ABSTRACT

The 5th annual Bone and The Oncologist New Updates (BONUS 5) conference, held at the National Arts Center, Ottawa, April 8–9, 2010, focused on innovative research into the mechanisms and consequences of increased bone turnover in the benign and metastatic settings alike. This year there was also a debate over the controversial use of bisphosphonates as an adjuvant treatment in patients with early-stage breast cancer. This meeting report highlights a few of the topics presented.

KEY WORDS

Bone, breast cancer, prostate cancer

1. INTRODUCTION

Since their inception in 2005, the Bone and The Oncologist New Updates (BONUS) meetings have covered a range of bone issues pertinent to health care professionals in oncology. The format of these single-day or day-and-a-half conferences has consisted of keynote lectures from national and international speakers. The topics covered have ranged from normal bone function to the effects of cancer and cancer therapies on normal and metastatic bone. A major part of the BONUS meetings has also been to concentrate on the wealth of local research experience and exciting Canadian initiatives that will shape basic, translational, and clinical research in the future.

The 5th annual BONUS conference (April 8–9, 2010) brought together clinicians, basic scientists, and others interested in the mechanisms and treatment of bone metastasis from breast cancer. Controversial topics about the adjuvant use of bisphosphonates to prevent breast cancer recurrences and the role of bone biomarkers and their clinical significance were discussed during the meeting. In an effort to encourage trainees to develop interest in bone research, five abstracts covering basic science and translational research were selected as oral poster presentations. Those abstracts can be found in the appendix to this article.

2. MEETING SESSIONS

2.1 Plenary Lecture: Intercellular Communication in Bone Metastasis

Presenter: Gurmit Singh, McMaster University and the Juravinski Cancer Centre, Hamilton, Ontario.

Bone is the most frequent site of metastasis for breast and prostate cancers, often resulting in pathologic changes in bone metabolism and in severe pain. Our understanding of the biologic mechanisms involved is limited, although the most popular view is that the tumour cells initiate the release of factors from bone, which in turn stimulates tumour cells to proliferate and causes further bone degradation. The application of this hypothesis, often called the “vicious cycle” model, has led to the development of many therapeutic strategies aimed at disrupting the role that osteoclasts play in the process. Unfortunately, the “vicious cycle” does not fully account for many of the clinical features observed in patients.

To complement the successes of the “vicious cycle,” other models need to be developed to push
forward in understanding this complex disease. An “intercellular communication hypothesis” has been developed that may be applied to this problem.

Many chemical factors released by tumour cells are known to interfere with the fine balance that exists in normal bone between bone resorption and formation. The amino acid and neurotransmitter glutamate has been shown to be a critical signalling molecule in bone homeostasis, and the cancer cell lines used to generate bone tumours in animals have been discovered to secrete significant amounts of glutamate *in vitro*. Furthermore, the molecular transport mechanism responsible for this glutamate release *in vitro* and *in vivo* has been identified, and its pharmacologic inhibition has been demonstrated. This aberrant chemical signal may be particularly disruptive in bone, because application of glutamate or modulation of its receptors and transporters significantly alters the differentiation and mature function of osteoclasts and osteoblasts alike. Because bone appears to be highly sensitive to glutamate disruption and is densely innervated with glutamate-sensitive neurons known to be involved in pain perception, it has been suggested that glutamate release by tumour cells is a plausible mechanism to account for many of the pathologic features of bone metastasis.

The suggestion that bone metastasis pathology may be the result of a “communication problem” represents a novel perspective in the understanding of cancer biology, and this concept may lead to practical targets for therapeutic intervention in bone metastasis and chronic bone cancer pain.

### 2.2 Plenary Lecture: Expression Profiling of Bone Metastases from Patients with Breast Cancer

**Presenter:** Peter M. Siegel, McGill University, Montreal, Quebec.

The most devastating aspect of breast cancer is the emergence of metastatic tumour cells that spread to distant organ sites. Indeed, 65%–75% of patients with advanced disease develop bone metastases. Numerous complications are associated with bone metastases, including pain, hypercalcemia, fracture, and spinal cord compressions, resulting in significant diminishment in the patient’s quality of life. These complications can interfere with treatment, allowing tumour cells to spread to additional sites that are associated with a worse patient prognosis.

The use of bisphosphonates has demonstrated utility in reducing skeletal-related events, but there are notable limitations to the effectiveness of these agents as treatments. Thus, a better understanding of the molecular regulators controlling breast cancer metastasis to bone will provide additional targets that can be exploited in combination with existing therapies.

Typically, human or mouse breast cancer cell lines are used to further an understanding of the molecular mediators that direct metastasis to bone. These approaches have been informative, but they rely on established cell models and often fail to identify factors that are expressed in the metastatic lesion *in situ*. To circumvent these limitations, trephine biopsies have been obtained from breast cancer patients with radiographically confirmed bone metastases. In an analysis of 20 independent samples from either unguided or computed tomography–guided trephine biopsies, 5 bone metastases were identified that contained lesions of sufficient size for laser-capture microdissection (LCM). In each case, the presence of tumour cells was confirmed by immunofluorescence staining with antibodies specific for CK8/18 or CK5. Based on immunohistochemistry for estrogen receptor, progesterone receptor, and the human epidermal growth factor receptor 2, 4 of the bone metastases were derived from luminal breast cancers and the 5th was a triple-negative breast cancer. To facilitate the analyses, 5 primary breast tumours (4 luminal and 1 triple-negative) were also subjected to LCM, and RNA from both sets of samples were amplified, labelled, and hybridized to Agilent Whole Human Genome microarrays (Agilent Technologies, Santa Clara, CA, U.S.A.).

Preliminary analysis of the resulting gene expression data has revealed that 117 genes were differentially expressed between the bone metastases and the primary tumours (filtered on a fold change ≥ 3; *p* < 0.005). Among those genes, several candidates belonging to the human ATP-binding cassette transporter family (drug resistance), Na+/K+ and Na+/H+ pumps, and genes encoding proteins involved in actin cytoskeleton remodelling and motility were selectively upregulated within bone metastatic lesions. Thus, potential candidates associated with the bone metastatic phenotype can be identified using clinical samples of breast cancer bone metastases, coupled with LCM and gene-expression profiling.

### 2.3 Plenary Lecture: Normal Bone and Prevention of Cancer Therapy–Induced Bone Loss


Bone integrity is an important contributor to optimal quality of life in women with breast cancer. Dual-energy X-ray absorptiometry (*DXA*) bone densitometry is currently used as the imaging of choice for the diagnosis osteoporosis and cancer-induced bone loss. However, since the late 1990s, this technique—although inexpensive and easily accessible—has been found to have several limitations. For example, *DXA* bone densitometry does not predict which patients will have a skeletal event; instead, it gives information on the relative risk of experiencing an event. Another limitation is that *DXA* is a difficult tool to use to follow response to treatment.
New imaging techniques that may provide researchers with more information about the microarchitecture of bone are currently being evaluated. One of those techniques is quantitative high-resolution computed tomography (QHRCT), which allows for better resolution of the cortical and trabecular bone microarchitecture. The importance of this new imaging modality is that it may correlate with skeletal events. Integrating a proper imaging modality into clinical trials can help in finding the answers to several long-standing questions, such as when to stop bisphosphonates in a patient with metastatic breast cancer, and whether bisphosphonates have an antitumour effect.

2.4 Do Bisphosphonates Have a Role in the Adjuvant Treatment of Breast Cancer?

**Presenters:** Robert Coleman, University of Sheffield, Norton, U.K., and Allan Lipton, Pennsylvania State University, University Park, Pennsylvania, U.S.A.

Bisphosphonates offer a potentially effective adjuvant therapy to reduce recurrence of bone metastases in breast cancer patients. Clinical trials are at an early stage, but initial results have shown some promise. A number of adjuvant trials of oral clodronate, pamidronate, and zoledronic acid have been published or are on going 1-7. Drs. Allan Lipton and Rob Coleman, both world leaders in this topic, debated the issue. Each speaker presented data both from preclinical studies and from subgroup analyses of studies including the three Zoledronic Acid—Letrozole Adjuvant Synergy Trials: Z-FAST (North America), ZO-FAST (Europe), and EzO-FAST (rest of the world).

The largest placebo-controlled trial reported to date remains that of Powles et al. of oral clodronate. This randomized placebo-controlled trial in 1069 women with primary operable breast cancer continues to show that, compared with placebo, clodronate resulted in a significantly lower incidence of bone metastasis: 45% lower during the first 2 years, and 31% lower during the 5-year study period. Overall survival time with clodronate was also a significantly longer than with placebo. After a 10.5-year follow-up, oral clodronate was still found to significantly improve overall survival.

Many studies are still ongoing, but two in particular will hopefully report soon. Accrual is complete for the National Surgical Adjuvant Breast and Bowel Project (NSABP) B34 trial, the fourth adjuvant clodronate trial. This double-blinded trial has randomized 3323 patients to either 1600 mg clodronate or placebo for 3 years. Also fully accrued is the 3360-patient AZURE (Adjuvant Zoledronic Acid to Reduce Recurrence) trial, which randomized patients with high risk or lymph-node-positive disease to either intravenous zoledronic acid or to control. The oncology community is waiting results from these and other studies.

2.5 Plenary Lecture: The Role of Biomarkers in Metastatic Disease and Prospects for Their Use in Early Breast Cancer

**Presenter:** Alexander Paterson, University of Calgary, Calgary, Alberta.

Bone biomarkers have gained interest, especially with regard to their ability to measure the rate of bone resorption and formation. Although that information is insufficient to permit earlier diagnosis of bone metastases than is possible with imaging and clinical assessments, the rate of bone turnover, especially bone resorption, provides powerful and clinically useful prognostic and predictive information. Patients with high rates of turnover are more likely to experience a skeletal-related event. Decreases in the levels of bone biomarkers have been associated with successful treatment with agents such as bisphosphonates.

Further studies are needed to evaluate whether bone biomarkers can predict which patients would benefit from bisphosphonate therapy and how long therapy should be continued. This information would optimize bisphosphonate use and provide a more patient-oriented and cost-effective treatment. Bone biomarkers may find their place in oncology not only in selecting patients for bone-targeted treatment, in monitoring response, and in individualizing care, but also in the development of new treatment approaches.

2.6 Current Radiotherapy Research in Bone Metastases

**Presenter:** Alysa Fairchild, Cross Cancer Institute, Edmonton, Alberta.

Although approximately twenty-five randomized clinical trials (RCTS) and three meta-analyses support the use of single-fraction radiotherapy (RT) in the treatment of uncomplicated bone metastases, practice differs significantly from published evidence. Some of the reasons postulated include toxicity, re-treatment issues, and geographic influences, all representing current controversies in RT. Dr. Fairchild presented a brief review of RT delivery methods and summarized the single-fraction studies, a National Cancer Institute of Canada–sponsored phase III RCT investigating repeat irradiation dosing, global patterns of practice, prevention of acute side effects (“pain flare”), and the emerging role of highly conformal RT.

2.7 Optimizing Bisphosphonate Therapy

**Presenter:** Eitan Amir, Princess Margaret Hospital and the University of Toronto, Toronto, Ontario.

Bisphosphonate therapy has become a standard of care for patients with malignant bone disease. In addition, preliminary clinical data suggest that bisphosphonates may prevent cancer-treatment-induced bone loss (CTIBL) and the development of malignant bone disease in patients with early-stage cancer.
There is a lack of correlation between bone mineral density and fracture rates in CTIBL, and new methods of predicting fracture risk are needed.

In the setting of metastatic bone disease, monitoring therapy remains difficult. Routine radiographs are sensitive, but not specific. Novel methods such as combined positron-emission tomography–computed tomography, possibly in combination with telopeptides, should be further studied.

Finally, dose scheduling for bisphosphonates should be optimized. Many low-risk patients are probably over-treated with the current dose recommendations, and ongoing studies are investigating dose-reduced schedules.

2.8 Can We Afford the Cost of Progress in the Age of New Targeted Therapies?

Presenter: George Dranitsaris, Consultant in Health and Biostatistics, Toronto, Ontario.

Globally, approximately 7.4 million people die annually from cancer, representing approximately 13% of deaths from all causes. As the population ages, we can expect a sharp increase in the number of new cancer cases over the next 10–20 years because cancer is more common in older people. Cancer is also associated with a substantial economic burden, both in terms of direct and indirect costs. In 19 European countries covered by the Organisation for European Cooperation and Development, the total annual expenditure for cancer care in 2004 was €54 billion (€120 per person). With respect to indirect costs, cancer was responsible for 16.7% of all disability adjusted life years (DALYs) lost in 25 European countries and for 12.5% of all DALYS lost in the United States and Canada. Despite the high impact of cancer on the number of DALYS, only 4%–6% of annual health care budgets are allocated for the treatment of cancer. An argument can therefore be made that, despite the rising cost of cancer care, not enough is being spent, thus limiting the availability of new treatments. This presentation reviewed the international literature addressing the important challenges associated with patient access to new cancer drugs. In addition, strategies currently used by countries around the world to contain rising drug costs were presented.

3. SUMMARY

The BONUS meetings continue to bring together people interested in bone disease and its implication in practice. The meetings continue to be an excellent forum to foster research and wide multidisciplinary interaction. We extend our thanks to all who made this meeting such a success, including our pharmaceutical sponsors. We will use the feedback from the BONUS 5 conference to make further program changes before we start organizing the meeting for 2011!

4. REFERENCES


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Biopsy Confirmation of Metastatic Disease in Breast Cancer: Results from a Large Prospective Study


Purpose: Decisions about treatment of women with metastatic breast cancer are based on estrogen receptor (ER), progesterone receptor (PgR), and HER2/new status of the primary tumour. This prospective study investigated concordance in receptor status between the primary tumour and distant metastases at various stages of progression, and assessed the impact of any discordance on patient management.

Materials and Methods: Biopsies of suspected metastatic lesions were obtained from patients and analyzed for ER/PgR and HER2/new and compared with the primary tumour.

Results: Of the 116 women enrolled in the study, 102 underwent biopsy. Of those 102 women, 52 (51%) were newly diagnosed with metastatic disease, 17 (17%) had received one line of metastatic treatment, and 35 (34%) had received 2 or more lines of treatment in the metastatic setting. Of samples taken, 86 (84%) were sufficient for analysis. Of the 86 biopsies, 5 (6%) showed benign disease, and 1 (1%) confirmed a different malignancy (indolent lymphoma). Changes in hormone receptor status were observed in 33%. Among the biopsies with changes, ER discordance was seen in 11%; PgR discordance, in 27%; and discordance in both ER and PgR in 5%. Loss of PgR was the most common change in hormone receptor status (25%). A 4% discordance in HER2/new was observed. Expression of HER2/new was gained in 3 patients (3%), and lost in 1 (1%). Biopsy results led to a change of management in 12% of patients. Among triple-negative primary tumours, metastases showed no observable changes in receptor expression.

Conclusions: This prospective biopsy study is the largest that we are aware of. Results demonstrate the presence of substantial discordance in receptor status between the primary tumour and metastases. The number needed to biopsy to alter immediate management was 8.5. Tissue confirmation should therefore be considered in all patients suspected to have metastatic recurrence or progression.

The Effect of Photodynamic Therapy on Structural Integrity and Skeletal Remodelling of Metastatically Involved Vertebrae, Alone and Subsequent to Bisphosphate Treatment

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Purpose: Vertebral metastases compromise spinal mechanical stability. Photodynamic therapy (PDT) has been shown to be effective at treating metastatic lesions secondary to breast cancer in an athymic rat model, and is proposed as a treatment for spinal metastasis. The objective of the present work was to determine the effect of PDT, alone or in combination with previously administered systemic bisphosphonates (BP), on the structural integrity and skeletal remodelling of both healthy and metastatically involved vertebrae.

Materials and Methods: Human breast carcinoma cells (M-T1) were inoculated into athymic rats (day 0). On day 7, a calcium green fluorochrome bone label was administered with or without 4-CCNU (60 mg/kg zoledronic acid). At day 14, xylene orange (a second fluorochrome bone label) was administered with or without a single PDT treatment targeting L2 (1.0 mg/kg benzylporphyrin derivative monooacid photosensitizer activated with 75 J light energy after a 15-minute drug–light interval). After euthanasia of the animals on day 21, L2 vertebrae were imaged by radiograph microcomputed tomography and processed for histology.

Results: Compared with untreated tumour-bearing controls, animals treated with PDT were found to have significantly less metastatic bone loss of the vertebrae. Tartrate-resistant acid phosphatase staining suggests that this finding may be a result of osteoclast destruction. Additional formation of osteoid was found on PDT-treated vertebrae on the cortical shell opposing the light pathway. Combined treatment with 4-CCNU and PDT further enhanced bone architecture in both metastatically involved and healthy bone. In contrast with 4-CCNU treatment alone, PDT caused tumour necrosis.

Conclusions: Overall, the ability of PDT to both ablate malignant tissue and to improve the structural integrity of vertebral bone motivates its consideration as a local minimally invasive treatment for spinal metastasis secondary to breast cancer.

Results from a Multicentre Study Exploring the Incidence of Hormone Receptor Discordance Between Primary Breast Cancers and Bone Metastases

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Purpose: The treatment of bone metastases in breast cancer patients is based on the hormone receptor status of the primary tumour. Discordant receptor expression between the primary tumour and metastases has been reported in about 20%-60% of cases. The present study prospectively explored the incidence of discordant receptor status in primary and metastatic bone disease, and evaluated the role of bone marrow biopsies for the reassessment of receptor status.

Methods: Patients with known bone metastases were approached to undergo a computed tomography (CT)-guided bone metastasis biopsy and a bone marrow aspirate and trephine. The estrogen receptor (ER) and progesterone receptor (PgR) status of these samples were assessed and compared with those of the primary breast cancer.

Results: The study has so far enrolled 25 patients. To date, results are available from 30 bone marrow aspirations and 25 CT-guided bone metastasis biopsies. Tumour cells were found in 13/30 bone marrow biopsy samples (45.3%) and in 16/25 of CT-guided biopsies (64%). Discordance between the primary and metastatic samples was seen in 14/30 patients (46.7%). Among the discordant samples, ER and PgR changed from positive to negative in 13 cases and from negative to positive in 1 case. In 7/25 analyzed cases (28%), malignant cells were identified both in bone metastasis samples and in bone marrow aspirates from the same patient. Among those 7 analyses, ER and PgR were concordant in 100% and 71% of cases.

Conclusions: This multicentre study confirms that patients are willing to undergo invasive biopsy procedures to assess metastatic sites of disease. Receptor discordance rates in this study were similar to those previously reported. There appeared to be good concordance between bone metastasis and bone marrow biopsies. Further prospective tissue studies are needed to better understand the mechanisms of these receptor changes and their significance with regard to cancer metastases.

Investigating a Role for CCN3 in the Promotion of Breast Cancer Metastasis to Bone

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Purpose: The most devastating aspect of breast cancer is the emergence of tumour cells that are spread (“metastasize”) to other organs and tissues. The skeleton is a common site for metastasis; however, the reasons bone is targeted are not fully understood.

Methods: We used mouse models to isolate breast cancer cell lines that aggressively metastasize to bone and compared those cells to cells that are weakly bone metastatic.

Results: Through gene expression profiling, we have determined that the protein CCN3 is expressed at higher levels in aggressively bone-metastatic cells than in cells that weakly metastasize to bone. We have verified that our bone metastatic cells overexpress CCN3 messenger RNA and that elevated levels of CCN3 protein are detected in the media of the bone-metastatic 4T1 subpopulations. Using primary cultures of mouse bone marrow cells, we confirmed that a recombinant CCN3 protein impaired osteoblast differentiation in a dose-dependant manner, resulting in an increase in the ratio of receptor activator for nuclear factor kB ligand (RANKL) to osteoprotegerin (OPG) that indirectly favours osteoclast formation. In addition, we provide evidence that CCN3 directly induces osteoclast differentiation of RANKL-primer RAW 267.4 monocytes. The CCN3 enhances osteoclast differentiation through its ability to induce calcium mobilization in the subsequent nuclear translocation of nuclear factor of activated T cells, a transcription factor essential for osteoclast differentiation. Ongoing studies are focused on whether CCN3 is necessary and sufficient to enhance bone metastasis in vivo. To determine if CCN3 expression is associated with relapse to bone, we are currently examining the expression of CCN3 in a tissue microarray consisting of 305 breast tumour samples. Immunohistochemical staining indicates that CCN3 is readily detectable in most breast cancer bone metastasis samples examined thus far.

Conclusions: Our data support an important role for CCN3 in modulating the differentiation capacity of bone resident cells to support osteoclast formation and the formation of osteolytic bone metastases.
Breast Cancer Cells Enhance Osteoclast Survival and Block the Apoptotic Action of Alendronate

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Purpose: Breast cancer commonly metastasizes to bone, where it stimulates formation of bone-resorbing osteoclasts. Bisphosphonates constitute an important treatment for osteolytic metastases. The goal of the present study was to assess the effects of soluble factors produced by breast cancer cells on osteoclast survival and responsiveness to bisphosphonates.

Materials and Methods: Mature osteoclasts derived from the murine monocytic cell line RAW 264.7 or from primary mouse bone marrow were cultured for 24–48 hours with 10% conditioned media (cm) from human (MDA-MB-231) or mouse (4T1) metastatic breast carcinoma cells (“untreated”) or with a pro-survival factor, receptor activator for nuclear factor κB ligand (RANKL).

Results: Cancer-derived factors maintained osteoclast survival and resorption at the levels comparable to those observed with RANKL. Alendronate induced osteoclast apoptosis in untreated and, to a smaller extent, in RANKL-treated cultures, resulting in a significant decrease in osteoclast number and size, induction of caspase-3 cleavage, and upregulation of Bim. However, in the presence of cancer-derived factors, alendronate was ineffective in inducing osteoclast apoptosis, resulting in only a modest decrease in osteoclast numbers and not in osteoclast size. The MDA-MB-231 cm prevented alendronate-induced cleavage of caspase-3 and upregulation of Bim. Macrophage colony-stimulating factor (m-CSF)–neutralizing antibody attenuated the effect of MDA-MB-231 cm by approximately 50%, but could not fully restore osteoclast responsiveness to alendronate. Inhibition of phospholipase C (PLC)–γ interfered with MDA-MB-231–induced downregulation of Bim and prevented anti-apoptotic action of cancer-derived factors on osteoclasts.

Conclusions: Our data suggest that soluble factors produced by the metastatic breast cancer cells promote osteoclast survival and block the apoptotic effect of bisphosphonates in a m-CSF– and PLC-dependent manner.

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