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Breast Cancer RESEARCH

REVIEW

Zoledronic acid effectiveness against breast cancer metastases – a role for estrogen in the microenvironment?

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Abstract

Zoledronic acid (ZA) is an imidazole-containing bisphosphonate that has been extensively studied as an osteoclast inhibitor. ZA decreases bone turnover and has been effective in limiting osteolysis in metastatic cancers, including breast cancer. Recent clinical trials that demonstrated enhancement of disease-free survival by bisphosphonates have prompted interest in bisphosphonates as anti-cancer agents. ZA, for example, increased disease-free survival in postmenopausal and in premenopausal, hormone-suppressed breast cancer patients. Intriguingly, however, there was a lack of an anti-cancer effect of ZA in premenopausal women without ovarian suppression. These observations have prompted the conjecture that anti-cancer effects of ZA are limited to estrogen-poor environments. This review explores possible mechanisms compatible with differences in ZA activity in premenopausal women compared with postmenopausal (or hormone-suppressed) women.

Background and introduction

Mechanisms and sites of activity of zoledronic acid

Bisphosphonates localize to bone by mimicking pyrophosphate and binding to hydroxyapatite in mineralized bone. Zoledronic acid (ZA) is the most potent nitrogen-containing bisphosphonate, and the majority of research studies and clinical trials have used ZA, which is the focus of this review.

After injection of a single dose of ZA its activity can be detected in bone 3 years later [1], but ZA plasma concentrations fall by 99% within 24 hours. ZA injection results in millimolar concentrations in bone, and at this high concentration ZA is directly toxic to osteoclasts and limits bone turnover and skeletal-related events arising from bone-metastatic cancer.

ZA blocks protein isoprenylation, a key step in many survival and proliferation pathways. ZA inhibits farnesyl diphosphonate synthase and, to a lesser degree, geranylgeranylpyrophosphate synthase, both vital enzymes in the mevalonate pathway involved in biosynthesis of cholesterol. These enzymes lead to prenylation and activation of key regulatory proteins, including farnesylation of Ras family proteins and geranylgeranylation of Rho family proteins. Disruption of Ras undermines intracellular vesicular transport and bone-resorptive capabilities of osteoclasts, ultimately leading to cell death. Although these effects of ZA have been most extensively studied in the bone microenvironment, ZA also has bone-extrinsic effects, including modulation of cell migration, angiogenesis, and immunity. Direct induction of cancer cell death by ZA has also been noted *in vitro*, albeit at high ZA concentrations that are not achieved outside bone. These effects of ZA and other bisphosphonates have been extensively reviewed [2].

Clinical efficacy of zoledronic acid, and potential hormone-dependent effects on breast cancer cells

In patients, ZA has been effective against both lytic and blastic bone disease, reducing bone symptoms and skeletal-related events in bone-metastatic prostate cancer, bladder cancer, hepatocellular cancer, breast cancer, lung cancer, and multiple myeloma [3]. The ability of ZA to decrease the cancer burden in bone is understandable, given its high concentration and long half-life in the bone environment. For instance, liberation of ZA from hydroxyapetite during bone turnover could allow accumulation of the high ZA concentrations required *in vitro* for direct anti-cancer cell effects. More surprising have been results from recent clinical studies identifying anti-cancer efficacy of ZA beyond the setting of bone metastases, leading to increased disease-free survival (DFS).

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In the Austrian Breast & Colorectal Cancer Study Group 12 (ABCSG12) study, 3 years of ZA added to endocrine therapy increased the DFS of premenopausal women with stage I/II breast cancer compared with endocrine therapy alone. All of the premenopausal women in the ABCSG12 trial received luteinizing hormone-releasing hormone agonist goserelin to suppress production of estrogen and progesterone. Production or function of estrogen was further inhibited by anastrozole or by tamoxifen. The study demonstrated a 36% reduction overall in the relative risk of disease progression among those patients taking ZA. The increased DFS was sustained after treatment cessation to 82 months [4]. Notably, the ABCSG12 trial demonstrated that ZA reduced both intra-osseous and extra-osseous breast cancer recurrence and locoregional recurrences in these premenopausal women on hormone suppression.

In contrast, the Adjuvant Zoledronic Acid to Reduce Recurrence (AZURE) trial indicated that addition of ZA to standard therapy did *not* prolong DFS in women with stage II/III breast cancer [5]. The AZURE study enrolled 2,259 premenopausal or perimenopausal women and 1,101 postmenopausal women, nearly all of whom received chemotherapy, and randomized them to adjuvant ZA treatment or placebo. ZA did not improve the overall survival or DFS of the entire cohort, but a prespecified subgroup analysis in the AZURE study indicated that postmenopausal women – but not premenopausal women – treated with ZA showed an increased DFS of a magnitude comparable with that observed in the ABGCS12 trial [5]. ZA was effective in preventing new secondary primary tumors and locoregional and non-skeletal distant recurrences in postmenopausal women in the AZURE trial.

Although not the primary endpoint, a DFS benefit of ZA treatment in postmenopausal women has also been supported by trials in which postmenopausal women with early-stage hormone-responsive breast cancer were treated with the aromatase inhibitor letrozole and were randomized to either immediate or delayed ZA (the ZO-Fast, Z-Fast, E-ZO-Fast and N03CC trials). Delayed ZA therapy was triggered by nontraumatic fracture or crossing a bone loss threshold. The results not only demonstrated improved bone mineral density (the primary endpoint) with immediate ZA treatment, but showed a benefit for DFS – in the ZO-Fast trial at 48 months [6] and in the Z-Fast trial at 12 to 48 months but not at 5 years [7] (the two other similar trials (E-ZO-Fast and N03CC) had insufficient data to evaluate recurrence at 12 months [8,9]). Additional information is presented in Table 1.

Collectively, these trials have led to several conclusions and potential interpretations. First, there is evidence of an anti-cancer effect of ZA that is not limited to bone. Second, ZA opposes breast cancer recurrence in sex-hormone-poor environments (that is, goserelin plus anti-estrogens or in postmenopausal women). Although the ABCSG12 and AZURE trials differ in enrollment criteria and the extent of adjuvant chemotherapy usage, the benefit of ZA in premenopausal women in the ABCSG12 trial and not in the AZURE trial has been interpreted as an inhibitory effect of sex hormones on the ability of ZA to augment DFS [5]. Third, the lack of anti-cancer effectiveness of ZA in premenopausal women could not be rescued by tamoxifen alone, given that 74% of subjects in each arm of the AZURE trial received tamoxifen plus chemotherapy [5] (R Coleman, personal communication). Opposition of ZA improvement of DFS could therefore be mediated by tamoxifen-insensitive estrogen receptor (ER) activity or through the activity of other, goserelin-sensitive, hormones. Finally, results from the AZURE trial showed that premenopausal women responded poorly to ZA – independent of the ER status of the tumor. ZA resistance in premenopausal women may therefore not arise from estrogen or progesterone signaling within the cancer cells themselves, but rather from sex hormone effects on the premenopausal microenvironment.

In this review we consider how these interpretations could be informed by preclinical *in vitro* and *in vivo* studies of ZA activity, focusing on the possible endocrine-dependent effects of ZA activity. In the AZURE trial, premenopausal women with either ER-positive or ER-negative tumors responded less well to ZA than did postmenopausal women [5]. This finding indicates that sex hormones did not solely antagonize ZA by acting on cancer cells but through hormonal manipulation of stroma or the tumor microenvironment, which we will discuss here. Although a number of hormone receptor pathways could be involved in the differential ZA responses, we will focus on estrogen, and only briefly discuss other hormones such as progesterone and inhibin.

Effects of zoledronic acid in bone

ZA, mimicking pyrophosphate, binds to hydroxyapatite in mineralized bone, and is directly toxic to osteoclasts – it limits bone turnover and skeletal-related events arising from bone-metastatic cancer. By inhibiting osteoclast-mediated bone resorption, ZA lowers bone marrow calcium and impedes the liberation of growth factors from the bone matrix that contribute to the vicious cycle of metastasis growth and bone breakdown. There are some data suggesting that bisphosphonates can have additional, osteoclast-independent effects. For example, ZA was shown to be effective against bone tumors in mice with nonfunctional osteoclasts [10]. However, ZA's primary effect on osteoclasts is supported by data in patients with advanced breast cancer showing that

Table 1. Zoledronic acid trials and disease-free survival

Trial	Hormonal status	ZA/hormonal intervention	Effect of ZA
Z-Fast [7] (*n* = 602)	Postmenopausal women	Immediate versus delayed ZA plus adjuvant letrazole	Decreased recurrence at 12 to 48 months, not at 60 months
ZO-Fast [6] (*n* = 868)	Postmenopausal women	Immediate versus delayed ZA plus adjuvant letrazole	Reduction in DFS (HR = 0.59) at 36 and 48 months. Disease recurrence reduced at bone and at nonbone sites
ABCSG12 [4] (*n* = 1,803)	Premenopausal ER/PR-positive stage 1/2 breast cancer	Phase 3 2×2 trial, goserelin ± tamoxifen or anastrozole ± ZA	Reduction in DFS (HR = 0.68) at 48 and 62 months. Disease recurrence reduced at bone and at nonbone sites
AZURE [5] (*n* = 3,360)	Premenopausal and postmenopausal stage 2/3 breast cancer	Phase 3 trial, standard adjuvant systemic therapy including hormonal ± ZA	Lack of effect on invasive DFS. Subgroup analysis indicated benefit in women ≥5 years postmenopausal (HR = 0.75)
AZURE subgroup [72] (*n* = 205)	Premenopausal and postmenopausal stage 2/3 breast cancer	Neoadjuvant chemotherapy ± ZA	Reduced residual invasive tumor size by 44%

ABCSG12, Austrian Breast & Colorectal Cancer Study Group 12; AZURE, Adjuvant Zoledronic Acid to Reduce Recurrence; DFS, disease-free survival; ER, estrogen receptor; HR, hazard ratio; PR, progesterone receptor; ZA, zoledronic acid.

ZA-treated breast cancer patients in whom ZA rapidly normalized bone turnover (manifested by N-telopeptide of type I collagen) had longer event-free survival and overall survival than those with continued high turnover [11].

In addition to the inhibition of osteoclast activity, ZA was shown to have many other activities within bone itself that could explain its ability to prevent recurrence of breast cancer. ZA downregulates adhesion molecules on bone marrow stromal cells and inhibits tumor cell adhesion to the bone matrix whether the tumor cells or the matrix are treated with bisphosphonate [12]. ZA also promotes osteoblast maturation and production of the rank ligand antagonist osteoprotegerin, an inhibitor of osteolysis [13]. Moreover, ZA has been shown to inhibit stromal IL-6 secretion and matrix metalloproteinase-1 production, effects that could block tumor growth in bone or at other sites.

There is increasing evidence that ZA could affect survival of disseminated tumor cells, whose prevalence predicts local and distant relapse [14]. Both neoadjuvant and postchemotherapy administration of ZA (and other bisphosphonates) have been shown to decrease disseminated tumor cells in marrow compared with pretreatment levels [15,16], lowering the risk of metastatic spread from micrometastases. This occurrence could represent an anti-cancer effect. Interestingly, osteoclasts and osteoblasts have been implicated in the maintenance of dormant leukemic clones in the marrow [17], which may also be the case for breast cancer. Moreover, breast tumor cell viability in the endosteal niche has been shown to involve heterotypic notch/jagged interactions with osteoblasts similar to those used to regulate hematopoietic stem cell numbers [18,19]. ZA-mediated shifts in osteoclast and osteoblast function may therefore lead to changes in the microenvironment that undermine support for disseminated cancer cells.

Estrogen actions in bone as a modulator of zoledronic acid effectiveness

As described above, the clinical data point towards a role for low-estrogen environments in high ZA efficacy. A possible caveat is the clinical observation that most of the premenopausal women in the AZURE trial who reaped no benefit from ZA were receiving tamoxifen. Conceivably, tamoxifen did not adequately block estrogen signaling that undermined ZA (discussed below); alternatively, the critical action of estrogen impairing the ZA anti-tumor effect occurred at a site where tamoxifen functioned as an agonist (for example, bone). Like estrogen, tamoxifen opposes bone turnover (albeit to a lesser degree [20]), and thus suppression of bone turnover by tamoxifen or estrogen may limit the magnitude of benefit accruing from ZA treatment because ZA has a greater survival benefit in patients with bone metastases with high basal levels of bone turnover [21].

Studies have not yet uncovered a mechanistic process that would link estrogen-suppressed bone turnover and decreased DFS among ZA-treated women. Bone metastases in premenopausal women may be less dependent on calcium and on cytokines liberated from bone and the matrix by osteoclasts than those in postmenopausal women. This could render the premenopausal women less sensitive to downstream effects of ZA that lower local calcium and cytokine levels. Another possibility is that the bone microenvironment in estrogen-exposed women better supports the survival and expansion of disseminated tumor cells in the endosteal niche. This idea

is supported by the findings that estrogen increases the number and activity of endosteal osteoblasts [22], which are critical mediators of stem cell dormancy and survival [23]; estrogen may thereby impede the ability of ZA to decrease disseminated tumor cells. To better understand whether estrogen and ZA interact at the level of dormancy, it will be important to measure the effect of ZA on the ability of estrogen-replete and estrogen-deprived endosteum to support cancer.

Estrogen deficiency, on the contrary, might bolster ZA action. Estrogen deficiency has been linked to increased expression of IL-1 (reviewed in [24]). There is early but intriguing evidence that could link IL-1α to the transition from latency to clinical disease. IL-1 has been shown to mobilize hematopoietic stem cells from the stem/progenitor niche. IL-1 is a TNFα target, and a recent report demonstrated that TNFα-dependent factors have converted latent breast cancer to symptomatic disease in a mouse model [25]. Both of these factors may activate cancer cells in the bone niche [25]. Conceivably, estrogen deficiency could thereby unmask latent disseminated breast cancer cells in the bone environment, making them susceptible to death from high local ZA concentrations.

Inhibin activity in bone – role in zoledronic acid efficacy?

Hormones other than steroids could engender a tumor microenvironment that is less responsive to ZA. The gonadal peptides inhibin A and inhibin B, which are highly expressed in premenopausal women but are blocked in postmenopausal or ovarian-suppressed premenopausal women, appear to sustain bone mass. Loss of inhibin has previously been suggested as a factor in the anti-cancer effect of ZA in postmenopausal women [26]. Briefly, decreased inhibin levels in perimenopause are associated with derepression of follicle-stimulating hormone production, deactivation of activins, and bone loss (reviewed in [27]). In both premenopausal women and postmenopausal women, inhibin levels are inversely correlated with bone turnover, independent of estrogen expression. The inhibin-poor environment of postmenopausal women (as in the AZURE, Z-FAST and ZO-FAST trials) or ovarian-suppressed women (as in the ABCSG12 trial) can therefore support the high bone turnover condition in which ZA is most effective (as discussed above in the context of estrogen deprivation). One caveat is that inhibin may not be eliminated from the tumor setting by goserelin or menopause because of breast cancer cell expression of inhibin A (in ductal carcinoma *in situ*) and inhibin B (in primary tumors and metastases) subunits [28]. Gonadal-independent expression of inhibins in the local breast cancer microenvironment could thus potentially offset the significance of endocrine suppression of inhibins. Clearly, additional studies are needed to understand a potential role for the inhibins in ZA activity.

Angiogenesis

Zoledronic acid effects on angiogenesis

Decreased tumor angiogenesis – associated with decreased endothelial proliferation in response to vascular endothelial growth factor (VEGF) – was observed in ZA-treated mice, and ZA-treated patients have decreased circulating levels of the pro-angiogenic molecule VEGF [29]. Indirect effects of ZA on angiogenesis arise from effects of ZA on macrophage polarization leading to a decrease in tumor-associated macrophages that promote vascularization and support circulating VEGF levels [30].

Candidate estrogen effects opposite to zoledronic acid on angiogenesis

Whereas ZA treatment decreased serum VEGF *in vitro* and *in vivo*, estrogen is known to cause transient upregulation of VEGF production in noncancerous cells [31], and to cause sustained low-level VEGF expression by cancer cells themselves *in vitro* and *in vivo* [32]. In mouse tumors comprised of species-specific breast cancer and stromal cells, Saarinen and colleagues demonstrated estrogen induction of the angiogenic cytokines in the stroma, implicating these as mediators of host angiogenesis [33]. In addition, estrogen has been reported to upregulate α_5-integrin [34] whereas ZA downregulates these integrins and adhesion in endothelial cells [35].

Immunity

Effects of zoledronic acid on macrophage polarization

While tumor infiltration by macrophages is common, the function of these macrophages can either be immune suppressive and tumor promoting (M2 phenotype) or be tumor suppressive (M1 phenotype). ZA seems to promote the tumor-suppressive phenotype; for example, it promoted a switch of pro-tumorigenic M2 macrophages in co-culture with prostate cancer cells to M1 polarization [36]. In an erb-B2 mouse model, Coscia and colleagues have shown that the ability of ZA to inhibit cancer was correlated with its ability to impair the recruitment of macrophages into tumors and to support M1 polarization of macrophages in tumors as manifested by decreased IL-10 and increased IFNγ production [30]. The contribution of macrophages to the anti-cancer effect of bisphosphonates has been reviewed recently [37].

Candidate estrogen and progesterone effects opposite to zoledronic acid on macrophage polarization

Estrogen has been posited as supportive of the M2 phenotype [38], consistent with reports that it downregulates the M1-promoting cytokine migration inhibitory factor [39]. Like estrogen, progesterone has been

found to promote alternative activation of macrophages [40]. Whereas ZA repolarizes macrophages to nitric-oxide-producing M1 macrophages, progesterone has been found to downregulate nitric oxide synthase activity in bone marrow macrophages [41]. Additional studies are necessary to fully understand the potential interaction between sex steroids, effect of ZA, and macrophage polarization.

Zoledronic acid effects on γδ T cells and natural killer cells

ZA increases the immunogenicity of cancer cells by increasing presentation of the prenyl phosphate antigens isoprenyl pyrophosphate and ApppI (resulting from isoprenyl pyrophosphate–AMP binding) on the cell surface [42]. Prenyl phosphate antigens promoted anti-tumor immunity by activating the tumor-suppressive γδ T-cell subset. γδ T-cell expansion and activation has been confirmed in cancer patients after ZA administration [43], leading to a phase I trial of ZA plus IL-2 to augment γδ T-cell activity in women with late-phase breast cancer. In addition to promoting IFNγ production by γδ T cells, ZA has been shown to induce IFNγ production by natural killer (NK) cells [44]. Anti-cancer effects of ZA have been eliminated in a mouse breast cancer model when IFNγ was knocked out [30]. The relative importance of macrophage-generated, NK cell-generated or γδ T-cell-generated IFNγ in ZA effectiveness against cancer is unknown.

Candidate progesterone and estrogen effects opposing zoledronic acid on γδ T cells or NK cells

Although limited data are available, there is preclinical evidence that estrogen can increase the growth of ER-negative tumors in immunodeficient mice by suppressing NK-cell cytotoxicity [45]. Estrogen has also been shown to increase levels of granzyme B inhibitor, leading to resistance of cancer cells to killing by NK cells [46]. There is also increasing evidence for progesterone playing a role in the regulation of γδ T cells. While expression of progesterone receptors on lymphocytes (particularly γδ T cells) has been studied primarily in pregnancy, signal transduction through progesterone receptors has recently been reported in peripheral T cells [47]. It has been hypothesized that responsive T cells could produce progesterone-induced blocking factor, an inhibitor of NK cell function [48]. Although far from established, whether progesterone or other sex hormones could oppose the activation of NK cells by ZA would be interesting.

Cell migration and invasion

Inhibition of cell migration by zoledronic acid

ZA decreases the migration of mesenchymal stem cells *in vitro* and lowers their production of CCL5 chemokine [49]. Mesenchymal stem cells migrate from bone marrow to the primary tumor, where they are induced to produce CCL5 that promotes breast cancer migration and metastases [50] – suggesting that anti-tumor effects of ZA could be mediated in part through suppression of mesenchymal stem cell movement and activity. ZA also decreases breast cancer invasion [51] and endothelial cell migration [52].

Promotion of migration by estrogen

In contrast to ZA, estrogen has been noted to increase endothelial cell migration [53]. Estrogen can directly increase cancer cell migration and also can increase the ability of mesenchymal stem cells to promote the migration of ER-positive MCF7 breast cancer cells [54]. These pro-migratory activities would probably be antagonized by ZA, however, because the migration is supported by prenylation of Rho and Rac [55].

Growth factor milieu

Zoledronic acid effects on pro-tumorigenic growth factor signaling

ZA inhibition of Ras prenylation is compatible with suppression of transduction by proliferative cytokines and mitogens. ZA has been reported to alter breast cancer cell responsiveness to growth factors *in vitro*. Fromigue and colleagues demonstrated that 1 μM ZA blocked the ability of insulin-like growth factor-1 and insulin-like growth factor-2 to support the survival of breast cancer cells cultured in serum-free medium [56]. This blocking could be critical since insulin-like growth factors are among the cytokines that are upregulated in the metastatic milieu. ZA has also been reported to inhibit the production of hepatocyte growth factor by macrophages [57]; given that hepatocyte growth factor supports breast cancer cell invasion, chemoresistance and DNA repair capability, this process could also be involved in modulation of the extra-osseous anti-tumor activity of ZA.

Candidate estrogen effects opposite to zoledronic acid on pro-tumorigenic growth factors

There is a well-established crosstalk between estrogen signaling and growth factor pathways, including reports on estrogen-mediated induction of a number of growth factors in peritumoral stroma. For example, estrogen has been noted to induce hepatocyte growth factor secretion by macrophages [58] and mammary fibroblasts [59]. Hepatocyte growth factor signaling through the c-met receptor activates mitogen-activated protein kinases independently of Ras, and could represent a rescue pathway around Ras inactivation by ZA [60].

The Kuperwasser group provides another example of indirect tumor-promoting effects of hormones acting on stroma or marrow rather than on cancer cells themselves.

Briefly, they showed that estrogen can promote growth of ER-negative tumors in a stromal-dependent fashion. Interestingly, enhanced growth of ER-negative breast cancer was transferable with bone marrow from estrogen-treated mice [61]. The relevance of these studies to human disease is still somewhat unclear given that the benefit of hormonal blockade as monotherapy clearly depends on the ER expression in the patient's tumor cells.

It is conceivable that cancer recurs in premenopausal women taking ZA because of enhanced formation of peripheral metastatic niches. The establishment of the peripheral metastatic niche has been linked to pro-tumorigenic conversion of stroma resulting from granulin-producing bone marrow cells [62]. Estrogen has been shown to upregulate pro-granulin expression in tumor cells and nontumor cells [63]. Conceivably, estrogen could increase granulin levels in migrating marrow cells to increase visceral metastasis. Further studies to determine the effect of ZA and sex hormones on the mobilization and function of granulin-expressing cells could be informative.

Resistance to zoledronic acid

There are limited reports of ZA-resistant cell lines arising from long-term low-dose exposure to ZA. An MCF-7 cell line resistant to ZA exhibited cross-resistance to several chemotherapeutic agents, and expressed an increased Bcl2/Bax ratio and increased ABC transporters BRCP and LRP [64]. Analysis of ZA-resistant osteosarcoma cells arising from culture in low-dose ZA disclosed a farnesyl diphosphate synthase-dependent resistance mechanism in one instance and a heat shock protein-27-dependent mechanism in the other [65,66]. These few reports suggest that intrinsic cancer cell resistance to ZA may be multifactorial. Whether extrinsic resistance to ZA arises through restoration of pro-tumorigenic paracrine or juxtacrine factors in the tumor microenvironment is unknown.

Estrogen modulation of hsp27

Estrogen has been shown to transcriptionally upregulate the hsp27 chaperone in both cancer cells [67] and osteoblasts [68], bolstering cell survival in the presence of apoptotic stimuli. Given the finding that hsp27 was required for acquired resistance of osteosarcoma cells to ZA [66], it is possible that heightened hsp27 in premenopausal women contributes to ZA resistance among those not treated with anti-endocrine therapy.

Estrogen and the prenylation pathway

It is worth considering whether estrogenic environments directly interfere with downstream signals of ZA. Dalenc and colleagues showed that neither tamoxifen nor the pure anti-estrogen ICI182780 (fulvestrant) affected farnesylation [69]. The ability of ZA to interfere with prenylation is therefore unlikely to differ in estrogen-rich and estrogen-poor environments.

Chemoresistance and integrin-5

No datasets that profile ZA-sensitive and ZA-resistant stroma or tumors are currently available to analyze for enrichment of estrogen-related pathways linked to ZA resistance. Although knowledge of estrogen-induced changes in stroma that cause chemoresistance is limited, much is known about the stromal role in supporting cancer resistance to chemotherapy. Stromal gene signatures have been identified that predict resistance to neoadjuvant chemotherapy for breast cancer [70]. To interrogate this signature for estrogen-directed transcripts, we conducted gene ontogeny and pathway analysis using GeneGo (Metacore, Inc., Philadelphia, PA, USA) software (RAS, unpublished observations). Our analysis indicated the strongest association of this resistance signature with signaling through α_5-integrins (Z score = 144, $P = 10^{-84}$). As noted earlier, integrin 5 is down-regulated by ZA and transcriptionally upregulated by estrogen.

Conclusion and questions for the future

While the mechanism of action is not yet known, adjuvant treatment with ZA does appear to improve DFS and overall survival in early-stage breast cancer patients with ovarian suppression. ZA effectiveness as an anti-tumor (as well as anti-osteolytic) agent could lead to broader incorporation of ZA (and next-generation bisphosphonates) in clinical practice. Optimal use of ZA in cancer will depend on identification of patient subgroups most likely to benefit, on delineation of mechanisms of ZA anti-tumor action that are consistent with its pharmacokinetics, and on identification of agents that can be optimally combined with ZA and/or can overcome resistance of cancers to ZA action. Progress will depend on correlated preclinical and clinical studies that uncover whether previously reported effects of ZA (for example, on γδ T-cell expansion, on circulating VEGF) differ as a function of hormonal status.

We note that there is support in the literature for complex and multifactorial interaction between ZA and hormones both within and outside the bone environment. Estrogen, acting through osteoblasts, could support a dormant tumor cell niche in bone, enabling disseminated cells to survive high intra-osseous ZA levels. ZA and estrogen could have antagonistic effects on cytokine stimulation of angiogenesis, on tumor-promoting or suppressing activation of macrophages, or on the mobilization and function of NK cells acting on tumors. These examples of candidate ZA/hormone interactions are summarized in Figure 1.

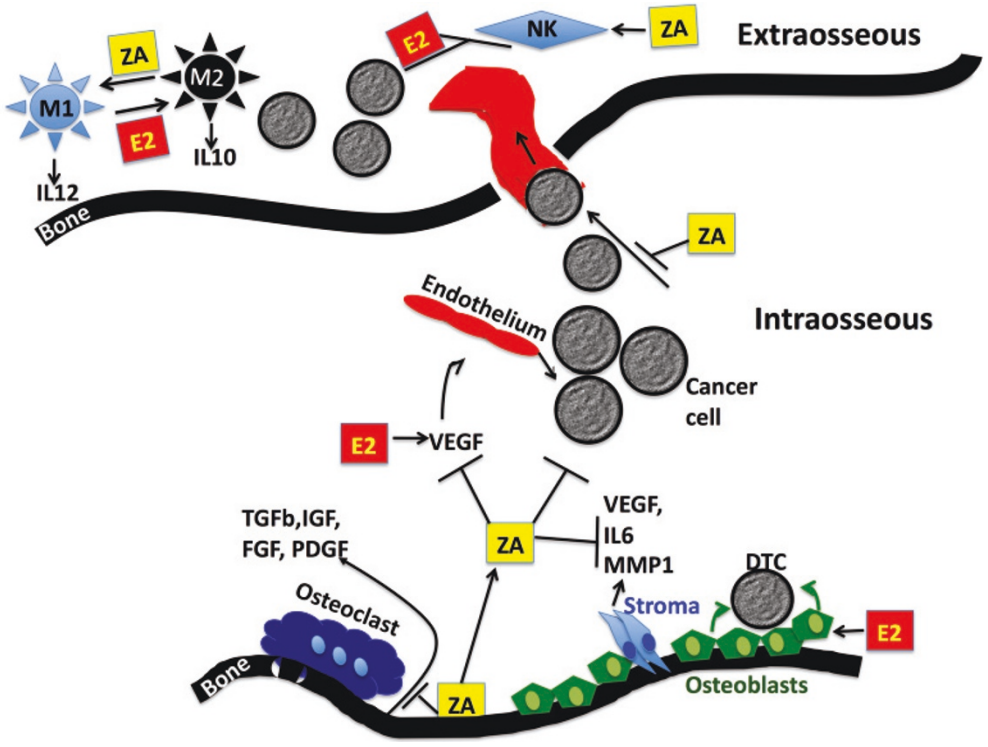

Figure 1. Potential sites of estrogen antagonism of zoledronic acid function. Bone-bound zoledronic acid (ZA) poisons osteoclasts and inhibits liberation of matrix-bound cytokines. Cytokines induced by stroma or macrophages are also inhibited by ZA but can be upregulated by estrogen. Estrogen also increases numbers of endosteal osteoblasts that can support disseminated tumor cells. Tumor cell migration to extra-osseous sites could be suppressed by anti-angiogenic and anti-migratory effects of ZA, while estrogen support of angiogenesis could promote cancer cell proliferation and dissemination. At extra-osseous sites, estrogen and ZA are proposed to have opposing effects on macrophage polarization and natural killer (NK) activity as described in the text. ZA up to 1 mM intra-osseous and 1 μM extra-osseous concentrations. DTC, quiescent disseminated tumor cell; E2, estrogen; FGF, fibroblast growth factor; IGF, insulin-like growth factor; M1 or M2, macrophages polarized to M1 or M2, respectively; MMP1, matrix metalloproteinase-1; PDGF, platelet-derived growth factor; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

One clinically relevant question is whether hormone-sensitive anti-cancer effects are specific to ZA or are a class effect or a general effect with bisphosphonates. The recently published NSABP-34 trial assigned women with operable primary breast cancer to adjuvant placebo or to the non-nitrogen-containing bisphosphonate clodronate for 3 years. Similar to the AZURE trial, no benefit in DFS or overall survival was detected in the clodronate arm, whereas treatment with clodronate increased DFS and the metastasis free-interval in the subset of women aged over 50 [71]. Clodronate lacks the ability of ZA to inhibit the mevalonate pathway, so this trial could highlight a different, common, endocrine-sensitive effect of these bisphosphonates.

Other clinically relevant questions include the following. Do other compounds that suppress bone turnover, such as denosumab, have this same effect, despite suppressing bone via a different mechanism? Are there patient-specific factors other than hormonal status that predict benefit from ZA? Do the benefits of adding ZA to endocrine therapy in postmenopausal women equal the benefits of adding chemotherapy for these women? Is there an additive or synergistic effect from ZA, endocrine therapy, and chemotherapy? Answers to these questions should help to integrate therapy with ZA into the standard of care for early-stage breast cancer.

Abbreviations

ABCSG12, Austrian Breast & Colorectal Cancer Study Group 12; AZURE, Adjuvant Zoledronic Acid to Reduce Recurrence; DFS, disease-free survival; ER, estrogen receptor; IFN, interferon; IL, interleukin; NK, natural killer; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; ZA, zoledronic acid.

Competing interests

The authors declare that they have no competing interests.

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