

Bisphosphonates in Breast Cancer: Clinical Activity and Implications of Preclinical Data

Rebecca Aft, MD, PhD

Dr. Aft is a Professor of Surgery in the Division of General Surgery at the Washington University School of Medicine in St. Louis, Missouri.

Address correspondence to:

Rebecca Aft, MD, PhD

Professor, Surgery

Division of General Surgery

Surgical Oncology and Endocrine

Surgery Section

Washington University School of Medicine

660 S. Euclid Avenue, Campus Box 8109

St. Louis, MO 63110

Phone: (314) 747-0063

Fax: (314) 454-5509

E-mail: aft@wudosis.wustl.edu

Abstract: Breast cancer is the most frequently diagnosed and second deadliest cancer among women. Bisphosphonates are stable pyrophosphate analogues used to treat skeletal-related events resulting from bone metastases. In the adjuvant setting, they have been shown to prevent aromatase inhibitor-associated and chemotherapy-induced bone loss. There is a growing body of evidence that bisphosphonates have direct and indirect anticancer activity in the preclinical and clinical settings. These include the inhibition of tumor growth; induction of apoptosis; synergism with chemotherapy; inhibition of tumor migration, invasion, and metastasis; reduction in disseminated tumor cells; inhibition of angiogenesis; stimulation of immune surveillance; and suppression of bone-derived growth factors. In addition to reducing the risk of breast cancer, bisphosphonate therapy has been shown to improve outcomes of early and metastatic breast cancer treatment. This review provides a brief overview of the current role of bisphosphonates in clinical practice and discusses their potential as anticancer agents.

Introduction

Breast cancer, the most frequently diagnosed and second deadliest cancer among women, is a global public health issue.¹ In 2008, approximately 1.38 million cases were diagnosed and 458,000 deaths occurred worldwide. Despite advances in early detection and in treatment options, all patients with breast cancer are at risk for disease recurrence, progression, and death. In the United States, the 5-year survival rate is 89% for all breast cancer patients.² For those diagnosed with metastatic disease, the 5-year survival rate is reduced to 23%. Multimodal management of breast cancer patients includes a combination of surgery, radiotherapy, chemotherapy, endocrine therapy, and targeted therapy.³ In addition, bisphosphonates (BPs) are used adjunctively in patients with evidence of lytic destruction of bone for the prevention of skeletal-related events (SREs). For patients with early-stage breast cancer, BPs are recommended for those at high risk for osteoporosis⁴ and, though not included in treatment guidelines, for the mitigation of therapy-induced bone loss.⁵ Most interestingly, there is a growing body of evidence demonstrating the anticancer activity of BPs.

Keywords

Anticancer, bisphosphonate, zoledronic acid, breast cancer

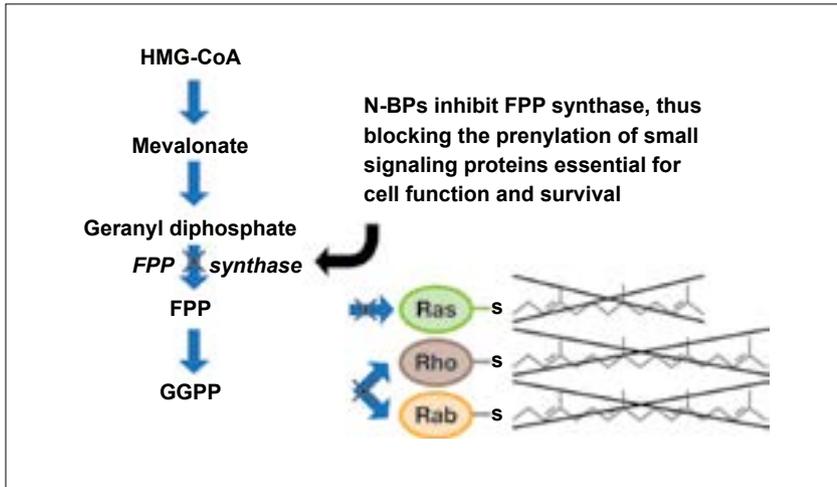


Figure 1. Mechanism of action of nitrogen-containing bisphosphonates.¹³

FPP=farnesyl diphosphate;
GGPP=geranylgeranyl diphosphate;
HMG-CoA=3-hydroxy-3-methylglutaryl-coenzyme A;
N-BPs=nitrogen-containing bisphosphonates.

This article is the first of a 2-part series; it provides a brief overview of the current role of BPs in clinical practice and discusses the preclinical data available to date. The second part of the series, which will discuss current clinical studies and future directions of bisphosphonate treatment, will appear in the April issue of *Clinical Advances in Hematology & Oncology*.

Clinical Activity of BPs

BPs are chemically stable analogues of inorganic pyrophosphates. They were developed in the 19th century,⁶ but it was not until 1968 that their biologic effects—inhibition of the precipitation and dissolution of calcium phosphate in vitro—were first reported.⁷ Bisphosphonates were initially hypothesized to regulate bone resorption in vivo, in a manner analogous to their in vitro effects. It was not until the 1990s that the cellular basis of BP regulation of bone resorption was demonstrated. At physiologic doses, BPs act almost exclusively on bone, owing to their high affinity for this tissue; they are deposited both in newly formed bone and on osteoclast surfaces.⁸

The first-generation BPs were non-nitrogen-containing (non-N-BPs) and exerted their effects by replacing terminal phosphates of adenosine triphosphate (ATP).⁹ These nonhydrolyzable ATP analogues likely promoted apoptosis by inhibiting ATP-dependent cellular activities.¹⁰⁻¹² The more potent, second- and third-generation nitrogen-containing BPs (N-BPs) inhibit the mevalonate pathway. Studies have shown that this leads to the suppression of the prenylation of small G proteins (Figure 1),¹³ leading to apoptosis when the affected proteins are unable to regulate core cellular processes.¹⁴⁻¹⁸ Recently, it was demonstrated that N-BPs suppress the farnesylation of the centromeric protein Cenp-F (mitosin).¹⁹ This appears to impair chromosome separation, resulting in a delay in cell cycle progression and inhibition of cell proliferation.

Outside of the mevalonate pathway, there is evidence that some N-BPs induce production of the nonhydrolyzable ATP-analogue ApppI, which is able to induce apoptosis similarly to that observed with non-N-BPs.²⁰

Bisphosphonates approved for use in cancer therapy include the non-N-BP clodronate and the N-BPs ibandronate (Boniva, Roche), pamidronate, and zoledronic acid. Though not approved for cancer indications, commercially available BPs include the non-N-BPs etidronate and tiludronate and the N-BPs alendronate and risedronate (Figure 2).

Bisphosphonates have been widely used for the management of SREs in patients with osteolytic metastasis, shown to occur in approximately 70% of breast cancer patients with metastatic disease.²¹ In these patients, osteoclast-induced bone resorption may lead to complications including hypercalcemia of malignancy, bone pain, pathologic fractures, spinal cord compression, radiotherapy, and surgical intervention. A summary of studies supporting BP use for these conditions can be found in Table 1.²²⁻³³ Bisphosphonates have also been introduced into clinical practice for the prevention of aromatase inhibitor (AI)-associated bone loss. AIs have become the standard of care for many postmenopausal patients with hormone receptor-positive early breast cancer, as a significant improvement in disease-free survival (DFS) when compared to tamoxifen has been demonstrated.³⁴⁻³⁸ However, the profound estrogen suppression associated with AI therapy³⁹⁻⁴¹ may cause an increase in bone turnover, acceleration of bone loss, and an increase in fracture risk.^{33,42-45} Evidence supporting the hypothesis that BP therapy is a viable option for preventing AI-associated bone loss can be found in Table 2.⁴⁶⁻⁵¹ BPs have also demonstrated benefit in the prevention of bone loss in premenopausal patients treated with adjuvant chemotherapy. In these patients, a significant sequelae of treatment is ovarian damage leading to changes in menses, including

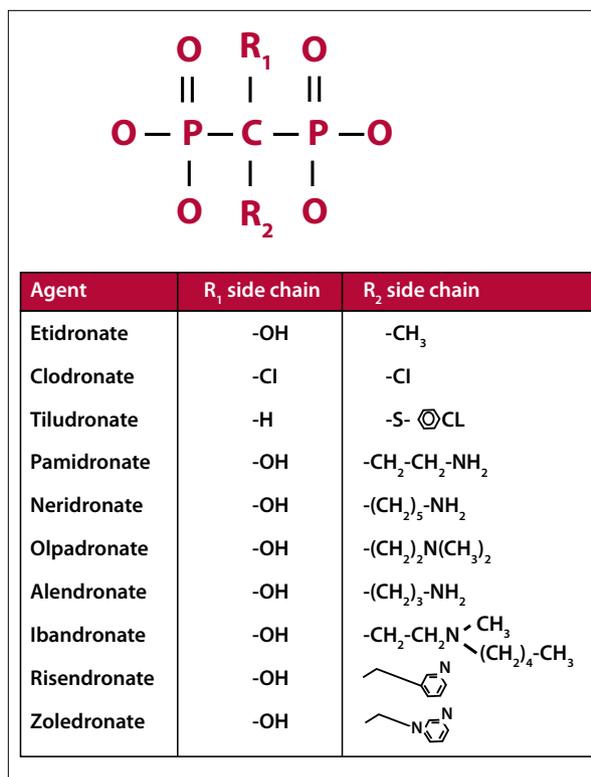


Figure 2. Structures of the bisphosphonates.

premature menopause.⁵² This has been associated with a rapid loss of bone mineral density, which increases the risk of osteoporosis and fractures.⁵³ Some, though not all, BPs have been shown to attenuate the effect of chemotherapy on bone mineral density (Table 3).⁵⁴⁻⁵⁹

New Directions: Anticancer Effects of BPs

There is an increasing body of evidence supporting direct and indirect anticancer actions of BPs. Direct anticancer activity has been demonstrated in the form of inhibition of tumor cell growth, induction of tumor cell apoptosis, and synergism with chemotherapy. Indirect anticancer effects attributed to BPs include inhibition of tumor migration, invasion, and metastasis; reduction in disseminated tumor cells (DTCs); inhibition of angiogenesis; stimulation of immune surveillance; and suppression of bone-derived growth factors (Figure 3).⁶⁰

In Vitro and Preclinical Studies of BPs

Inhibition of Tumor Cell Growth and Induction of Apoptosis (Monotherapy)

Bisphosphonates as monotherapy have been shown to have antiproliferative activity. Ibandronate at high concentrations (10⁻⁴ M) induced apoptosis of the estrogen

receptor (ER)-negative MDA-MB-231 breast cancer cells.⁶¹ Likewise, treatment with zoledronic acid, pamidronate, ibandronate, or clodronate (each at 10⁻⁴, 10⁻⁶, and 10⁻⁸ M) reduced cell viability that was time and dose dependent and irreversible in ER-positive MCF-7 and T47D breast cancer cells.⁶² Ibandronate and zoledronic acid were the most efficacious. These findings were supported by other studies that demonstrated that BPs reduce cell growth and viability of multiple breast cancer cell lines, with zoledronic acid being the most potent.^{63,64}

The mechanism by which BPs induce apoptosis has been partially elucidated. Bcl-2 is a protein that confers resistance to apoptosis, and it is hypothesized that it prevents the release of cytochrome c, resulting in the inhibition of caspase activation and leading to the inhibition of apoptosis.⁶⁵ In accordance with the role of BPs in promoting apoptosis, BP activity has been associated with the induction of a caspase-dependent signaling pathway. In the MDA-MB-231 cell line, pamidronate downregulated bcl-2 expression,^{63,66} whereas the zoledronic acid-induced reduction in cell viability was reversed by bcl-2 overexpression.⁶⁷ Zoledronic acid induced caspase activation via the cleavage of pro-caspase-3, whereas preincubation with a caspase-3 selective inhibitor prevented zoledronic acid-induced apoptosis.⁶⁷ Likewise, the mevalonate pathway intermediate geranylgeraniol reversed the caspase-3 activation and decreased zoledronic acid-induced apoptosis in 4T1/luc mouse breast cancer cells.⁶⁸ Ibandronate increased caspase-3 activity and DNA fragmentation in MDA-MB-231 cells, and this was inhibited by addition of the caspase inhibitor Z-VAD-FMK.⁶¹ The increase in caspase-3 activity leads to the degradation of the caspase substrate poly (ADP)-ribose polymerase, which in turn inhibits DNA repair and promotes apoptosis, as demonstrated with pamidronate and zoledronic acid.⁶³

Inhibition of Tumor Cell Growth and Induction of Apoptosis (Synergism With Chemotherapy)

Synergistic effects have been observed when cytotoxic agents are combined with N-BPs in vitro and in animal models. Treatment of the MCF-7 cells with zoledronic acid (10 μM) and paclitaxel (2 μM) for 72 hours resulted in a 5-fold increase in apoptosis compared with zoledronic acid alone and a 4-fold increase compared with paclitaxel alone.⁶⁹ At a more clinically relevant dose of 1 μM, zoledronic acid induced a 4.1% level of apoptosis, compared with 1.26% (paclitaxel) and 0.26% (zoledronic acid) with either agent alone.⁷⁰ Induction of apoptosis in MCF-7 cells was also greater with the combination of doxorubicin and zoledronic acid compared with either agent alone.⁷¹ However, BP combinations are not equally efficacious. In the aforementioned study, doxorubicin and alendronate also acted synergistically to induce apoptosis, though to a lesser degree than zoledronic acid, whereas doxorubicin

Table 1. Bisphosphonate Therapy in Women With Stage IV Breast Cancer Receiving Chemotherapy or Hormone Therapy

Bisphosphonate	Comparator	N	Treatment	Results
Clodronate ²²	Placebo	144	≤12 months	Decrease in the onset of new bone events ($P=.05$), pain intensity ($P=.01$), and in requirement for analgesics ($P=.02$).
Clodronate ²³	No additional therapy	100	Daily for 2 years	Decrease in the number of skeletal events, time to first skeletal event ($P=.015$), and time to first fracture ($P=.023$). No statistical difference in time to first radiotherapy ($P=.069$).
Oral and IV ibandronate* ²⁴	–	913	q3–4w for ≤24 weeks	Improvement in bone pain severity in 70% of patients with pain at baseline.
IV ibandronate ²⁵	Placebo	466	q3–4w for ≤24 months	Decrease in skeletal morbidity period rate ($P=.004$), vertebral fractures ($P=.023$), and requirement for radiotherapy ($P=.012$). Improvement in bone pain score and no statistical difference in nonvertebral fractures and events requiring surgery.
Oral ibandronate ²⁶	Placebo	564	Daily for ≤96 weeks	Reduction in bone pain ($P=.019$). Less increase in analgesic use ($P=.019$).
IV pamidronate ²⁷	Placebo	382	q3–4w for 12 cycles	Decrease in skeletal complication ($P=.005$), pathologic fracture ($P=.01$), radiation to bone ($P=.001$), surgery to bone ($P=.01$), and hypercalcemia ($P=.02$). Increase in time to first skeletal complication ($P=.005$) and time to first nonvertebral pathologic fracture ($P=.01$), time to first radiotherapy to bone ($P=.001$), time to first bone surgery ($P=.01$), and time to first hypercalcemic episode ($P=.02$). No significant differences in time to new vertebral pathologic fractures.
IV pamidronate ²⁸	Placebo	382	q3–4w for ≤24 months	Reduction in any skeletal complication ($P<.001$), nonvertebral pathologic fracture ($P<.001$), radiotherapy to bone ($P<.001$), surgery to bone ($P=.003$), and hypercalcemia ($P=.005$). Improvement in time to increase in pain severity ($P=.43$). Smaller increase in analgesic use ($P=.011$). No statistical difference in vertebral pathologic fracture.
IV pamidronate ²⁹	Placebo	754	q3–4w for ≤24 cycles	Reduction in skeletal morbidity rate ($P<.001$), time to first skeletal complication ($P<.001$), radiation to bone ($P<.001$), time to requirement for radiotherapy ($P<.001$), pathologic fracture ($P=.002$), time to new pathologic fracture ($P=.003$), surgery to bone ($P=.008$), and hypercalcemia ($P=.001$). No statistical difference in spinal cord compression.
IV zoledronic acid ³⁰	Pamidronate	280 [†]	q4w for ≤10 months	Similarly reduced SREs and need for radiotherapy.
IV zoledronic acid ³¹	Pamidronate	1,130 [†]	q3–4w for 24 months	Decrease in skeletal complications ($P=.025$) and skeletal complications in hormonally treated breast cancer pts ($P=.009$). Increase in time to first SRE in hormonally treated breast cancer pts ($P=.047$).
IV zoledronic acid ^{‡32}	None	31	q4w for 3 months	Decrease in worst pain and average pain score at week 8 vs baseline ($P<.001$).
Clodronate (meta-analysis) ³³	Placebo	330	Oral 1,600 mg/d for 1–2 years	No statistical improvement in overall, bone metastasis-free, or nonskeletal metastasis-free survival.

IV=intravenous; q3/4w=every 3/4 weeks; SRE=skeletal-related event.

*Postmarketing surveillance study.

†Breast cancer subset.

‡Second-line after clodronate or pamidronate.

Table 2. Bisphosphonate Therapy in Women Receiving Adjuvant Endocrine Therapy for Hormone Receptor-Positive Early-Stage Breast Cancer

Bisphosphonate	Comparator	N	Treatment	Results
Zoledronic acid (ABCSCG-12) ⁴⁶	Placebo	1,803 (903 in AI arm)	4 mg every 6 months for 3 yrs	Substudy showed zoledronic acid completely eliminated cancer treatment-induced bone loss.
Upfront zoledronic acid (ZO-FAST) ⁴⁷	Delayed-start zoledronic acid	1,065	Every 6 months for 5 yrs immediately or upon decrease in T-score or nontraumatic fracture	<i>At 12 months</i> Increase in LS BMD in upfront group and decrease in delayed-start group. Significant difference in LS ($P<.0001$) and TH ($P<.0001$) BMD. Reduction in serum BSAP ($P<.0001$) in upfront group; increase in serum BSAP ($P<.0001$) in delayed-start group. Similar incidence of fractures.
Upfront zoledronic acid (Z-FAST) ⁴⁸	Delayed-start zoledronic acid	602	Every 6 months for 5 yrs immediately or upon decrease in T score or nontraumatic fracture	Increase in LS and TH BMD in upfront group and decrease in delayed-start group through 61 months ($P<.0001$ [LS] and $P<.001$ [TH]). Suppression of serum BSAP over 61 months (P significant at all time points).
Upfront zoledronic acid (combined Z-FAST and ZO-FAST) ⁴⁹	Delayed-start zoledronic acid	1,667		<i>At 12 months</i> Increase in LS and TH BMD in upfront group and decrease in delayed-start group ($P<.0001$ for both). Significant change in LS and TH and TH BMD ($P<.0001$ for both) from baseline. Increase in serum BSAP in upfront group ($P<.0001$) and decrease in delayed-start group ($P=.0011$) from baseline.
Risedronate ⁵⁰	Placebo	38 high risk; 154 moderate risk	Every week for 2 yrs	<i>High-risk group</i> Increase in LS ($P=.0006$) and TH ($P=.0104$) BMD at 24 months vs baseline. Decrease in sCTX, P1NP, and BSAP from baseline to 12 months ($P<.0001$ for all). <i>Moderate-risk group</i> Significant difference in BMD change in LS ($P<.0001$) and TH ($P<.0001$) vs placebo. Decrease in sCTX, P1NP, and bALP at 12 months vs placebo ($P<.0001$ for all).
Ibandronate (ARIBON) ⁵¹	Placebo	50		Increase and stabilization of LS and TH BMD in ibandronate group and decrease in LS and TH BMD in placebo group. Significant differences at both sites and each time point ($P<.01$). Reduction in uNTX, sCTX, and sBALP in ibandronate group and increase in uNTX, sCTX, and sBALP in placebo group. Significant difference between treatment arms for each marker ($P<.001$).

AI=aromatase inhibitor; BMD=bone mineral density; BSAP=bone-specific alkaline phosphatase; LS=lumbar spine; P1NP=serum procollagen type 1 amino-terminal propeptide; sBALP=serum bone alkaline phosphatase; sCTX=serum C-telopeptide of type I collagen; TH=total hip; uNTX=urinary NH₂-terminal peptide of type I collagen.

and clodronate did not combine beneficially. In another study, ibandronate did not enhance the ability of paclitaxel or docetaxel to induce apoptosis of MDA-MB-231 cells.⁷² In contrast, combinations of ibandronate and the cytotoxic agents cyclophosphamide/methotrexate/5-fluorouracil, epirubicin/cyclophosphamide, epirubicin/

paclitaxel, and epirubicin/docetaxel were effective in inhibiting the growth of primary breast cancer cells, but less so than combinations with zoledronic acid.⁷³ In addition to BP-chemotherapy combinations, the sequence of administration appears to play a role in therapeutic efficacy. In one study, maximal induction of apoptosis,

Table 3. Bisphosphonate Therapy in Premenopausal Women Receiving Adjuvant Chemotherapy

Chemotherapy	Bisphosphonate	Comparator	N	Treatment	Results
CMF ⁵⁴	Oral clodronate	No additional treatment	148	Daily for 3 years	Reduced bone loss at FN ($P=.0005$), slight increase in LS ($P=.017$). Reduced bone loss at FN and LS at 2 years in 43 amenorrheic pts.
Investigator discretion ⁵⁵	IV pamidronate	Placebo	40	Every 3 months for 1 year	Stabilization of LS BMD vs decrease in placebo group. Significant change in LS BMD at 6 and 12 mos ($P=.003$) including a slight increase in the risedronate group. No significant difference in TH. Significant change in LS ($P=.0084$) BMD at 6 and 12 months and TH BMD at 12 months ($P=.026$) in amenorrheic pts.
Investigator discretion ⁵⁶	IV zoledronic acid	Placebo	101	Every 3 weeks for 1 year	Increase in LS, FN, and TH BMD at 12 months, return to baseline at 24 months in zoledronic acid arm. Decrease in LS, FN, and TH BMD at 12 and 24 months in placebo group ($P<.001$ vs zoledronic acid arm at all sites and time points).
AC → T ⁵⁷	IV zoledronic acid	No additional treatment or delayed zoledronic acid	110	At 0 and 6 months	Significant change in LS and FN BMD at 6 and 12 months ($P<.001$ for both).
A, T, or C ⁵⁸	Oral risedronate	Placebo	216	Every week for 1 year	No significant difference in the prevention of bone loss.
AT ⁵⁹	IV zoledronic acid	Placebo	30	Every 3 weeks for 1 year	Significant decrease in LS and FN BMD with no bisphosphonates, and significant increase in FN BMD with zoledronic acid ($P<.01$).

A=anthracycline; BMD=bone mineral density; C=cyclophosphamide; F=fluorouracil; FN=femoral neck; IV=intravenous; LS=lumbar spine; M=methotrexate; T=taxane; TH=total hip.

(6.1%) was achieved when MCF-7 cells were treated with 2 nM paclitaxel on day 1 followed by 25 μ M zoledronic acid on day 2 as opposed to the reverse sequence (2.4%) or coadministration (3.75%).⁷⁰ In MCF-7 cells, maximal induction of apoptosis required exposure to doxorubicin prior to zoledronic acid.⁷¹

The findings of these in vitro studies were supported by findings of in vivo studies. Ibandronate (4 mg/d) demonstrated additive anticancer effects in combination with doxorubicin in a metastatic tumor model formed by intracardiac implantation of MDA-MB-231 cells in nude mice.⁷⁴ The combination reduced tumor burden in bone, though not in adrenal tissue, compared with either agent alone. The reason for these differential effects remains unclear but may have to do with the ability of BPs to deposit preferentially in bone. In a similar mouse model, zoledronic acid (0.2 mg per mouse) in combination with the antibiotic doxycycline resulted in a 74% decrease in total tumor burden compared with placebo ($P<.05$).⁷⁵ Finally, when MDA-MB-436 cells were injected into the tibiae of immunocompromised mice, sequential doxorubicin and zoledronic acid (100 μ g/kg) treat-

ment resulted in a reduction in tumor burden, an increase in apoptosis, and a reduction in tumor proliferation compared with either agent alone.⁷⁶

Inhibition of Tumor Cell Migration, Invasion, and Metastasis

The effects of BPs on tumor cell dissemination have also been studied. In vitro, pretreatment of breast (MCF-7 and MDA-MB-231) and prostate (PC-3) tumor cells with BPs caused a dose-dependent inhibition of adhesion to unmineralized and mineralized osteoblastic extracellular matrices, with the following rank order of potency: ibandronate > NE-10244 (analogue of risedronate) > pamidronate > clodronate.⁷⁷ A subsequent study showed that BPs caused a dose-dependent inhibition of breast (MDA-MB-231), prostate (PmPC3), and osteosarcoma (MG-63) tumor cell invasion through Matrigel (BD Biosciences), with the following rank order of potency: zoledronic acid > ibandronate > NE-10244 > clodronate.⁷⁸ In these studies, BPs did not induce cytotoxic effects,^{77,78} and they interfere with the production of matrix metallopro-

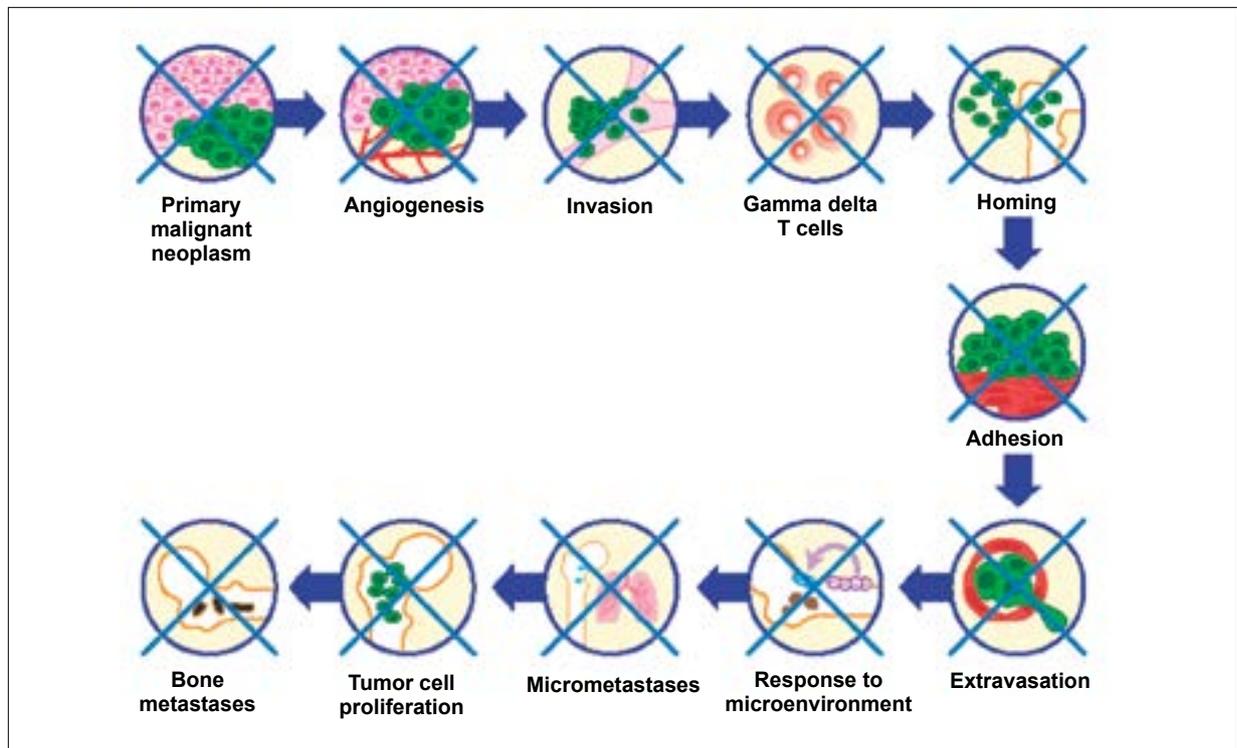


Figure 3. Bisphosphonates inhibit multiple steps in the metastatic cascade.

Adapted with permission from Macmillan Publishers Ltd. Mundy GR.⁶⁰

teinases⁷⁸ by tumor cells at concentrations that inhibited tumor cell invasion; however, they inhibited proteolytic activity. In another study, a clinically relevant concentration (1 μM) of zoledronic acid inhibited invasion of MDA-MB-231 cells through Matrigel in a process that was mediated by disorganization of actin cytoskeleton due to Ras homolog gene family member A inhibition related to its defective prenylation.⁷⁹ Zoledronic acid also inhibited the chemotactic effect induced by stromal cell-derived factor-1, a chemokine greatly involved in cancer metastasis to bone. It also reduced cyclooxygenase-2 expression and, consequently, the secretion of prostaglandin E2. Prostaglandin E2 can contribute to bone protection, as it stimulates osteoclast-mediated bone resorption. Finally, synergism with chemotherapy has also been demonstrated. Exposure of MDA-MB-231 cells to paclitaxel or docetaxel resulted in dose-dependent inhibition of tumor cell adhesion and invasion of mineralized bone matrices.⁷² When the cells were treated with ibandronate (1 μM) prior to taxane exposure, inhibition of adhesion was further increased by 38–59% whereas inhibition of invasion was enhanced by 70–78%, compared with taxane treatment alone. The effect of the reverse treatment sequence, shown to be more efficacious in inhibiting apoptosis, is unknown.

In the mouse model of metastatic breast cancer formed by intracardiac injection of MDA-MB-231 cells, administration of ibandronate (4 mg/mouse/day) after bone metastases were established inhibited the progression of osteolytic bone metastases.⁶¹ In contrast, ibandronate failed to inhibit MDA-MB-231 tumor formation and had no effect on apoptosis in MDA-MB-231 breast cancer cells implanted orthotopically in the mammary fat pads. Thus, the effects of ibandronate on apoptosis in MDA-MB-231 breast cancer cells appears to be restricted to bone, where ibandronate selectively deposits. In a subsequent study, zoledronic acid inhibited visceral metastases in a mouse model in which orthotopic implantation of 4T1/luc breast cancer cells spontaneously metastasize to multiple organs including bone, lung, and liver.⁶⁸ Repeated injections of zoledronic acid (0.5 or 5 $\mu\text{g}/\text{mouse}$) reduced metastatic foci in bone, lung, and liver, prolonging overall survival (OS). Interestingly, zoledronic acid increased the number of apoptotic 4T1/luc cells colonized in the bone but not in the lung.

Antiangiogenic Effects

As reviewed in Hanahan and Folkman, angiogenesis is a multistep process involving endothelial cell proliferation, adhesion, and formation of new capillary tubes; agents

with antiangiogenic activity have proven efficacious as anticancer agents.⁸⁰ Bisphosphonates are thought to exert their antitumor activity in part through inhibition of angiogenesis. In the *in vitro* model of human umbilical vein endothelial cells, clodronate at 1–30 μM reduced endothelial cell growth in a dose-dependent manner.⁸¹ Similarly, exposure of endothelial cells to clodronate, ibandronate, and risedronate at 100 μM inhibited proliferation, as did zoledronic acid in a dose-dependent manner (0.0001–100 μM).⁸² At 100 μM , clodronate, ibandronate, risedronate, and zoledronic acid all inhibited capillary-like tube formation, and zoledronic acid was additionally shown to induce endothelial cell apoptosis.⁸² Studies using human dermal microvascular endothelial cells showed similar results.⁸³ Compared with no treatment, zoledronic acid at 25 and 50 μM inhibited proliferation and caused an increase in cells in the S phase. However, apoptosis was not induced at these doses. In addition, paclitaxel 4 nM and zoledronic acid 25 μM administered simultaneously induced accumulation of cells in the S phase and apoptosis in comparison with either treatment alone or treatment in sequence. The combination also induced a decrease in tubule number and inhibited human dermal microvascular endothelial cell migration, compared with control treatment. Interestingly, zoledronic acid has also been shown to have a dose-dependent biphasic effect on endothelial cell adhesion to various integrins and migration.⁸⁴ Zoledronic acid induced cell adhesion at 1–3 μM and migration at 0.3–10 μM , but inhibited cell adhesion at 30–100 μM and migration at 30 μM . In contrast, pamidronate did not stimulate adhesion at lower concentrations, but inhibited attachment at higher concentrations. Other studies supported a more selective mechanism, with zoledronic acid inhibiting adhesion to some but not all integrins.⁸⁵ In this study, clodronate had no effect. Bisphosphonates also modulated growth factor-induced cell proliferation and morphogenesis. Clodronate inhibited the formation of capillary-like tubules induced by fibroblast growth factor (FGF)-2 treatment.⁸¹ Zoledronic acid inhibited the proliferation of human umbilical vein endothelial cells stimulated with fetal calf serum (half maximal inhibitory concentration [IC_{50}] 4.1 μM), basic FGF (bFGF, IC_{50} 4.2 μM), and at higher concentrations, vascular endothelial growth factor (VEGF, IC_{50} 6.9 μM).⁸⁴

Animal studies support the *in vitro* findings. Systemic administration of zoledronic acid (10 and 100 $\mu\text{g}/\text{kg}/\text{day}$) to mice inhibited angiogenesis induced by subcutaneous implants impregnated with bFGF in a dose-dependent manner, as measured by a reduction in blood content and tissue weight.⁸⁴ In contrast, zoledronic acid was less potent against VEGF-induced

angiogenic response, and pamidronate was less potent than zoledronic acid. In another study, daily or weekly administration of zoledronic acid at a cumulative dose of 98–100 $\mu\text{g}/\text{kg}$ (equivalent to 4 mg IV in humans) or clodronate at 530 $\mu\text{g}/\text{kg}/\text{day}$ (equivalent to 1,600 mg orally in humans) were effective in reducing bone destruction and skeletal tumor burden in an animal model of bone metastasis caused by MDA-MB-231 breast cancer cells, though clodronate was less effective.⁸⁶ A single dose of either agent was ineffective. Frequent administration of low-dose chemotherapy (metronomic therapy), such as that described above, has been shown to have profound antiangiogenic effects.⁸⁷ The antiangiogenic and antitumor effects of clinically achievable doses of zoledronic acid outside the bone were also investigated in a mouse model.⁸⁸ BALB-neuT mice, which develop metastatic breast tumors, received saline or zoledronic acid 100 $\mu\text{g}/\text{kg}$ weekly for 4 weeks, then every 3 weeks thereafter. There was a reduction in VEGF production at the tumor site and in circulating VEGF, as well as a reduction in the number of tumor-associated macrophages, in the mice treated with zoledronic acid compared with those treated with saline. In addition, zoledronic acid-treated mice demonstrated a significant reduction in tumor multiplicity and tumor growth rate and improvement in tumor-free survival and OS. It should be noted that zoledronic acid alone or in combination with paclitaxel does not appear to have deleterious effects on normal microvasculature, suggesting a tumor-specific effect.⁸³

Modulation of Immune Surveillance ($\gamma\delta$ T Cells)

T cells bearing the gamma delta receptor ($\gamma\delta$ T cells), the majority of which are of the $\text{V}\gamma\text{9V}\delta\text{2}$ subtype, have been shown to recognize transformed cells and potently kill malignant cells. Studies in this area are mainly in non-breast cancer cells. One study assessed the ability of BPs to stimulate $\gamma\delta$ T cells and generate antiplasma cell activity.⁸⁹ Treatment of peripheral blood mononuclear cells (PBMCs) with clinically relevant concentrations of the N-BPs alendronate, ibandronate, and pamidronate in the presence of interleukin (IL)-2 induced a dose-dependent expansion of $\text{V}\gamma\text{9V}\delta\text{2}$ T cells, whereas non-N-BPs did not.⁸⁹ In addition, a pamidronate-treated $\gamma\delta$ T-cell line exhibited strong lytic activity against lymphoma and myeloma cell lines, whereas pamidronate-treated bone marrow mononuclear cells from multiple myeloma patients caused a reduction in malignant plasma cell survival that was correlated with $\gamma\delta$ T cell activation.⁸⁹ Another study evaluated the cytotoxicity of $\gamma\delta$ T cells expanded *ex vivo* on various cancer cell lines.⁹⁰ Incubation with zoledronic acid 1 μM plus IL-2 for 14 days increased the absolute number of $\gamma\delta$ T cells up to 768-fold, par-

ticularly the V γ 9V δ 2 subset. In addition, zoledronic acid 1 μ M was 3 times more effective than a similar concentration of pamidronate at expanding $\gamma\delta$ T cells. Interestingly, small cell lung cancer and fibrosarcoma cell lines pretreated with zoledronic acid 5 μ M were more sensitive to lysis by $\gamma\delta$ T cells compared with untreated cell lines. This finding was supported by experiments in mice xenografted with SBC-5 lung cancer cells. Antitumor activity of $\gamma\delta$ T cells was significantly enhanced by pretreatment of mice with zoledronic acid 80 mg/kg, suggesting that cytotoxicity of $\gamma\delta$ T cells may require N-BP pretreatment of the target cells.

Peripheral blood monocytes appear to be responsible for $\gamma\delta$ T-cell activation induced by N-BPs.⁹¹ N-BPs indirectly activate V γ 9V δ 2 T cells through inhibition of farnesyl pyrophosphate synthase and intracellular accumulation of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Treatment of human PBMCs with zoledronic acid 1 μ M induced accumulation of IPP/DMAPP selectively in monocytes, and zoledronic acid–pulsed monocytes activated $\gamma\delta$ T cells in a contact-dependent manner. It also appears that mevalonate metabolites agonize the activation of $\gamma\delta$ T cells. Proapoptotic actions of zoledronic acid are prevented by the mevalonate pathway inhibitor geranylgeraniol.⁶⁹ In addition, blockade of hydroxy-methylglutaryl-CoA reductase (HMGR), the rate-limiting enzyme of the mevalonate pathway, prevents the accumulation of mevalonate metabolites and recognition by $\gamma\delta$ T cells.⁹² Conversely, induction of mevalonate metabolites by overexpression of HMGR or by treatment with N-BPs allows tumor cells to acquire the ability to stimulate the same $\gamma\delta$ T-cell population.

Suppression of Bone-Derived Growth Factors

Breast and prostate cancers frequently metastasize to bone. The bone matrix is abundant in growth factors released during the continuous bone remodeling process, and these stimulate the proliferation and survival of tumor cells, according to a review by Mundy.⁶⁰ There is evidence that BPs antagonize the stimulatory effects of growth factors on breast cancer cells. Treatment of MCF-7 and T47D cells with insulin-like growth factor (IGF) I or II or FGF-2 showed growth stimulatory effects; however, addition of BPs attenuated such effects to varying degrees.⁹³ For example, clodronate, ibandronate, pamidronate, and zoledronic acid (all at 10⁻⁶ M) inhibited the stimulatory effects of FGF-2 on MCF-7 cells by 86–99%. Results were less pronounced with IGFs, with reductions reaching 20–68%. In contrast, the growth stimulatory effect of IGF-II on T47D cells was completely inhibited by clodronate and ibandro-

nate, but less so by pamidronate and zoledronic acid. The stimulatory effect of FGF-2 on this cell line was unaffected by BPs, whereas that of IGF-I was completely abrogated by all 4 BPs. The mechanism by which BPs inhibit growth factor activity appears to be in part the modulation of hypoxia-inducible factor (HIF)-1 α and VEGF protein expression.⁹⁴ Treatment of MCF-7 cells with clodronate 50 μ M or pamidronate 50 μ M suppressed IGF-I–induced HIF-1 α and VEGF expression and promoted HIF-1 α degradation. Accordingly, both BPs abrogated angiogenesis induced by IGF-I–stimulated MCF-7 cells.

Implications of Preclinical Data

Taken as a whole, preclinical studies support the notion that BPs have significant direct and indirect antitumor properties. However, all BPs do not appear to have equivalent potency. Several studies signal a trend toward antitumor potency that is proportional to the antiresorptive potency of the BP. Accordingly, zoledronic acid, which has been the most thoroughly studied analogue, appears to be the most potent. Antitumor activity has been demonstrated with single-agent BPs, though in certain circumstances this activity appears to be potentiated when BPs are administered in a sequence-specific manner in conjunction with chemotherapy. Interestingly, there is some indication that this is true for some (apoptosis) but not all (invasion) anticancer mechanisms. The ability to modulate some but not all anticancer mechanisms may yet prove to be a general phenomenon.

The translation of preclinical findings to the clinical setting should be interpreted with caution. Although a number of studies use clinically relevant BP doses, the high doses of BPs used to achieve antitumor activity in other studies cannot be administered safely in the clinical setting. The standard dose for the treatment of bone metastasis from solid tumors for approved agents is zoledronic acid 4 mg IV every 3–4 weeks, pamidronate IV 90 mg every 4 weeks, ibandronate 50 mg orally daily, and clodronate 1,600 mg orally daily. In addition, the high affinity of BPs for bone and their rapid clearance from the general circulation means that visceral tissues, and presumably tumor cells, may be subjected to limited BP exposure. Thus, it is possible that in the clinical setting, the exposure of tumor cells to the sustained levels of BPs required for in vitro antitumor activity in the preclinical setting is not achieved. Despite these limitations, in animal models, activity outside the bone has been demonstrated with some BPs (zoledronic acid).

In summary, the body of in vitro and animal data provides support for a potential antitumor role for BPs in breast cancer that warrants further clinical studies.

References

- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin*. 2011;61:000-000.
- Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin*. 2010;60:277-300.
- NCCN Clinical Practice Guidelines in Oncology. Breast Cancer. V2. 2010.
- Hillner BE, Ingle JN, Chlebowski RT, et al. American Society of Clinical Oncology 2003 update on the role of bisphosphonates and bone health issues in women with breast cancer. *J Clin Oncol*. 2003;21:4042-4057.
- Burstein HJ, Prestrud AA, Seidenfeld J, et al. American Society of Clinical Oncology clinical practice guideline: update on adjuvant endocrine therapy for women with hormone receptor-positive breast cancer. *J Clin Oncol*. 2010;28:3784-3796.
- Menschutkin M. Ueber die Einwirkung des Chloracetyls auf phosphorige Saure. *Ann Chem Pharm*. 1865;133:317-320.
- Fleisch H, Russell RG, Bisaz S, Casey PA, Muhlbauer RC. The influence of pyrophosphate analogues (diphosphonates) on the precipitation and dissolution. *Calcif Tissue Res*. 1968;Suppl:10-10a.
- Masarachia P, Weinreb M, Balena R, Rodan GA. Comparison of the distribution of 3H-alendronate and 3H-etidronate in rat and mouse bones. *Bone*. 1996;19:281-290.
- Pelorgeas S, Martin JB, Satre M. Cytotoxicity of dichloromethane diphosphonate and of 1-hydroxyethane-1,1-diphosphonate in the amoebae of the slime mould *Dictyostelium discoideum*. A 31P NMR study. *Biochem Pharmacol*. 1992;44:2157-2163.
- Frith JC, Monkkonen J, Blackburn GM, Russell RG, Rogers MJ. Clodronate and liposome-encapsulated clodronate are metabolized to a toxic ATP analog, adenosine 5'-(beta, gamma-dichloromethylene) triphosphate, by mammalian cells in vitro. *J Bone Miner Res*. 1997;12:1358-1367.
- Lehenkari PP, Kellinsalmi M, Napankangas JB, et al. Further insight into mechanism of action of clodronate: inhibition of mitochondrial ADP/ATP translocase by a nonhydrolyzable, adenine-containing metabolite. *Mol Pharmacol*. 2002;61:1255-1262.
- Selander KS, Monkkonen J, Karhukorpi EK, Harkonen P, Hannuniemi R, Vaananen HK. Characteristics of clodronate-induced apoptosis in osteoclasts and macrophages. *Mol Pharmacol*. 1996;50:1127-1138.
- Russell RG. Bisphosphonates: mode of action and pharmacology. *Pediatr*. 2007;119(suppl 2):S150-S162.
- Alakangas A, Selander K, Mulari M, et al. Alendronate disturbs vesicular trafficking in osteoclasts. *Calcif Tissue Int*. 2002;70:40-47.
- Hughes DE, Wright KR, Uy HL, et al. Bisphosphonates promote apoptosis in murine osteoclasts in vitro and in vivo. *J Bone Miner Res*. 1995;10:1478-1487.
- Luckman SP, Coxon FP, Ebetino FH, Russell RG, Rogers MJ. Heterocycle-containing bisphosphonates cause apoptosis and inhibit bone resorption by preventing protein prenylation: evidence from structure-activity relationships in J774 macrophages. *J Bone Miner Res*. 1998;13:1668-1678.
- Luckman SP, Hughes DE, Coxon FP, Graham R, Russell G, Rogers MJ. Nitrogen-containing bisphosphonates inhibit the mevalonate pathway and prevent post-translational prenylation of GTP-binding proteins, including Ras. *J Bone Miner Res*. 1998;13:581-589.
- Rogers MJ, Frith JC, Luckman SP, et al. Molecular mechanisms of action of bisphosphonates. *Bone*. 1999;24(suppl 5):73S-79S.
- Brown HK, Ottewill PD, Coleman RE, Holen I. The kinetochore protein Cenp-F is a potential novel target for zoledronic acid in breast cancer cells. *J Cell Mol Med*. 8 Dec 2009. doi:10.1111/j.1582-4932.2009.00995.x.
- Raikkonen J, Crockett JC, Rogers MJ, Monkkonen H, Auriola S, Monkkonen J. Zoledronic acid induces formation of a pro-apoptotic ATP analogue and isopentenyl pyrophosphate in osteoclasts in vivo and in MCF-7 cells in vitro. *Br J Pharmacol*. 2009;157:427-435.
- Manders K, van de Poll-Franse LV, Creemers GJ, et al. Clinical management of women with metastatic breast cancer: a descriptive study according to age group. *BMC Cancer*. 2006;6:179.
- Tubiana-Hulin M, Beuzebec P, Mauriac L, et al. Double-blinded controlled study comparing clodronate versus placebo in patients with breast cancer bone metastases [in French]. *Bull Cancer*. 2001;88:701-707.
- Kristensen B, Ejlersen B, Groenvold M, Hein S, Loft H, Mouridsen HT. Oral clodronate in breast cancer patients with bone metastases: a randomized study. *J Intern Med*. 1999;246:67-74.
- Diel IJ, Kurth AH, Sittig HB, et al. Bone pain reduction in patients with metastatic breast cancer treated with ibandronate-results from a post-marketing surveillance study. *Support Care Cancer*. 2010;18:1305-1312.
- Body JJ, Diel IJ, Lichinitser MR, et al. Intravenous ibandronate reduces the incidence of skeletal complications in patients with breast cancer and bone metastases. *Ann Oncol*. 2003;14:1399-1405.
- Body JJ, Diel IJ, Bell R, et al. Oral ibandronate improves bone pain and preserves quality of life in patients with skeletal metastases due to breast cancer. *Pain*. 2004;111:306-312.
- Hortobagyi GN, Theriault RL, Porter L, et al. Efficacy of pamidronate in reducing skeletal complications in patients with breast cancer and lytic bone metastases. Protocol 19 Aredia Breast Cancer Study Group. *N Engl J Med*. 1996;335:1785-1791.
- Hortobagyi GN, Theriault RL, Lipton A, et al. Long-term prevention of skeletal complications of metastatic breast cancer with pamidronate. Protocol 19 Aredia Breast Cancer Study Group. *J Clin Oncol*. 1998;16:2038-2044.
- Lipton A, Theriault RL, Hortobagyi GN, et al. Pamidronate prevents skeletal complications and is effective palliative treatment in women with breast carcinoma and osteolytic bone metastases: long term follow-up of two randomized, placebo-controlled trials. *Cancer*. 2000;88:1082-1090.
- Berenson JR, Rosen LS, Howell A, et al. Zoledronic acid reduces skeletal-related events in patients with osteolytic metastases. *Cancer*. 2001;91:1191-1200.
- Rosen LS, Gordon D, Kaminski M, et al. Long-term efficacy and safety of zoledronic acid compared with pamidronate disodium in the treatment of skeletal complications in patients with advanced multiple myeloma or breast carcinoma: a randomized, double-blind, multicenter, comparative trial. *Cancer*. 2003;98:1735-1744.
- Clemons MJ, Dranitsaris G, Ooi WS, et al. Phase II trial evaluating the palliative benefit of second-line zoledronic acid in breast cancer patients with either a skeletal-related event or progressive bone metastases despite first-line bisphosphonate therapy. *J Clin Oncol*. 2006;24:4895-4900.
- Coates AS, Keshaviah A, Thurlimann B, et al. Five years of letrozole compared with tamoxifen as initial adjuvant therapy for postmenopausal women with endocrine-responsive early breast cancer: update of study BIG 1-98. *J Clin Oncol*. 2007;25:486-492.
- Coombes RC, Kilburn LS, Snowdon CF, et al. Survival and safety of exemestane versus tamoxifen after 2-3 years' tamoxifen treatment (Intergroup Exemestane Study): a randomised controlled trial. *Lancet*. 2007;369:559-570.
- Forbes JF, Cuzick J, Buzzdar A, Howell A, Tobias JS, Baum M. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 100-month analysis of the ATAC trial. *Lancet Oncol*. 2008;9:45-53.
- Jakesz R, Jonat W, Gnant M, et al. Switching of postmenopausal women with endocrine-responsive early breast cancer to anastrozole after 2 years' adjuvant tamoxifen: combined results of ABCSG trial 8 and ARNO 95 trial. *Lancet*. 2005;366:455-462.
- Jones SE, Seyanaev C, Hasenburg A, et al. Results from the first planned analysis of the TEAM (tamoxifen exemestane adjuvant multinational) prospective randomized phase III trial in hormone sensitive postmenopausal early breast cancer. Presented at: the 2008 San Antonio Breast Cancer Symposium; December 11, 2008; San Antonio, Texas.
- Mouridsen H, Giobbie-Hurder A, Goldhirsch A, et al. Letrozole therapy alone or in sequence with tamoxifen in women with breast cancer. *N Engl J Med*. 2009;361:766-776.
- Dixon JM, Renshaw L, Young O, et al. Letrozole suppresses plasma estradiol and estrone sulphate more completely than anastrozole in postmenopausal women with breast cancer. *J Clin Oncol*. 2008;26:1671-1676.
- Geisler J, Helle H, Ekse D, et al. Letrozole is superior to anastrozole in suppressing breast cancer tissue and plasma estrogen levels. *Clin Cancer Res*. 2008;14:6330-6335.
- Goss PE, Hadji P, Subar M, Abreu P, Thomsen T, Banke-Bochita J. Effects of steroidal and nonsteroidal aromatase inhibitors on markers of bone turnover in healthy postmenopausal women. *Breast Cancer Res*. 2007;9:R52.
- Coleman RE, Banks LM, Girgis SI, et al. Skeletal effects of exemestane on bone-mineral density, bone biomarkers, and fracture incidence in postmenopausal women with early breast cancer participating in the Intergroup Exemestane Study (IES): a randomised controlled study. *Lancet Oncol*. 2007;8:119-127.
- Eastell R, Adams JE, Coleman RE, et al. Effect of anastrozole on bone mineral density: 5-year results from the anastrozole, tamoxifen, alone or in combination trial 18233230. *J Clin Oncol*. 2008;26:1051-1057.

44. Howell A, Cuzick J, Baum M, et al. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. *Lancet*. 2005;365:60-62.
45. Perez EA, Josse RG, Pritchard KI, et al. Effect of letrozole versus placebo on bone mineral density in women with primary breast cancer completing 5 or more years of adjuvant tamoxifen: a companion study to NCIC CTG MA.17. *J Clin Oncol*. 2006;24:3629-3635.
46. Gnant MF, Mlineritsch B, Luschin-Ebengreuth G, et al. Zoledronic acid prevents cancer treatment-induced bone loss in premenopausal women receiving adjuvant endocrine therapy for hormone-responsive breast cancer: a report from the Austrian Breast and Colorectal Cancer Study Group. *J Clin Oncol*. 2007;25:820-828.
47. Bundred NJ, Campbell ID, Davidson N, et al. Effective inhibition of aromatase inhibitor-associated bone loss by zoledronic acid in postmenopausal women with early breast cancer receiving adjuvant letrozole: ZO-FAST Study results. *Cancer*. 2008;112:1001-1010.
48. Brufsky A, Harker WG, Beck JT, et al. The effect of zoledronic acid on aromatase inhibitor-associated bone loss in postmenopausal women with early breast cancer receiving adjuvant letrozole: the Z-FAST study 5-year final follow-up. Poster presented: at the 2009 San Antonio Breast Cancer Symposium; December 12, 2009; San Antonio, Texas.
49. Brufsky A, Bundred N, Coleman R, et al. Integrated analysis of zoledronic acid for prevention of aromatase inhibitor-associated bone loss in postmenopausal women with early breast cancer receiving adjuvant letrozole. *Oncologist*. 2008;13:503-514.
50. Van Poznak C, Hannon RA, Mackey JR, et al. Prevention of aromatase inhibitor-induced bone loss using risendronate: the SABRE trial. *J Clin Oncol*. 2010;28:967-975.
51. Lester JE, Dodwell D, Purohit OP, et al. Prevention of anastrozole-induced bone loss with monthly oral ibandronate during adjuvant aromatase inhibitor therapy for breast cancer. *Clin Cancer Res*. 2008;14:6336-6342.
52. Bines J, Oleske DM, Cobleigh MA. Ovarian function in premenopausal women treated with adjuvant chemotherapy for breast cancer. *J Clin Oncol*. 1996;14:1718-1729.
53. Shapiro CL, Manola J, Leboff M. Ovarian failure after adjuvant chemotherapy is associated with rapid bone loss in women with early-stage breast cancer. *J Clin Oncol*. 2001;19:3306-3311.
54. Saarto T, Blomqvist C, Valimaki M, Makela P, Sarna S, Elomaa I. Chemical castration induced by adjuvant cyclophosphamide, methotrexate, and fluorouracil chemotherapy causes rapid bone loss that is reduced by clodronate: a randomized study in premenopausal breast cancer patients. *J Clin Oncol*. 1997;15:1341-1347.
55. Fuleihan GH, Salamoun M, Mourad YA, et al. Pamidronate in the prevention of chemotherapy-induced bone loss in premenopausal women with breast cancer: a randomized controlled trial. *J Clin Endocrinol Metab*. 2005;90:3209-3214.
56. Hershman DL, McMahon DJ, Crew KD, et al. Prevention of bone loss by zoledronic acid in premenopausal women undergoing adjuvant chemotherapy persist up to one year following discontinuing treatment. *J Clin Endocrinol Metab*. 2010;95:559-566.
57. Ahn JH, Jung KH, Kim SB, et al. Zoledronic acid prevents bone loss in premenopausal women with early breast cancer undergoing adjuvant chemotherapy: a phase III study of Korean Cancer Study Group (KCSG-BR06-01). Poster presented at: the 2009 San Antonio Breast Cancer Symposium; December 11, 2009; San Antonio, Texas.
58. Hines SL, Mincey BA, Sloan JA, et al. Phase III randomized, placebo-controlled, double-blind trial of risendronate for the prevention of bone loss in premenopausal women undergoing chemotherapy for primary breast cancer. *J Clin Oncol*. 2009;27:1047-1053.
59. Aft R, Naughton M, Trinkaus K, et al. Effect of zoledronic acid on disseminated tumour cells in women with locally advanced breast cancer: an open label, randomised, phase 2 trial. *Lancet Oncol*. 2010;11:421-428.
60. Mundy GR. Metastasis to bone: causes, consequences and therapeutic opportunities. *Nat Rev Cancer*. 2002;2:584-593.
61. Hiraga T, Williams PJ, Mundy GR, Yoneda T. The bisphosphonate ibandronate promotes apoptosis in MDA-MB-231 human breast cancer cells in bone metastases. *Cancer Res*. 2001;61:4418-4424.
62. Fromiguet O, Lagneaux L, Body JJ. Bisphosphonates induce breast cancer cell death in vitro. *J Bone Miner Res*. 2000;15:2211-2221.
63. Senaratne SG, Pirianov G, Mansi JL, Arnett TR, Colston KW. Bisphosphonates induce apoptosis in human breast cancer cell lines. *Br J Cancer*. 2000;82:1459-1468.
64. Verdijk R, Franke HR, Wolbers F, Vermes I. Differential effects of bisphosphonates on breast cancer cell lines. *Cancer Lett*. 2007;246:308-312.
65. Marzo I, Susin SA, Petit PX, et al. Caspases disrupt mitochondrial membrane barrier function. *FEBS Lett*. 1998;427:198-202.
66. Hockenbery D, Nunez G, Millman C, Schreiber RD, Korsmeyer SJ. Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. *Nature*. 1990;348:334-336.
67. Senaratne SG, Mansi JL, Colston KW. The bisphosphonate zoledronic acid impairs Ras membrane [correction of impairs membrane] localisation and induces cytochrome c release in breast cancer cells. *Br J Cancer*. 2002;86:1479-1486.
68. Hiraga T, Williams PJ, Ueda A, Tamura D, Yoneda T. Zoledronic acid inhibits visceral metastases in the 4T1/luc mouse breast cancer model. *Clin Cancer Res*. 2004;10:4559-4567.
69. Jagdev SP, Coleman RE, Shipman CM, Rostami HA, Croucher PI. The bisphosphonate, zoledronic acid, induces apoptosis of breast cancer cells: evidence for synergy with paclitaxel. *Br J Cancer*. 2001;84:1126-1134.
70. Neville-Webbe HL, Evans CA, Coleman RE, Holen I. Mechanisms of the synergistic interaction between the bisphosphonate zoledronic acid and the chemotherapy agent paclitaxel in breast cancer cells in vitro. *Tumour Biol*. 2006;27:92-103.
71. Neville-Webbe HL, Rostami-Hodjegan A, Evans CA, Coleman RE, Holen I. Sequence- and schedule-dependent enhancement of zoledronic acid induced apoptosis by doxorubicin in breast and prostate cancer cells. *Int J Cancer*. 2005;113:364-371.
72. Mignetto S, Boissier S, Delmas PD, Clezardin P. Additive antitumor activities of taxoids in combination with the bisphosphonate ibandronate against invasion and adhesion of human breast carcinoma cells to bone. *Int J Cancer*. 1999;83:263-269.
73. Vogt U, Bielawski KP, Bosse U, Schlotter CM. Breast tumour growth inhibition in vitro through the combination of cyclophosphamide/methotrexate/5-fluorouracil, epirubicin/cyclophosphamide, epirubicin/paclitaxel, and epirubicin/docetaxel with the bisphosphonates ibandronate and zoledronic acid. *Oncol Rep*. 2004;12:1109-1114.
74. Michigami T, Hiraga T, Williams PJ, et al. The effect of the bisphosphonate ibandronate on breast cancer metastasis to visceral organs. *Breast Cancer Res Treat*. 2002;75:249-258.
75. Duivenvoorden WC, Vukmirovic-Popovic S, Kalina M, Seidlitz E, Singh G. Effect of zoledronic acid on the doxycycline-induced decrease in tumour burden in a bone metastasis model of human breast cancer. *Br J Cancer*. 2007;96:1526-1531.
76. Ottewill PD, Woodward JK, Lefley DV, Evans CA, Coleman RE, Holen I. Anticancer mechanisms of doxorubicin and zoledronic acid in breast cancer tumor growth in bone. *Mol Cancer Ther*. 2009;8:2821-2832.
77. Boissier S, Mignetto S, Frappart L, et al. Bisphosphonates inhibit prostate and breast carcinoma cell adhesion to unmineralized and mineralized bone extracellular matrices. *Cancer Res*. 1997;57:3890-3894.
78. Boissier S, Ferreras M, Peyruchaud O, et al. Bisphosphonates inhibit breast and prostate carcinoma cell invasion, an early event in the formation of bone metastases. *Cancer Res*. 2000;60:2949-2954.
79. Denoyelle C, Hong L, Vannier JP, Soria J, Soria C. New insights into the actions of bisphosphonate zoledronic acid in breast cancer cells by dual RhoA-dependent and -independent effects. *Br J Cancer*. 2003;88:1631-1640.
80. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell*. 1996;86:353-364.
81. Ribatti D, Maruotti N, Nico B, et al. Clodronate inhibits angiogenesis in vitro and in vivo. *Oncol Rep*. 2008;19:1109-1112.
82. Fournier P, Boissier S, Filleur S, et al. Bisphosphonates inhibit angiogenesis in vitro and testosterone-stimulated vascular regrowth in the ventral prostate in castrated rats. *Cancer Res*. 2002;62:6538-6544.
83. Michailidou M, Brown HK, Lefley DV, et al. Microvascular endothelial cell responses in vitro and in vivo: modulation by zoledronic acid and paclitaxel? *J Vasc Res*. 2010;47:481-493.
84. Wood J, Bonjean K, Ruetz S, et al. Novel antiangiogenic effects of the bisphosphonate compound zoledronic acid. *J Pharmacol Exp Ther*. 2002;302:1055-1061.
85. Bezzi M, Hasim M, Bieler G, Dormond O, Ruegg C. Zoledronic acid sensitizes endothelial cells to tumor necrosis factor-induced programmed cell death: evidence for the suppression of sustained activation of focal adhesion kinase and protein kinase B/Akt. *J Biol Chem*. 2003;278:43603-43614.
86. Daubine F, Le Gall C, Gasser J, Green J, Clezardin P. Antitumor effects of clinical dosing regimens of bisphosphonates in experimental breast cancer bone metastasis. *J Natl Cancer Inst*. 2007;99:322-330.

87. Pasquier E, Kavallaris M, Andre N. Metronomic chemotherapy: new rationale for new directions. *Nat Rev Clin Oncol*. 2010;7:455-465.
88. Coscia M, Quaglino E, Iezzi M, et al. Zoledronic acid repolarizes tumour-associated macrophages and inhibits mammary carcinogenesis by targeting the mevalonate pathway. *J Cell Mol Med*. 2010;14:2803-2815.
89. Kunzmann V, Bauer E, Feurle J, Weissinger F, Tony HP, Wilhelm M. Stimulation of gammadelta T cells by aminobisphosphonates and induction of antiplasma cell activity in multiple myeloma. *Blood*. 2000;96:384-392.
90. Sato K, Kimura S, Segawa H, et al. Cytotoxic effects of gammadelta T cells expanded ex vivo by a third generation bisphosphonate for cancer immunotherapy. *Int J Cancer*. 2005;116:94-99.
91. Roelofs AJ, Jauhainen M, Monkkonen H, Rogers MJ, Monkkonen J, Thompson K. Peripheral blood monocytes are responsible for gammadelta T cell activation induced by zoledronic acid through accumulation of IPP/DMAPP. *Br J Haematol*. 2009;144:245-250.
92. Gober HJ, Kistowska M, Angman L, Jenö P, Mori L, De Libero G. Human T cell receptor gammadelta cells recognize endogenous mevalonate metabolites in tumor cells. *J Exp Med*. 2003;197:163-168.
93. Fromiguet O, Kheddoumi N, Body JJ. Bisphosphonates antagonise bone growth factors' effects on human breast cancer cells survival. *Br J Cancer*. 2003;89:178-184.
94. Tang X, Zhang Q, Shi S, et al. Bisphosphonates suppress insulin-like growth factor 1-induced angiogenesis via the HIF-1alpha/VEGF signaling pathways in human breast cancer cells. *Int J Cancer*. 2010;126:90-103.

Clinical Advances in
HEMATOLOGY & ONCOLOGY™

On the Web: Complete content of each issue
 available as free PDF downloads:

- Review Articles
- Clinical Case Studies
- Advances Columns
- Hem/Onc News
- Educational Supplements
- CMExpress:
Online CME Credits
- Archived Issues
- Author Guidelines

www.clinicaladvances.com

The Mechanism of Action of Bisphosphonates in Breast Cancer

Rebecca Aft, MD, PhD

Dr. Aft is a Professor of Surgery in the Division of General Surgery at the Washington University School of Medicine in St. Louis, Missouri.

Address correspondence to:
Rebecca Aft, MD, PhD
Professor, Surgery
Division of General Surgery
Surgical Oncology and Endocrine
Surgery Section
Washington University School of
Medicine
St. Louis, MO
Phone:
Fax:
E-mail: aftr@wudosis.wustl.edu

Abstract: Breast cancer is the most frequently diagnosed and deadliest cancer among women. Bisphosphonates are stable pyrophosphate analogues used to treat skeletal-related events resulting from bone metastases. In the adjuvant setting, they have been shown to prevent aromatase inhibitor-associated and chemotherapy-induced bone loss. There is a growing body of evidence that bisphosphonates have direct and indirect anticancer activity in the preclinical and clinical settings. These include the inhibition of tumor growth; induction of apoptosis; synergism with chemotherapy; inhibition of tumor migration, invasion, and metastasis; reduction in disseminated tumor cells; inhibition of angiogenesis; stimulation of immune surveillance; and suppression of bone-derived growth factors. In addition to reducing the risk of breast cancer, bisphosphonate therapy has been shown to improve outcomes of early and metastatic breast cancer treatment. This review provides a brief overview of the current role of bisphosphonates in clinical practice and discusses their potential as anti-cancer agents.

Introduction

This is the second part of a 2 part series on bisphosphonates in breast cancer. The first part was published in the March issue....

Clinical Studies

Antiangiogenic Effects

Preclinical data demonstrating inhibitory properties of BPs on endothelial cell proliferation, adhesion, and capillary formation strongly support an antiangiogenic mechanism, although translation to anticancer activity in the clinical setting is just beginning to emerge. Several studies were designed to quantitate changes in circulating angiogenic cytokines after single or multiple doses of BPs. In an early study, 25 cancer patients (11 with breast cancer) with bone metastasis were treated with a single infusion of pamidronate 90 mg.⁹⁵ VEGF levels decreased significantly

Keywords

Anticancer, bisphosphonate, zoledronic acid, mechanism of action

from baseline (-19.2%; $P=.019$) after 1 day, continued to decline on day 2 (-25.2%; $P=.001$), and persisted through day 7 (-25.2%; $P=.03$). In contrast, the levels of interferon γ and IL-6 decreased significantly from baseline ($P=.003$ and $P=.007$, respectively) on day 1, but were not significantly different from baseline on day 7. In a similar study, 30 cancer patients (17 with breast cancer) with metastatic bone disease were treated with a single infusion of zoledronic acid 4 mg.⁹⁶ An increase in the number of patients with reduced circulating VEGF levels was observed, from 36.7% (11/30) on day 1 to 63.3% (19/30) on day 21. This was associated with a decrease in VEGF levels from baseline that was significant by day 2 (-23%; $P=.0298$) and long lasting, reaching a 34% decrease by day 21 ($P=.001$). Interestingly, a study of 18 breast cancer patients with bone metastasis who were treated with a single dose of zoledronic acid 4 mg had slightly different results.⁹⁷ An 11.8% decrease ($P=.03$) in VEGF levels was observed 2 days after treatment. However, 7 days after BP infusion, VEGF levels rose to 11.9% above basal levels, though the increase was not statistically significant ($P=.07$). Changes in bFGF, interferon γ , and interleukins were not significant at any time point. Longer term metronomic therapy was also investigated in 26 patients with solid tumors (4 with breast cancer) and bone metastasis.⁹⁸ Zoledronic acid 1 mg weekly was administered for 4 weeks followed by 4 mg every 28 days for 3 cycles. VEGF levels were 29.7% lower ($P=.038$) than baseline 7 days after a single dose of zoledronic acid. The decreased levels persisted throughout the remainder of the study period and remained statistically significant. Thus, it appears that the antiangiogenic effects of BPs may be long lasting. However, the ability of BPs to modulate various angiogenic factors appears to vary, and how this differential activity translates into anticancer activity remains to be elucidated.

Modulation of Immune Surveillance ($\gamma\delta$ T Cells)

As in the preclinical setting, the anticancer activity of BPs associated with $\gamma\delta$ T-cell activation/proliferation in the clinical setting has been investigated mainly in nonbreast or solid tumors. One study enrolled 19 patients with low-grade non-Hodgkin lymphoma or multiple myeloma with the goal of determining the effective dose for $\gamma\delta$ T-cell proliferation.⁹⁹ The first 10 patients received a single dose of pamidronate 90 mg followed by increasing doses of IL-2 (0.25 to 3×10^6 IU/m²) from day 3 to day 8. Since none of the patients showed a measurable $\gamma\delta$ T-cell response and since stable disease in 1 patient was the best response, the subsequent 9 patients were selected by positive in vitro proliferation of $\gamma\delta$ T cells. Treatment was similar to that for the first group, though IL-2 was administered on days 1–6. In vivo proliferation of $\gamma\delta$ T cells was observed in

5 of 9 patients, 3 of whom achieved a partial response and 1 of whom achieved stable disease. In line with this, another study evaluated the effect of low-dose zoledronic acid on V γ 9V δ 2 cells in 9 patients (3 with breast cancer) with metastasis to the bone who showed positive in vitro proliferation of V γ 9V δ 2 T cells.¹⁰⁰ Peripheral blood mononuclear cells were collected at various time points for analysis. After IL-2 treatment in vitro, zoledronic acid induced an expansion of effector V γ 9V δ 2 T cells and a decrease in naïve and memory V γ 9V δ 2 T cells from PBMCs collected at 1 month, and more so at 3 months. Thus, zoledronic acid appears to induce maturation of V γ 9V δ 2 T cells toward an effector phenotype, which may enhance antitumor response. A third study examined the effects of zoledronic acid on immunologic parameters of therapeutically terminal advanced breast cancer patients.¹⁰¹ Ten patients received low-dose IL-2 followed by zoledronic acid 4 mg every 21 days for 1 year and PBMCs were obtained at various time points. Differentiation of V γ 9V δ 2 T cells toward an effector/memory-like phenotype was observed in all patients and remained robust at 12 months in 3 of 10 patients. Of these 3 patients, 1 achieved a partial response and 2 achieved stable disease. Thus, while these data indicate an anticancer activity associated with the activation of V γ 9V δ 2 T cells, it appears that prescreening for proliferation may be useful if not necessary to identify potentially responsive patients.

Effects on Disseminated Tumor Cells

The first step in the metastatic process is the early dissemination of cells from the primary tumor. Though DTCs in the bone marrow may remain dormant for prolonged periods, their presence is an independent prognostic indicator for increased risk of distant metastasis and death.^{102,103} Preliminary clinical data support the hypothesis that antineoplastic effects of BPs may occur through a reduction in DTCs. In a phase II trial, 120 women with newly diagnosed stage II/III breast cancer were randomized to receive 4 cycles of neoadjuvant epirubicin/docetaxel and 2 cycles of adjuvant epirubicin/docetaxel concomitantly with zoledronic acid 4 mg every 3 weeks or no zoledronic acid for 1 year.⁵⁹ The primary endpoint was the number of patients with detectable DTCs after 3 months. At baseline, DTCs were detected in 43.3% (26/60) of patients in the zoledronic acid group and 48.3% (28/58) of patients in the control group. At 3 months, 30.4% (17/56) of patients receiving zoledronic acid versus 47.2% (25/53) of patients in the control group had detectable DTCs ($P=.054$). Patients with no initial detectable DTCs were also more likely to remain negative for DTCs at 3 months when treated with zoledronic acid (87.1%) than without zoledronic acid (60.0%; $P=.03$). Disease-free survival and

OS were assessed at a median follow-up of 61.9 months, and no differences were observed between trial arms in the overall population. However, there was a significant improvement in DFS and OS among patients with ER-negative, human epidermal growth factor receptor 2 (HER2)-negative disease ($P=.013$).¹⁰⁴ In another study, 45 patients with stage I–III breast cancer who were positive for DTCs and had completed neoadjuvant or adjuvant therapy received zoledronic acid 4 mg monthly for 2 years.¹⁰⁵ The primary endpoint was the reduction in DTCs. An analysis of 32 patients demonstrated a reduction in DTCs from a mean of 25.4/mL at baseline to 8/mL ($P=.0017$) after 1 year of therapy. A third study was conducted in 96 stage I–III breast cancer patients who were positive for DTCs.¹⁰⁶ Patients were randomly assigned to receive monthly zoledronic acid 4 mg or no zoledronic acid for 2 years in conjunction with adjuvant chemotherapy with or without hormone therapy or hormone therapy alone. The aim of the study was to evaluate the reduction in DTCs. Results showed that 66.7% of patients treated with zoledronic acid were DTC negative after 12 months, whereas only 35.1% of those who did not receive zoledronic acid had a DTC-negative status ($P=.0009$). A nonsignificant trend for a reduction in the number of DTCs in the zoledronic acid group compared with the control group ($P=.066$) was also observed. These studies clearly demonstrate that zoledronic acid affects reduction in DTCs. It remains unclear how this translates into a reduction in the risk of distant metastasis and death; however, it appears that zoledronic acid may benefit a subset of difficult-to-treat patients.

Metastatic Breast Cancer

There are limited data with regard to the ability of BPs to delay disease progression and improve survival in the metastatic setting. In a randomized trial conducted by the Aredia Multinational Cooperative Group, patients with bone metastasis were randomized to receive chemotherapy plus pamidronate 45 mg every 3 weeks ($n=143$) or chemotherapy alone ($n=152$).¹⁰⁷ Patients remained in the active phase of the trial until disease progression in bone. Chemotherapy was administered at the discretion of the investigator. Results showed that the addition of pamidronate increased the time to disease progression (TTP), a primary endpoint, by 48% (249 days for chemotherapy plus pamidronate vs 168 days for chemotherapy alone; $P=.02$). Another study compared the safety and efficacy of pamidronate and zoledronic acid in 1,130 patients with bone metastasis secondary to stage IV breast carcinoma or stage III multiple myeloma.¹⁰⁸ Patients were randomized to receive zoledronic acid 4 mg or 8 mg or pamidronate 90 mg every 3–4 weeks for 24 months. During the course of the trial, the 8-mg dose of zoledronic acid

was reduced to 4 mg for safety reasons. A retrospective subset analysis was undertaken to determine the correlation between normalization of elevated baseline levels of N-telopeptides of type I collagen (NTX) at 3 months and OS at 24 months. The analysis included 328 patients who received zoledronic acid and for whom bone resorption marker data were available. Results demonstrated that at 3 months, NTX levels had normalized in 149 of the 196 patients with elevated NTX levels at baseline. Of the remaining patients, 31 had persistently elevated NTX levels and 16 had died. Patients with normalized NTX levels at 3 months had a significantly lower risk of death at 24 months than those with persistently elevated NTX levels. Median survival was 790 days for patients whose NTX levels normalized, compared with 446 days for patients whose NTX levels remained elevated. In contrast to these studies, a meta-analysis of 4 advanced breast cancer trials showed no significant benefit in OS (hazard ratio [HR], 0.71; confidence interval [CI], 0.40–1.26), bone metastasis-free survival (HR, 0.68; CI, 0.34–1.36), or non-skeletal metastasis-free survival (HR, 0.95; CI, 0.31–2.91) in patients receiving clodronate therapy compared with those who received no active treatment, as demonstrated by the CIs that crossed 1.0.¹⁰⁹

The above studies provide preliminary clinical evidence for anticancer activity in the metastatic setting. Studies to date indicate that anticancer activity may be limited to N-BPs and that biomarkers for response may provide a useful tool for assessing potential response. A number of ongoing studies should clarify possible anticancer activity of the N-BPs in the metastatic setting. These studies include an assessment of the ability of zoledronic acid to delay disease progression and prolong OS in patients with metastatic breast cancer (NCT01129336, NCT00458796, NCT00365105). In particular, the Z-ACT trial (NCT01129336) is evaluating the anti-tumor effects of zoledronic acid in patients with newly diagnosed HER2-negative metastatic breast cancer, with progression-free survival (PFS) as the primary endpoint. Secondary endpoints include TTP, time to bone metastases, and OS.

Adjuvant Treatment of Breast Cancer

Three large prospective studies have shown improved outcomes with the addition of zoledronic acid to conventional neoadjuvant or adjuvant therapy (Figure 4). The randomized Austrian Breast and Colorectal Cancer Study Group (ABCSSG)-12 trial (NCT00295646) evaluated the effect of adding zoledronic acid to endocrine therapy.¹¹³ In this 4-arm trial, 1,803 premenopausal women with hormone-responsive stage I/II breast cancer were randomized to receive goserelin 3.6 mg every 28 days plus either tamoxifen 20 mg daily or anastrozole 1 mg daily, with or

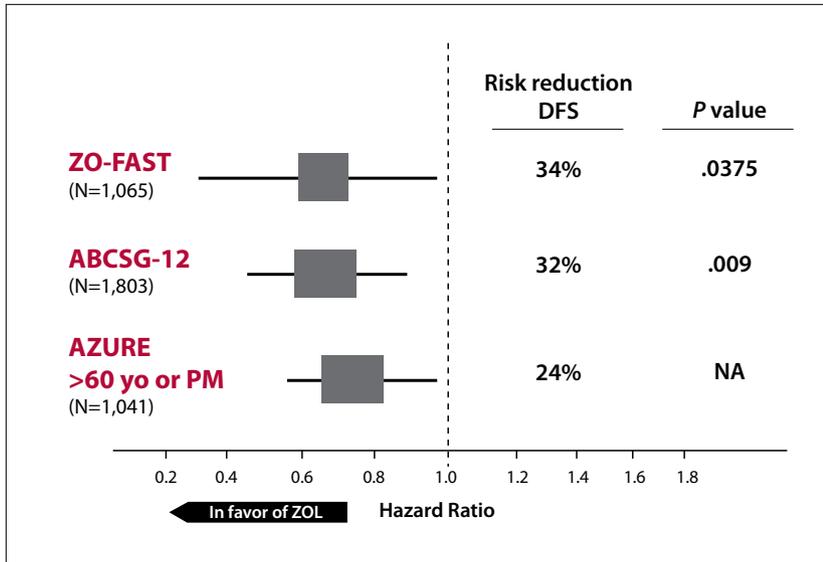


Figure 4. Improvement in disease-free survival with the addition of zoledronic acid to conventional therapy.¹¹⁰⁻¹¹²

PM=postmenopausal; yo=year old; ZOL=zoledronic acid.

without zoledronic acid 4 mg every 6 months for 3 years (7 total doses). The addition of zoledronic acid resulted in a 36% reduction in the risk of disease progression, the primary endpoint, compared with endocrine therapy alone ($P=.01$) after a median follow-up of 47.8 months. At a median follow-up of 62 months, the risk of disease progression was maintained (32% reduction; $P=.008$).¹¹⁴ The reduction in recurrences was observed locally and distantly both in and outside the bone. The risk of death was also reduced but did not reach statistical significance, although it approached significance in women older than 40 years ($P=.057$). The AZURE (Adjuvant Zoledronic Acid to Reduce Recurrence) trial (NCT00072020) evaluated the addition of zoledronic acid to adjuvant or neoadjuvant chemotherapy or endocrine therapy in 3,360 patients with stage II/III breast cancer.¹¹⁰ Patients received chemotherapy with or without zoledronic acid 4 mg every 3–4 weeks for 6 doses, every 3 months for 8 doses, and every 6 months for 5 doses (19 doses in 5 years). The primary endpoint was DFS, defined as recurrent locoregional or distant disease. In an interim analysis, zoledronic acid was not associated with a significant improvement in DFS in the overall population. However, in a subgroup analysis, zoledronic acid improved OS by 29% ($P=.017$) and reduced DFS events in and outside the bone in women who were more than 5 years postmenopausal. A subset of 195 patients who received neoadjuvant therapy with zoledronic acid for up to 6 doses were also assessed for pathologic tumor response, specifically residual invasive tumor size (RITS), at surgery.¹¹⁵ By multivariate analysis, the adjusted mean RITS was 12 mm lower in the zoledronic acid group (15.5 mm) than in the group not receiving zoledronic acid (27.4 mm; $P=.006$). In a

third study, the Zometa-Femara Adjuvant Synergy Trials (Z-FAST/ZO-FAST/E-ZO-FAST), which were designed to investigate the bone-protective effects of zoledronic acid, an exploratory analysis was conducted to assess the anticancer potential of zoledronic acid. A total of 2,194 postmenopausal women with hormone-responsive early breast cancer received letrozole 2.5 mg daily.¹¹⁶ Patients were randomized to receive zoledronic acid 4 mg administered every 6 months for 5 years starting either upon randomization (up-front) or upon a predetermined measure of bone loss (delayed start). After 36 months of follow-up, a 34% reduced incidence of DFS events with up-front zoledronic acid treatment compared with delayed treatment was observed in the ZO-FAST trial ($P=.0375$).¹¹¹ These studies show a DFS benefit or OS benefit in postmenopausal women or women who have chemical ovarian suppression.

Pamidronate has also shown anticancer efficacy. In a small nonrandomized trial, patients with primary breast cancer and 4 or more positive nodes receiving adjuvant therapy were assigned to receive pamidronate 45 mg every 2 weeks for 4 cycles ($n=33$) or no additional therapy ($n=57$).¹¹⁷ The primary endpoints were the reduction or delay of bone metastasis. Results showed that pamidronate significantly reduced the incidence of bone metastases ($P=.005$) and significantly improved bone metastasis-free survival ($P=.029$). In another study, 429 perimenopausal women with primary operable stage I–III breast cancer were assigned to receive pamidronate ($n=258$) or no BP therapy ($n=171$) concomitantly with adjuvant chemotherapy.¹¹⁸ The incidence of bone metastasis was 2.3% in the pamidronate group and 8.7% in the control group. The incidence of metastasis at other sites was lower in the

Table 4. Ongoing Trials Evaluating Anticancer Activity of Antiresorptives In Breast Cancer

Study	Agent	Region	Planned Accrual (Closed/Open)
Adjuvant			
NSABP B-34 (NCT00009945)	Clodronate	Canada/US	3,400 (Closed)
SUCCESS	Zoledronic acid	Germany	3,754 (Closed)
SWOG 0307 (NCT00127205)	Clodronate vs ibandronate vs zoledronic acid	US	6,097 (Closed)
AZAC	Zoledronic acid	France	600 (Open)
HOBOE (NCT00412022)	Zoledronic acid	Italy	1,050 (Open)
NATAN (NCT00512993)	Postoperative zoledronic acid	Germany, Austria	693 (Closed)
GAIN (NCT00196872)	Ibandronate	Germany	3,024 (Open)
ICE (NCT00196859)	Ibandronate	Germany	1,500 (Open)
Metastatic			
Optimize 2 (NCT00320710)	Zoledronic acid	US	705 (Open)
BisMARK (NCT00458796)	Zoledronic acid	UK	1,500 (Open)
CALGB 70604 (NCT00869206)	Zoledronic acid	US	1,538 (Open)
Z-ACT (NCT01129336)	Zoledronic acid	US	300 (Open)

pamidronate group than in the control group, but the difference was not statistically significant. Rates of OS and DFS were equivalent in the 2 groups.

A meta-analysis of 3 early breast cancer trials examining the effect of clodronate on OS has been conducted.¹⁰⁹ No significant benefit in OS (HR, 0.75; CI, 0.31–1.82), bone metastasis-free survival (HR, 0.68; CI, 0.38–1.23), or nonskeletal metastasis-free survival (HR, 0.89, CI; 0.40–1.98) was observed in patients who received clodronate therapy compared with those who received no active treatment.

As in the metastatic setting, N-BPs appear to have greater anticancer potential than non-N-BPs in the adjuvant setting. Zoledronic acid and pamidronate have demonstrated efficacy in the bone. Outside the bone, zoledronic is efficacious, though pamidronate may be less so. Current evidence from clinical trials suggests that N-BPs have anticancer activity in the subpopulation of breast cancer patients who are 5 years or more postmenopausal or older than 40 years with ovarian suppression (Figure 4). Several large studies to further assess the anticancer effects of BPs in breast cancer patients are ongoing. Among them, the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-34 trial (NCT00009945) will determine whether clodronate administered for 3 years, alone or in addition to adjuvant chemotherapy and/or hormone therapy, will improve DFS. The Southwest Oncology Group 0307 study (NCT00127205) is comparing zoledronic acid with clodronate and ibandronate

in terms of improving DFS and OS. In the HOBOE (A Study of Hormonal Adjuvant Treatment Effect on Bone Mineral Density in Early Breast Cancer Patients) study (NCT00412022) comparing tamoxifen (or triptorelin in premenopausal patients), letrozole, and letrozole plus zoledronic acid, DFS in premenopausal patients is a primary endpoint (Table 4).

Breast Cancer Risk

Three studies have investigated whether BPs are able to reduce breast cancer risk. A retrospective analysis examined the association between oral BP use and invasive breast cancer in postmenopausal women enrolled in the Women's Health Initiative.¹¹⁹ In this study, 18.2% (2,816/154,768) of participants were oral BP users at entry. After an average of 7.8 years of follow-up, invasive breast cancer incidence was significantly lower, by 32%, in BP users ($P < .01$), as was the incidence of ER-positive invasive cancers ($P = .02$). A similar but nonsignificant trend was seen for ER-negative invasive cancers. The incidence of ductal carcinoma in situ was higher in BP users (HR, 1.58; 95% CI, 1.08–2.31; $P = .02$), suggesting that BPs may interfere with the progression of breast cancer development. A second population-based case-control study comprising 2,936 patients with incident-invasive breast cancer and 2,975 controls older than 70 years was conducted to evaluate the association between BPs and breast cancer.¹²⁰ Results showed a 33% reduction in breast cancer risk among BP users compared with nonus-

ers (odds ratio 0.67 [CI=0.51–0.89]). Risk reduction was greatest with increasing duration of BP use (P -trend=.01) and in women who were not obese (P -interaction=.005). Another population-based case-control study, the Breast Cancer in Northern Israel Study, evaluated 4,039 postmenopausal patients with breast cancer and age-, clinic-, and ethnic-group matched controls.¹²¹ BP use for at least 1 year before diagnosis was associated with a significant 39% reduction in breast cancer risk (odds ratio 0.61; CI, 0.50–0.76). In this study, breast cancer risk did not change with longer duration of BP use, and breast tumors that developed during BP therapy tended to have a more favorable prognosis (more ER-positive and well differentiated).

Implications of Clinical data

Taken together, clinical studies support the notion that BPs have antitumor properties. Data from several small clinical trials suggest that BP can modify angiogenic factors, immune surveillance, and disseminated tumor cells detected in bone marrow, which provide possible mechanisms for the favorable effects observed on recurrence and survival observed in early and late stage breast cancer. Moreover, emerging data suggest that BPs used for osteoporosis prevention may inhibit breast cancer development.

The development of metastatic disease is a complex multi-step process. Recent data suggest that there is cross-talk between tumor cells and the bone marrow, which results in the release of growth factors as well as bone marrow-derived cells, which play critical roles in both tumor growth and metastases development.¹³³ Interference with this process by alteration of the bone marrow microenvironment with BP treatment may provide an explanation for the effects of BP treatment on tumor development, local-regional recurrence, and metastases. Future experiments directed at understanding the tumor-bone marrow interaction will lead to new insights into the effects of BPs.

Safety of BPs

In clinical trials, therapy with N-BPs has been associated mainly with mild to moderate adverse events; severe adverse events have been rare.^{27,31,122,123} The most common adverse effects associated with N-BPs (>25%) were nausea, fatigue, anemia, bone pain, constipation, fever, vomiting, and dyspnea. Noteworthy adverse reactions to N-BPs are renal toxicity, hypercalcemia of malignancy, acute-phase reactions, mineral/electrolyte abnormalities, injection-site reactions, and ocular abnormalities (uveitis/scleritis). As reviewed by Ruggiero and Mehroratra, osteonecrosis of the jaw is a rare adverse reaction, with a reported incidence of

0.8–12%.¹²⁴

Beyond BPs

Improvements in our understanding of the molecular and cellular basis of metastasis have resulted in the development of novel therapies. Normal bone remodeling is tightly balanced between bone resorption by osteoclasts and bone formation by osteoblasts. The formation of bone metastases alters the balance of bone remodeling.⁶⁰ Denosumab is a fully human monoclonal antibody that targets the receptor activator for nuclear-factor- κ B ligand, one of the principal regulators of osteoclast differentiation, function, and survival.¹²⁵ Denosumab has demonstrated beneficial effects on SREs.¹²⁶ No PFS or OS advantage compared with zoledronic acid has been observed. Further investigation into the mechanisms of its potential anticancer effects, as has been demonstrated with BPs, as well as its safety profile compared with BPs, is warranted. The D-CARE (Study of Denosumab as Adjuvant Treatment for Women With High Risk Early Breast Cancer Receiving Neoadjuvant or Adjuvant Therapy) trial (NCT01077154) is investigating the effect of adjuvant denosumab in women at high risk for disease recurrence. Src is a positive regulator of osteoclasts and a negative regulator of osteoblasts. Src inhibitors (dasatinib [Spryvel, Bristol Myers-Squibb], bosutinib [Wyeth], and saracatinib [AstraZeneca]) are in early stages of clinical testing for breast cancer. Preliminary data from studies in patients with advanced breast cancer suggest clinical benefits with dasatinib as a single agent in ER-positive¹²⁷ and triple-negative tumors,¹²⁸ as well as in combination with palitaxel¹²⁹ and capecitabine.¹³⁰ Studies with saracatinib have shown very little activity.^{131,132}

Conclusion

Bone metastases add to the burden of breast cancer, with patients experiencing considerable skeletal morbidity, including severe bone pain, pathologic fractures, spinal cord compression, and hypercalcemia of malignancy, all of which substantially reduce the patient's quality of life. Non-N-BPs inhibit bone resorption, thereby reducing the risk of skeletal complications. Consequently, N-BPs have become the standard treatment for SREs and bone pain, as well as for chemotherapy- and AI-associated bone loss.

Emerging preclinical and clinical evidence indicates that BPs negatively affect multiple processes that support tumor growth and proliferation and formation of metastases. The data suggest that these effects can occur directly through inhibition of farnesyl diphosphate synthase and indirectly by release of cytokines such as IPP.

BP treatment likely leads to an alteration of the bone marrow microenvironment, which not only affects the lodging of DTCs, but interrupts the interaction between the host tumor and the bone marrow, which appears to play an important role in release of growth factors and bone marrow-derived cells, which ultimately provide support for local tumor progression, metastatic niche formation, tumor self-seeding, and metastases development. However, the process of tumor growth and metastasis is complex, and BPs may be effective in some but not all pathways and may depend on the host environment.

Clinical benefit in OS, DFS, and TTP has been demonstrated with BP treatment in the adjuvant setting (Figure 4). Zoledronic acid administered during adjuvant therapy significantly reduces the risk of disease progression and recurrence locally and systemically in premenopausal women with ovarian suppression, in women who are 5 years or more postmenopausal, and in postmenopausal women receiving treatment with an AI. Pamidronate has been shown to reduce bone metastasis. Additional studies to confirm the antitumor activity of N-BPs are warranted, and results from a number of large trials with DFS as the primary endpoint are expected in the next few years (Table 4).

In the metastatic setting, N-BPs have been shown to significantly improve TTP and OS. However, there have been only a limited number of small trials, and large randomized studies with disease progression, if not OS, as primary the endpoint are needed. In the neoadjuvant setting, zoledronic acid has been shown to significantly reduce residual tumor size. Emerging data suggest that BP treatment for osteoporosis leads to a reduction in breast cancer risk.

Ongoing clinical trials will further elucidate the patient populations most likely to benefit from BP treatment, optimal BP dosing, combination therapy, treatment duration, and markers of response. Current evidence suggests that with appropriate safety considerations, the addition of BPs, particularly N-BPs, to standard therapy may provide anticancer benefits. To date, the combination of zoledronic acid and standard therapy has been the most widely investigated combination of an N-BP and standard therapy.

References

- Garcia M, Jemal A, Ward EM, et al. *Global Cancer Facts & Figures 2007*. Atlanta, GA: American Cancer Society; 2007.
- Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin*. 2010;60:277-300.
- NCCN Clinical Practice Guidelines in Oncology. Breast Cancer. V2. 2010.
- Hillner BE, Ingle JN, Chlebowski RT, et al. American Society of Clinical Oncology 2003 update on the role of bisphosphonates and bone health issues in women with breast cancer. *J Clin Oncol*. 2003;21:4042-4057.
- Burstein HJ, Prestrud AA, Seidenfeld J, et al. American Society of Clinical Oncology clinical practice guideline: update on adjuvant endocrine therapy for women with hormone receptor-positive breast cancer. *J Clin Oncol*. 2010;28:3784-3796.
- Menschutkin M. Ueber die Einwirkung des Chloracetyls auf phosphorige Saure. *Ann Chem Pharm*. 1865;133:317-320.
- Fleisch H, Russell RG, Bisaz S, Casey PA, Muhlbauer RC. The influence of pyrophosphate analogues (diphosphonates) on the precipitation and dissolution. *Calcif Tissue Res*. 1968;Suppl:10-10a.
- Masarachia P, Weinreb M, Balena R, Rodan GA. Comparison of the distribution of 3H-alendronate and 3H-etidronate in rat and mouse bones. *Bone*. 1996;19:281-290.
- Pelorgeas S, Martin JB, Satre M. Cytotoxicity of dichloromethane diphosphonate and of 1-hydroxyethane-1,1-diphosphonate in the amoebae of the slime mould *Dictyostelium discoideum*. A 31P NMR study. *Biochem Pharmacol*. 1992;44:2157-2163.
- Frith JC, Monkkonen J, Blackburn GM, Russell RG, Rogers MJ. Clodronate and liposome-encapsulated clodronate are metabolized to a toxic ATP analog, adenosine 5'-(beta, gamma-dichloromethylene) triphosphate, by mammalian cells in vitro. *J Bone Miner Res*. 1997;12:1358-1367.
- Lehenkari PP, Kellinsalmi M, Napankangas JP, et al. Further insight into mechanism of action of clodronate: inhibition of mitochondrial ADP/ATP translocase by a nonhydrolyzable, adenine-containing metabolite. *Mol Pharmacol*. 2002;61:1255-1262.
- Selander KS, Monkkonen J, Karhukorpi EK, Harkonen P, Hannuniemi R, Vaananen HK. Characteristics of clodronate-induced apoptosis in osteoclasts and macrophages. *Mol Pharmacol*. 1996;50:1127-1138.
- Russell RG. Bisphosphonates: mode of action and pharmacology. *Pediatrics*. 2007;119(suppl 2):S150-S162.
- Alakangas A, Selander K, Mulari M, et al. Alendronate disturbs vesicular trafficking in osteoclasts. *Calcif Tissue Int*. 2002;70:40-47.
- Hughes DE, Wright KR, Uy HL, et al. Bisphosphonates promote apoptosis in murine osteoclasts in vitro and in vivo. *J Bone Miner Res*. 1995;10:1478-1487.
- Luckman SP, Coxon FP, Ebetino FH, Russell RG, Rogers MJ. Heterocycle-containing bisphosphonates cause apoptosis and inhibit bone resorption by preventing protein prenylation: evidence from structure-activity relationships in J774 macrophages. *J Bone Miner Res*. 1998;13:1668-1678.
- Luckman SP, Hughes DE, Coxon FP, Graham R, Russell G, Rogers MJ. Nitrogen-containing bisphosphonates inhibit the mevalonate pathway and prevent post-translational prenylation of GTP-binding proteins, including Ras. *J Bone Miner Res*. 1998;13:581-589.
- Rogers MJ, Frith JC, Luckman SP, et al. Molecular mechanisms of action of bisphosphonates. *Bone*. 1999;24(suppl 5):73S-79S.
- Brown HK, Ottewill PD, Coleman RE, Holen I. The kinetochore protein Cenp-F is a potential novel target for zoledronic acid in breast cancer cells. *J Cell Mol Med*. 8 Dec 2009. doi:10.1111/j.1582-4932.2009.00995.x.
- Raikkonen J, Crockett JC, Rogers MJ, Monkkonen H, Auriola S, Monkkonen J. Zoledronic acid induces formation of a pro-apoptotic ATP analogue and isopentenyl pyrophosphate in osteoclasts in vivo and in MCF-7 cells in vitro. *Br J Pharmacol*. 2009;157:427-435.
- Manders K, van de Poll-Franse LV, Creemers GJ, et al. Clinical management of women with metastatic breast cancer: a descriptive study according to age group. *BMC Cancer*. 2006;6:179.
- Tubiana-Hulin M, Beuzebec P, Mauriac L, et al. Double-blinded controlled study comparing clodronate versus placebo in patients with breast cancer bone metastases [in French]. *Bull Cancer*. 2001;88:701-707.
- Kristensen B, Ejlersen B, Groenvold M, Hein S, Loft H, Mouridsen HT. Oral clodronate in breast cancer patients with bone metastases: a randomized study. *J Intern Med*. 1999;246:67-74.
- Diel IJ, Kurth AH, Sittig HB, et al. Bone pain reduction in patients with metastatic breast cancer treated with ibandronate—results from a post-marketing surveillance study. *Support Care Cancer*. 2010;18:1305-1312.
- Body JJ, Diel IJ, Lichinitser MR, et al. Intravenous ibandronate reduces the incidence of skeletal complications in patients with breast cancer and bone metastases. *Ann Oncol*. 2003;14:1399-1405.
- Body JJ, Diel IJ, Bell R, et al. Oral ibandronate improves bone pain and preserves quality of life in patients with skeletal metastases due to breast cancer. *Pain*. 2004;111:306-312.
- Hortobagyi GN, Theriault RL, Porter L, et al. Efficacy of pamidronate in reducing skeletal complications in patients with breast cancer and lytic bone metastases. Protocol 19 Aradia Breast Cancer Study Group. *N Engl J Med*. 1996;335:1785-1791.

28. Hortobagyi GN, Theriault RL, Lipton A, et al. Long-term prevention of skeletal complications of metastatic breast cancer with pamidronate. Protocol 19 Aredia Breast Cancer Study Group. *J Clin Oncol*. 1998;16:2038-2044.
29. Lipton A, Theriault RL, Hortobagyi GN, et al. Pamidronate prevents skeletal complications and is effective palliative treatment in women with breast carcinoma and osteolytic bone metastases: long term follow-up of two randomized, placebo-controlled trials. *Cancer*. 2000;88:1082-1090.
30. Berenson JR, Rosen LS, Howell A, et al. Zoledronic acid reduces skeletal-related events in patients with osteolytic metastases. *Cancer*. 2001;91:1191-1200.
31. Rosen LS, Gordon D, Kaminski M, et al. Long-term efficacy and safety of zoledronic acid compared with pamidronate disodium in the treatment of skeletal complications in patients with advanced multiple myeloma or breast carcinoma: a randomized, double-blind, multicenter, comparative trial. *Cancer*. 2003;98:1735-1744.
32. Clemons MJ, Dranitsaris G, Ooi WS, et al. Phase II trial evaluating the palliative benefit of second-line zoledronic acid in breast cancer patients with either a skeletal-related event or progressive bone metastases despite first-line bisphosphonate therapy. *J Clin Oncol*. 2006;24:4895-4900.
33. Coates AS, Keshaviah A, Thurlimann B, et al. Five years of letrozole compared with tamoxifen as initial adjuvant therapy for postmenopausal women with endocrine-responsive early breast cancer: update of study BIG 1-98. *J Clin Oncol*. 2007;25:486-492.
34. Coombes RC, Kilburn LS, Snowdon CF, et al. Survival and safety of exemestane versus tamoxifen after 2-3 years' tamoxifen treatment (Intergroup Exemestane Study): a randomised controlled trial. *Lancet*. 2007;369:559-570.
35. Forbes JF, Cuzick J, Buzdar A, Howell A, Tobias JS, Baum M. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 100-month analysis of the ATAC trial. *Lancet Oncol*. 2008;9:45-53.
36. Jakesz R, Jonat W, Gnant M, et al. Switching of postmenopausal women with endocrine-responsive early breast cancer to anastrozole after 2 years' adjuvant tamoxifen: combined results of ABCSG trial 8 and ARNO 95 trial. *Lancet*. 2005;366:455-462.
37. Jones SE, Seyanaev C, Hasenburg A, et al. Results from the first planned analysis of the TEAM (tamoxifen exemestane adjuvant multinational) prospective randomized phase III trial in hormone sensitive postmenopausal early breast cancer. Presented at: the 2008 San Antonio Breast Cancer Symposium; December 11, 2008; San Antonio, Texas.
38. Mouridsen H, Giobbie-Hurder A, Goldhirsch A, et al. Letrozole therapy alone or in sequence with tamoxifen in women with breast cancer. *N Engl J Med*. 2009;361:766-776.
39. Dixon JM, Renshaw L, Young O, et al. Letrozole suppresses plasma estradiol and estrone sulphate more completely than anastrozole in postmenopausal women with breast cancer. *J Clin Oncol*. 2008;26:1671-1676.
40. Geisler J, Helle H, Ekse D, et al. Letrozole is superior to anastrozole in suppressing breast cancer tissue and plasma estrogen levels. *Clin Cancer Res*. 2008;14:6330-6335.
41. Goss PE, Hadji P, Subar M, Abreu P, Thomsen T, Banke-Bochita J. Effects of steroidal and nonsteroidal aromatase inhibitors on markers of bone turnover in healthy postmenopausal women. *Breast Cancer Res*. 2007;9:R52.
42. Coleman RE, Banks LM, Girgis SI, et al. Skeletal effects of exemestane on bone-mineral density, bone biomarkers, and fracture incidence in postmenopausal women with early breast cancer participating in the Intergroup Exemestane Study (IES): a randomised controlled study. *Lancet Oncol*. 2007;8:119-127.
43. Eastell R, Adams JE, Coleman RE, et al. Effect of anastrozole on bone mineral density: 5-year results from the anastrozole, tamoxifen, alone or in combination trial 18233230. *J Clin Oncol*. 2008;26:1051-1057.
44. Howell A, Cuzick J, Baum M, et al. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. *Lancet*. 2005;365:60-62.
45. Perez EA, Josse RG, Pritchard KI, et al. Effect of letrozole versus placebo on bone mineral density in women with primary breast cancer completing 5 or more years of adjuvant tamoxifen: a companion study to NCIC CTG MA.17. *J Clin Oncol*. 2006;24:3629-3635.
46. Gnant MF, Mlineritsch B, Luschin-Ebengreuth G, et al. Zoledronic acid prevents cancer treatment-induced bone loss in premenopausal women receiving adjuvant endocrine therapy for hormone-responsive breast cancer: a report from the Austrian Breast and Colorectal Cancer Study Group. *J Clin Oncol*. 2007;25:820-828.
47. Bundred NJ, Campbell ID, Davidson N, et al. Effective inhibition of aromatase inhibitor-associated bone loss by zoledronic acid in postmenopausal women with early breast cancer receiving adjuvant letrozole: ZO-FAST Study results. *Cancer*. 2008;112:1001-1010.
48. Brufsky A, Harker WG, Beck JT, et al. The effect of zoledronic acid on aromatase inhibitor-associated bone loss in postmenopausal women with early breast cancer receiving adjuvant letrozole: the Z-FAST study 5-year final follow-up. Poster presented at the 2009 San Antonio Breast Cancer Symposium; December 12, 2009; San Antonio, Texas.
49. Brufsky A, Bundred N, Coleman R, et al. Integrated analysis of zoledronic acid for prevention of aromatase inhibitor-associated bone loss in postmenopausal women with early breast cancer receiving adjuvant letrozole. *Oncologist*. 2008;13:503-514.
50. Van Poznak C, Hannon RA, Mackey JR, et al. Prevention of aromatase inhibitor-induced bone loss using risenedronate: the SABRE trial. *J Clin Oncol*. 2010;28:967-975.
51. Lester JE, Dodwell D, Purohit OP, et al. Prevention of anastrozole-induced bone loss with monthly oral ibandronate during adjuvant aromatase inhibitor therapy for breast cancer. *Clin Cancer Res*. 2008;14:6336-6342.
52. Bines J, Oleske DM, Cobleigh MA. Ovarian function in premenopausal women treated with adjuvant chemotherapy for breast cancer. *J Clin Oncol*. 1996;14:1718-1729.
53. Shapiro CL, Manola J, Leboff M. Ovarian failure after adjuvant chemotherapy is associated with rapid bone loss in women with early-stage breast cancer. *J Clin Oncol*. 2001;19:3306-3311.
54. Saarto T, Blomqvist C, Valimaki M, Makela P, Sarna S, Elomaa I. Chemical castration induced by adjuvant cyclophosphamide, methotrexate, and fluorouracil chemotherapy causes rapid bone loss that is reduced by clodronate: a randomized study in premenopausal breast cancer patients. *J Clin Oncol*. 1997;15:1341-1347.
55. Fuleihan GH, Salamoun M, Mourad YA, et al. Pamidronate in the prevention of chemotherapy-induced bone loss in premenopausal women with breast cancer: a randomized controlled trial. *J Clin Endocrinol Metab*. 2005;90:3209-3214.
56. Hershman DL, McMahon DJ, Crew KD, et al. Prevention of bone loss by zoledronic acid in premenopausal women undergoing adjuvant chemotherapy persist up to one year following discontinuing treatment. *J Clin Endocrinol Metab*. 2010;95:559-566.
57. Ahn JH, Jung KH, Kim SB, et al. Zoledronic acid prevents bone loss in premenopausal women with early breast cancer undergoing adjuvant chemotherapy: a phase III study of Korean Cancer Study Group (KCSG-BR06-01). Poster presented at: the 2009 San Antonio Breast Cancer Symposium; December 11, 2009; San Antonio, Texas.
58. Hines SL, Mincey BA, Sloan JA, et al. Phase III randomized, placebo-controlled, double-blind trial of risenedronate for the prevention of bone loss in premenopausal women undergoing chemotherapy for primary breast cancer. *J Clin Oncol*. 2009;27:1047-1053.
59. Aft R, Naughton M, Trinkaus K, et al. Effect of zoledronic acid on disseminated tumour cells in women with locally advanced breast cancer: an open label, randomised, phase 2 trial. *Lancet Oncol*. 2010;11:421-428.
60. Mundy GR. Metastasis to bone: causes, consequences and therapeutic opportunities. *Nat Rev Cancer*. 2002;2:584-593.
61. Hiraga T, Williams PJ, Mundy GR, Yoneda T. The bisphosphonate ibandronate promotes apoptosis in MDA-MB-231 human breast cancer cells in bone metastases. *Cancer Res*. 2001;61:4418-4424.
62. Fromiguet O, Lagneaux L, Body JJ. Bisphosphonates induce breast cancer cell death in vitro. *J Bone Miner Res*. 2000;15:2211-2221.
63. Senaratne SG, Pirianov G, Mansi JL, Arnett TR, Colston KW. Bisphosphonates induce apoptosis in human breast cancer cell lines. *Br J Cancer*. 2000;82:1459-1468.
64. Verdijk R, Franke HR, Wolbers F, Vermes I. Differential effects of bisphosphonates on breast cancer cell lines. *Cancer Lett*. 2007;246:308-312.
65. Marzo I, Susin SA, Petit PX, et al. Caspases disrupt mitochondrial membrane barrier function. *FEBS Lett*. 1998;427:198-202.
66. Hockenbery D, Nunez G, Millman C, Schreiber RD, Korsmeyer SJ. Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. *Nature*. 1990;348:334-336.
67. Senaratne SG, Mansi JL, Colston KW. The bisphosphonate zoledronic acid impairs Ras membrane [correction of impairs membrane] localisation and induces cytochrome c release in breast cancer cells. *Br J Cancer*. 2002;86:1479-1486.
68. Hiraga T, Williams PJ, Ueda A, Tamura D, Yoneda T. Zoledronic acid inhibits visceral metastases in the 4T1/luc mouse breast cancer model. *Clin Cancer Res*. 2004;10:4559-4567.
69. Jagdev SP, Coleman RE, Shipman CM, Rostami HA, Croucher PI. The bisphosphonate, zoledronic acid, induces apoptosis of breast cancer cells: evidence

- for synergy with paclitaxel. *Br J Cancer*. 2001;84:1126-1134.
70. Neville-Webbe HL, Evans CA, Coleman RE, Holen I. Mechanisms of the synergistic interaction between the bisphosphonate zoledronic acid and the chemotherapy agent paclitaxel in breast cancer cells in vitro. *Tumour Biol*. 2006;27:92-103.
71. Neville-Webbe HL, Rostami-Hodjegan A, Evans CA, Coleman RE, Holen I. Sequence- and schedule-dependent enhancement of zoledronic acid induced apoptosis by doxorubicin in breast and prostate cancer cells. *Int J Cancer*. 2005;113:364-371.
72. Magnetto S, Boissier S, Delmas PD, Clezardin P. Additive antitumor activities of taxoids in combination with the bisphosphonate ibandronate against invasion and adhesion of human breast carcinoma cells to bone. *Int J Cancer*. 1999;83:263-269.
73. Vogt U, Bielawski KP, Bosse U, Schlotter CM. Breast tumour growth inhibition in vitro through the combination of cyclophosphamide/methotrexate/5-fluorouracil, epirubicin/cyclophosphamide, epirubicin/paclitaxel, and epirubicin/docetaxel with the bisphosphonates ibandronate and zoledronic acid. *Oncol Rep*. 2004;12:1109-1114.
74. Michigami T, Hiraga T, Williams PJ, et al. The effect of the bisphosphonate ibandronate on breast cancer metastasis to visceral organs. *Breast Cancer Res Treat*. 2002;75:249-258.
75. Duivenvoorden WC, Vukmirovic-Popovic S, Kalina M, Seidlitz E, Singh G. Effect of zoledronic acid on the doxycycline-induced decrease in tumour burden in a bone metastasis model of human breast cancer. *Br J Cancer*. 2007;96:1526-1531.
76. Ottewill PD, Woodward JK, Lefley DV, Evans CA, Coleman RE, Holen I. Anticancer mechanisms of doxorubicin and zoledronic acid in breast cancer tumor growth in bone. *Mol Cancer Ther*. 2009;8:2821-2832.
77. Boissier S, Magnetto S, Frappart L, et al. Bisphosphonates inhibit prostate and breast carcinoma cell adhesion to unmineralized and mineralized bone extracellular matrices. *Cancer Res*. 1997;57:3890-3894.
78. Boissier S, Ferreras M, Peyruchaud O, et al. Bisphosphonates inhibit breast and prostate carcinoma cell invasion, an early event in the formation of bone metastases. *Cancer Res*. 2000;60:2949-2954.
79. Denoyelle C, Hong L, Vannier JB, Soria J, Soria C. New insights into the actions of bisphosphonate zoledronic acid in breast cancer cells by dual RhoA-dependent and -independent effects. *Br J Cancer*. 2003;88:1631-1640.
80. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell*. 1996;86:353-364.
81. Ribatti D, Maruotti N, Nico B, et al. Clodronate inhibits angiogenesis in vitro and in vivo. *Oncol Rep*. 2008;19:1109-1112.
82. Fournier P, Boissier S, Filleur S, et al. Bisphosphonates inhibit angiogenesis in vitro and testosterone-stimulated vascular regrowth in the ventral prostate in castrated rats. *Cancer Res*. 2002;62:6538-6544.
83. Michailidou M, Brown HK, Lefley DV, et al. Microvascular endothelial cell responses in vitro and in vivo: modulation by zoledronic acid and paclitaxel? *J Vasc Res*. 2010;47:481-493.
84. Wood J, Bonjean K, Ruetz S, et al. Novel antiangiogenic effects of the bisphosphonate compound zoledronic acid. *J Pharmacol Exp Ther*. 2002;302:1055-1061.
85. Bezzi M, Hasnim M, Biele R, Dormond O, Ruegg C. Zoledronic acid sensitizes endothelial cells to tumor necrosis factor-induced programmed cell death: evidence for the suppression of sustained activation of focal adhesion kinase and protein kinase B/Akt. *J Biol Chem*. 2003;278:43603-43614.
86. Daubine F, Le Gall C, Gasser J, Green J, Clezardin P. Antitumor effects of clinical dosing regimens of bisphosphonates in experimental breast cancer bone metastasis. *J Natl Cancer Inst*. 2007;99:322-330.
87. Pasquier E, Kavallaris M, Andre N. Metronomic chemotherapy: new rationale for new directions. *Nat Rev Clin Oncol*. 2010;7:455-465.
88. Coscia M, Quaglini E, Iezzi M, et al. Zoledronic acid repolarizes tumour-associated macrophages and inhibits mammary carcinogenesis by targeting the mevalonate pathway. *J Cell Mol Med*. 2010;14:2803-2815.
89. Kunzmann V, Bauer E, Feurle J, Weissinger F, Tony HP, Wilhelm M. Stimulation of gammadelta T cells by aminobisphosphonates and induction of antiplasma cell activity in multiple myeloma. *Blood*. 2000;96:384-392.
90. Sato K, Kimura S, Segawa H, et al. Cytotoxic effects of gammadelta T cells expanded ex vivo by a third generation bisphosphonate for cancer immunotherapy. *Int J Cancer*. 2005;116:94-99.
91. Roelofs AJ, Jauhainen M, Monkkonen H, Rogers MJ, Monkkonen J, Thompson K. Peripheral blood monocytes are responsible for gammadelta T cell activation induced by zoledronic acid through accumulation of IPP/DMAPP. *Br J Haematol*. 2009;144:245-250.
92. Gober HJ, Kistowska M, Angman L, Jenö P, Mori L, De Libero G. Human T cell receptor gammadelta cells recognize endogenous mevalonate metabolites in tumor cells. *J Exp Med*. 2003;197:163-168.
93. Fromigue O, Kheddoumi N, Body JJ. Bisphosphonates antagonise bone growth factors' effects on human breast cancer cells survival. *Br J Cancer*. 2003;89:178-184.
94. Tang X, Zhang Q, Shi S, et al. Bisphosphonates suppress insulin-like growth factor 1-induced angiogenesis via the HIF-1alpha/VEGF signaling pathways in human breast cancer cells. *Int J Cancer*. 2010;126:90-103.
95. Santini D, Vincenzi B, Avvisati G, et al. Pamidronate induces modifications of circulating angiogenic factors in cancer patients. *Clin Cancer Res*. 2002;8:1080-1084.
96. Santini D, Vincenzi B, Dicuonzo G, et al. Zoledronic acid induces significant and long-lasting modifications of circulating angiogenic factors in cancer patients. *Clin Cancer Res*. 2003;9:2893-2897.
97. Ferretti G, Fabi A, Carlini P, et al. Zoledronic-acid-induced circulating level modifications of angiogenic factors, metalloproteinases and proinflammatory cytokines in metastatic breast cancer patients. *Oncology*. 2005;69:35-43.
98. Santini D, Vincenzi B, Galluzzo S, et al. 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;13:4482-4486.
99. Wilhelm M, Kunzmann V, Eckstein S, et al. Gammadelta T cells for immune therapy of patients with lymphoid malignancies. *Blood*. 2003;102:200-206.
100. Dieli F, Gebbia N, Poccia F, et al. Induction of gammadelta T-lymphocyte effector functions by bisphosphonate zoledronic acid in cancer patients in vivo. *Blood*. 2003;102:2310-2311.
101. Meraviglia S, Eberl M, Vermijlen D, et al. In vivo manipulation of Vgamma9Vdelta2 T cells with zoledronate and low-dose interleukin-2 for immunotherapy of advanced breast cancer patients. *Clin Exp Immunol*. 2010;161:290-297.
102. Braun S, Vogl FD, Naume B, et al. A pooled analysis of bone marrow micro-metastasis in breast cancer. *N Engl J Med*. 2005;353:793-802.
103. Janni W, Rack B, Schindlbeck C, et al. The persistence of isolated tumor cells in bone marrow from patients with breast carcinoma predicts an increased risk for recurrence. *Cancer*. 2005;103:884-891.
104. Aft R, Trinkaus K, Weibaecker K. Effect of zoledronic acid on disease-free survival and overall survival in women with clinic stage II/III undergoing neoadjuvant chemotherapy for breast cancer. Poster presented at: the 2010 San Antonio Breast Cancer Symposium; December 12, 2010; San Antonio, Texas.
105. Lin A, Park J, Melisko M, et al. Zoledronic acid as adjuvant therapy for women with early stage breast cancer and occult tumor cells in bone marrow. Poster presented at: the 2007 San Antonio Breast Cancer Symposium; December 15, 2007; San Antonio, Texas.
106. Solomayer EF, Gebauer G, Hirmler P, et al. Influence of zoledronic acid on disseminated tumor cells (DTC) in primary breast cancer patients. *Cancer Res*. 2008;69(suppl 2):170s-171s. Abstract 2048.
107. Conte PF, Latreille J, Mauriac L, et al. Delay in progression of bone metastases in breast cancer patients treated with intravenous pamidronate: results from a multinational randomized controlled trial. The Aredia Multinational Cooperative Group. *J Clin Oncol*. 1996;14:2552-2559.
108. Lipton A, Cook RJ, Major P, Smith MR, Coleman RE. Zoledronic acid and survival in breast cancer patients with bone metastases and elevated markers of osteoclast activity. *Oncologist*. 2007;12:1035-1043.
109. Ha TC, Li H. Meta-analysis of clodronate and breast cancer survival. *Br J Cancer*. 2007;96:1796-1801.
110. Coleman R. Adjuvant treatment with zoledronic acid in stage II/III breast cancer. The AZURE trial (BIG 01/04). Paper presented at: the 2010 San Antonio Breast Cancer Symposium; December 10, 2010; San Antonio, Texas.
111. De Boer RH, Bundred N, Eidtmann H, et al. The effect of zoledronic acid on aromatase inhibitor-associated bone loss in postmenopausal women with early breast cancer receiving adjuvant letrozole: the ZO-FAST study 5-year final follow-up. Poster presented at: the 2010 San Antonio Breast Cancer Symposium; December 11, 2010; San Antonio, Texas.
112. Gnant M, Mlineritsch B, Stoeger H, et al. Mature results from ABCSG-12: adjuvant ovarian suppression combined with tamoxifen or anastrozole, alone or in combination with zoledronic acid, in premenopausal women with endocrine responsive breast cancer. *J Clin Oncol* (ASCO Annual Meeting Abstracts). 2010;28:Abstract 533.
113. Gnant M, Mlineritsch B, Schippering W, et al. Endocrine therapy plus zoledronic acid in premenopausal breast cancer. *N Engl J Med*. 2009;360:679-691.
114. Gnant M, Mlineritsch B, Stoeger H, et al. The carry-over effect of adjuvant zoledronic acid: comparison of 48- and 62-month analyses of ABCSG-12 suggests

that the benefits of combining zoledronic acid with adjuvant endocrine therapy persist long after completion of therapy. Poster presented at: the 2010 San Antonio Breast Cancer Symposium; December 11, 2010; San Antonio, Texas.

115. Coleman RE, Winter MC, Cameron D, et al. The effects of adding zoledronic acid to neoadjuvant chemotherapy on tumour response: exploratory evidence for direct anti-tumour activity in breast cancer. *Br J Cancer*. 2010;30;102:1099-1105.

116. Coleman R, Bundred N, DeBoer RH, et al. Impact of zoledronic acid in postmenopausal women with early breast cancer receiving adjuvant letrozole: Z-FAST, ZO-FAST, and E-ZO-FAST. Poster presented at: the 2009 San Antonio Breast Cancer Symposium; December 12, 2009; San Antonio, Texas.

117. Kokufu I, Kohno N, Takao S. Adjuvant pamidronate (PMT) therapy for the prevention of bone metastases in breast cancer (BC) patients with four or more positive nodes. *J Clin Oncol* (ASCO Annual Meeting Abstracts). 2004;23:Abstract 530.

118. Jung J, Hwang G, Lee Y. Pamidronate as adjuvant treatment for prevention of bone metastases in breast cancer. *J Clin Oncol* (ASCO Annual Meeting Abstracts). 2005;23:Abstract 888.

119. Chlebowski RT, Chen Z, Cauley JA, et al. Oral bisphosphonate use and breast cancer incidence in postmenopausal women. *J Clin Oncol*. 2010;28:3582-3590.

120. Newcomb PA, Trentham-Dietz A, Hampton JM. Bisphosphonates for osteoporosis treatment are associated with reduced breast cancer risk. *Br J Cancer*. 2010;102:799-802.

121. Rennert G, Pinchev M, Rennert HS. Use of bisphosphonates and risk of postmenopausal breast cancer. *J Clin Oncol*. 2010;28:3577-3581.

122. Zometa [package insert]. East Hanover, NJ: Novartis Pharmaceutical Corporation; 2011.

123. Aredia [package insert]. East Hanover, NJ: Novartis Pharmaceutical Corporation; 2008.

124. Ruggiero SL, Mehrotra B. Bisphosphonate-related osteonecrosis of the jaw: diagnosis, prevention, and management. *Annu Rev Med*. 2009;60:85-96.

125. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. *Nature*. 2003;423:337-342.

126. Stopeck AT, Lipton A, Body JJ, et al. Denosumab compared with zoledronic acid for the treatment of bone metastases in patients with advanced breast cancer: a randomized, double-blind study. *J Clin Oncol*. 2010;28:5132-5139.

127. Mayer E, Baurain J, Sparano J, et al. Dasatinib in advanced HER2/neu amplified and ER/PR-positive breast cancer: Phase II study CA180088. *J Clin Oncol* (ASCO Annual Meeting Abstracts). 2009;27:Abstract 1011.

128. Finn RS, Bengala C, Ibrahim N, et al. Phase II trial of dasatinib in triple-negative breast cancer: results of study CA 180059. Poster presented at: the 2008 San Antonio Breast Cancer Symposium; December 12, 2008; San Antonio, Texas.

129. Morris PG, Abbruzzi A, D'Andrea G, et al. A phase I-II trial of dasatinib in combination with weekly paclitaxel for patients with metastatic breast carcinoma. Poster presented at: the 2010 San Antonio Breast Cancer Symposium; December 12, 2010; San Antonio, Texas.

130. Cortes J, Specht J, Gradishar W, et al. Dasatinib plus capecitabine for advanced breast cancer: safety and efficacy in phase 1 CA 180-004. Poster presented at: the 2009 San Antonio Breast Cancer Symposium; December 11, 2009; San Antonio, Texas.

131. Pegram M, Silva O, Higgins C, et al. Src kinase inhibition with AZD0530 (saracatinib) plus anastrozole in postmenopausal hormone receptor positive (HR+) metastatic breast cancer. Poster presented at the 2010 San Antonio Breast Cancer Symposium; December 10, 2010; San Antonio, Texas.

132. Traina TA, Sparano JA, Caravelli J, et al. Phase II trial of saracatinib in patients (pts) with ER/PR-negative metastatic breast cancer (MBC). *J Clin Oncol* (ASCO Annual Meeting Abstracts). 2010;28:Abstract 1086.

133. Coghlin C, Murray GI. Current and emerging concepts in tumor metastases. *J Pathol*. 2010;222:1-15.