

# 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](mailto:aft@wudosis.wustl.edu)

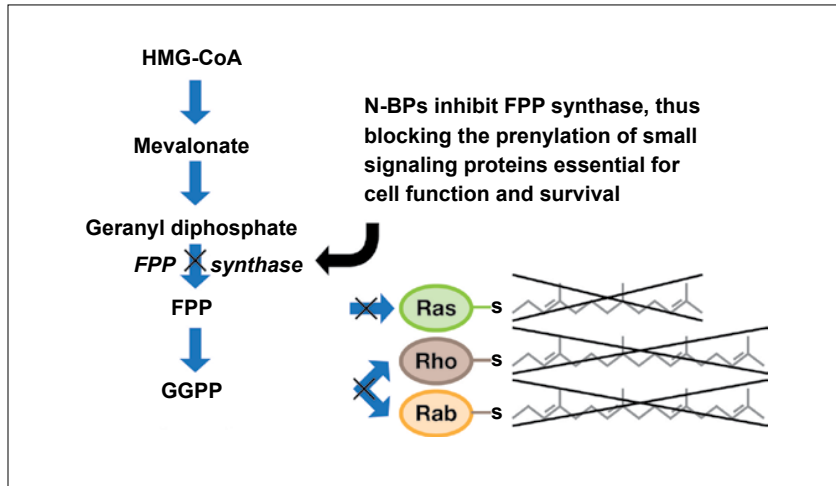
**Abstract:** Breast cancer is the most frequently diagnosed and second deadliest cancer among women. Bisphosphonates are stable pyrophosphate analogues used to treat skeletal-related events resulting from bone metastases. In the adjuvant setting, they have been shown to prevent aromatase inhibitor-associated 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.<sup>1</sup> 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.<sup>2</sup> 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.<sup>3</sup> 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 osteoporosis<sup>4</sup> and, though not included in treatment guidelines, for the mitigation of therapy-induced bone loss.<sup>5</sup> 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.<sup>13</sup>

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

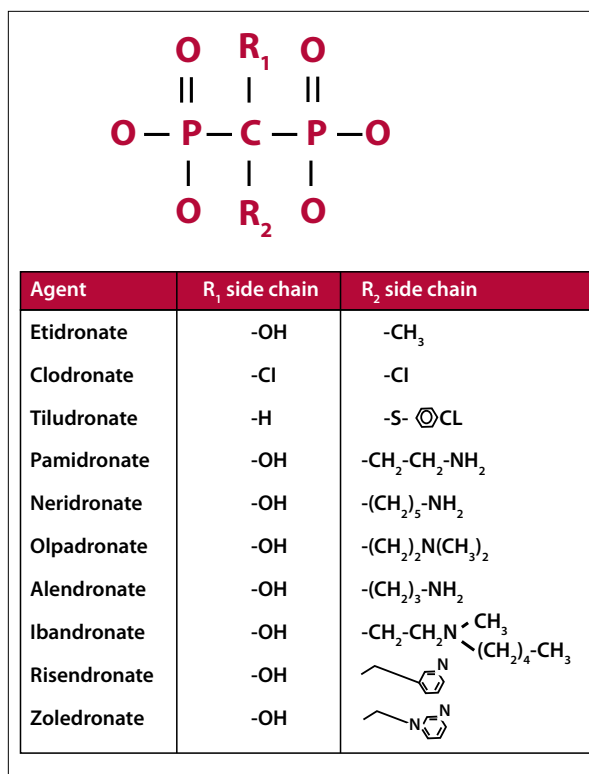
BPs are chemically stable analogues of inorganic pyrophosphates. They were developed in the 19th century,<sup>6</sup> but it was not until 1968 that their biologic effects—inhibition of the precipitation and dissolution of calcium phosphate in vitro—were first reported.<sup>7</sup> Bisphosphonates were 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.<sup>8</sup>

The first-generation BPs were non-nitrogen-containing (non-N-BPs) and exerted their effects by replacing terminal phosphates of adenosine triphosphate (ATP).<sup>9</sup> These nonhydrolyzable ATP analogues likely promoted apoptosis by inhibiting ATP-dependent cellular activities.<sup>10-12</sup> 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),<sup>13</sup> leading to apoptosis when the affected proteins are unable to regulate core cellular processes.<sup>14-18</sup> Recently, it was demonstrated that N-BPs suppress the farnesylation of the centromeric protein Cenp-F (mitosin).<sup>19</sup> 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.<sup>20</sup>

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.<sup>21</sup> 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.<sup>22-33</sup> 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.<sup>34-38</sup> However, the profound estrogen suppression associated with AI therapy<sup>39-41</sup> may cause an increase in bone turnover, acceleration of bone loss, and an increase in fracture risk.<sup>33,42-45</sup> Evidence supporting the hypothesis that BP therapy is a viable option for preventing AI-associated bone loss can be found in Table 2.<sup>46-51</sup> 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.<sup>52</sup> This has been associated with a rapid loss of bone mineral density, which increases the risk of osteoporosis and fractures.<sup>53</sup> Some, though not all, BPs have been shown to attenuate the effect of chemotherapy on bone mineral density (Table 3).<sup>54-59</sup>

### 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).<sup>60</sup>

### 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<sup>-4</sup> M) induced apoptosis of the estrogen

receptor (ER)-negative MDA-MB-231 breast cancer cells.<sup>61</sup> Likewise, treatment with zoledronic acid, pamidronate, ibandronate, or clodronate (each at 10<sup>-4</sup>, 10<sup>-6</sup>, and 10<sup>-8</sup> M) reduced cell viability that was time and dose dependent and irreversible in ER-positive MCF-7 and T47D breast cancer cells.<sup>62</sup> 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.<sup>63,64</sup>

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.<sup>65</sup> 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,<sup>63,66</sup> whereas the zoledronic acid-induced reduction in cell viability was reversed by bcl-2 overexpression.<sup>67</sup> 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.<sup>67</sup> Likewise, the mevalonate pathway intermediate geranylgeraniol reversed the caspase-3 activation and decreased zoledronic acid-induced apoptosis in 4T1/luc mouse breast cancer cells.<sup>68</sup> 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.<sup>61</sup> 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.<sup>63</sup>

#### *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.<sup>69</sup> 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.<sup>70</sup> Induction of apoptosis in MCF-7 cells was also greater with the combination of doxorubicin and zoledronic acid compared with either agent alone.<sup>71</sup> 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 <sup>22</sup>	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 <sup>23</sup>	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* <sup>24</sup>	–	913	q3–4w for ≤24 weeks	Improvement in bone pain severity in 70% of patients with pain at baseline.
IV ibandronate <sup>25</sup>	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 <sup>26</sup>	Placebo	564	Daily for ≤96 weeks	Reduction in bone pain ( $P=.019$ ). Less increase in analgesic use ( $P=.019$ ).
IV pamidronate <sup>27</sup>	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 <sup>28</sup>	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 <sup>29</sup>	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 <sup>30</sup>	Pamidronate	280 <sup>†</sup>	q4w for ≤10 months	Similarly reduced SREs and need for radiotherapy.
IV zoledronic acid <sup>31</sup>	Pamidronate	1,130 <sup>†</sup>	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 <sup>‡32</sup>	None	31	q4w for 3 months	Decrease in worst pain and average pain score at week 8 vs baseline ( $P<.001$ ).
Clodronate (meta-analysis) <sup>33</sup>	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 (ABCSG-12) <sup>46</sup>	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) <sup>47</sup>	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) <sup>48</sup>	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) <sup>49</sup>	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 <sup>50</sup>	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) <sup>51</sup>	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 NH<sub>2</sub>-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.<sup>72</sup> 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.<sup>73</sup> 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 <sup>54</sup>	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 <sup>55</sup>	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 <sup>56</sup>	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 <sup>57</sup>	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 <sup>58</sup>	Oral risedronate	Placebo	216	Every week for 1 year	No significant difference in the prevention of bone loss.
AT <sup>59</sup>	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.

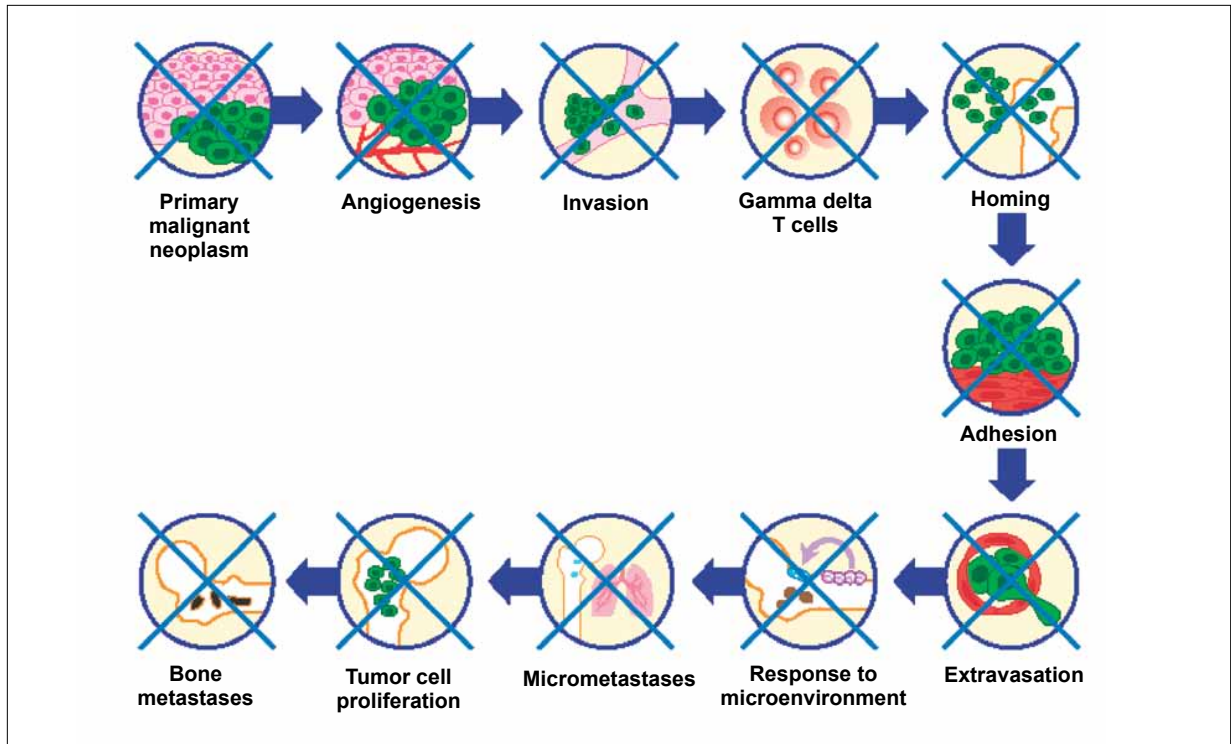
(6.1%) was achieved when MCF-7 cells were treated with 2 nM paclitaxel on day 1 followed by 25  $\mu$ M zoledronic acid on day 2 as opposed to the reverse sequence (2.4%) or coadministration (3.75%).<sup>70</sup> In MCF-7 cells, maximal induction of apoptosis required exposure to doxorubicin prior to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

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.<sup>74</sup> 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 ( $P<.05$ ).<sup>75</sup> Finally, when MDA-MB-436 cells were injected into the tibiae of immunocompromised mice, sequential doxorubicin and zoledronic acid (100  $\mu$ 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.<sup>76</sup>

#### ***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.<sup>77</sup> 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.<sup>78</sup> In these studies, BPs did not induce cytotoxic effects,<sup>77,78</sup> 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.<sup>60</sup>

teinases<sup>78</sup> by tumor cells at concentrations that inhibited tumor cell invasion; however, they inhibited proteolytic activity. In another study, a clinically relevant concentration (1  $\mu\text{M}$ ) 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.<sup>79</sup> 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.<sup>72</sup> When the cells were treated with ibandronate (1  $\mu\text{M}$ ) 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.<sup>61</sup> 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.<sup>68</sup> 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.<sup>80</sup> 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  $\mu\text{M}$  reduced endothelial cell growth in a dose-dependent manner.<sup>81</sup> Similarly, exposure of endothelial cells to clodronate, ibandronate, and risedronate at 100  $\mu\text{M}$  inhibited proliferation, as did zoledronic acid in a dose-dependent manner (0.0001–100  $\mu\text{M}$ ).<sup>82</sup> At 100  $\mu\text{M}$ , clodronate, ibandronate, risedronate, and zoledronic acid all inhibited capillary-like tube formation, and zoledronic acid was additionally shown to induce endothelial cell apoptosis.<sup>82</sup> Studies using human dermal microvascular endothelial cells showed similar results.<sup>83</sup> Compared with no treatment, zoledronic acid at 25 and 50  $\mu\text{M}$  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  $\mu\text{M}$  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.<sup>84</sup> Zoledronic acid induced cell adhesion at 1–3  $\mu\text{M}$  and migration at 0.3–10  $\mu\text{M}$ , but inhibited cell adhesion at 30–100  $\mu\text{M}$  and migration at 30  $\mu\text{M}$ . 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.<sup>85</sup> 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.<sup>81</sup> Zoledronic acid inhibited the proliferation of human umbilical vein endothelial cells stimulated with fetal calf serum (half maximal inhibitory concentration [ $\text{IC}_{50}$ ] 4.1  $\mu\text{M}$ ), basic FGF (bFGF,  $\text{IC}_{50}$  4.2  $\mu\text{M}$ ), and at higher concentrations, vascular endothelial growth factor (VEGF,  $\text{IC}_{50}$  6.9  $\mu\text{M}$ ).<sup>84</sup>

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.<sup>84</sup> 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.<sup>86</sup> 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.<sup>87</sup> The antiangiogenic and antitumor effects of clinically achievable doses of zoledronic acid outside the bone were also investigated in a mouse model.<sup>88</sup> 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.<sup>83</sup>

#### ***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 V $\gamma$ 9V $\delta$ 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.<sup>89</sup> 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 V $\gamma$ 9V $\delta$ 2 T cells, whereas non-N-BPs did not.<sup>89</sup> 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.<sup>89</sup> Another study evaluated the cytotoxicity of  $\gamma\delta$  T cells expanded *ex vivo* on various cancer cell lines.<sup>90</sup> Incubation with zoledronic acid 1  $\mu\text{M}$  plus IL-2 for 14 days increased the absolute number of  $\gamma\delta$  T cells up to 768-fold, par-

ticularly the V $\gamma$ 9V $\delta$ 2 subset. In addition, zoledronic acid 1  $\mu$ 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  $\mu$ 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.<sup>91</sup> N-BPs indirectly activate V $\gamma$ 9V $\delta$ 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  $\mu$ 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.<sup>69</sup> 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.<sup>92</sup> 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.<sup>60</sup> 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.<sup>93</sup> For example, clodronate, ibandronate, pamidronate, and zoledronic acid (all at 10<sup>-6</sup> 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 $\alpha$  and VEGF protein expression.<sup>94</sup> Treatment of MCF-7 cells with clodronate 50  $\mu$ M or pamidronate 50  $\mu$ M suppressed IGF-I–induced HIF-1 $\alpha$  and VEGF expression and promoted HIF-1 $\alpha$  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.

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