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Current Perspective

Adjuvant bisphosphonates in breast cancer: Are we witnessing the emergence of a new therapeutic strategy?

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ABSTRACT

Great strides have been made over the last 20 years in the treatment of breast cancer and, despite an increasing incidence, the number of deaths has fallen sharply since the late 1980s. The widespread use of new adjuvant therapies including trastuzumab, taxanes and aromatase inhibitors should decrease this even further. However, for women, metastatic breast cancer still remains the number one cause of cancer death in Europe, and the detection and treatment of micrometastatic disease represent the most important challenge in breast cancer management.

Bone is the most frequent site of distant relapse, accounting for 30–40% of all first recurrence. In addition to the well-recognised release of bone cell activating factors from the tumour, a wealth of pre-clinical data indicates that the release of bone-derived growth factors and cytokines into the microenvironment can both attract cancer cells to the bone surface and facilitate their growth and proliferation. Bisphosphonates are potent inhibitors of bone osteolysis and may interrupt this so-called 'viscous cycle', thereby impeding both the development of bone metastases and the survival of dormant cells in the marrow microenvironment for the subsequent dissemination to extra-osseus sites. Additionally, the potent amino-bisphosphonates may also have direct effects on tumour cells, especially when administered in combination with chemotherapy.

Clinical trial results with the early oral bisphosphonate and clodronate were judged to be inconclusive but the recent data with zoledronic acid suggest that bone targeted treatments may indeed modify the course of the disease. The results of ongoing large metastasis prevention trials are however, required before routine use of adjuvant bisphosphonates can be recommended.

1. Introduction

Approximately 1.15 million new cases of breast cancer are diagnosed each year, more than the combined incidence of colorectal and cervix uteri cancer — the second and third most common cancers in women. Breast cancer is also the leading cause of cancer mortality amongst women, accounting for approximately 411,000 deaths each year.[1] With the lifetime risk of developing breast cancer as high as 1 in 8 women in some Western countries, and a 5-year prevalence of

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approximately 4.5 million cases worldwide, the global burden of breast cancer is clearly substantial.[2]

Although most patients present with disease that appears to be localised to the breast, a significant proportion of women will eventually develop metastatic breast cancer. Therefore, new strategies for metastasis prevention are required which modify the tumour microenvironment by, for example, either inhibiting vascularization or, of relevance to this review, rendering the host environment less conducive to the survival of tumour cells. Bone is the most frequent site of distant relapse, accounting for 30–40% of first recurrence and up to 80% of patients with metastatic breast cancer will develop bone secondaries.[3] Targeting the bone microenvironment is therefore, an attractive therapeutic strategy.

2. Pathophysiology of bone metastases

Bone is not an inert organ. During adult life, normal bone undergoes a continuous remodelling process of resorption and formation. This is normally a tightly coordinated process regulated by multiple genes in which initial osteoclast resorption takes place in discrete 'packets', known as bone remodelling units.[4] This is followed by a more prolonged phase of bone formation mediated by osteoblasts to repair the defect. During this period, new osteoid tissue is laid down and calcium and other minerals are deposited giving bones their hardness and final form. When healthy, there is a steady-state balance, or 'coupling' of osteoclastic bone resorption and osteoblastic bone formation.

Balanced coupling of osteoblastic bone formation and osteoclastic bone resorption is lost when tumour cells enter the bone microenvironment. Cancer cells produce a range of growth factors and cytokines that increase osteoclast activity through release from osteoblasts to stromal cells of receptor activator of nuclear factor kappa beta (RANK) ligand.[5] Within normal bone, the secretion of osteoprotegerin (OPG) by osteoblasts neutralises RANK ligand, and prevents the stimulatory effects of RANK ligand on osteoclasts. However, in bone harbouring cancer cells there is a loss of this regulatory control. The imbalance is further compounded by the release of transforming growth factor-beta (TGF-β) and insulin-like growth factor type one (IGF-1) by resorbing bone that in pre-clinical models have been shown to attract circulating cancer cells to the bone surface. These growth factors also facilitate the tumour cells' growth and proliferation[6] (Fig. 1), and have been suggested to act as survival factors for breast cancer cells, further promoting tumour production of bone cell activating growth factors and cytokines,[8] and thus creating a vicious cycle of osteolytic bone destruction.[7] Interrupting these interactions would be expected to impede both the development of bone metastases and the survival of dormant cells in the marrow microenvironment, thus leaving fewer viable cells available for the subsequent dissemination to extra-osseus sites.

3. Pharmacology of bisphosphonates

Bisphosphonates are stable analogues of pyrophosphate in which the oxygen atom linked to two phosphate groups (P–O–P) is replaced by a geminal central carbon atom (P–C–P) to prevent hydrolysis. Side chains R^1 and R^2 are attached to the carbon atom. The R^1 chain influences the bisphosphonates' affinity for bone, acting as the 'bone hook' and the structure of the R^2 portion determines the anti-resorptive ability.

Bisphosphonates bind avidly to hydroxyapatite bone mineral surfaces, especially within resorption cavities. Osteoclast production of acid and lysozymes leads to profound local changes in pH, promoting the release of the bisphosphonate

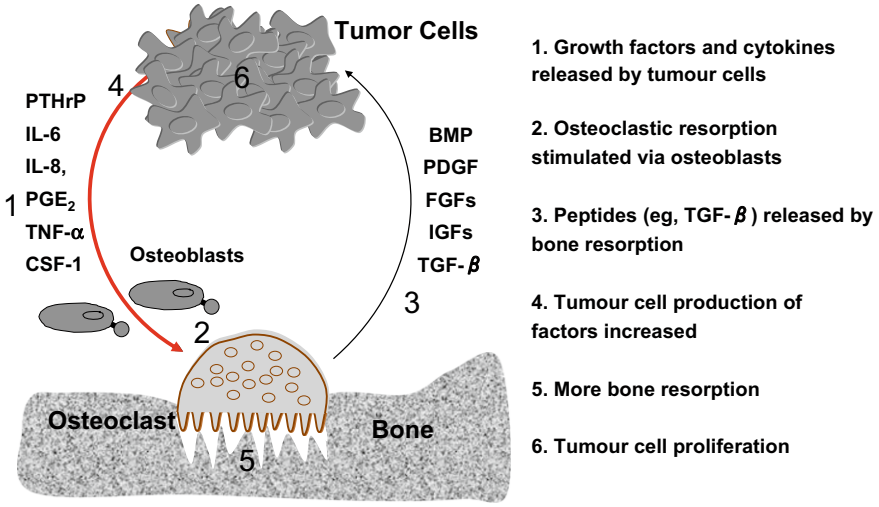

Fig. 1 – Cartoon of interactions between tumour cells and bone cells (osteoblasts and osteoclasts) in the bone microenvironment. Tumour derived growth factors may include parathyroid hormone related protein (PTHrP), interleukins 6 (IL-6) and 8 (IL-8), prostaglandin E2 (PGE2), tumour necrosis factor alpha (TNF-a) and colony stimulating factor 1 (CSF-1). Bone derived growth factors include transforming growth factor beta (TGF-b), insulin-like growth factor type one (IGF-1), fibroblast growth factors (FGF), platelet derived growth factor (PDGF) and bone morphogenic proteins (BMPs).

from the bone surface to the subsequent uptake by local osteoclasts, primarily through endocytosis, where they inhibit cellular activity and promote apoptosis.[8]

Nitrogen-containing bisphosphonates inhibit the mevalonate pathway, the main target being farnesyl diphosphate (FPP) synthase.[9] Inhibition of the mevalonate pathway leads to a loss of important prenylated proteins which are required for post-translation lipid modification (that is, prenylation) of signalling GTPases, such as Ras, Rho and Rac. These regulate a variety of key osteoclast cell functions including the control of endosomes, integrin signalling, membrane ruffling and control of cell morphology. Loss of these proteins leads to the induction of osteoclast apoptosis. In addition build up of proximal metabolites in the mevalonate including the ATP analogue known as ApppI may have positive effects on immune function including the expansion of V9γΔT lymphocytes.[10] By contrast, non-nitrogen-containing bisphosphonates are metabolically incorporated into non-hydrolysable analogues of ATP, ultimately, albeit at high concentrations, also leading to osteoclast apoptosis and inhibition of osteolysis.

For over a century bisphosphonates have been utilised for a range of industrial processes that take advantage of their property of inhibiting calcium carbonate precipitation, such as descaling agents and toothpaste additives. However, it was not until 1968 that bisphosphonates were shown to have biological effects. Many bisphosphonates have been investigated in the clinic and a number of them are commercially available today for the treatment of bone disease. In oncology zoledronic acid, pamidronate, ibandronate and clodronate are the most widely used agents, whilst alendronate and residronate currently dominate in the treatment of benign bone diseases.

4. Potential anti-tumour effects of bisphosphonates

There is increasing evidence accumulating that bisphosphonates may directly effect tumour cells, in addition to their known effects upon osteoclasts.[11] The potency of anti-tumour effects *in vitro* generally mirrors the potency of the anti-resorptive ability. Bisphosphonates induce apoptosis of tumour cells and inhibit tumour cell growth *in vitro* of a wide variety of tumour cell types.[11] Nitrogen-bisphosphonates also inhibit adhesion and invasion of various human tumour cell lines onto and into the bone matrix.[12] The mechanism underlying the induction of tumour cell apoptosis and inhibition of invasion also appears to be primarily through the inhibition of the mevalonate pathway.[11]

More recently, possible anti-angiogenic effects of bisphosphonates have been discovered, neo-angiogenesis being a prerequisite for cancer cell growth and spread. Osteoblastic cells in the bone marrow produce both vascular endothelial growth factor (VEGF) and basic fibroblastic growth factor (b-FGF) and vascularisation is needed for osteoclastic bone resorption. Santini et al.[13] observed that pamidronate was able to induce significant decreases in serum VEGF of cancer patients with a variety of solid tumours that had spread to bone. Zoledronic acid and pamidronate have also been shown to decrease b-FGF and to a lesser extent, VEGF-induced proliferation of vascular tissue in a murine soft tissue model of angiogenesis.[14] This indicates that nitrogen-bisphosphonates may have anti-angiogenic potential outside the bone microenvironment, with zoledronic acid the more potent inhibitor of the two compounds on angiogenesis.

Of particular clinical interest is the potential for bisphosphonates to enhance the anti-tumour activity of cytotoxic agents.[15] Additionally, we have shown that clinically relevant concentrations of doxorubicin and zoledronic acid induce sequence-dependent synergistic apoptosis of cancer cells.[16] Consistently, and across several malignant cell lines, enhanced apoptosis and reduced proliferation were seen when cells were treated first with doxorubicin and then exposed to the bisphosphonate 24 h later. The drugs alone, in the reverse sequence and even given synchronously had little or no effects.[16]

Subsequently, we evaluated this observation in an *in vivo* soft tissue tumour model. Again, sequence dependent synergy between clinically relevant doses of doxorubicin and zoledronic acid was observed with complete inhibition of tumour growth associated with evidence of enhanced apoptosis and reduced proliferation and angiogenesis.[17] The clinical relevance of these observations is now being addressed in a neo-adjuvant study in breast cancer. Patients are randomised to anthracycline based chemotherapy alone, or followed 24 h later by an infusion of zoledronic acid 4 mg. Acute changes in apoptosis, proliferation and angiogenesis are being evaluated in serial biopsy samples.

5. Adjuvant breast cancer trial experience with bisphosphonates

5.1. *Clodronate*

The early pre-clinical observations[18] formed the basis for three randomised clinical trials with oral clodronate in the adjuvant setting in the 1990s.[19–22] In addition there have been several trials investigating the potential role of bisphosphonates as secondary adjuvant therapy[23,24] in patients with locoregional-recurrence or extraskeletal metastases to try and delay or prevent the subsequent development of bone metastases.

In the largest adjuvant trial, Powles et al.[19] evaluated oral clodronate in 1069 patients with primary operable stages I–III breast cancer. Patients received oral clodronate (1600 mg/day) or placebo for 2 years, starting within 6 months of primary treatment (surgery, radiotherapy and tamoxifen). In the most recent analysis oral clodronate significantly reduced the risk of bone metastases compared to placebo both during the 2 year medication period (HR = 0.546, $P = 0.031$), and at the planned analysis time of 5 years (HR = 0.692, $P = 0.043$). Oral clodronate also significantly improved overall survival (HR = 0.768, $P = 0.048$). Clodronate was generally well tolerated, with mild-to-moderate diarrhoea being the most frequently reported adverse event.

In an earlier study from Germany, Diel et al.[20] recruited 302 patients with primary breast cancer who also had immunocytochemical evidence of malignant epithelial cells in the bone marrow, a known risk factor for distant metastases. Patients were randomly assigned to receive clodronate at a dose of 1600 mg per day orally for 2 years (157 patients) or standard

follow-up (145 patients). The incidence of both osseous and visceral metastases was significantly lower in the clodronate group than in the control group ($P = 0.003$ for both osseous and visceral metastases). In a re-analysis at a follow-up time of 103 ± 12 months,[21] the incidence of osseous and visceral metastases was found to be similar in both groups. Although the advantage in terms of disease-free survival with clodronate was no longer statistically significant, a significant overall survival advantage for the clodronate-treated group persisted ($p < 0.01$).

In a third study Saarto et al.[22] randomised 299 women with node-positive breast cancer to oral clodronate 1600 mg daily for three years ($n = 149$) or a control group ($n = 150$). Unlike in the other two studies, a preliminary analysis suggested that clinical outcomes including overall survival were worse in the clodronate treated patients. However, more recent data from this study with a ten year follow-up period were more reassuring. Here the incidence in bone metastases was similar (44 (32%) versus 42 (29%)), although the frequency of non-skeletal recurrences (visceral and local) remained higher in the clodronate group. There is no obvious biological rationale for a worsening of outcome with an adjuvant bisphosphonate to explain these results. An imbalance in prognostic factors and a somewhat unconventional use of endocrine treatments due to secondary randomisations, plus the small size of the trial are more plausible reasons for the outcomes observed. Significantly more patients with ER negative and progesterone receptor (PR) negative cancers (and therefore, a worse prognosis) were randomised to the clodronate group compared to the control group. Indeed, multivariate analysis of factors influencing outcomes showed that study treatment no longer had a negative influence on outcome.

5.2. *Pamidronate*

Several non-randomised trials of intravenous pamidronate have suggested this agent may inhibit the development of bone metastases.[25,26] However, the study designs make interpretation of the findings very difficult. More recently the results of a randomised evaluation of oral pamidronate on the adjuvant setting were published.[27] No benefits associated with this oral bisphosphonate in a study of over 1000 patients could be identified. However, there is considerable uncertainty surrounding the bioavailability of the formulation used. Indeed the development of oral pamidronate was halted in the late 1990s due to the lack of efficacy in advanced cancers such as multiple myeloma, clinical settings where other bisphosphonates, both oral and parenteral, have shown clear benefits.

5.3. *Zoledronic acid*

This is the most potent bisphosphonate and is widely used in the treatment of metastatic bone disease from a range of tumour types. Several pilot studies have suggested that this agent is able to clear disseminated tumour cells (DTC) from the bone marrow (Fig. 2), although the clinical relevance of these observations is uncertain.[28,29]

Recently, the Austrian Breast Cancer Study Group (ABCSG) has reported results from an adjuvant study (ABCSG XII) in good prognosis endocrine sensitive pre-menopausal breast cancer. In this study all 1800 patients received ovarian suppression with goserelin and were then randomised in a two by two factorial design to receive either tamoxifen or anastrazole, each with or without zoledronic acid 4 mg infusions every 6 months for 3 years.[30]

This schedule of zoledronic acid was selected primarily to evaluate the effects of zoledronic acid on bone mineral density, but in addition disease outcomes were compared. No significant differences in disease-free survival (DFS) were seen between the two adjuvant endocrine strategies. However, the administration of zoledronic acid was associated with a significant 35% improvement in DFS. Intriguingly the benefits (54 events versus 83 events) were not confined to the frequency of bone metastases but extended to all distant meta-

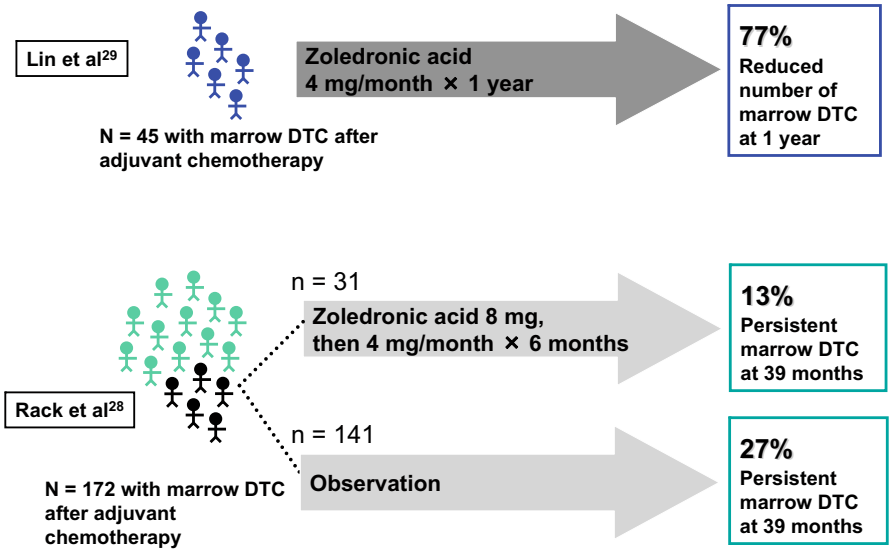

Fig. 2 – Schematic representation of pilot studies evaluating effects of zoledronic acid on bone marrow disseminated tumour cells (DTCs). Studies by Lin et al.[29] and Rack et al.[28] suggest antitumour-effects within the bone microenvironment.

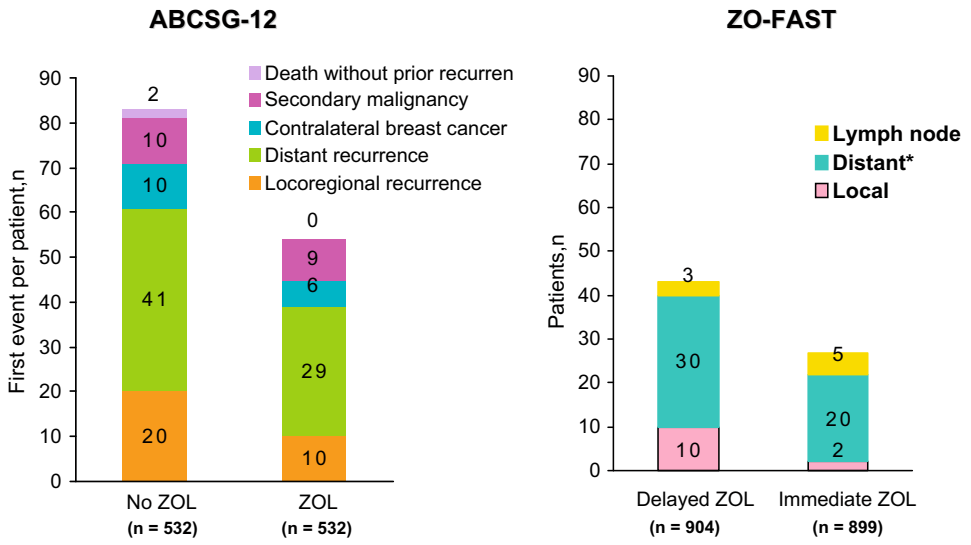

Fig. 3 – Summary of results from the ABCSG-12 and ZO-FAST studies evaluating endocrine therapy +/− zoledronic acid 4 mg every six months. In both studies treatment with zoledronic acid was associated with a reduction in both distant and loco-regional recurrence rates.

static sites and also loco-regional recurrence rates (Fig. 3A). The mechanisms underlying such an effect are unclear but the most likely explanation is an inhibitory effect on dormant tumour cells in the bone marrow microenvironment which have the potential to not only cause bone metastases but also the subsequent spread to other sites. Direct anticancer effects seem unlikely with such infrequent dosing and in the absence of chemotherapy. The impact of 6 monthly zoledronic acid on immune function is unknown.

Supportive data have been presented from the Z-FAST/ZO-FAST studies that, although not fully published, suggest a similar improvement in DFS with the same 6 monthly administration of zoledronic acid in an endocrine sensitive population of post-menopausal women.[31,32] Here, patients received endocrine treatment with letrozole plus either immediate treatment to prevent bone loss with zoledronic acid or delayed treatment if required due to a rapid bone loss or osteoporotic fracture. In a non-protocol specified or event driven analysis performed after 3 years of follow-up a 41% risk reduction for recurrence was reported (22 DFS events versus 40 DFS events). Again benefits extended across both distant and loco-regional sites (Fig. 3).[32]

Toxicity with 6 monthly zoledronic acid is negligible and no cases of osteonecrosis of the jaw (ONJ) have been reported in either ABCSG XII or the Z-FAST/ZO-FAST studies.[30–32]

5.4. *Ongoing adjuvant trials*

Despite the encouraging data with oral clodronate, clarification on the role of clodronate was required if regulatory approval or routine use were to be considered. A large randomised controlled trial (B-34) run under the auspices of the National Surgical Adjuvant Breast and Bowel Project (NSABP) has completed accrual. The study compares 1600 mg/day of oral clodronate with placebo on disease progression in 3400 patients with stages I–III breast cancer. Due to the large number of stage I patients included, the event rate is very low but ultimately this study should provide the definitive assessment for the role of clodronate in this setting.

The adjuvant zoledronic acid to reduce recurrence (AZURE) trial has randomised 3360 relatively high risk patients with stage II (node positive) or III breast cancer. Recruitment was completed in early 2006. This study evaluates DFS and bone metastasis free survival in patients treated with standard anticancer therapy alone compared to those treated with standard therapy plus zoledronic acid (4 mg), administered monthly for 6 doses, every 3 months for eight doses, and then every 6 months for five doses to complete 5 years of treatment. Other end-points include overall survival and the incidence of skeletal morbidity.

The added potency of zoledronic acid may have beneficial effects, not only through the inhibition of bone resorption and reduction in growth factors and cytokines in the bone marrow microenvironment which appear to promote the development of a metastasis, but also through direct effects on tumour cells in the bone marrow and possible synergy with adjuvant chemotherapy. Indeed a retrospective evaluation of neoadjuvant response rates within the breast has suggested that zoledronic acid does add to neoadjuvant chemotherapy with a higher pathological complete remission rate and smaller residual tumours in those with incomplete response.[33] Efficacy data in terms of effects on DFS are expected in 2010. Zoledronic acid does not appear to have significantly increased the toxicity of adjuvant treatments in AZURE,[34] although there is a low incidence of osteonecrosis of the jaw associated with the intensive schedule used in this study. To date nine cases of ONJ have been confirmed.[35]

The South West Oncology Group (SWOG) is recruiting to a large randomised three-arm trial (SWOG 0307/Intergroup) designed to identify the best choice of bisphosphonate between intensive intravenous treatment and two oral strategies. Centres throughout North America are randomising 4500 patients with stages I, II or IIIA breast cancer to receive one of i.v. zoledronic acid (4 mg via a 15-minute i.v. infusion every month for

Table 1 – Summary of ongoing metastasis prevention studies with zoledronic acid showing study acronym, number and type of patients, study treatment and primary end-point. BC, breast cancer; PC, prostate cancer; NSCLC, non-small cell lung cancer; ZOL, zoledronic acid; Tam, tamoxifen; ANA, anastrozole; IBA, ibandronate; CLO, clodronate; FEC-DOC, fluorouracil, epirubicin, cyclophosphamide followed by docetaxel; GEM, gemcitabine; AD, androgen deprivation; ADT, androgen deprivation therapy; DFS, disease-free survival; EFS, event free survival; PSA-RFS, freedom from increase in prostate specific antigen; OS, overall survival.

Name	Patients	Treatment arms	Primary end-point
ABCSG-12	1803 BC pts (Stages I, II)	TAM; ANA; TAM + ZOL (4 mg q 6 mo); ANA + ZOL (4 mg q 6 mo)	DFS at 130 events
AZURE	3360 BC pts (Stages II, III)	Standard therapy + ZOL (4 mg q 1 mo; q 3 mo; q 6 mo)	DFS at 940 events
SUCCESS	3754 BC pts (Stages I, II, III)	FEC + DOC then endocrine therapy + ZOL 3 or 5 year; FEC + DOC + GEM then endocrine therapy + ZOL 3 or 5 year	DFS at 5 years
SWOG 0307	4500 BC pts (Stages I, II, III)	ZOL (4 mg q 1 mo; q 3 mo); CLO (1600 mg q d); IBAN (50 mg q d)	DFS at 3 years
NATAN	654 BC pts (Stages II, III)	Standard therapy + ZOL (4 mg q 1 mo; q 3 mo; q 6 mo)	EFS at 5 years
ZEUS	1,434 PC pts (No distant mets)	ZOL (4 mg q 3 mo); No ZOL	Proportion of pts with bone mets at 4 years
RADAR	1071 PC pts (Stage T2b-4)	Short-term AD + ZOL (4 mg q 3 mo) Intermediate-term AD + zOl (4 mg q 3 mo)	PSA-RFS at 5 years
2419 Study	446 NSCLC pts (Stage III)	ZOL (4 mg q 1 mo); No ZOL	Time to occurrence of bone mets at 2 years
STAMPEDE	3300 PC pts (high-risk)	ADT and 1, No additional therapy; 2, Taxotere; 3, ZOL; 4, Celecoxib; 5, Celecoxib + ZOL; 6, Taxotere + ZOL + Celecoxib	Failure-free survival, OS (multiple phases)

six doses, then every 3 months), oral clodronate (1600 mg/day) or oral ibandronate (50 mg/day). The primary end-point is disease-free survival, and secondary end-points include overall survival, bone mineral density, quality of life and bone markers as predictors of recurrent disease. This study assumes that either AZURE and/or B34 will show an advantage for an adjuvant bisphosphonates and clarify the choice of agent.

Table 1 summarises the large number of ongoing metastasis prevention studies with zoledronic acid in breast, prostate and lung cancers encompassing >20,000 patients. In addition there are ongoing studies with other bone targeted agents including ibandronate and denosumab. Incorporated into the current adjuvant bisphosphonate trials are correlative studies investigating the use of biomarkers that may identify patients at especially high risk for metastases. This could lead to the selection of breast cancer patients who might benefit most from adjuvant bisphosphonates by evaluating tumour characteristics, bone marrow findings or urine or serum markers that predict who is at highest risk for bone recurrence. Bisphosphonates are relatively expensive drugs and a more targeted use would contribute to cost effectiveness.

6. Conclusions

There is a wealth of pre-clinical as well as increasing clinical trial evidence to suggest that bone targeted treatment with bisphosphonates are able to modify the bone microenvironment and thereby reduce the risk of metastasis. There may also be direct antitumour-effects especially when administered with chemotherapy. It seems likely that we are on the threshold of a shift in adjuvant treatment but positive results from AZURE and/or NSABP B34 are required before bisphosphonates can be considered a routine standard of care even in endocrine sensitive breast cancer where the data to date are strongest.

Conflict of interest statement

I have received consultancy fees from Novartis, Amgen, and Pfizer; speaker fees from Novartis, Roche, Pfizer, AstraZeneca, and Amgen; research funding from Novartis and Astra Zeneca, and given expert testimony on behalf of Novartis.

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