MEETING REPORTS

Meeting Report from the 17th Scientific Meeting of the International Bone and Mineral Society

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OSTEOBLASTS

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Amongst the most exciting aspects of the meeting for osteoblast biology were the advances reported in defining transcriptional and signaling pathways regulating osteoblast differentiation and function.

As understanding of the regulation of osteoclast formation and activity by osteoblast lineage cells increases (reviewed in (1)) and new information on the bone niche for hematopoietic stem cells (HSCs), the progenitors of osteoclasts grows (2), interest in a reciprocal pathway, i.e., regulation of osteoblastogenesis and bone formation by osteoclasts and other blood-derived cells, is also increasing. It has long been known that hematopoietic lineages regulate osteoblast formation and activity in vitro (see, e.g., (3;4)) and an increasing body of data, much of which comes from genetically modified mice, supports the view that resorption-formation coupling for bone remodeling in vivo may involve osteoclast-derived signals that regulate bone formation (5). In support of this concept, the anabolic effect of parathyroid hormone (PTH) was shown to be significantly impaired in mice with impaired osteoclast formation or function, i.e., interleukin 6 (IL6) (-/-) mice or mice co-treated with PTH and salmon calcitonin (an acute blocker of osteoclast activation) (6). Much remains to be done to separate the direct effects of these cytokines and hormones on formation from indirect effects due to changes in osteoclastogenesis and resorption, but the data add to the intriguing possibility that an active osteoclast-derived signal(s) is a coupling factor that regulates osteoblast lineage cells. In addition, these studies increase the need for better understanding of the bone niche for HSCs. As highlighted in the opening lecture (2;7), osteoblasts/bone, bone marrow and the sympathetic nervous system appear to integrate signals to regulate HSCs. Given the growing number of studies on nervous system control of bone remodeling, spearheaded by those in which the adipocyte-derived hormone leptin was found to regulate both osteoclast and osteoblast lineage cells through its action on the ventromedial hypothalamus and subsequently via the sympathetic nervous system to osteoblasts (8), we should anticipate additional insights into the regulatory loops integrating the sympathetic nervous system, HSCs and their progeny including osteoclasts, and osteoblasts.

Members of the leukemia inhibitory factor (LIF)/IL6 family, including IL6, IL11, oncostatin M (OSM) and cardiotoxin-1 (CT-1), have long been studied as positive regulators of osteoclastogenesis and bone resorption. However, a wide variety of data indicate that at least some of the cytokines that signal through the gp130 receptor are...
also positive and negative regulators of osteoblast lineage cells and bone formation (9;10). New data were presented on OSMR null mice that display increases in several bone parameters (i.e., bone mineral density (BMD), bone volume (BV/TV), trabecular number and thickness (TbN and TbTh) in the tibia, femur and vertebra, concomitant with reduced osteoclast surface and bone resorption, reduced bone formation and increased marrow adipogenesis (11). In keeping with earlier reports that the OSMR stimulates osteoprogenitor differentiation in the rat calvaria cell culture model (12), OSM was found to increase osteoblast differentiation and mineralization and inhibit adipocyte differentiation in a multipotent mouse stromal cell line (Kusa 4b10). CT-1 also abrogated adipocyte differentiation and increased osteoblast differentiation and mineralization in Kusa 4b10 cells, in keeping with results in stromal cell cultures from CT-1 null mice (13). Interestingly, CT-1 appears to enhance mineralization by upregulating C/EBPδ, which cooperates with Runx2 to activate osteocalcin transcription (14;15). These studies add to a list that includes LIF (16;17) and IL11 (18) as members of this cytokine family with anabolic effects on bone resulting at least in part from effects on differentiation, and perhaps commitment, of osteoblasts and other mesenchymal precursors (19). They also emphasize the importance of further dissecting the multiple skeletal effects of this complex – and sometimes functionally redundant and promiscuous – family of ligands and receptors, at least some of which appear to have biphasic effects on osteoblasts at different developmental stages (12).

The important concept that a single regulator may have opposite effects at different osteoblast developmental stages was underscored by presentations addressing the mechanisms by which members of the Wnt signaling pathway regulate bone mass. Wnts are a family of 19 secreted proteins that bind to a membrane receptor complex composed of Frizzled (FZD) G-protein coupled receptors (GPCRs) and low-density lipoprotein (LDL) receptor-related proteins (LRPs) and activate intracellular signaling pathways. The best characterized is the canonical or Wnt/ß-catenin pathway that signals through LRP-5 or LRP-6 and leads to inhibition of glycogen synthase kinase (GSK)-3ß and sufficient to exert most of the effects of ∆FosB in osteoblasts, was shown to physically interact with not only ∆2∆FosB but also with Runx2 and to strongly repress transcriptional activity of Runx2 and osteoblast differentiation in vitro (23). However, Zfp521 overexpression in osteoblasts in transgenic mice markedly increased bone formation. This suggests that Zfp521 inhibits early osteoblast differentiation but promotes late stages of osteoblast maturation and bone formation by antagonizing Runx2 activity. Such developmental stage-specific regulation was further highlighted in a study on FIAT, a leucine zipper protein that heterodimerizes with ATF4 and inhibits ATF4 transcriptional activity on genes such as osteocalcin (24). Transgenic mice overexpressing FIAT in osteoblasts are osteopenic, with reduced bone mineral density, reduced BV/TV, and impaired bone strength, resulting from impaired osteoblast activity (24). It was now reported that FIAT also binds to Fra-1 and inhibits transcriptional activation by a Fra-1/c-Jun heterodimer, without affecting transcription mediated by a c-Jun homodimer, suggesting that FIAT modulates early osteoblast activity by interacting with ATF4, and regulates later osteoblast function through inhibition of Fra-1 (25). Other studies on mice with gain-of-function/loss-of-function of Atf4 and osteoblast-specific ablation of Nf1 support the role of ATF4 as a critical transcriptional mediator of osteogenesis, Nfi signaling in osteoblasts and an osteoblast autonomous role of Nf1 in bone remodeling (26).

The need for additional data to address developmental versus postnatal osteoblast regulatory mechanisms was underscored by presentations addressing the mechanisms by which members of the Wnt signaling pathway regulate bone mass. Wnts are a family of 19 secreted proteins that bind to a membrane receptor complex composed of Frizzled (FZD) G-protein coupled receptors (GPCRs) and low-density lipoprotein (LDL) receptor-related proteins (LRPs) and activate intracellular signaling pathways. The best characterized is the canonical or Wnt/ß-catenin pathway that signals through LRP-5 or LRP-6 and leads to inhibition of glycogen synthase kinase (GSK)-3ß and
subsequent stabilization of β-catenin, which translocates to the nucleus and activates lymphoid-enhancer binding factor (LEF)/T cell-specific transcription factors (TCFs); Wnts also activate noncanonical pathways (reviewed in (27)). To date, knowledge of which of the 19 secreted Wnts influence bone mass remains unclear since knockout of many Wnts in mice results in embryonic or neonatal lethality. Thus, a comparison of mice with targeted inactivation of Wnt 10b and Wnt16, both of which are viable, is of interest (28). It was confirmed that Wnt10b knockout mice have slightly lower cancellous bone mass (29) but that Wnt16 knockout mice display reduced cortical bone diameter and thickness, indicating an apparent site specificity of bone regulatory activities of these two Wnts. On the other hand, in another approach, secreted frizzled related protein 3 (sFRP3), which acts as a decoy receptor for Wnts and antagonizes the Wnt/frizzled signaling pathway, was overexpressed by Phi 31 integrase strategy in adult mice and found to increase femur BMD, increase BV/TV and TrN, and increase mechanical strength (30). The data imply that sustained Wnt signaling in adult mice could be deleterious to bone. Similarly, it is striking that the phenotype of mice with β-catenin ablated either in osteoblast precursors or in differentiated osteoblasts is much more severe than that of a global Lrp5 knockout (31;32), raising important questions about possible redundancies of regulation of β-catenin by LRP5 and LRP6 in osteoblasts and their precursors (33). The data presented also point to the need for more studies to dissect the role that Wnt/Lrp-independent pathways play in regulating β-catenin functions and the role that other regulators of β-catenin play in the bone phenotypes. In this regard, β-catenin activity is regulated by a complex of proteins, including Axin2, a scaffolding protein, which binds GSK-3β and promotes β-catenin degradation. It is already known that lack of Axin2 results in neonatal skeletal defects resembling craniosynostosis due to loss of Axin2’s ability to negatively regulate both expansion of osteoprogenitors and maturation of osteoblasts, the former related to the fact that β-catenin promotes cell division by stimulating cyclin D1 in osteoprogenitors, and the latter related to Wnt-dependent BMP signaling (34). Bone volume was shown to increase with age, and mineral apposition and bone formation rates were increased, in adult mice lacking Axin2 expression (35). Analysis of osteoblast differentiation in Axin2(-/-) bone marrow stromal cell cultures showed increased proliferation and differentiation, no change in β-catenin localization, but increased basal Smad1 and 5 phosphorylation and increased bone nodule formation in response to BMP-4, suggesting that Axin2 negatively regulates bone mass in adult, but not growing mice through a mechanism involving inhibition of BMP signaling in osteoblasts. It is interesting that sclerostin, the SOST gene product, was initially thought to function as a BMP antagonist, but is now known to be a circulating antagonist of canonical Wnt signaling by binding to the Wnt co-receptors LRP5 and LRP6 (36). Sclerostin is expressed almost exclusively in osteocytes and mutations in sclerostin have been found to underlie sclerosing bone disease. Data were summarized showing that downregulation of sclerostin participates in the anabolic effects of PTH and mechanical loading, and that administration of neutralizing sclerostin antibodies increases bone formation, suggesting that stimulating canonical Wnt signaling by decreasing sclerostin activity increases bone formation and bone mass in the adult (36). Taken together, these data suggest that the developing and growing skeleton may be regulated differently than the adult skeleton by different members of the Wnt pathway and their regulators and highlights the need for much more information about both age-related and skeletal site-specific effects of members of this pathway.

The lineage relationships between adipocytes and osteoblasts vis-à-vis mesenchymal cell commitment and differentiation (see above) are but one side of the story on the coupling between fat and bone. New data on the relationships between fat, energy metabolism and bone mass included data on bone effects of adiponectin, the most abundant adipokine and a regulator of energy homeostasis, glucose and lipid metabolism, which is reduced in obesity, insulin resistance and
type 2 diabetes. In spite of an anabolic effect in vitro, adiponectin-deficient mice were shown to have increased TrN and BV/TV, indicating that adiponectin has a negative effect on bone mass in vivo (37). The question was also flipped around to ask whether the skeleton, in particular osteoblasts, regulate energy metabolism (38;39). Based on evidence from mice with osteoblast-specific ablation of osteotesticular phosphatase (OST-PTP), the answer appears to be yes! An elegant series of studies with multiple genetically modified mouse lines provided support for the novel hypothesis that OST-PTP lies upstream in a genetic pathway regulating osteocalcin in osteoblasts and that osteocalcin is a hormone that regulates adiponectin and insulin. The idea of osteocalcin, long-studied as a non-collagenous matrix molecule and amongst the most specific of markers of mature osteoblasts, as a hormonal regulator of obesity was without a doubt the most novel and startling new osteoblast data presented at the meeting.

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

HORMONES AND BONE

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While a few decades ago the IBMS meeting was still known as the International Conference on Calcium Regulating Hormones, and before that as the Parathyroid Conference, the number of oral and poster presentations in Montreal dealing primarily with the classical calciotropic hormones (PTH, calcitonin, and vitamin D) was only a minor fraction of the whole meeting, and was replaced by new hormones, new humoral factors and an exciting number of new genes and proteins involved in calcium/phosphate/bone homeostasis.

Parathyroid Hormone

PTH (1-34) is by now a well-known therapeutic bone anabolic agent, whereas intact PTH (1-84) is less effective (40-42). The search for better analogs is still ongoing and Fugu parathyroid hormone (43) was found in rat models of osteoporosis to have a slightly better bone formation/resorption profile than hPTH (1-34) (44), but the real endpoint should be bone mass and strength and fracture incidence in humans.

While PTH receptor signaling is that of a simple classical G-protein coupled receptor (GPCR), it is in fact extremely complex. PTH-R1 traffic and thus PTH sensitivity is dependent on a Na/H exchanger regulatory factor 1 (NHERF) that tethers the PTH-R to the cytoskeleton and inhibits the binding of arrestin and thereby decreases ligand-induced receptor internalization (45). β-arrestin was discovered initially as an intracellular protein capable of inactivating ligand activated GPCRs. β-arrestin 2 KO mice are therefore more sensitive to endogenous or exogenous PTH, but not all intracellular signaling pathways are equally affected. For example, SOST inhibition by PTH was abolished in β-arrestin2 (Arrb2) KO mice (46).

Klotho

The klotho gene codes for a single-pass transmembrane protein with expression limited to the distal tubule of the kidney, the parathyroid gland and choroid plexus of the brain. Gene deficiency shortens the life span of mice (and vice versa) and causes all the metabolic symptoms of FGF-23 deficiency (hypercalcemia due to 1,25(OH)2D excess, hyperphosphaturia and hypophosphatemia, and premature aging (47)). Klotho binds to FGF receptors and is an essential cofactor for multiple FGF-Rs. It is now reported that the extracellular domain of klotho circulates as a hormone and when infused in mice causes phosphaturia independently of FGF-23 actions. Klotho therefore functions as a (pro)hormone circulating in pM concentrations in mouse serum (47). The vitamin D hormone upregulates klotho and TRPV5 epithelial channels and the glucuronidase activity of klotho is necessary for deglycosylation of TRPV5 to become fully active (48).

Estrogens downregulate klotho expression in the kidney, as shown in aromatase-deficient mice before and after estradiol
treatment (49). PTH, klotho and FGF-23 hormones are all phosphaturic by downregulation of Na/P transporter 2 activity, while klotho and FGF-23 inhibit, and PTH stimulates, the renal 1α-hydroxylase (CYP27B1) (47).

Androgens

The effects of androgen deficiency in rodents and men have received greater attention, albeit more recently, than the effects of estrogens on bone (50). The effects of orchidectomy in adult (10-yr-old) cynomolgus monkeys (51;52) are now described. Bone turnover measured histologically or biochemically clearly increases after orchidectomy, whereas BMD (trabecular and cortical bone) decreased from 4 mo after orchidectomy onwards. Androgen deficiency thus causes bone effects similar to those caused by estrogen deficiency in monkeys and these effects are very similar to the effects observed previously in rodents (mice and rats) and men (50).

Bone as an Endocrine Tissue

In contrast to the original concept of specialized endocrine glands, it is now well-accepted that other tissues and cells frequently have mixed functions, including genuine hormone secretion or prohormone conversion. Osteoblasts are known to activate/inactivate “phosphatonin”s by Phex, and are moreover the main source of FGF-23. Now a new study reports that osteoblasts are regulating energy metabolism through at least one and probably more hormones (38;53), as part of a feedback system since hormones and the central nervous system are regulating bone metabolism (leptin and its signaling from the hypothalamus regulate trabecular bone homeostasis). Based on unique osteoblast gene profiling, investigators knocked out (total and osteoblast-specific) the protein phosphatase OST-PTP or Esp, and observed β-cell hyperplasia, hyperinsulinemia and neonatal and postnatal hypoglycemia, as well as increased insulin sensitivity probably mediated by increased adiponectin secretion by adipocytes. A hormonal signal, or at least a prohormone activation, must therefore take place in osteoblasts, signaling to both pancreatic islets and adipocytes. Moreover, these Esp KO mice are resistant to drug- and high fat intake-induced obesity, as they consume more energy than normal mice. Overexpression of Esp causes the opposite phenotype of insulin resistance and impaired glucose tolerance. Reanalyzing osteocalcin KO mice generated by the same group a decade ago (54), a similar “metabolic syndrome”-like phenotype in such KO mice was identified. By generating Esp KO mice also lacking one osteocalcin gene, the hypoglycemic phenotype of Esp mice could be corrected, indicating that osteocalcin can function as a hormonal signal linking osteoblasts with adipocytes and the endocrine pancreas (38;53). These observations open totally new perspectives on the skeleton as part of the control loop regulating energy metabolism. The same osteoblasts are now also considered to be the home (“niche”) for nurturing bone marrow stem cells (55;56) and add yet another dimension to the essential role of bone cells. Many questions remain open, however, as the link between the bone cell phosphatase, Esp, and osteocalcin is missing, pointing towards the existence of yet another hormonal factor. Moreover, it remains to be shown how essential or redundant the new signaling is for the epidemic of metabolic syndrome related to changing lifestyles (including a lack of exercise and energy consumption, (chronic) inflammation and high fat/caloric intake).

Glucocorticoids

Pharmacological doses of glucocorticoids are well known as a possible “devil” for bone due to a reduction in bone formation, decreased bone mass and strength, and a rapid increase in fractures. Children might even be more sensitive as glucocorticoids may compromise bone accrual. However, glucocorticoids are used to treat diseases that may intrinsically affect bone (accrual) by inflammatory cytokines. Comparing prospective (1 yr) bone loss in several groups of children treated with glucocorticoids, a study reported that most children with Crohn’s disease already had marked deficits in bone before the start of
glucocorticoids, and aggravation after 1 yr of glucocorticoid therapy (57). In contrast, children with nephrotic syndrome had normal bone before glucocorticoid therapy and only marginal deleterious effects on (trabecular) bone, or even positive (cortical bone) effects, after 1 yr of glucocorticoid treatment. Therefore, apart from the negative consequences on growth, glucocorticoid effects on bone density largely depend on the underlying disease for which glucocorticoids are needed (57).

Interleukins

Many inflammatory cytokines have a bad reputation for bone (IL-1, IL-6, INFα). Some interleukins, however, have a positive effect on bone homeostasis due to inhibition of osteoclast function. This was known for IL-12 and is now extended to IL-23 as revealed by IL-23 KO mice (58). Interleukins may also negatively affect chondrocytes and thus play a role in osteoarthritis. Transgenic mice overexpressing IL-18 (known to be increased in aging humans) exhibited accelerated joint cartilage degradation (59).

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

CANCER, BONE AND BEYOND: MOLECULAR MECHANISMS AND TRANSITION TO THERAPY

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The Ying-Yang of the TGF-β Superfamily in Bone Metastases: BMP7 as Bone Metastases Prevention?

Bone metastases are common in breast and prostate cancer patients with advanced disease. Knowledge of pathophysiology has increased in recent years, but effective therapy for this devastating complication of cancer remains suboptimal. In the Cancer and Bone session, Buijs and colleagues shed more light into the pathophysiology of bone metastases (60;61). Most importantly, they apply this knowledge to develop novel therapy for bone metastases using bone morphogenetic protein 7 (BMP7).

In the bone microenvironment, tumor cells interact with bone cells to disrupt normal bone remodeling, causing abnormal new bone formation or bone destruction, characteristic of osteoblastic and osteolytic metastases, respectively. This imbalance increases patient morbidity from pathologic fractures, intractable bone pain, spinal cord compression and hypercalcemia. Bone osteotropism of cancers has been attributed to the characteristics of cancer cells to survive and grow in the fertile soil of the bone microenvironment. Mineralized bone matrix is a major storehouse of growth factors, such as transforming growth factor-β (TGF-β), which is released and activated by tumor stimulation of osteoclastic bone resorption. TGF-β plays a central role in this feed-forward stimulation of tumor growth in bone by increasing tumor production of prometastatic factors. Here, the investigators implicate an additional role of TGF-β in this process to mediate epithelial to mesenchymal transition (EMT). The TGF-β signaling pathway in tumor cells represents a promising therapeutic target; different modalities to block TGF-β signaling are under investigation in mice and in humans. The new research demonstrates that, in breast cancer cells, BMP7 is an antagonist of the TGF-β pathway and can inhibit osteolytic metastases due to MDA-MB-231 breast cancer. It does so in part by mediating mesenchymal to epithelial transition (MET).

Here is the evidence offered to support the ying-yang of TGF-β and BMP pathways in breast cancer bone metastases: 1). BMP7 expression was inversely related to the tumorigenic potential and degree of EMT in different breast cancer cell lines. 2). The aggressive breast cancer line, MDA-MB-231, expresses very little BMP7, but when BMP7 is overexpressed, there are fewer osteolytic lesions when administered via intracardiac or local inoculation of tumor cells. 3). BMP7 treatment of mice in which tumors were inoculated into the tibia had reduced tumor growth in bone. Tumors from mice treated with BMP7 had increased expression of the epithelial marker of
pancytokeratin in bone mets. 4). Dose-dependent activation of the TGF-β signaling pathway was inhibited by BMP7 in a Smad-dependent manner. 5). BMP7 mRNA expression is lower in primary tumors associated with bone metastases than in primary breast tumors associated with metastases outside the skeleton.

Unlike in their similar work in prostate cancer and melanoma (62;63), the investigators found that the effect of BMP7 was not bone-specific since it also reduced orthotopic mammary fat pad tumor growth. Nonetheless, BMP7 could be used as a TGF-β antagonist in the treatment of osteolytic metastases from breast cancer. These data are consistent with those derived from breast cancer and melanoma bone metastases in which inhibition of TGF-β signaling by either small-molecule inhibitors or overexpression of the inhibitory Smad7 reduced bone metastases.

Collectively, the findings suggest that decreased BMP7 expression during carcinogenesis in the human breast contributes to the acquisition of a bone metastatic phenotype. Because exogenous BMP7 can still counteract the breast cancer growth at the primary site and in bone, BMP7 may represent a novel therapeutic molecule for repression of local and bone metastatic growth of breast cancer.

Other Highlights

Hedgehog signaling in bone metastases

Parathyroid hormone-related protein (PTHrP) can mediate local osteolysis in breast cancer bone metastases. Gli2, a Hedgehog (Hh) signaling transcription factor, is expressed by osteolytic breast cancer cells and regulates PTHrP promoter activity and expression. Over-expression of Gli2 increases tumor burden in bone and bone destruction (64) while dominant negative Gli2 decreases PTHrP expression and tumor-induced osteolysis.

Cyclopamine, a Hh inhibitor, blocks tumor cell growth in several tumor types. Since Gli2 expression by osteolytic breast cancer cells is mediated through canonical Hh signaling, investigators hypothesized that inhibitors of Hh signaling, such as cyclopamine, will decrease PTHrP and osteolysis. However, the researchers found no effect of cyclopamine on these parameters in a mouse model of MDA-MB-231 breast cancer bone metastases. This was no surprise as the tumors cells did not express the Hh signaling receptor, Smo (65). Thus, they concluded that modalities to target Gli2 directly would have more benefit than Hh receptor antagonists to inhibit tumor-induced osteolysis.

Similarly, in the prostate cancer arena, another group showed that cyclopamine did not reduce bone metastases due to PC-3 prostate cancer (66). However, they found that Gli1 was upregulated in bone metastases; Gli2 was not investigated. These studies indicate that further investigation of the Hh pathway in cancer metastases to bone is needed.

A new rat syngeneic model of prostate cancer osteoblastic metastases

Prostate cancer is associated with the formation of osteoblastic metastases. Animal models that mimic human prostate carcinoma skeletal metastasis are few. A new syngeneic model in Sprague-Dawley rats was developed by the direct injection of rat AT6-1 prostate tumor cells into the femur (67). Although AT6-1 cells had some characteristics of osteoblasts (alkaline phosphatase, Runx2, type I collagen, osteocalcin and bone sialoprotein markers) and osteoclasts (TRAP, cathepsin K, and calcitonin receptor markers), the cancer cells could not be induced to form mineralized nodules or express alkaline phosphatase activity in vitro. In vivo, AT6-1 cells inoculated into the distal femora in rats caused disorganized bone formation and resorption, evident on radiograph and micro-CT, resembling the pattern of osteoblastic metastases in humans with prostate cancer. Osteoclastic bone resorption is a critical component of osteoblastic disease, so the AT6-1 model was used to evaluate the effect of the bisphosphonate zoledronic acid on tumor growth in bone. High doses of zoledronic acid (100 mg/kg twice a week) significantly slowed tumor growth in bone. In
*in vitro*, zoledronic acid inhibited AT6-1 cell proliferation by a mechanism independent of caspase 3 activation and induced cell cycle arrest. This new syngeneic prostate cancer model of osteoblastic disease could be developed to test pathophysiology and treatment modalities.

**Tumor cell resistance to bisphosphonates: role of farnesyl diphosphate (FPP) synthase**

Bisphosphonates have been shown to have direct effects on tumor cells, mostly via *in vitro* methods. Amino-bisphosphonates act to inhibit osteoclastic bone resorption via the HMG-CoA reductase pathway, but whether this class of bisphosphonates acts on this pathway in tumor cells is unclear. Further, can cells (osteoclasts and/or tumor cells) develop resistance to amino-bisphosphonates? Here, investigators show that zoledronic acid alters the growth of osteosarcoma cells, but that these cells can develop resistance via farnesyl diphosphate (FPP) synthase of the HMG-CoA pathway (68). Specifically, zoledronic acid induced cell cycle arrest in S-G2/M phases and osteosarcoma cell death, but this was not associated with a multidrug resistance phenotype and was restricted to the nitrogen-containing bisphosphonates. Osteosarcoma lines that were resistant to this effect of zoledronic acid had increased expression of FPP synthase. Knockdown of FPP synthase with siRNA sensitized the osteosarcoma cells to zoledronic acid and this could be reversed by geranyl geraniol, a molecule downstream of FPP synthase in the pathway. Further, the investigators found that there was heterogeneity of FPP synthase expression in osteosarcoma lines and human osteosarcoma tumors. These findings have important implications for the characterization of the emerging, yet ill-described concept of bisphosphonate resistance.

**Targeting the RANKL pathway in bone metastases**

Bisphosphonate therapy reduces skeletal morbidity in patients with breast cancer bone metastases, but does not cure disease. Furthermore, complications with bisphosphonate therapy, as well as possible resistance, create a need for antiresorptive agents with different mechanisms of action. Since RANKL is a common final mediator of osteoclast formation and differentiation, this pathway represents another therapeutic approach to target cancer-induced bone diseases. Research now shows that OPG prevents tumor growth and tumor-induced osteolysis by the RANKL inhibitor osteoprotegerin (OPG); an effect that translated into increased survival in mice with bone metastases due to MDA-MB-231 breast cancer (69). Mouse RANKL protein is increased in MDA-MB-231 tumor-bearing bones. Further, this new work reveals that RANKL inhibition in mice with OPG-Fc treatment: 1) blocked MDA-231 tumor-induced osteolysis; 2) decreased tumor growth in bone; 3) caused tumor cell apoptosis; and 4) led to an overall improvement in survival endpoints. These data support the use of RANKL inhibition therapy in the treatment of breast cancer bone metastases and the rationale for its use in on-going clinical trials.

**Conflict of Interest:** The author reports that she is a consultant to Novartis and Amgen.

**TREATMENT OF OSTEOPOROSIS**

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The current dominance of bisphosphonates in the treatment of osteoporosis was reflected in the abstracts presented at the meeting. Further data were presented from the recently published zoledronate phase 3 program (70). A re-analysis of the fracture data across age categories and different geographies (Asia, Eastern Europe, Western Europe, Latin America and North America), showed consistent anti-fracture efficacy for the three categories of fracture considered (vertebral, clinical, and hip) (71). Results were not statistically significant in every single sub-category (since there was not adequate power to achieve this) but there were no trends that suggested differences in anti-fracture efficacy across these groupings. Histomorphometric and micro-CT analyses with the bone biopsies from this study were also presented (72). These demonstrated the expected changes
of reduced activation frequency and increased trabecular bone volume, though the latter finding has not always been statistically significant with other agents from this class. Unexpectedly, mineral apposition rate was significantly increased in the zoledronate group. This is possibly a chance finding, but it could be interpreted to suggest that the degree of osteoblast suppression is less with annual dosing of a bisphosphonate than it is with daily or weekly administration. Tetracycline label was demonstrable in all but one of the 82 biopsies obtained from patients treated with zoledronate, the unlabelled sample being incomplete and fragmented.

Further data were also presented from the BONE study of ibandronate (73). Hip structural analysis was performed on pre- and post-study femur DXA scans. In the ibandronate groups, hip structural parameters improved significantly, but were generally unchanged or deteriorated in the placebo group. There were also beneficial trends in indices of trabecular micro-architecture assessed by micro-CT in 80 subjects from this trial, though these effects do not appear to reach conventional levels of statistical significance. However, the changes suggest that the trabeculae are more plate-like than rod-like in the ibandronate-treated subjects, and that their trabecular density is higher. A meta-analysis pooling data from four randomized, controlled trials of ibandronate was also presented (Harris et al., presented as a late-breaking abstract in the Hot Topics session). This analysis assessed the anti-fracture efficacy of a wide range of annual dosages of ibandronate and concluded that there was consistent evidence of non-vertebral anti-fracture efficacy associated with the use of the highest doses (absorbed doses of 10–12 mg/year).

There was also consideration of the adverse events associated with bisphosphonate use. Bisphosphonate adverse effects in the context of pediatric practice were reviewed (74). Concerns remain regarding interference with normal growth and metaphyseal remodeling, particularly in children with milder forms of osteogenesis imperfecta. Delayed healing of osteotomy sites has been demonstrated, though this does not appear to be a problem after spontaneous fractures. A population of more than 400 children treated with pamidronate has been carefully monitored, for evidence of osteonecrosis of the jaw (ONJ), and no cases have been found. The effects of bisphosphonates on fracture healing were also the focus of rat studies reported from Australia (75). The animals pre-treated with pamidronate showed marginal reductions in long bone growth, a normal rate of fracture healing and an increase in callus volume. The problem of ONJ was also the subject of an original presentation (76). Investigators from Germany have established a register that seeks to record all cases in that country, currently having almost 500 affected patients. Of these, only eight have osteoporosis and no malignancy, suggesting an incidence of ONJ in osteoporosis subjects of the order of 1/100,000 patient-years.

An important trial assessing the interaction of calcium with bisphosphonate treatment was also reported (77). 700 elderly women with low bone density were randomized to calcium 1 g/day, alendronate 10 mg/day, or calcium plus alendronate. At two years, the changes in spine and hip bone density were comparable in the two alendronate groups, and significantly greater than those in the calcium monotherapy group. While addition of calcium supplementation to alendronate did not significantly increase BMD compared with alendronate alone, it did result in a small though statistically significant additional reduction in urine NTX, but not in bone-specific alkaline phosphatase. All subjects had a dietary calcium intake of at least 800 mg/day and received vitamin D 400 IU/day as well. This suggests that the routine co-prescription of calcium with bisphosphonates is not necessary in women with calcium intakes at this level.

Another study (78) compared the efficacy of alendronate 70 mg/week alone, with alendronate co-administered with alfalcaldiol 1 µg/day. At one year, turnover markers were more suppressed in those taking both agents and the increases in spine and hip BMD were also greater. However, the turnover and BMD changes
with alendronate monotherapy were somewhat less than had been reported previously.

In other presentations, the effects of ultra-low dose transdermal estrogen (14 µg/day) were presented. The effects on both markers and BMD tended to be greater in those with the lowest baseline serum estradiol levels, suggesting the possibility of targeting this low dose therapy to patients with low endogenous estrogens. Looking to the future (36), the role of sclerostin in bone biology was reviewed. Sclerostin is a naturally occurring inhibitor of bone formation produced in the osteocyte, and is an attractive target for the development of novel treatments for osteoporosis. The role of myostatin in muscle biology was reviewed, drawing particularly upon the myostatin null mouse (79). The conclusion was that myostatin inhibitors could be developed with a view to improving both bone and muscle regeneration, increasing muscle strength and preventing falls and fractures in the elderly.

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