Bisphosphonates in the adjuvant treatment of young women with breast cancer: the estrogen rich is a poor candidate!

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ABSTRACT

During the last 2 decades the role of bisphosphonates (BPs) to reduce skeletal-related events from bone metastases in breast cancer has been well defined. Several preclinical studies have strongly suggested that BPs may also provide an anti-cancer effect in early breast cancer. Indeed, the use of adjuvant BPs represents a unique approach that attempts at eradicating occult tumor micro-metastases residing in the bone marrow via targeting the bone microenvironment to render it less favorable for cancer cell growth. Although, this concept has been tested clinically for more than 15 years, no final consensus has been reached as for the routine use of BPs in the adjuvant phase of breast cancer, owing to conflicting results of randomized studies. Nevertheless, accumulating evidence from recent trials has indicated a therapeutic benefit of adjuvant BPs—particularly zoledronic acid—in women with established menopause, with no or perhaps detrimental effects in premenopausal women. Indeed, this hypothesis has opened a new chapter on the role of estrogen-poor microenvironment as a potential pre-requisite for the anti-tumor effects of BPs in the adjuvant phase of breast cancer. In this review, we will emphasize the biological rational of using BPs to target bone microenvironment in patients with early breast cancer and we will explore mechanistic differences; related to bisphosphonates effects in premenopausal versus postmenopausal women and how the endocrine environment would influence the anticancer potential of these compounds.

KEY WORDS

Adjuvant; bisphosphonates (BPs); anti-tumor activity; premenopausal; breast cancer
and promote osteoclast apoptosis. BPs also increase production of osteoprogerin (OPG) by osteoblasts (14). OPG is a secreted soluble receptor, that functions as a decoy receptor for RANKL, which is a pivotal molecule for osteoclastic activation. Hence, OPG is considered as a natural inhibitor of osteoclastogenesis, that induces suppression of physiological and pathological bone resorption (5,6). Of note, BPs are cleared rapidly from the blood stream via their avid binding to mineralized bone and by renal filtration of unbound drug (15). As these agents do not readily cross the plasma membrane, the intracellular concentration of BPs in most tissues is very low.

**Anti-cancer effects of BPs in breast cancer**

Extensive in vitro and animal data suggests that BPs may act as antitumor agents and can reduce skeletal tumor burden (15,16). However, and in view of their high affinity for bone mineral and very low concentration in other tissues, the evidence for their in...
vivo antitumor activity outside the bone is less convincing (17-19). BPs exert direct antitumor effects via inhibition of tumor cell adhesion, invasion, and proliferation, in addition to induction of tumor cell apoptosis (15,16). A major molecular target inhibited by nitrogen containing BP (N-BP) like ZA, pamidronate, and ibandronate is farnesyl pyrophosphate synthase (FPPS), a key enzyme in the mevalonate pathway (20,21). This is an important metabolic pathway required for producing steroids, maintaining cell-membrane integrity, regulating cellular metabolism and is also crucial for the prenylation of regulatory proteins involved in many intracellular signaling pathways that control cell proliferation. Inhibition of the mevalonate pathway will ultimately cause osteoclasts to undergo apoptosis (20,21) (Figure 2). The mevalonate pathway is also an important part of the metabolic and proliferative processes in cancer cells. Compared to other BPs, ZA has been shown to be the most potent inhibitor of FPPS activity in cancer cells, which correlates with its highest anti-osteoclastic activity in vitro and in vivo (22). The N-BPs may also act indirectly on tumor cells through anti-angiogenic (23) and immuno-modulatory mechanisms (24-26). The later is especially attributed to their ability to accumulate in macrophages and monocytes which share the same ontogeny with osteoclasts (24). Therapeutic doses of ZA has been shown to modulate monocyte, macrophage and dendritic cell function and improve the γδ T-cell anti- cancer properties (16,22,25,27).

Although the exact mechanism(s) responsible for the observed anti-tumor effects of BPs remains unclear, recent data from animal studies strongly suggested that the main in vivo effect of clinically relevant doses of BPs on breast cancer cells, is mediated via inhibition of osteoclast-mediated bone resorption rather than a direct cytotoxic effect (28). This supports the argument that tumor growth can be effectively inhibited in the clinic by targeting the bone microenvironment and not necessarily via a direct cytotoxic effect against the primary tumor.
It has been known for a while that dormant tumor cells (DTCs) in the bone marrow (BM), can provide a major source of late relapse in patients with early breast cancer (EBC). A significant correlation between DTC in bone marrow or circulating tumor cells in the blood stream and poor prognosis has been demonstrated in several studies (29). Indeed, the BM microenvironment can provide an ideal sanctuary site for these cancer cells to evade systemic anticancer therapy (30).

Two distinct protective interactions within the bone marrow have been described as an endosteal niche and a vascular niche (31). The endosteal niche allows DTC to interact with osteoblasts, which are critical mediators of stem cell dormancy and survival. The vascular niche facilitates DTC to interact with hematopoietic stem cells. Meads et al. has shown that the hematopoietic stem cell can induce environmentally mediated drug resistance (EM-DR), which protects the tumor cells from the cytotoxic effects of chemotherapy as well as the physiologic mediators of cell death (32). Although, the specific signals responsible for reactivation of DTC are still unclear (33,34), yet it has been postulated that DTC in the BM can be activated by osteoclast-mediated release of bone derived growth factors (34), to form metastases at other osseous and non osseous sites, while serving also as a source of local recurrences (‘tumor self-seeding’ phenomenon) (35).

In several phase II clinical studies, including women with high risk, early-stage breast cancer, both ZA and ibandronate, in combination with standard adjuvant therapy, could effectively reduce DTC number and persistence in bone marrow compared with standard therapy alone (36-39). Although, the prognostic impact of such reduction of DTC has never been addressed in these studies, yet this should definitely bring enthusiasm to incorporate BPs into the adjuvant treatment regimens in EBC, in an attempt to interfere with the unique support that bone microenvironment provides to cancer cell survival. Altering the BM microenvironment by adjuvant BPs therapy would—at least theoretically—render it less conducive to cancer cell survival, and therefore may provide a unique mechanism to prevent cancer recurrence in EBC (16,28,34).

The first generation of clinical studies testing the anti-tumor role of BPs in early breast cancer evaluated oral clodronate in 3 randomized trials. The long term follow-up data have shown conflicting outcome, with 2 studies (48,49) demonstrating a significant benefit at some follow up periods, while in the 3rd trial the ten-year DFS was significantly lower in the clodronate group compared to the control arm (45% vs. 58%, P=0.01, respectively) (50). A meta-analysis of the three trials has shown that clodronate did not provide any significant benefit in bone metastasis-free survival, or DFS (51). Therefore, no real take home message could have been concluded from these trials.

Later on, the ABCSG-12 and the ZO-FAST trials, have strongly concluded for a therapeutic benefit of adjuvant ZA...
in women with poor estrogen microenvironment at the time of their breast cancer treatment. The ABCSG12 study (52), included 1,803 premenopausal women with stage I/II breast cancer, who were randomized to receive 3 years of ZA versus observation, added to endocrine therapy (luteinizing hormone-releasing hormone agonist to suppress the ovarian function and anastrozole or tamoxifen). The study demonstrated a 36% reduction in the relative risk of disease progression among those patients taking ZA. Importantly, and unlike the earlier clodronate studies, the therapeutic gain obtained by ZA was maintained at 84 months median follow-up, with a significant benefit in DFS (HR=0.72; P=0.014) and OS (HR=0.63; P=0.049) (53). The ZO-FAST trial included 1,065 Stage I-IIIa, ER positive postmenopausal patients who were treated with letrozole and were randomized to either immediate or delayed ZA (54). Delayed ZA therapy was administered in case of non-traumatic fracture or crossing a bone loss threshold. At 5 years follow up, a DFS benefit (which was a secondary endpoint) of immediate ZA treatment has been reported (HR=0.66; log-rank P value=0.0375) with a trend for an OS gain (HR=0.69; P value=0.196). Of notice, the patients in the above 2 trials were treated with endocrine therapies known to induce a profound estrogen poor environment and significant bone loss. The patients in the 2 trials have received a small dose of ZA (once/6 months), that was good enough to prevent bone loss in the treated patients (which was a secondary end point for the ABCSG-12 trial and a primary end points for the ZO-FAST trial).

Unfortunately, the 2 studies cannot really answer the question related to the benefit of adjuvant BPs in other adjuvant settings (i.e., in women with estrogen rich microenvironment or in women with ER negative EBC). However, the exclusive benefit of adjuvant ZA in women with estrogen poor environment was subsequently concluded from the Azure trial, which was a randomized phase III study addressing the role of adjuvant ZA (5 years of ZA in a gradual tapering fashion) in chemotherapy treated stage II/III breast cancer. Of notice the Azure study failed to show that adding ZA to chemotherapy improves disease-free survival in the overall patient population (which was its primary endpoint). However, in a pre-specified subgroup analysis, the postmenopausal patients (5 years or more) had an significant DFS benefit with the addition of ZA (Adjusted HR=0.75; 95% CI: 0.59-0.96; P=0.02) (55). The restricted benefit of BPs adjuvant treatment in postmenopausal women was further suggested by 2 subsequent phase III studies: NSABP B-34 (3,323 patients randomized to receive oral clodronate 1,600 mg or placebo daily for 3 years and GAIN trial [3,023 randomized to receive oral ibandronate (50 mg daily for 2 years) or observation] (56,57). In line with AZURE trial, these 2 studies failed to show improvement in DFS, which was their primary end point. Still, again prespecified subgroup analysis suggested that BPs might perform better in patients who are ≥50 years (in NSABP B-34) and ≥60 years (in GAIN ), or in other wards those who would have achieved complete ovarian suppression at the time of BPs treatment.

**Bisphosphonates in the adjuvant treatment of young breast cancer patients: is it ready for a prime time?**

With the exception of the ABCSG 12 and the ZOFAST (and its sister trials Z-FAST and E-ZO-FAST (58,59)), the majority of clinical trials addressing the anti-cancer role of adjuvant BPs in EBC, were designed on “the one size fits all” approach (Table 1) as they included a very heterogeneous patient population in terms of the disease phenotypes, menstrual status, and type of the standard adjuvant treatment given to their patients, which in our opinion was a major reason for their hard to interpret results. Furthermore, the 3 largest studies, AZURE, B34 and GAIN, had used different types of BPs for a variable treatment period (ranging from 2 to 5 years) and adopted different definitions of menopause. This would certainly pose many difficulties towards their combined analysis. Nevertheless, a meta-analysis of these 3 trials together with other 3 trials that specifically evaluated the effects of adjuvant BPs on DFS according to menopausal status was recently presented (60). The authors reported no beneficial effect in the entire population of EBC treated by BPs compared to the control arm, with a significant DFS benefit in the subgroup of women with established menopause [HR=0.81 (0.69-0.95)]. However, an alarming conclusion was made in this meta-analysis, which suggested an apparent harm of adjuvant BPs in pre- and perimenopausal women. Importantly, this observation has been previously highlighted by AZURE study in which there was a significant detrimental effect of ZA on the rate of non-skeletal metastases in premenopausal women, that was independent of the ER status of the tumor [HR=1.32 (95% CI: 1.09-1.59)], and that was never discussed by the authors (55). Interestingly an older Finnish trial had also made a similar conclusion, when clodronate was given in the adjuvant setting, where the frequency of non-skeletal recurrences was significantly higher in the clodronate group versus the control group especially in ER negative patients (DFS at 10 years were 25% vs. 58%, P=0.004, respectively). Importantly, in this particular study, the only subgroup where no adverse effect of clodronate was seen, were postmenopausal ER positive patients (50). Of interest, some preclinical studies have also indicated that adjuvant BPs may enhance the development of non-skeletal metastases, if given without a concomitant anticancer drugs (like the situation in the long term BPs treatment in ER negative breast cancer) (19). This particular observation was strongly emphasized as a worrying issue when BPs are to used in the prevention setting (4,50). Till further evidence emerges, this potentially detrimental effect of adjuvant BPs in premenopausal and/or ER negative EBC could be considered due to chance. Still we wish to raise...
some critical questions in this context: what could be putatively tumor promoting when a high dose of ZA (as adopted in the AZURE) is given in the adjuvant phase of BC in premenopausal women? Is it the estrogen rich microenvironment or is it the ER negative phenotype or both? In fact, there is a lot of potential speculations to explain the lack of response to ZA in estrogen rich microenvironment (61). Of notice estrogen and BPs may interact at the level of BM cancer cell dormancy. The estrogen-rich bone microenvironment appears to better support the survival and expansion of DTC in the endosteal niche. This observation is supported by the findings that estrogen increases the number and activity of endosteal osteoblasts, which are critical mediators of stem cell dormancy and survival (30,62). This may imply that the ability of BPs to decrease DTC is offset by the high level of oestrogen in premenopausal women.

Finally, we believe that the altered immune profile in response
to ZA that may explain a preferential benefit of this drug in relation to the disease phenotype. As mentioned earlier, standard doses of ZA have been consistently reported to induce selective stimulation of γδ T-cells which exert a beneficial anti-tumor function in vivo (16,22,25,26). Clinically, γδ T-cell expansion and activation has been confirmed in cancer patients after ZA administration. Recently, Benzaid et al. (27) showed that only the ER positive, HER2 negative breast cancer cell lines are sensitive to the immune-mediated attack by γδ T-cells. This may suggest that ER positive phenotypes are more likely to have a therapeutic benefit from adjuvant ZA. It may be assumed that premenopausal women have more ER negative disease (data not shown by the AZURE authors), which is less sensitive to γδ T-cell-mediated cytotoxicity.

Another immunologically significant molecule affected by ZA is OPG, which as mentioned earlier is a potent inhibitor of bone resorption. The ability of OPG to inhibit osteolysis suggests that OPG can have an inhibitory effect on cancer-induced bone disease and metastasis (5,6). Both ZA (in a dose dependent fashion) (14) and estrogen have been reported to increase the serum level of OPG (63-65), which is one of the suggested mechanisms for their anti-resorptive function. Interestingly, OPG may promote tumor cell survival though its ability to enhance angiogenesis and to inhibit TRAIL induced apoptosis (66-68). TRAIL (TNF-related apoptosis-inducing ligand) is an important molecule mediating major antitumor effects of the immune system (66). Importantly, in several cancer types, elevated levels of serum OPG were significantly associated with poor prognosis (69,70). Of note, it has been shown that OPG preferentially protects ER negative breast cancer cell lines from TRAIL-induced apoptosis (66-68).

In conclusion, a number of clinical trials and animal studies have strongly suggested that the benefits of adjuvant bone targeted treatments on risks of recurrence or death in EBC are restricted to women with established menopause (72). We strongly believe that this statement is clinically and biologically correct. However, while we are focusing on ‘the estrogen poor soil’, as a prerequisite for a preferential benefit of adjuvant BPs, the properties of ‘the seed’ may be also valuable or even crucial in this context, where the ER positive and not the ER negative breast cancer phenotype may be expected to derive the maximum benefit of these agents. To this end we would certainly recommend the use of low dose of ZA (at 4 mg/6 months) in all ER positive premenopausal women whose treatment regimens includes LHRH agonist, or those who develop complete ovarian suppression following adjuvant chemotherapy. At this dose level of ZA, the associated bone loss will be effectively prevented in the treated patients, which will be the ideal approach to maintain their bone health. Furthermore ZA at this dose can effectively interrupt the cross talk between DTC and the estrogen poor bone microenvironment, a step that has been reported to potentially improve DFS in EBC. Importantly, the ABCSG-12 which is the only study that included a pure premenopausal population (median age 45 years) has recently reported in a preplanned subgroup analyses based on age (≤40 years or >40 years), that ZA significantly improved DFS by 34% in women over 40 years of age (n=1,390; HR=0.66; P=0.013), while it did not improve the DFS in women who were 40 years of age or younger (n=413) (53). The authors have attributed this to the assumption that women over 40 years of age may achieve more complete ovarian suppression. While this statement is certainly valid for women treated by adjuvant chemotherapy, it cannot be applied to the population included in the ABCSG 12 (less than 10% received chemotherapy only during the neoadjuvant phase). Furthermore, the results in women ≤40 years of age were concluded from a total of 77 DFS events at 84 months, which looks as insufficient evidence to preclude ZA benefit in these women. As the anti-tumor effects of adjuvant BPs might be exclusively observed in patients with estrogen depletion and accelerated bone loss, or in other words in those patients with a susceptible soil, then we confidently assume that it is the menopausal status rather than age that will determine the benefit of adjuvant BPs in young women. Taken together, the biological concept that one size does not fit all, seems to be very true when it comes to the role of BPs in premenopausal women with EBC.

Acknowledgements

Disclosure: The authors declare no conflict of interest.
References


