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Breast cancer bone metastasis and current small therapeutics

Muzaffer Cicek · Merry Jo Oursler

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Abstract Patients with advanced breast cancer frequently develop metastasis to bone. Bone metastasis results in intractable pain and a high risk of fractures due to tumor-driven bone loss (osteolysis), which is caused by increased osteoclast activity. Osteolysis releases bone-bound growth factors including transforming growth factor beta (TGF-β). The widely accepted model of osteolytic bone metastasis in breast cancer is based on the hypothesis that the TGF-β released during osteolytic lesion development stimulates tumor cell parathyroid hormone related protein (PTHrP), causing stromal cells to secrete receptor activator of NFκB ligand (RANKL), thus increasing osteoclast differentiation. Elevated osteoclast numbers results in increased bone resorption, leading to more TGF-β being released from bone. This interaction between tumor cells and the bone microenvironment results in a vicious cycle of bone destruction and tumor growth. Bisphosphonates are commonly prescribed small molecule therapeutics that target tumor-driven osteoclastic activity in osteolytic breast cancers. In addition to bisphosphonate therapies, steroidal and non-steroidal antiestrogen and adjuvant therapies with aromatase inhibitors are additional small molecule therapies that may add to the arsenal for treatment of osteolytic breast cancer. This review focuses on a brief discussion of tumor-driven osteolysis and the effects of small molecule therapies in reducing osteolytic tumor progression.

M. Cicek (✉) · M. J. Oursler
Endocrine Research Unit, Mayo Clinic College of Medicine,
Rochester, MN 55901, USA
e-mail: Cicek.muzaffer@mayo.edu

M. J. Oursler
e-mail: Oursler.merryjo@mayo.edu

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1 Introduction

Among women, breast cancer is the most common type of malignancy and is the second overall cause of death in developed countries. According to The American Cancer Society, each year nearly 175,000 women are diagnosed with breast cancer and it is estimated that approximately 43,300 women will die from breast cancer this year. Metastasis to the spine, ribs, pelvis, and proximal long bones are frequently seen pathological lesions in advanced breast cancer, leading to debilitating skeletal complications such as osteolysis, intractable bone pain, and pathologic fracture [1]. Moreover, once breast tumor cells metastasize to bone, mortality increases to 70% [1]. Fortunately, early diagnosis and effective chemical and radiotherapies have significantly reduced the mortality rate from primary breast cancer. However, current therapies remain only palliative for advanced metastatic breast cancer patients and new therapies that specifically target both metastasis to bone and osteolysis are needed. Over one hundred years ago, Stephen Paget [2] proposed the seed and soil hypothesis in which tumor cells (the seeds) travel to tissues throughout the body. These tissues (the soils) provide a spectrum of milieus in which the tumor cells could survive and proliferate. The hypothesis is that different types of tumor cells would be able to expand and colonize different tissues based on the characteristics of the different tumors. Today, molecular evidence supports the seed and soil hypothesis that circulating breast cancer cells lodge in fertile sites such as the bone marrow stroma and commence growth as micro-metastases. As compared to other organs, bone has a

dynamic and complex structure that provides a suitable microenvironment for breast cancer cells, making bone a frequent site of metastasis.

The pathophysiology of osteolysis is dependent on a break-down of the coordination of two predominant cell types, bone resorbing osteoclasts and bone forming osteoblasts [3]. Bone structure is controlled in part by a balance between osteoclastic and osteoblastic activity. These interactions alter bone during growth or maintain bone during adulthood and appear to be subverted by tumor cells lodged in bone marrow. Although most cancers that metastasize to bone are a mixture of osteolytic (bone degrading) and osteoblastic (bone forming), breast cancers tend to be primarily osteolytic. For many years, it was hypothesized that the tumor cells themselves degraded bone to cause osteolysis, but there is now ample evidence that tumors drive bone degradation by stimulating osteoclastic activity [3–5]. Studies have supported that tumors stimulate osteoclasts differentiation, increase the activity of each osteoclasts, and also prolong the life-span of the osteoclasts [4, 5]. Since a significant problem both in terms of patient suffering and advancing tumor progression is caused by tumor-driven osteolysis, reducing osteoclastic activity is an important target in seeking new therapies to slow disease progression. It is clear that therapies that repress osteoclast differentiation and/or target osteoclast survival would be of great benefit in repressing osteolysis and slowing tumor progression.

2 Factors involved in bone metastasis

Breast cancer cells lodged in bone marrow produce multiple factors that influence osteoclast formation and activity [4, 6–10]. During bone resorption, osteoclasts release growth factors including TGF-β, insulin-like growth factors (IGFs), fibroblast growth factor (FGF), platelet derived growth factor (PDGF) and bone morphogenic proteins (BMPs) from bone matrix [11–14]. These factors support further tumor growth by increasing proliferation, survival, and angiogenesis [15, 16]. During normal bone turnover, osteoclast differentiation requires RANKL and M-CSF whereas osteoprotogerin (OPG), a decoy receptor for RANKL, represses differentiation [17–19]. Bhatia et al. [20] documented that RANKL expression was observed in 90% of nonneoplastic breast tissue yet it was observed in only 62% of nonmetastatic infiltrating ductal carcinoma (IDC), 31% of metastatic IDC, and 2% of osteolytic breast cancer bone metastasis. Thus, tumor cell production of RANKL seems unlikely to be a driving force behind tumor-induced increased osteoclastogenesis. The cognate receptor for RANKL, RANK, is expressed in both neoplastic and nonneoplastic tissues [20]. Jones et al. [21] has found that RANKL can stimulate migration of epithelial cancer cells that express RANK. Interestingly, they also found that blocking RANKL in an animal model of tumor metastasis selectively blocked tumor cell metastasis to bone, but not other tissues. Thus the roles of RANKL in tumor progression are complex and many aspects of this remain to be resolved.

There are two possible ways that tumor cells could stimulate osteoclast differentiation without providing RANKL to drive the process. Tumor cells could stimulate local stroma to increase RANKL production or other cytokines could be produced by the tumor cells to drive RANKL-independent osteoclast differentiation. Evidence is accumulating that tumor cells exploit both of these scenarios to increase osteolysis.

Guise et al. [22] documented that PTHrP stimulated osteoblasts and stromal cells to increase RANKL and suppress OPG expression, supporting that tumor-derived PTHrP could indirectly activate osteoclastogenesis via osteoblasts (see Fig. 1). To further examine the role of PTHrP in tumor metastasis, Saito et al. [23] injected an aggressive bone metastatic breast cancer cell line into mice and tested the effects of an anti-PTHrP antibody on tumor development. Bone metastasis progression and associated osteolytic destruction were severely suppressed by the PTHrP antibodies. Dominant negative (dn) TGF-βRII transfected into breast cancer cells reduced TGF-β-mediated stimulation of PTHrP expression and metastasis to bone while over-expression of wild type PTHrP in MDA-MB-231 cells expressing dn TGF-βRII restored bone metastasis [24, 25]. These data support a critical role for TGF-β and PTHrP in tumor progression although data have also suggested that Interleukin-8 (IL-8), not PTHrP, is important in some analyses (see Fig. 1) [26]. Clinical studies of breast cancer patients have found that plasma PTHrP levels are elevated in approximately 50% of patients with hypercalcemia [27, 28]. This raises the possibility that bone metastasis-associated hypercalcemia could be caused by PTHrP-mediated elevated osteoclast differentiation and resulted in increased bone resorption in some patients. To examine whether plasma level of PTHrP could be a biomarker for tumor progression, primary tumors of 526 patients with breast cancers were examined by immunohistochemistry for PTHrP expression and patient survival was tracked for a median 10 year following diagnosis and treatment [29]. Seventy nine percent of the patients with tumors that stained positively for PTHrP had improved survival. Moreover, patients with PTHrP positive primary tumors were less likely to develop bone metastasis. These unexpected results suggested that increased production of PTHrP by breast cancer is correlated with less invasive phenotype. Studies have found that tumor cells express other factors that could stimulate osteoclast formation and

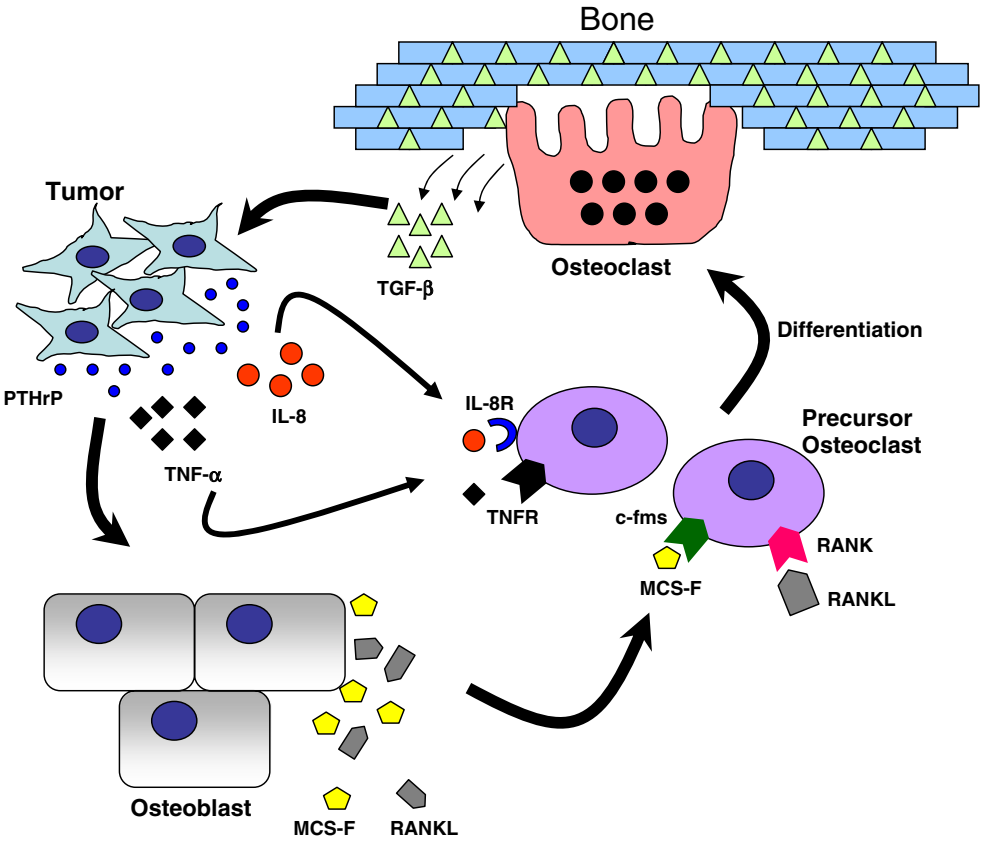

Fig. 1 The widely accepted model of osteolytic bone metastasis in breast cancer is illustrated. The hypothesis that the TGF-β released from bone matrix during osteolysis stimulates PTHrP in tumor cells. Tumor-produced PTHrP stimulates osteoblasts and stromal cells to express RANKL, MCSF and to suppress OPG expression. In addition, tumor-derived TNF-α and IL-8 can also stimulate osteoclast differentiation independent of RANKL. These data may suggest that other factors synthesized by tumor besides PTHrP or RANKL may stimulate osteoclast differentiation and increase osteolysis

subsequently osteolytic activity such as IL-6, IL-8, IL-11, tumor necrosis factor alpha (TNF-α), and macrophage colony stimulating factor (M-CSF) [4, 6–10]. This may support a direct stimulation of osteoclast differentiation as TNF-α and IL-8 can stimulate osteoclast differentiation independent of RANKL [26, 30]. These data may suggest that tumor cell production of other factors besides PTHrP or RANKL may be involved in stimulating osteoclast differentiation.

3 Mechanism of osteolytic breast cancer bone metastasis

Bone marrow has a complex vasculature that provides a conduit for tumor cell access, a mechanism for removal of metabolic waste, and a source for nutrients and growth factors to stimulate growth. In addition, bone is a storage compartment for significant levels of several growth factors, including TGF-β [11, 13]. Although osteoclasts secrete active TGF-β, osteoclast-mediated bone degradation also releases bone matrix-associated TGF-β [31, 32]. As noted above, several studies have documented that TGF-β is a critical growth factor in breast cancer cell metastasis to bone (reviewed in [33, 34]). Early in tumor progression, TGF-β suppresses tumor cells whereas, in later stages, TGF-β promotes tumor development by advancing invasion, metastasis, and angiogenesis [35, 36]. Thus, tumor driven osteolysis leads to increased TGF-β in the tumor microenvironment, which would enhance tumor progression. Canonical TGF-β signaling involves receptor-mediated phosphorylation of SMAD2 and/or SMAD3, either of which dimerize with SMAD4. This complex moves into the nucleus to alter gene transcription [37]. Evidence is mounting that TGF-β also activates MAPK pathways as well [38, 39]. TGF-β-mediated stimulation of PTHrP expression as well as its promotion of osteolytic metastases are driven by both SMAD and MAPK signaling pathways [39, 40]. Deckers et al. [41] have shown that blocking SMAD4 signaling in tumor cells decreases many aspects of tumor development including advancement of tumor-induced osteolysis, indicating an important role for SMAD signaling in tumor progression. Although micro-tumor establishment and advancement were reduced in tumor cells lacking SMAD4, large lesions were not similarly affected, suggesting that SMAD signaling in micrometastases is more critical than later stages of tumor progression. In support of this, mouse models have suggested that TGF-β antagonism may be an effective therapeutic target [42]. In addition to promoting invasion, metastasis, and angiogenesis, TGF-β secreted by breast cancer cells suppresses late stages of osteoblast differentiation and it is possible that TGF-β is an integral component

by which tumor cells suppress osteoblast differentiation [43, 44]. Suppression of osteoblasts would contribute to the net bone loss observed during osteolytic lesion progression by uncoupling resorption from formation.

4 Small molecule therapies targeting breast cancer bone metastasis

In this section we will focus on small molecule therapies used in the treatment of breast cancers that metastasize to bone. Potentially, therapies could target a variety of pathways including angiogenesis, tumor cell cycle progression, tumor cell migration to bone, tumor-mediated osteolysis, and tumor cell apoptosis. Small molecules such as bisphosphonates, anti-estrogens, and aromatase inhibitors can interrupt the molecular mechanisms that promote tumor progression and the discussion below will focus on these therapies.

Bisphosphonates Bisphosphonates (BPs) are a class of pyrophosphate analogues that bind with high affinity to hydroxyapatite crystals in mineralized bone and target osteoclasts to block bone resorption, reducing fracture risk in postmenopausal women with progressive osteoporosis [45]. In addition, BPs have successfully been used in the treatment of malignant hypercalcemia and skeletal metastasis in breast and prostate cancers [46]. Recent animal and human studies suggest that BPs not only reduce osteolysis and bone pain associated with metastasis in breast cancer, but also decrease tumor burden in bone [47, 48]. BPs (non nitrogen containing) are metabolized to form cytotoxic ATP-analogues after internalized by tumor cells and osteoclasts. These toxic metabolites inhibit ATP-dependent regulatory enzymes, protein tyrosine phosphatases that transfer phosphate from adenosine triphosphate to tyrosine amino acid [49–51]. Nitrogen-containing BPs (N-BP) target the mevalonate pathway by inhibiting the enzyme farnesyl pyrophosphate (FPP) synthase [52]. Inhibition of FPP reduces prenylation of GTP-binding proteins, which are essential for signal transduction in 3-Hydroxy-3-Methyl-Glutaryl coenzyme A metabolism. FPP is involved in supporting osteoclast survival as well as cholesterol biosynthesis [52, 53]. In addition to their potent anti-osteoclast effects, recent *in vitro* studies have demonstrated that BPs inhibited cell viability and induced apoptosis in the breast cancer cell lines MCF-7 and MDA-MB-231 [54–59]. During tumor invasion, matrix metalloproteinases (MMPs) play a significant role as they have ability to break down the extracellular matrix and basement membranes, which facilitates blood vessels to access the tumor sites [60–62]. Boissier et al. [60] investigated the effect of BPs on proteolytic activity of breast and prostate cancer cell MMPs and found that BPs did not reduce the expression of MMPs but significantly inhibits proteolytic activity of MMPs. Therefore anti-tumor effect of BPs may be possible through MMP inhibition. Clinically, BPs have been widely used in the treatment of breast cancer patients with osteolytic tumors for the past 20 years [63–66].

Clodronate was the first generation of BPs used in the treatment of breast cancer bone metastasis. Cancer patients treated with oral Clodronate exhibited a significant reduction in hypercalcemia and vertebral and nonvertebral fractures [67]. In addition, radiotherapy reduced bone pain in patients treated with Clodronate and Clodronate adjuvant therapy also reduced visceral and bone metastases in breast cancer patients [68].

Pamidronate, a second generation BPs, was reported in 1997 to be the most successful and widely used intravenous BP for the treatment of bone metastasis in patients with breast carcinoma [69]. The efficacy and safety of Pamidronate for treatment of bone metastasis of breast cancer were established in the late 1990s [66]. Theriaulth et al. [66] assessed the efficacy of Pamidronate in reducing skeletal morbidity in 372 patients with osteolytic bone metastasis and found that 2-hour intravenous infusion of pamidronate (90 mg) every 4 weeks as a supplement to hormonal therapy significantly reduces skeletal morbidity from osteolytic metastasis. Newer nitrogen-containing Residronate and Ibandronate inhibit prenylation of proteins, including the GTP-binding protein Ras, with farnesyl or geranylgeranyl isoprenoid groups and to lead apoptosis of osteoclasts [70]. Residronate and Ibandronate potentially prevent bone loss in breast cancer patients with bone metastasis, significantly reducing skeletal morbidity [71–73]. Ibandronate was also shown to suppress bone metastasis through promotion of apoptosis of metastatic cancer cells as well as of osteoclasts, supporting a dual action on tumor cells and osteoclasts [74].

A third-generation BP, Zoledronate, minimizes the destructive consequences of bone metastases and exerts a profound effect on tumor-induced osteolysis and tumor growth in bone [75]. Studies comparing Pamidronate and Zoledronate found that they both worked equally well but Zoledronate has a slight advantage as it takes less time to inject [76]. Results from a randomized patient trial demonstrated that 4 mg i.v. every 3–4 weeks for 12 months of Zoledronate is effective at decreasing the skeletal morbidity of breast cancer metastasis to bone [76]. In addition, patients receiving Zoledronate therapy showed significant reduction in pain [77]. Even though the mechanism of action of Zoledronate remains unresolved, growing evidence showed that it also inhibits tumor cell adhesion to the extracellular matrix, invasion, and angiogenesis [62, 78]. Zoledronate inhibits membrane localization of Ras in two breast cancer cell lines suggesting that

farnesylation of GTP-binding Ras protein may also be inhibited by Zoledronate [79]. Evidence from animal models demonstrates that Zoledronate may reduce skeletal tumor burden and prevent metastasis to bone [75].

Unfortunately, BP therapy has not succeeded in appreciably prolonging the lifespan of patients, perhaps because treatment does not appear to slow tumor growth in soft tissues [74]. The American Society of Clinical Oncology guidelines reported that, in breast cancer patients, there is no significant improvement in patients' life expectancy with BP treatment although BPs are considered an effective supportive therapy to reduce pain in patients with osteolytic cancer [80]. Whether BPs have anti-tumor effect along with anti-osteoclastic effect is poorly understood and requires further study. Further studies are also needed to fully elucidate these biochemical mechanisms and to determine if the anti-tumor potential of bisphosphonates translates to the clinical setting. This raises the question whether a therapy that both reduces osteoclast activity and represses tumor cell growth directly will be achievable.

Antiestrogens Excessive estrogen exposure promotes breast carcinogenesis by increasing tumor cell proliferation and suppressing DNA repair mechanism and drugs that target estrogen signaling have been used successfully for the treatment of early and advance stage of breast cancer [81]. Current anti-estrogen treatments in breast cancer malignancy are based on two different strategies. These are: (a) antagonizing the estrogen binding to estrogen receptor and (b) inhibiting estrogen biosynthesis. Selective estrogen receptor modulators (SERMs) have been developed to repress estrogen effects on tumor cells. The SERM Tamoxifen has been used for many years in the treatment of both early and advanced breast cancer [82, 83]. Mechanistically, Tamoxifen competes with estrogen for binding to the estrogen receptor (ER), which inhibits receptor activation [84–86]. Clinical studies indicated that Tamoxifen not only prevent the development of breast cancer in high risk women but also protect patients against contra-lateral breast cancer metastases [87–90]. Adjuvant Tamoxifen therapy has been shown to be beneficial to patients with advanced breast cancer since treatment significantly prolong disease-free and overall survival in postmenopausal women with early stage breast cancer [91]. As with other anti-estrogen therapies, patients with higher estrogen receptor levels respond better to Tamoxifen treatment [86]. However many patients who responded to Tamoxifen therapy frequently become Tamoxifen resistant [86]. As Tamoxifen treatment is associated with rare but serious adverse effects, including endometrial cancer and thromboembolism [83], use of new generations of Tamoxifen analogs with reduced site effects is rising in the treatment of early and advance stage of breast cancer. Raloxifene is a second generation of SERM with high affinity for ER and has been shown to reduce the incidence of malignancy in clinical studies [92, 93]. Raloxifen therapy was first approved for the prevention and treatment of osteoporosis because it suppresses bone remodeling to the premenopausal level, maintaining the function of osteoblasts and osteocytes. Raloxifene suppresses osteoclastogenesis and inhibits expression of TNF-α-induced IL-1β, but not IL-6 [94]. On the other hand, it also positively affects osteoblasts survival, suggesting that Raloxifene has not only an antiresorptive role, but also an osteoblast stimulatory role, which may improve bone densities in patients [94]. Clinical trials indicate that Raloxifen can be used for prevention and treatment of osteoporosis in postmenopausal patients with invasive breast cancer [95, 96]. Recently, Vogel et al. [97] confirmed the benefit of Raloxifene in reducing the potential risk of invasive breast cancer and lowering the risk of thromboembolism in a clinical trial. Surprisingly, the risk of osteoporotic fracture remained similar in both Tamoxifen and Raloxifen given group [97]. The Raloxifene analog Arzoxifene also has high binding affinity for ERs and is being developed for prevention and treatment of breast cancer [98, 99]. In addition to Arzoxifene, Lasofoxifene and Toremifene are emerging SERMs that demonstrate high affinity for the ER in ER positive breast cancers [100, 101]. Lasofoxifene has the potential to be used as a therapeutic for postmenopausal women who have osteoporosis and is currently being tested to prevent osteoporosis in patients with advanced breast cancer patients [100, 102, 103]. Furthermore, Toremifene, which is analog of Tamoxifen, shows a similar efficacy and toxicity profile to Raloxifen as it has efficacy in the treatment of metastatic breast cancer in postmenopausal women [104]. The steroidal anti-estrogen, Fulvestrant (ICI 182780) is a new type of ER antagonist that is effective in tumors with reduced ER levels in a small clinical trial [105]. Fulvestrant is now approved as a treatment for postmenopausal women with ER positive metastatic breast cancer if Tamoxifen treatment fails [106].

Aromatase inhibitors In contrast to the above discussed SERMs, aromatase inhibitors target aromatase activity, blocking the conversion of androgens to estrogens and reducing estrogen levels in tissue and plasma [107]. Anastrozole (Arimidex), Letrozole (Femera), and Exemestane (Aromasin) are adjuvant therapies being used to treat advanced breast cancers as aromatase inhibitors are considered second-line therapies for Tamoxifen-relapsed patients [108]. They are also regarded to be first-line therapies for the patients who are resistant to Tamoxifen treatment [108]. Aromatase expression, and thus estrogen synthesis, is seen in the ovaries, adipose tissue, brain, placenta, bone, fetal liver and smooth muscle cells [109].

There is also increased aromatase expression in breast tumors and adipotic stromal tissue adjacent to tumors [110, 111]. Due to effects of estrogen suppression on bone metabolism, aromatase inhibitor use is commonly restricted to postmenopausal women.

Anastrozole is a non-steroidal aromatase inhibitor that has been effective in postmenopausal women with advanced breast cancer and visceral metastases and is approved by FDA for the first and second-line early and metastatic breast cancer [112, 113]. A multi-center clinical trial included postmenopausal women who had primary therapy as well as surgery for invasive breast cancer [114]. Anastrozol was as effective as Tamoxifen and the combination study did not improve disease-free survival beyond individual treatment [112]. However Anastrozole treated subjects had significantly reduced site effects compared to Tamoxifen treated subjects. Anastrozole is considered at least equally effective as Tamoxifen when utilized as first-line therapy in metastatic breast cancer [112]. Letrozole, another non-steroidal aromatase inhibitor, was the first hormonal therapy to significantly reduce metastasis when given after standard Tamoxifen treatment of postmenopausal patients [115]. Women receiving Letrozole had a lower incidence of contra lateral breast cancer compared to Tamoxifen treated women. When Letrozole is used subsequent to Tamoxifen treatment, node-positive patients had improved disease-free survival compared to patients not receiving Letrozole treatment [116]. However, Letrozole did not improve the survival of node negative patients compared to those receiving only Tamoxifen. This study suggests that postmenopausal women with ER positive tumors who have completed 5 years of adjuvant Tamoxifen therapy should be considered for Letrozole treatment. Aromatase inhibitors are considered as the second-line therapies for Tamoxifen-relapsed breast cancer and are currently under consideration as first-line therapies as they show promise as future treatments of invasive breast cancer. Women with metastatic breast cancer who were given Letrozole as first line treatment had a significantly higher response rate, longer time to progression, and improved one and 2-year survival rates compared with women given Tamoxifen [117–119]. Reports from a recent clinical study suggested that adjuvant treatment with Letrozole reduced the risk of recurrent disease especially in metastatic sites in postmenopausal women with ER positive tumors when compared to Tamoxifen [120]. The side effects of thromboembolism, endometrial cancer, and vaginal bleeding were common in the Tamoxifen-treated group and a higher incidence of skeletal and cardiac events and hypercholesterolemia were seen in the Letrozole-treated group [120]. The steroidal aromatase inhibitor Exemestane is currently used as a second-or third-line treatment option in postmenopausal patients whose disease has progressed following Tamoxifen therapy. Both anti-estrogen therapies, SERMs and aromatase inhibitors, are only effective to ER positive tumors, which limits the application of endocrine therapy solely for breast cancer patients with ER-positive. Although these therapies show promise in repressing tumor progression, it should be taken into consideration that therapies by aromatase inhibitors do not replace the bone lost in advanced stage of metastatic breast cancer. This, combined with the effects of inhibiting estrogen effects on bone metabolism, means that patients taking aromatase inhibitor treatment need to have their bone densities carefully monitored [121].

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