, taxanes are a fairly new yet widely used breast cancer treatment. It was also mentioned that
thalidomide has produced ONJ in patients with multiple myeloma, which has been attributed to the antiangiogenic effect of thalidomide.

At this time, conservative management of ONJ is advocated. Physicians are encouraged to use the following link to report all cases of ONJ after the use of bisphosphonates:


Aromatase Inhibitors in Cancer Treatment-Induced Bone Diseases

Seventy percent of breast cancers are estrogen receptor (ER)+. Therefore, medical therapy for breast cancer is aimed at blocking estrogen action at its receptor (tamoxifen) or preventing estrogen synthesis (aromatase inhibitors). Patients with ER+ tumors and women at high risk for developing breast cancer will be offered anti-estrogen treatment to suppress metastases. Aromatase inhibitors (AIs) block the conversion of androstenedione and testosterone to estrone and estradiol. They are only used in post-menopausal women, and are superior to tamoxifen in the metastatic setting. The profound estrogen deficiency from AI therapy, however, has negative consequences for bone.

The Arimidex, Tamoxifen alone or in Combination (ATAC) trial evaluated anastrozole (A), tamoxifen (T) or the combination (C) for adjuvant treatment of postmenopausal women with early breast cancer (102). Disease-free survival was significantly improved in A versus T, however, an analysis at 33 months showed a fracture rate of 5.8% in A, 3.7% in T and 4.6% in C. In addition, BMD at the lumbar spine and total hip were increased in T but decreased in A (103).

A second clinical trial that compared the AI letrozole to placebo in early-stage breast cancer in postmenopausal women showed that letrozole, after 5 years of treatment with tamoxifen, significantly improved disease-free survival compared to placebo (104). After a median duration of 2.4 years of follow-up, there was a new diagnosis of osteoporosis in 5.8% of the letrozole group versus 4.5% in the placebo group (P=0.07). The fracture rate was 3.6% in the letrozole group and 2.9% in the placebo group (P=0.24) (104). Another clinical trial demonstrated that the AI exemestane, after 2-3 years of tamoxifen, significantly improved disease-free survival compared to 5 years of tamoxifen in postmenopausal women with primary breast cancer (105). Fractures were reported in 3.1% of the exemestane group and in 2.3% of the tamoxifen group, but this was not statistically significant (P=0.08) (105).

ABCSG-12 is a multicenter, randomized Phase III clinical trial that compared the LH-RH agonist goserelin (G), in combination with either the AI anastrozole (A) or tamoxifen (T), in premenopausal patients with hormone-responsive breast cancer (106). A secondary endpoint was the effect of complete endocrine blockade, with or without concomitant treatment with the BP zoledronic acid (ZA), on BMD. Patients were randomized to 4 treatment groups: G/T, G/T/ZA, G/A, or G/A/ZA. After 3 years, patients in the combination groups without ZA had a 14.4% decrease in mean BMD. The mean change in BMD was significantly worse in the G/A group (-17.4%) than in the G/T group (-11.6%). With the addition of ZA, treatment-induced bone loss did not occur in either group (106).

A multicenter open-label randomized clinical trial evaluated the effect of ZA upfront (4 mg q 6 months) versus delayed ZA in postmenopausal women with hormone-responsive breast cancer who were initiating AI therapy (letrozole) (107). Delayed ZA was administered if the lumbar spine T score decreased <-2 SD or if a fracture occurred. After 6 months, mean lumbar spine BMD increased by 1.55% in the upfront ZA group but decreased by 1.78% in the delayed ZA group. Total hip mean BMD increased by 1.02% in the upfront ZA group but decreased by 1.40% in the delayed ZA group (107). These differences were not statistically significant. In addition, a clinical
trial is currently underway to evaluate the effect of AMG 162 (RANKL Ab) versus placebo in postmenopausal women with breast cancer and osteopenia who are being treated with an AI.

The mechanism of AI-induced bone loss is not entirely understood. Although it has been established that decreased estrogen levels stimulate bone resorption, decreased estrogen levels also result in hypothalamic-induced increases in FSH and LH; FSH may directly stimulate osteoclasts. AIs also interrupt hypothalamic growth hormone secretion; reduced levels of IGF-1 may play a role in AI-induced bone loss. In addition, T cells may exert an indirect effect on bone resorption. If AI-induced bone loss is different from menopausal bone loss, are different therapies needed? What are the long-term effects of AI-induced bone loss? Could AI–induced bone loss alter progression to bone metastases, bone marrow micrometastases or metastasis growth? Further studies are needed to answer these questions.

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CURRENT AND FUTURE DIRECTIONS IN TREATMENT

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Novel Mechanisms and Applications of Bisphosphonate Treatment

The two main classes of bisphosphonates, non-nitrogen containing (etidronate, clodronate) and nitrogen-containing (the amino-bisphosphonates pamidronate, alendronate, risedronate, and zoledronate), have common pharmacological properties: they are not metabolized, 50% of each dose rapidly binds to bone, and 50% is rapidly excreted into the urine. Very low and transient levels are reached in soft tissues. An acute phase reaction is experienced by approximately 30% of patients who receive their first dose of amino-bisphosphonates. Nitrogen-containing bisphosphonates inhibit farnesyl diphosphate (FPP) synthase which, in turn, inhibits prenylation of small signaling proteins essential for cell function and survival. This results in osteoclast apoptosis. X-ray crystallographic studies show that zoledronic acid binds to the active sites of human FPP synthase (108). Zoledronic acid is held rigid at this site, which may explain its potency. Apppl (a new endogenous ATP analog) (109) production, induced by zoledronic acid inhibition of FPP synthase, inhibits mitochondrial ADP/ATP translocase, resulting in apoptosis of tumor cells.

Bisphosphonates have antitumor and antiangiogenic properties that may also contribute to their efficacy against bone metastases. In vitro, bisphosphonates decrease tumor cell proliferation and viability, increase apoptosis, and decrease tumor cell adhesion and invasion of the extracellular matrix. In addition, in vitro, bisphosphonates increase the efficacy of glucocorticoids, radiation and “taxoids.” In vitro, bisphosphonates also enhance the cytostatic effect of epirubicin/docetaxel on primary breast cancer cell lines, and the combination of doxorubicin and high doses of zoledronic acid augments apoptosis of tumor cell lines. In vitro studies also demonstrated a synergistic cytotoxic effect of zoledronate and XRT on human breast cancer (110) and C4-2 prostate cancer cell lines. In vivo, zoledronic acid reduced angiogenesis in rat prostate (111). This study showed that tissue distribution of a single dose of zoledronic acid was primarily in bone and prostate, which could explain the antiangiogenic effect in the prostate. Other investigators showed that zoledronic acid inhibited angiogenesis in cervical tissue by reducing MMP-9 expression (112).

Animal models show that bisphosphonates may have antitumor effects on soft tissues. Zoledronic acid reduced lung, liver and bone metastases due to 4T1 mouse mammary tumor (113). Minodrinate reduced tumor growth and prolonged survival in mice bearing melanoma tumors. Zoledronic acid
reduced cervical tumor volume in transgenic K14-HPN16 mice treated with estrogen. Recent evidence indicates that nitrogen-containing bisphosphonates have immunomodulatory effects that may enhance anti-tumor potential. The acute phase response of nitrogen-containing bisphosphonates is associated with stimulation and proliferation of gammadelta T cells, increased production of cytotoxic cytokines and anti-tumor activity in vivo. Human gammadelta T cells expanded ex vivo are cytotoxic to small cell lung tumors and fibrosarcomas in nude mice (114). Zoledronic acid significantly stimulated the proliferation of gammadelta T cells, and gammadelta T cells required pre-treatment with zoledronic acid for cytotoxic activity against target cells.

Adjuvant Therapy of Bisphosphonates to Prevent Metastatic Disease

Bisphosphonates reduce skeletal morbidity in patients with breast cancer, myeloma, prostate cancer and other solid tumors. Emerging data from clinical trials indicate that the prospects for the prevention of bone metastases and the complications thereof are good (115). Oral clodronate has been shown to prevent bone metastases in patients with breast cancer (116).

The rationale for using bisphosphonates in the adjuvant setting is to prevent bone metastases, by reducing tumor-induced osteolysis at the bone site. However, emerging preclinical data indicate that increased bone resorption or bone turnover may increase tumor growth in bone. Recent clinical studies indicate that fracture risk is higher in postmenopausal breast cancer survivors (117). Thus, bisphosphonate treatment should also prevent bone loss and reduce fracture risk in this population, in addition to preventing bone metastases. Five-year results were recently updated from the largest double-blind placebo-controlled multi-center trial in 1069 patients with primary operable breast cancer (stages I-III) who received oral clodronate 1600 mg or placebo once daily in addition to standard adjuvant therapy for 2 years (118). In this study, clodronate-treated women had fewer bone metastases and better survival. Bone mineral density, upon entry into the study, did not predict for the development of bone metastases, but women who had the highest concentrations of PINP, which represents higher bone turnover, were more likely to develop bone metastases. Inhibition of bone resorption may thus prevent bone metastases and osteoporosis, the two skeletal insults inflicted upon breast cancer patients.

Ongoing and larger clinical trials of bisphosphonate treatment in the adjuvant setting should provide more data to guide clinical care. Such trials include a larger clodronate trial (NSABP-B34), an IV zoledronic acid trial, as well as trials to compare oral clodronate with oral ibandronate and IV zoledronic acid.

Therapy Directed Against RANK Ligand

The RANK/RANKL/OPG axis was reviewed (31) as a mediator of osteoclast differentiation, activation and survival through complex signaling cascades. In addition, RANK signaling in tumor cells may contribute to tumor growth and osteolysis (15). The emerging role of this axis in skeletal complications of malignancy, as well as in cancer biology, was a continued theme throughout the meeting. In preclinical models of malignancy-associated skeletal disease, both OPG and RANK-Fc (a soluble form of RANK that neutralizes RANKL), have been effective against hypercalcemia of malignancy, breast and prostate cancer bone metastases, as well as multiple myeloma. Targeting the RANK/RANKL/OPG axis represents the newest frontier in the treatment of skeletal complications of malignancy. Although Fc-OPG and AMGN-007 have been tested in human phase I clinical trials, the current anti-RANKL treatment is AMG-162, a fully human anti-human RANKL monoclonal antibody (IgG2). A single subcutaneous injection of AMG162 causes rapid and potent suppression of bone turnover markers in breast cancer and multiple myeloma patients. In postmenopausal women, bone mineral
density increased at all sites (hip, spine, wrist, and total body). AMG162 is currently in clinical trials in patients with breast and prostate cancer, as well as in multiple myeloma. Possible advantages of AMG162 over bisphosphonates in the treatment of skeletal complications of malignancy include easy and infrequent dosing via subcutaneous route; additional benefit to block RANK signaling in tumor cells; and no uptake of the drug in bone. However, only direct comparison of AMG162 to bisphosphonates will provide evidence of superiority, as well as evidence whether such therapy will also be associated with the complication of osteonecrosis of the jaw.

**Treatment of Bone Cancers By Killing Tumor Cells and Osteoclasts**

Most therapy against skeletal complications of malignancy has been directed against the osteoclast or the tumor cells, but either therapy alone has been insufficient to cure bone cancers. Here, investigators described a unique experimental approach to target both the tumor cells and the osteoclast (119-121). The approach utilizes the yeast cytosine deaminase (CD)/5fluorocytosine (5FC) prodrug system in which CD converts 5FC to the chemotherapeutic agent 5-fluorouracil. The prodrug system can result in both direct killing of CD expressing cells as well as bystander killing of adjacent non-transduced cells. Osteoclast-specific delivery of the therapy was achieved using a transgenic mouse in which the CD gene was regulated by the TRAP promoter. These transgenic mice were inoculated with sarcoma cells and treated with 5FC once osteolytic tumors developed. This resulted in elimination of tumor and reduction in osteoclast number. These studies raise the possibility that the osteoclast can be used as a gene delivery system to sites of bone cancer.

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