IBMS BoneKEy. 2009 June;6(6):210-217
http://www.bonekey-ibms.org/cgi/content/full/ibmske;6/6/210
doi: 10.1138/20090381

PERSPECTIVES

Insights into the Antitumor Effects of Bisphosphonates from Preclinical Models and Potential Clinical Implications

Philippe Clézardin

INSERM, UMR U664, Laennec School of Medicine, University of Lyon, Lyon, France

Abstract

Since the observation that bisphosphonates act as potent inhibitors of osteoclast-mediated bone resorption, there is now a growing body of preclinical and clinical evidence that they may also exhibit direct and indirect antitumor activities through inhibition of tumor cell functions, enhancement of the cytotoxic activity of chemotherapy agents, stimulation of antitumor immune reactions and inhibition of tumor angiogenesis. Current insights and fronts of ongoing research on the antitumor effects of bisphosphonates in animal models of human cancers are presented in this review. Moreover, the clinical importance of these experimental findings is also discussed in light of the adjuvant use of bisphosphonates in several recent, large, phase III clinical trials in patients with early breast cancer. *IBMS BoneKEy*. 2009 June;6(6):210-217.

Keywords: Bisphosphonate; Zoledronate; Breast; Cancer; Angiogenesis; Disseminated tumor cell; γδ T cell

Introduction

As cancer becomes more advanced, it tends to spread throughout the body, with the bones being a common site of spread for many cancers. Spread of cancer to the bone from its original site is referred to as bone metastasis. The reasons why cancer cells metastasize to bone remain poorly understood, yet skeletal metastases can be fatal and may rapidly impede the quality of life of patients by causing pathological fractures, hypercalcemia, bone pain, nerve compression and loss of mobility (1). Cancer cells are, on their own, rarely able to destroy bone (1). Instead, they alter the functions of osteoclasts and osteoblasts, leading to the formation of skeletal lesions (1).

Bisphosphonates are structural analogues of pyrophosphates. Chemically, bisphosphonates are all characterized by two phosphonate groups linked to a central carbon atom, forming a P-C-P structure (2). Two side chains (referred to as R_1 and R_2) are covalently bound to the carbon atom of the common P-C-P structure. This common backbone, and the R_1 side chain (preferably a hydroxyl group), allow bisphosphonates to bind avidly to hydroxyapatite crystals in the skeleton (2). The R_2 side chain determines the potency of bisphosphonates to inhibit osteoclast-mediated bone resorption (2). Bisphosphonates that lack a nitrogen functional group in the R_2 side chain (*e.g.*, etidronate or clodronate) cause the intracellular accumulation of non-hydrolyzable, cytotoxic ATP analogues that subsequently induce osteoclast apoptosis (2). Bisphosphonates with an R_2 side chain containing a nitrogen atom either in an alkyl chain (*e.g.*, pamidronate, alendronate, ibandronate) or within a heterocyclic ring (*e.g.*, risedronate, minodronate or zoledronate) target osteoclast farnesyl diphosphate synthase (FPPS), a key enzyme in the mevalonate pathway, and inhibit its activity to varying degrees depending on the overall molecular structure of these nitrogen-containing bisphosphonates (N-BPs) (2). In addition, N-BPs can also interfere with an enzyme that is downstream of FPPS in the mevalonate pathway, the geranylgeranyl diphosphate

synthase (GGPPS) (2). As a consequence, cells become depleted of FPP and GGPP, which are required for the prenylation of small GTPases (such as Ras and Rho) that are essential for osteoclast function (2). Moreover, N-BPs induce the production of a unique cytotoxic adenosine triphosphate analogue (ApppI) that can directly induce osteoclast apoptosis (3).

Because of their potent antiosteoclastic activity, N-BPs have demonstrated clinical utility in the treatment and prevention of skeletal complications due to bone metastases in patients with solid tumors (breast, prostate, lung) and multiple myeloma (4). Bisphosphonates are also effective in preventing bone loss associated with the use of aromatase inhibitors in postmenopausal women and premenopausal women with early breast cancer. A similar protective effect of bisphosphonates on bone loss has been observed after androgen deprivation therapy in men with prostate cancer (4). Very recently, the addition of the bisphosphonate zoledronate to adjuvant endocrine therapy has been shown to not only prevent bone loss, but also to significantly improve disease-free survival in premenopausal women with estrogen-responsive early breast cancer (5). The benefit of zoledronate with respect to disease-free survival cannot be explained by the antiresorptive activity of this compound, and it has been proposed that zoledronate might exert some antitumor effects (5). Indeed, there is extensive *in vitro* and *in vivo* preclinical evidence that N-BPs have both direct and indirect antitumor effects (6). The following is an overview of current insights and fronts of ongoing research on the antitumor activities of N-BPs in animal models of human cancers. The clinical importance of these experimental findings is also discussed.

Direct Inhibitory Effects of N-BPs on Soft Tissue Tumors and Visceral Metastases

N-BPs have been shown to decrease the release of tumor-promoting growth factors from bone and to delay skeletal tumor growth by inhibiting osteoclast-mediated bone resorption (6). However, beside the fact that N-BPs may render the bone a less favorable microenvironment for tumor cell colonization by inhibiting bone resorption, there are now studies providing evidence that N-BPs also directly inhibit the growth of soft tissue tumors and visceral metastases.

The primary biochemical effect of N-BPs in cancer cells is on the mevalonate pathway through inhibition of FPPS, which impairs the prenylation of small G-proteins (Ras, Rho) and induces IPP and ApppI formation (6;7). These biochemical effects of N-BPs lead to the inhibition of tumor cell functions, such as adhesion, migration, invasion and proliferation, and to the induction of tumor cell apoptosis *in vitro* (6;7). Therefore, if N-BPs are administered during the early stages of cancer, metastasis may be prevented or reduced. For example, a 4T1 breast cancer murine model demonstrated that zoledronate and ibandronate significantly inhibit cellular invasion, thereby reducing the spontaneous formation of distant metastases to bone and visceral organs (lung, liver) compared with vehicle-treated mice (6). Similarly, alendronate inhibits Caov-3 ovarian cancer cell invasion in visceral organs *in vivo* (6). In addition, experiments using animals bearing subcutaneous or orthotopic tumors reveal that N-BPs (zoledronate, minodronate) can also block cancer cell cycle progression of non-small-cell lung carcinomas (6;8) or induce cancer cell apoptosis in mesothelioma, melanoma, osteosarcoma, and in breast and bladder carcinomas (6;9). Although N-BPs exhibit direct anti-tumor activity in these animal models (6;8;9), it is fair to say that high doses of N-BPs have been used in most of these studies (6), and that such high doses are incompatible with approved bisphosphonate-dosing regimens for patients with metastatic disease. This is the reason why we recently examined if a clinically relevant dosing regimen of zoledronate or clodronate could achieve meaningful antitumor effects in an animal model of bone metastasis (10). We reasoned that a chemotherapeutic approach that emphasizes dose density (*i.e.*, the administration of low doses of a bisphosphonate over short treatment

intervals) rather than dose escalation could be an effective way to minimize skeletal tumor burden. We found that bisphosphonate therapy at a high dosage with a long dosing interval reduces osteolysis by inhibiting bone resorption, whereas therapy at a low dosage with a more frequent dosing interval can also directly affect the growth of tumor cells resident in bone (10). In the same vein, the treatment of mice bearing subcutaneous LM8 osteosarcoma tumors with a clinically relevant dosage of zoledronate over short treatment intervals has been shown to inhibit the spontaneous formation of lung metastases in these animals (9). In the light of these findings (9;10), we believe that a frequent low-dose therapy with N-BPs facilitates the prolonged exposure of cancer cells to the drug, thus enabling a direct anti-tumor effect through a long-lasting (or even irreversible) inhibition of FPPS activity and sustained accumulation of the pro-apoptotic molecule ApppI within cancer cells. Interestingly, the administration of zoledronate over short treatment intervals inhibits lung metastasis formation at a dose that does not affect growth of the primary tumor, suggesting that the inhibitory effect of zoledronate on lung metastasis formation is attributable to inhibition of migration and invasion of LM8 osteosarcoma cells (9). This contention is supported by the fact that submicromolar concentrations of N-BPs that inhibit tumor cell adhesion and invasion do not inhibit tumor cell proliferation *in vitro* (6).

In vivo studies have also explored the potential for synergistic antitumor effects when a bisphosphonate is combined with cytotoxic drugs such as docetaxel, paclitaxel, imatinib mesylate, and ifosfamide (6). For example, zoledronate in combination with imatinib mesylate or paclitaxel demonstrated increased inhibition of tumor growth and reduced incidence of lymph node metastasis in a prostate cancer cell (PC-3MM2ST1571) model of bone metastases (11). Moreover, recent preclinical studies investigating the sequential combination of doxorubicin and zoledronate in breast cancer models demonstrated the most potent antitumor effects when doxorubicin was administered before zoledronate (12;13). The sequential treatment of mice with doxorubicin and then zoledronate resulted in an almost complete growth inhibition of subcutaneous human MDA-MB-436 breast tumor xenografts, whereas treament with doxorubicin or zoledronate alone or zoledronate followed by doxorubicin did not decrease tumor burden (13). These results (6;11-13) are interesting in two ways. First, they suggest that combinations of a cytotoxic agent and a N-BP could potentially provide clinical benefits in patients with cancer. Second, they indicate that the timing and sequence of cytotoxic chemotherapy and a N-BP may play a role in determining anticancer efficacy.

Indirect Inhibitory Effects of N-BPs on Soft Tissue Tumors and Visceral Metastases

Emerging data suggest that N-BPs have immunomodulatory effects via induction of gammadelta ($\gamma\delta$) T cells, which are known mediators of the immune response against tumor cells that express major histocompatibility complex (MHC) class 1 chain-related antigens A and B (MICA and MICB) (6). A specific subset of these $\gamma\delta$ T cells, referred to as Vγ9Vδ2 T cells, is strongly activated by phosphoantigens from bacteria and parasites and by IPP from eukaryotic cells. Because N-BPs are potent inhibitors of the IPP-processing enzyme FPPS, IPP accumulates within the cytoplasm and is then exposed at the surface of N-BP-treated cells, thereby leading to the activation of Vγ9Vδ2 T cells through recognition of IPP by the $\gamma\delta$ T-cell receptor (TCR) (6). Several preclinical and clinical studies have demonstrated that N-BPs stimulate expansion and activation of $\gamma\delta$ T cells when used in the presence of low doses of IL-2. In preclinical models, the cytotoxic activity of $\gamma\delta$ T cells *in vitro* was enhanced by N-BPs, and zoledronate enhances $\gamma\delta$ T cell antitumor activity in a murine lung cancer xenograft model (6;14). A phase I clinical trial in metastatic hormone-refractory prostate cancer patients

(N = 18) treated with zoledronate in combination with IL-2 has shown encouraging data with good immunologic (differentiation of γδ T cells) and clinical responses (partial response or stable disease) when compared to those observed in patients with metastatic prostate carcinoma treated with zoledronate alone (15). Similarly, tumor stabilization or even partial regression has been reported in patients with multiple myeloma treated with zoledronate in combination with low doses of IL-2, due to the expansion of Vγ9Vδ2 T cells (6). A recent study in 23 patients with breast cancer receiving a single dose of zoledronic acid (4 mg) also demonstrated a long-lasting activation of γδ T cells (16). Additional studies are ongoing to better characterize the clinical implications of γδ T-cell activation.

Angiogenesis, the formation of new blood vessels from existing ones, is a fundamental step in tumor development that involves a series of events, including endothelial cell proliferation and migration and endothelial cells' realignment to form new capillaries. In addition, bone marrow-derived endothelial progenitor cells mobilize to the bloodstream and contribute to the vascularization of primary tumors and pre-metastatic niches (17). Angiogenesis inhibition therefore results in an indirect antitumor effect. Several N-BPs have been reported to inhibit vascular endothelial cell functions *in vitro* and *in vivo* (6). In addition, N-BPs suppress tumor-associated angiogenesis in mouse models of breast carcinoma, cervical carcinoma and melanoma by blocking vascular endothelial growth factor (VEGF) signaling in endothelial cells, thereby inhibiting tumor growth and improving survival (6;13;18). In accordance with these *in vivo* findings, N-BPs (pamidronate, zoledronate) were reported to reduce circulating levels of VEGF in metastatic breast cancer patients (6). Moreover, a small study in patients with bone metastases (N = 26) demonstrated that a repeated low-dose therapy with zoledronate (1 mg weekly for 4 weeks) induced an early and sustained decrease of plasma levels of VEGF compared with a single 4-mg monthly dose of zoledronate (19). These findings support previous results from our pre-clinical study showing that the administration of a bisphosphonate at a low dosage over short treatment intervals enables a direct antitumor effect (10), and they raise the exciting possibility that, despite their rapid accumulation in bone, some N-BPs could be potent antiangiogenic agents.

Emerging Clinical Data on the Antitumor Activity of Bisphosphonates in Early Breast Cancer

The observation that the addition of oral clodronate to standard treatment reduced the incidence of bone metastases in patients with primary breast cancer gave the first hints that bisphosphonates might prevent metastases in breast cancer (20). More recently, results have been reported on the benefit of adjuvant therapy with zoledronate on inhibition of disseminated tumor cells (DTCs) in the bone marrow from patients with early-stage breast cancer (21-24). Residual DTCs from a primary breast cancer can lay dormant in the bone marrow for extended periods of time before developing into bone lesions or mobilizing to cause disease recurrence at other distant sites (25). In this respect, preliminary clinical evidence from 435 patients enrolled in 4 early breast cancer studies shows that zoledronate plus standard therapy significantly reduced the prevalence of DTCs in the bone marrow compared with standard therapy alone (21-24).

Importantly, the antitumor activity of zoledronate demonstrated in the preclinical setting has also been translated to the bedside recently in several large phase III clinical trials in patients with early breast cancer (ABCSG-12, ZO-FAST, neoadjuvant AZURE). The ABCSG-12 trial accrued 1,803 premenopausal women with early-stage breast cancer receiving ovarian suppression (goserelin) plus hormonal therapy (tamoxifen or anastrozole) alone or in combination with zoledronate (4 mg every 6 months) for 3 years (5). The addition of zoledronate to endocrine therapy resulted in

a reduction of 36% (P = 0.01) in the relative risk of disease progression when compared with endocrine therapy alone (5). It is important to note that the reduced risk of recurrence upon adjuvant therapy with zoledronate was not limited to bone sites, but was also seen in the contralateral breast, and in locoregional, lymph node and distant relapses (5). Moreover, a 40% reduction of disease-free survival by zoledronate was also observed in the ZO-FAST 36 months update (26). The ZO-FAST trial accrued 2,193 postmenopausal women with early breast cancer who were due to receive adjuvant therapy with an aromatase inhibitor (letrozole) for 5 years and who had a starting T-score of ≥ -2 for bone mineral density (26). Patients were randomized to receive immediate or delayed zoledronate at a dosing regimen of 4 mg every 6 months. Women in the immediate group were given the N-BP at the start of letrozole therapy while women in the delayed group were only given zoledronate if their T score fell below –2, or if there was a clinical or asymptomatic fracture at 36 months. Looking at the time to disease recurrence, immediate zoledronate treatment was shown to significantly decrease the risk for disease-free survival events (HR: 0.588, P = 0.03) (26). The reasons why adjuvant zoledronate treatment significantly improves disease-related outcomes in the ABCSG-12 and ZO-FAST trials are unknown. However, given that zoledronate is administered at a 4-mg dosage over long treatment intervals (every 6 months), the benefit of zoledronate with respect to disease-free survival is likely explained by indirect antitumor mechanisms. For example, zoledronate may render the bone marrow a less favorable environment for DTC colonization by inhibiting the release of bone-derived growth factors during bone resorption. It could also interfere with the normal functions of bone marrow-derived endothelial progenitor cells, thereby inhibiting angiogenesis at the primary tumor site or in distant relapses. Furthermore, it could block the engraftment of cancer (stem) cells in the stem cell niches in bone. The osteoblastic and vascular niches in bone are playing important roles in regulating the mobilization of hematopoietic stem cells (27) and similar signaling pathways may regulate the retention and dormancy of stem cells and cancer cells in these niches (28). For instance, the calcium-sensing receptor (CaSR) facilitates the retention of hematopoietic stem cells in the osteoblastic niche (29) and extracellular ionic calcium also promotes the localization of CaSR-expressing cancer cells in bone (30). It is therefore conceivable that, in an adjuvant setting, N-BPs interfere with the retention of cancer cells in osteoblastic niches by inhibiting the release of ionic calcium from bone mineral during bone resorption. Beside these potential indirect anti-tumor effects of N-BPs in the clinic, an exploratory analysis of the AZURE trial showed that in patients receiving neo-adjuvant chemotherapy (N = 205), the addition of zoledronate also led to a possible direct anti-tumor effect (31). The AZURE trial recruited 3,360 women with stage II/III breast cancer to determine whether treatment with zoledronate in addition to (neo) adjuvant therapy improves disease-related outcomes. A retrospective exploratory evaluation of the subgroup of AZURE patients who received neoadjuvant chemotherapy in combination with zoledronate (4 mg every 3-4 weeks) showed that there was a tumor volume reduction of 33% (P = 0.002) and 88% improvement in complete pathological response (P = 0.03) when compared to that observed in patients receiving neoadjuvant chemotherapy alone (31). Although underlying antitumor mechanisms are unknown, zoledronate could possibly act synergistically with the neoadjuvant chemotherapy on inhibition of primary tumor growth and/or exert antiangiogenic or antitumor immune reactions.

Conclusion

Since the observation that N-BPs act as potent inhibitors of osteoclast-mediated bone resorption, there is now a growing body of preclinical and clinical evidence that N-BPs may also exhibit direct and indirect antitumor activities. Data from large breast cancer trials are now demonstrating zoledronate's ability to improve disease

outcomes through its antitumor effects. There are several ongoing clinical trials (AZURE, SUCCESS, AZAC, NATAN, GAIN, ICE, NSABP B34, SWOG 0307) that are assessing the benefits of the bisphosphonates zoledronate, ibandronate, and clodronate in early breast cancer. These studies will provide additional information on bisphosphonates' antitumor impact in breast cancer in the near future.

Conflict of Interest: The author reports receiving research support from Novartis (Basel, Switzerland) and Procter & Gamble Pharmaceuticals (Mason, OH, USA).

Peer Review: This article has been peer-reviewed.

References

1. Clézardin P, Teti A. Bone metastasis: pathogenesis and therapeutic implications. *Clin Exp Metastasis*. 2007;24(8):599-608.

2. Coxon F. An update on the pharmacology of bisphosphonates and analogues with lower bone affinity. *IBMS BoneKEy*. 2008 October;5(10):357-69.

3. Mönkkönen H, Auriola S, Lehenkari P, Kellinsalmi M, Hassinen IE, Vepsäläinen J, Mönkkönen J. A new endogenous ATP analog (ApppI) inhibits the mitochondrial adenine nucleotide translocase (ANT) and is responsible for the apoptosis induced by nitrogen-containing bisphosphonates. *Br J Pharmacol*. 2006 Feb;147(4):437-45.

4. Coleman RE. Risks and benefits of bisphosphonates. *Brit J Cancer.* 2008 June 3;98(11):1736-40.

5. Gnant M, Mlineritsch B, Schippinger W, Luschin-Ebengreuth G, Pöstlberger S, Menzel C, Jakesz R, Seifert M, Hubalek M, Bjelic-Radisic V, Samonigg H, Tausch C, Eidtmann H, Steger G, Kwasny W, Dubsky P, Fridrik M, Fitzal F, Stierer M, Rücklinger E, Greil R; ABCSG-12 Trial Investigators, Marth C. Endocrine therapy plus zoledronic acid in premenopausal breast cancer. *N Engl J Med*. 2009 Feb 12;360(7):679-91.

6. Stresing V, Daubiné F, Benzaid I, Mönkkönen H, Clézardin P. Bisphosphonates in cancer therapy. *Cancer Lett*. 2007 Nov 8;257(1):16-35.

7. Mönkkönen H, Kuokkanen J, Holen I, Evans A, Lefley DV, Jauhiainen M, Auriola S, Mönkkönen J. Bisphosphonate-induced ATP analog formation and its effect on inhibition of cancer cell growth. *Anticancer Drugs*. 2008 Apr;19(4):391-9.

8. Li YY, Chang JW, Chou WC, Liaw CC, Wang HM, Huang JS, Wang CH, Yeh KY. Zoledronic acid is unable to induce apoptosis, but slows tumor growth and prolongs survival for non-small-cell lung cancers. *Lung Cancer*. 2008 Feb;59(2):180-91.

9. Koto K, Horie N, Kimura S, Murata H, Sakabe T, Matsui T, Watanabe M, Adachi S, Maekawa T, Fushiki S, Kubo T. Clinically relevant dose of zoledronic acid inhibits spontaneous lung metastasis in a murine osteosarcoma model. *Cancer Lett*. 2009 Feb 18;274(2):271-8.

10. Daubiné F, Le Gall C, Gasser J, Green J, Clézardin P. Antitumor effects of clinical dosing regimens of bisphosphonates in experimental breast cancer bone metastasis. *J Natl Cancer Inst*. 2007 Feb 21;99(4):322-30.

11. Kim SJ, Uehara H, Yazici S, He J, Langley RR, Mathew P, Fan D, Fidler IJ. Modulation of bone microenvironment with zoledronate enhances the therapeutic effects of STI571 and paclitaxel against experimental bone metastasis of human prostate cancer. *Cancer Res*. 2005 May 1;65(9):3707-15.

12. Ottewell PD, Deux B, Mönkkönen H, Cross S, Coleman RE, Clézardin P, Holen I. Differential effect of doxorubicin

and zoledronic acid on intraosseous versus extraosseous breast tumor growth in vivo. *Clin Cancer Res*. 2008 Jul 15;14(14):4658-66.

13. Ottewell PD, Mönkkönen H, Jones M, Lefley DV, Coleman RE, Holen I. Antitumor effects of doxorubicin followed by zoledronic acid in a mouse model of breast cancer. *J Natl Cancer Inst*. 2008 Aug 20;100(16):1167-78.

14. Sato K, Kimura S, Segawa H, Yokota A, Matsumoto S, Kuroda J, Nogawa M, Yuasa T, Kiyono Y, Wada H, Maekawa T. Cytotoxic effects of gammadelta T cells expanded ex vivo by a third generation bisphosphonate for cancer immunotherapy. *Int J Cancer*. 2005 Aug 10;116(1):94-9.

15. Dieli F, Vermijlen D, Fulfaro F, Caccamo N, Meraviglia S, Cicero G, Roberts A, Buccheri S, D'Asaro M, Gebbia N, Salerno A, Eberl M, Hayday AC. Targeting human gammadelta T cells with zoledronate and interleukin-2 for immunotherapy of hormone-refractory prostate cancer. *Cancer Res*. 2007 Aug 1;67(15):7450-7.

16. Santini D, Martini F, Fratto ME, Galluzzo S, Vincenzi B, Agrati C, Turchi F, Piacentini P, Rocci L, Manavalan JS, Tonini G, Poccia F. In vivo effects of zoledronic acid on peripheral gammadelta T lymphocytes in early breast cancer patients. *Cancer Immunol Immunother*. 2009 Jan;58(1):31-8.

17. Kaplan RN, Psaila B, Lyden D. Bone marrow cells in the 'pre-metastatic niche': within bone and beyond. *Cancer Metastasis Rev*. 2006 Dec;25(4):521-9.

18. Melani C, Sangaletti S, Barazzetta FM, Werb Z, Colombo MP. Amino-biphosphonate-mediated MMP-9 inhibition breaks the tumor-bone marrow axis responsible for myeloid-derived suppressor cell expansion and macrophage infiltration in tumor stroma. *Cancer Res*. 2007 Dec 1;67(23):11438-46.

19. Santini D, Vincenzi B, Galluzzo S, Battistoni F, Rocci L, Venditti O, Schiavon G, Angeletti S, Uzzalli F, Caraglia M, Dicuonzo G, Tonini G. Repeated intermittent low-dose therapy with zoledronic acid induces an early, sustained, and long-lasting decrease of peripheral vascular endothelial growth factor levels in cancer patients. *Clin Cancer Res*. 2007 Aug 1;13(15 Pt 1):4482-6.

20. Diel IJ, Solomayer EF, Costa SD, Gollan C, Goerner R, Wallwiener D, Kaufmann M, Bastert G. Reduction in new metastases in breast cancer with adjuvant clodronate treatment. *N Engl J Med*. 1998 Aug 6;339(6):357-63.

21. Rack BK, Jueckstock J, Genss EM, Schoberth A, Schindbeck C, Strobl B, Heinrigs M, Rammel G, Zwingers T, Sommer H, Friese K, Janni W. Effect of zoledronate on persisting isolated tumor cells in the bone marrow of patients without recurrence of early breast cancer. *Breast Cancer Res Treat.* 2007;106(Suppl 1):S40. Abstract 511.

22. Lin AY, Park JW, Scott J, Melisko M, Goga A, Moasser MM, Moore DH, Rugo HS. Zoledronic acid as adjuvant therapy for women with early stage breast cancer and disseminated tumor cells in bone marrow. *J Clin Oncol.* 2008 May 20;26(15 Suppl):20s. Abstract 559.

23. Aft R, Watson M, Ylagan L, Chavez-MacGregor M, Trinkaus K, Zhai J, Naughton M, Weilbaecher K. Effect of zoledronic acid on bone marrow micrometastases in women undergoing neoadjuvant chemotherapy for breast cancer. *J Clin Oncol.* 2008 May 20;26(15 Suppl):46s. Abstract 1021.

24. Solomayer EF, Gebauer G, Hirnle P, Janni W, Lück HJ, Becker S, Huober J, Kraemer B, Wackwitz B, Fehm T.

Influence of zoledronic acid on disseminated tumor cells (DTC) in primary breast cancer patients. *Cancer Res*. 2009 Jan 15;69(Suppl 1):171s. Abstract 2048.

25. Pantel K, Brakenhoff RH, Brandt B. Detection, clinical relevance and specific biological properties of disseminating tumour cells. *Nat Rev Cancer*. 2008 May;8(5):329-40.

26. Eidtmann H, Bundred NJ, DeBoer R, Llombart A, Davidson N, Neven P, von Minckwitz G, Miller J, Schenk N, Coleman R. The effect of zoledronic acid on aromatase inhibitor associated bone loss in postmenopausal women with early breast cancer receiving adjuvant letrozole: 36 months follow-up of ZO-FAST. *Cancer Res*. 2009 Jan 15;69(Suppl 1):74s. Abstract 44.

27. Yin T, Li L. The stem cell niches in bone. *J Clin Invest*. 2006 May;116(5):1195-201.

28. Psaila B, Lyden D. The metastatic niche: adapting the foreign soil. *Nat Rev Cancer.* 2009 April;9(4):285-93.

29. Adams GB, Chabner KT, Alley IR, Olson DP, Szczepiorkowski ZM, Poznansky MC, Kos CH, Pollak MR, Brown EM, Scadden DT. Stem cell engraftment at the endosteal niche is specified by the calcium-sensing receptor. *Nature*. 2006 Feb 2;439(7076):599-603.

30. Liao J, Schneider A, Datta NS, McCauley LK. Extracellular calcium as a candidate mediator of prostate cancer skeletal metastasis. *Cancer Res*. 2006 Sept 15;66(18):9065-73.

31. Winter MC, Thorpe HC, Burkinshaw R, Beevers SJ, Coleman RE. The addition of zoledronic acid to neoadjuvant chemotherapy may influence pathological response - exploratory evidence for direct anti-tumor activity in breast cancer. *Cancer Res*. 2009 Jan 15;69(Suppl 1):330s. Abstract 5101.