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Review

# Combining Src inhibitors and aromatase inhibitors: A novel strategy for overcoming endocrine resistance and bone loss

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ABSTRACT

Aromatase inhibitors have largely replaced tamoxifen as the first-line treatment for postmenopausal women with metastatic, hormone receptor-positive (HR+) breast cancer. However, many patients develop clinical resistance with prolonged treatment, and oestrogen deprivation following aromatase inhibition can result in loss of bone mineral density. Furthermore, most patients with metastatic breast cancer develop bone metastases, and the resulting adverse skeletal-related events are a significant cause of patient morbidity. Src, a non-receptor tyrosine kinase, is a component of signalling pathways that regulate breast cancer cell proliferation, invasion and metastasis as well as osteoclast-mediated bone turnover. Preclinical evidence also suggests a role for Src in acquired endocrine resistance. As such, Src inhibition represents a logical strategy for the treatment of metastatic breast cancer. *In vitro*, combination therapy with Src inhibitors and endocrine agents, including aromatase inhibitors, has been shown to inhibit the proliferation and metastasis of both endocrine-responsive and endocrine-resistant breast cancer cell lines more effectively than either of the therapy alone. Src inhibition has also been shown to suppress osteoclast formation and activity. Combination therapy with aromatase inhibitors and Src inhibitors therefore represents a novel approach through which the development of both acquired resistance and bone pathology could be delayed. Data from clinical trials utilising such combinations will reveal if this strategy has the potential to improve patient outcomes.


## 1. Introduction

Advanced or metastatic breast cancer (MBC) is the leading cause of death from cancer among women in Europe, accounting for 18% of all female cancer deaths.[1] The majority of advanced breast cancers express the oestrogen receptor (ER) and/or progesterone receptor (PgR) and are therefore likely to be hormone sensitive.[2] Oestrogen binding to the ER stimulates breast cancer cell proliferation either directly or indirectly by stimulating growth factor production. Classically, oestrogen-bound ER activates gene expression either through direct interaction with oestrogen response elements located in the promoter regions of target genes or indirectly through protein–protein interactions with additional transcription factors. However, oestrogen binding to the ER also has non-genomic effects by promoting the assembly of ER-dependent signalling complexes which ultimately influence gene expression through the activation of cell signalling

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pathways that act on downstream targets (Fig. 1).[3] Treatment strategies for patients with metastatic hormone receptor-positive (HR+) breast cancer are therefore initially concerned with preventing ER activation.

In clinical practice, endocrine treatment of HR+ breast cancer is directed by the patient's menopausal stage. In premenopausal women, the level of oestrogen, which is produced primarily by the ovaries, is high. Therapies therefore include ovarian ablation through surgery or radiation, ovarian suppression using luteinising hormone-releasing hormone (LHRH) agonists or ER blockade using selective oestrogen receptor modulators (SERMs) such as tamoxifen or fulvestrant (Faslodex, AstraZeneca).[4] Following ovarian ablation, treatment may include aromatase inhibitors (AIs), including anastrozole (Arimidex, AstraZeneca), letrozole (Femara, Novartis) and exemestane (Aromasin, Pfizer), that inhibit the conversion of androgens to oestrogens (Table 1). In postmenopausal women, oestrogen is produced by non-ovarian tissues and treatment involves using SERMs or AIs. Numerous studies have demonstrated that AIs offer significant survival benefits over tamoxifen in the adjuvant setting, with a reduced risk of thromboembolic events (reviewed in Ref.[5]). As a result, AIs have been approved worldwide and are also recommended by various guidelines for the first- and second-line treatment of postmenopausal women with ER-positive (ER+) MBC.[6–8] However, because a deficiency of oestrogen, secondary to aromatase inhibition, is known to promote osteoporosis and increase the risk of fracture,[9] concerns exist regarding the effects of AIs on bone health in the adjuvant setting.

Src, a non-receptor tyrosine kinase, has a role in tumour processes such as growth, invasion and metastasis, and importantly, in regulating normal and pathologic bone activities. Recent evidence has also suggested a role for Src as a modulator of hormone receptor signalling and endocrine response in breast cancer. Because of these roles, Src has been identified as a candidate target for breast cancer therapy and several Src inhibitors are now in clinical development. This review will evaluate the rationale for the dual inhibition of aromatase and Src in patients with HR+ MBC. In addition, relevant preclinical data and emerging clinical data with therapeutic Src inhibitors in breast cancer will be discussed.

## 2. Bone health in postmenopausal breast cancer

In postmenopausal women with MBC, bone health can be adversely affected both by bone metastases and AI-induced decreases in bone density. Bone metastases occur in 65–75% of

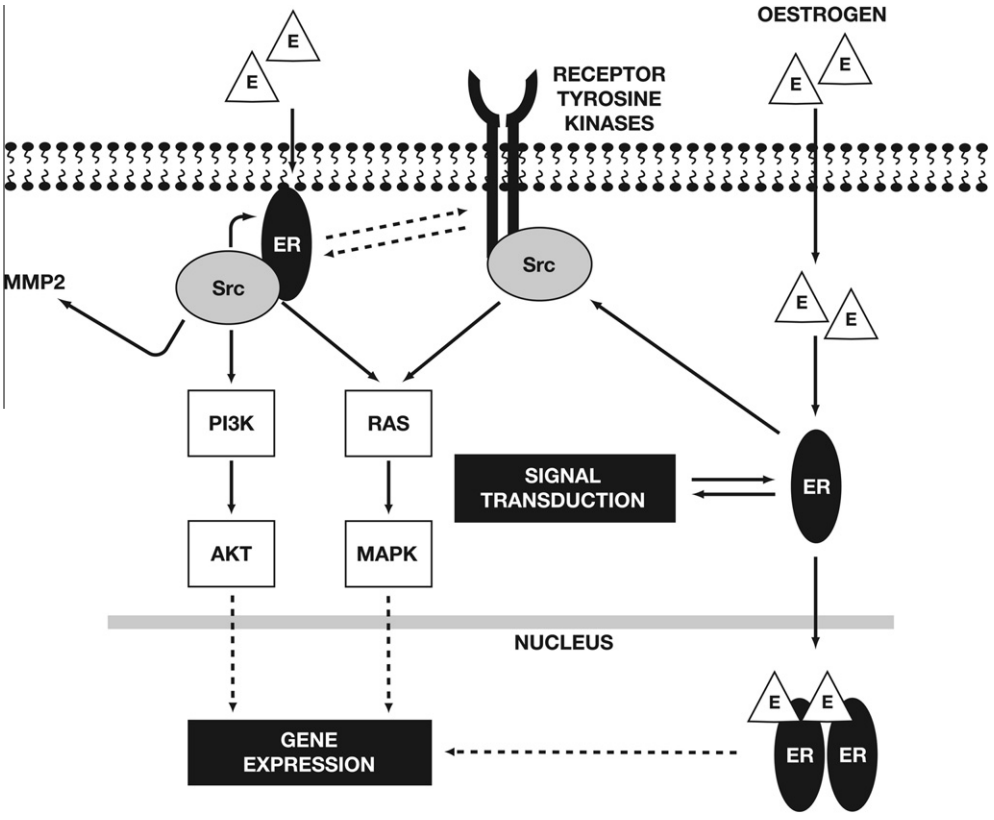


**Fig. 1 – Oestrogen (E) has direct and indirect effects on breast cancer cell proliferation. Oestrogen binds to oestrogen receptors (ERs) which dimerise and subsequently interact with DNA sequences to regulate gene transcription. Oestrogen stimulation also triggers the association of ERs with Src, activating cell signalling pathways such as the phosphoinositide 3-kinase (PI3K)/AKT and mitogen-activated protein kinase (MAPK) pathways, key components of cell survival and proliferation and the production of matrix metalloproteinase 2 (MMP2). In addition, Src is an important mediator of many pathways downstream of receptor tyrosine kinases such as the epidermal growth factor receptor and HER2. Activation of such growth factor receptors results in phosphorylation of ER, promoting its association with Src and initiation of both receptor tyrosine kinase activation and downstream signalling events.**

**Table 1 – Aromatase inhibitors used in the treatment of hormone receptor-positive breast cancer.**

| Agent | Manufacturer | Mechanism of action | Approved indication |
|---|---|---|---|
| Anastrozole (Arimidex®) | AstraZeneca | Non-steroidal aromatase inhibitor | • Adjuvant treatment of postmenopausal women with HR+ early breast cancer<br>• First-line treatment of postmenopausal women with HR+ or HR-unknown locally advanced or metastatic breast cancer<br>• Second-line treatment of advanced breast cancer in postmenopausal women with progression following tamoxifen therapy |
| Letrozole (Femara®) | Novartis | Non-steroidal aromatase inhibitor | • Adjuvant treatment of postmenopausal women with HR+ early breast cancer<br>• Extended adjuvant treatment of postmenopausal women with HR+ early breast cancer who are within three months of completing five years of tamoxifen treatment<br>• First-line treatment of postmenopausal women with ER+ or ER-unknown metastatic breast cancer<br>• Second-line treatment of advanced breast cancer in postmenopausal women with disease progression following antioestrogen therapy |
| Exemestane (Aromasin®) | Pfizer | Steroidal aromatase inhibitor | • Adjuvant treatment of postmenopausal women with ER+ early breast cancer who have received 2–3 years of tamoxifen treatment<br>• Second-line treatment of advanced breast cancer in postmenopausal women with disease progression following tamoxifen therapy |

*Abbreviations:* ER, oestrogen receptor; HR, hormone receptor; PgR, progesterone receptor.

patients with MBC, causing significant morbidity.[10] In the normal bone remodelling process, bone resorption by osteoclasts and bone formation by osteoblasts occurs in equilibrium. The presence of metastatic cells disrupts this equilibrium, leading to the development of lesions that, in breast cancer, have a predominantly osteolytic phenotype. Increased osteolysis leads to skeletal-related events (SREs), defined as pathologic fracture, spinal cord compression, hypercalcemia of malignancy, bone pain and the requirement for surgery or radiation therapy to bone.[11] Oestrogen normally suppresses bone resorption by inhibiting osteoclast activity and inducing apoptosis.[12] In postmenopausal women, the aromatase enzyme converts peripheral tissue androgens to oestrogen, meaning that low levels of circulating oestrogen, critical for bone health, are present. However, AIs suppress circulating oestrogen to almost undetectable levels, resulting in significant effects on bone physiology.[13]

The effects of AIs on bone health have been analysed within a number of clinical studies. The Tamoxifen Exemestane Adjuvant Multicentre (TEAM) trial in postmenopausal women with HR+ breast cancer compared the efficacy and safety of 5 years of adjuvant exemestane or ≈2.5 years of tamoxifen followed by 2.5 years of exemestane. With 2.75 years of follow-up, exemestane was associated with improved disease-free survival and time to first distant metastasis compared with tamoxifen.[14] However, in patients with ER+ early breast cancer, markers of bone loss were significantly increased with exemestane and significantly decreased with tamoxifen.[15] In a study of exemestane, letrozole, anastrozole or placebo in healthy postmenopausal women, markers of bone resorption were increased in all treatment groups. Interestingly, this study also found that exemestane increased serum levels of bone formation markers, which was hypothesised to be due to its steroidal structure and the androgenic activity of its primary metabolite.[16]

Reducing the risk of SREs in patients with bone metastases or AI-associated bone loss is an important treatment goal. Bisphosphonates such as zoledronic acid (Zometa, Novartis) bind to the bone surface and prevent osteoclast-mediated bone resorption.[17] In premenopausal women with HR+ breast cancer, adding zoledronic acid to endocrine therapy significantly improves disease-free survival compared with endocrine therapy alone.[18] The ongoing Zometa-Femara Adjuvant Synergy Trial (Z-FAST) is evaluating the efficacy and safety of zoledronic acid treatment in postmenopausal women with early HR+ breast cancer receiving adjuvant letrozole. With 36 months of follow-up, results from the trial indicate that up-front initiation of zoledronic acid with letrozole more effectively prevents and reverses AI-induced bone loss than delayed administration.[19] Denosumab is a monoclonal antibody that specifically inhibits receptor activator of NF-κB ligand (RANKL), which is required for osteoclast formation, function and survival. In a phase 3 study of patients with non-metastatic ER+ breast cancer who were receiving adjuvant AI therapy, denosumab treatment consistently increased bone mineral density across multiple skeletal sites compared with placebo treatment,[20] and when compared with zoledronic acid in patients with breast cancer metastatic to bone, denosumab was superior in delaying or preventing SREs.[21] These data demonstrate the therapeutic value of agents with antiosteoclast effects.

## 3. Rationale for targeting Src in HR+ MBC

### 3.1. Role of Src in tumour growth, migration and invasion

Src activity and/or expression is elevated in breast cancer cell lines and in breast tumour tissue compared with non-tumour tissue.[22,23] A correlation between elevated activity of Src and breast cancer progression has been suggested, with a trend for increased Src activity from pre-invasive to invasive disease.[24] In a recent study, high levels of activated Src were found in primary breast cancer tissues compared with normal tissues. Interestingly, evaluation of activated Src in tumour tissues revealed an association with HER2 expression, high tumour grade and elevated proliferation. Moreover, high levels of activated Src were associated with distant metastasis and shortened survival in ER+ patients.[25] Because Src is involved in signalling pathways that regulate normal cellular growth, proliferation, angiogenesis, motility and survival, aberrant Src activity can lead to tumourigenesis. For example, Src activation alters cell–cell adhesion and induces metastasis by modulating the cadherin cell adhesion system.[26] In addition, Src is involved in the process of epithelial–mesenchymal transition (EMT), which is associated with tumour metastasis *in vivo*.[27] Src inhibition also impairs the migration, adhesion and spreading of the ER+ MCF-7 breast cancer cell line, which is associated with reduced activation of proteins involved in integrin signalling and adhesion dynamics, such as focal adhesion kinase (FAK).[28] The antimetastatic potential of Src inhibition has been further demonstrated in a mouse model of MBC, which showed that inhibiting Src reduced the incidence of bone and visceral metastases, resulting in decreased morbidity and mortality.[29]

### 3.2. Role of Src in osteoclast activity

High levels of Src are found within active osteoclasts. These cells attach tightly to bone and secrete proteases and acid to dissolve the bone mineral.[30,31] Thus, by positively regulating osteoclast activity in addition to suppressing osteoblast function, Src signalling is implicated as playing a central role in the process of bone remodelling as well as being associated with the capacity of breast cancer to metastasise to bone.[32] Mice with targeted disruption of the *Src* gene have defective bone development[33] and inhibition or disruption of Src enhances osteoblast differentiation and bone-forming activity.[34] *In vitro*, reversible inhibition of Src activity inhibits human osteoclast formation, differentiation and activity, preventing osteoclast precursor cells from migrating from the osteoblast layer to the bone surface and forming resorption pits.[35,36] In addition, when prostate cancer cells were injected into tibiae of mice, Src inhibition increased the bone mineral density of normal bone and bone harbouring metastatic lesions.[37]

### 3.3. Role of Src in growth factor and hormone signalling pathways

Src is associated with and activated by a variety of growth factor receptors involved in the growth and survival of human breast cancer cells.[38,39] A physical association between Src and the HER2 receptor, which is amplified in ~20% of human breast carcinomas and is thought to be involved in tumour initiation and early progression,[40] was reported in a subset of HER2-expressing human breast cancer cell lines.[22] In addition, Src inhibition prevents oestrogen-mediated stimulation of the AKT and PI3-kinase pathways, preventing cell cycle progression,[41] and in response to epidermal growth factor (EGF), androgen or oestrogen stimulation, Src forms a complex with the ER and androgen receptor (AR), initiating Src-dependent signalling, DNA synthesis and cytoskeletal changes (Fig. 1).[42] Post-translational modification of the ER plays a key role in regulating oestrogenic signalling; subsequently, the ability of Src to phosphorylate the ER increases the affinity of the ER for oestradiol (E2),[43] augments transcriptional activation of the E2–ER complex and enhances tamoxifen-mediated ER activation.[44] The fact that Src can mediate interactions between ER and growth factor signalling pathways is of particular importance because cross-talk between these pathways is implicated in endocrine resistance (see below).[45]

### 3.4. Role of Src in hormone resistance

Studies have demonstrated that Src is a key signalling molecule in the growth of antioestrogen-resistant cells. For example, synergistic interaction between EGF receptor, ER and Src enhances the responsiveness of MCF-7 breast cancer cells to mitogenic stimulation by both EGF and oestrogen, allowing cells to survive and proliferate in the presence of tamoxifen.[46] Similarly, in a recent study, the expression of constitutively active Src attenuated the sensitivity of MCF-7 cells to tamoxifen.[25] Moreover, the acquisition of tamoxifen resistance in breast cancer cells is accompanied by an increase in Src kinase activity which is associated with enhanced migration and a more aggressive phenotype.[25,47–49] In addition, both oestrogen and Src impair the inhibitory action of p27, a cell cycle inhibitor required by antioestrogens to induce cell cycle arrest.[50] Importantly, endocrine agents such as anastrozole stimulate Src activity in breast cancer cells, both *in vitro* and *in vivo*, further highlighting the potential for using Src inhibitors.[50]

## 4. Src inhibitors in breast cancer

Because of the role of Src in tumourigenesis, hormone signalling and osteoclast activity, Src inhibitors have potential for the treatment of solid tumours, particularly breast cancer. Preclinical studies have investigated the activity of Src inhibitors that are currently in clinical development against breast cancer cells and osteoclasts.

Dasatinib (SPRYCEL®, Bristol-Myers Squibb) is a potent inhibitor of Src and Src-family kinases (SFKs).[51] When tested against a panel of breast cancer cell lines, dasatinib demonstrated greatest growth inhibition against cell lines characterised by microarray to be broadly representative of triple-negative disease (i.e. negative for ER, PgR and HER2 receptors).[52] Moreover, a baseline gene expression signature correlating with *in vitro* sensitivity to dasatinib has been identified in breast cancer cell lines, and expression of this signature was frequently detected in samples from patients with triple-negative breast cancer.[53] Interestingly, preclinical studies

also show substantial synergy when dasatinib is used in combination with cytotoxic chemotherapy (cisplatin and deoxy-5-fluorouridine) in triple-negative cell lines.[54] However, dasatinib also inhibits the growth of breast cancer cell lines with high EGFR expression and an aggressive phenotype. In EGFR-overexpressing cells, dasatinib treatment resulted in cell-cycle arrest, apoptosis through caspase activation and inhibition of cell migration and invasion.[55] Dasatinib has also demonstrated synergistic inhibition of breast cancer cell growth in combination with bisphosphonates such as zoledronic acid[56] and synergistic inhibition of cell growth, migration and invasion in combination with doxorubicin.[57] *In vitro*, therapeutic concentrations of dasatinib inhibited human osteoclast differentiation and activity[36,58] and in a rat model of bone resorption, dasatinib rapidly suppressed serum calcium levels.[59] Finally, in mice inoculated with bone metastatic cells derived from a triple-negative breast cancer cell line, dasatinib prevented the formation of osteolytic metastases.[60]

Bosutinib (formerly SKI-606, Wyeth) is an inhibitor of Src and SFKs plus AXL that potently inhibited the proliferation, invasion and migration of breast cancer cell lines *in vitro*, although bosutinib showed relatively weak antiproliferative effects against some cell lines.[61–63] Similarly, Vultur and colleagues showed that although bosutinib decreased breast cancer cell motility and invasion, cell proliferation and survival were unaffected.[64] In a mouse xenograft model of breast cancer, mice treated with bosutinib developed significantly smaller tumours and had a significantly reduced incidence and size of metastases compared with control animals.[63]

Saracatinib (formerly AZD0530, AstraZeneca) is an inhibitor of Src and SFKs, plus c-terminal Src kinase (CSK).[65] *In vitro*, saracatinib significantly inhibited human breast cancer cell migration but had limited effects of cell proliferation.[66,67] In an *in vitro* bone model, saracatinib inhibited the differentiation and activity of human osteoclasts and blocked the migration of osteoclast precursor cells to the bone surface, thereby reducing osteoclast-mediated bone resorption.[35] The antiosteoclastic effects of saracatinib observed *in vitro* were supported in a recent study of bone turnover in healthy men, in which saracatinib treatment resulted in a dose-dependent decrease in markers of bone resorption, indicating inhibition of osteoclast activity.[68]

Clinical studies of therapeutic Src inhibitors have been performed in patients with breast cancer, although bone-specific effects have not been reported to date. In a phase I study of patients with advanced breast cancer who received escalating doses of dasatinib in combination with capecitabine, 11 of 27 patients had a partial response (confirmed in six), and 9 patients had stable disease.[69] In phase II studies of patients with advanced breast cancer who received dasatinib monotherapy, low numbers of partial responses or stable disease were reported in patients with triple-negative, HR+ or HER2-amplified disease.[70,71] In a phase II trial of bosutinib in 73 patients with breast cancer, including patients with HR+, HER2-amplified or triple-negative disease, two had partial response and 12 patients had stable disease at 24 weeks.[72] Phase II trials of saracatinib monotherapy in breast cancer are currently recruiting patients, including a study of patients with unresectable, locally advanced or metastatic disease that is ER and PgR negative (NCT00559507) and a study of bone marker effects in patients with bone metastases from breast or prostate cancer (NCT00558272).

Preliminary results from these clinical trials indicate that single-agent Src inhibitor treatment has modest antitumour effects in patients with HR+ breast cancer, possibly because of the complexity of endocrine signalling pathways. One explanation for these observations could be that following Src inhibitor treatment, compensatory prosurvival pathways might be activated through feedback mechanisms. For example, prolonged *in vitro* treatment of breast cancer cells with saracatinib was associated with activation of MEK/MAPK and PI3K/AKT/MTOR pathways, both of which are downstream mediators of Src activation.[50] Combined endocrine/Src inhibitor therapy could therefore be more efficacious in the treatment of HR+ MBC, particularly for patients whose tumours have metastasised to bone or who have treatment-associated bone loss.

## 5. Preclinical studies combining endocrine agents with Src inhibitors

Despite significant improvements in the efficacy of endocrine therapy for breast cancer, initial or acquired resistance remains a major clinical issue. Many growth factor pathways and oncogenes are aberrantly activated and utilised by breast cancer cells to bypass normal endocrine responsiveness.[73] High levels of activated Src in ER+ breast cancer tissue are associated with reduced response to tamoxifen.[25] Preclinical data demonstrate that dual targeting of Src and the ER increases endocrine responses in HR+ breast cancer cells and restores sensitivity in endocrine-resistant cells. Src inhibition enhanced the inhibitory effects of both tamoxifen and oestrogen deprivation on the growth of MCF-7 cells *in vitro*, with maximal effects observed when tamoxifen treatment and Src inhibition were combined, including cells with elevated Src activity.[25,49] Specific effects of combination treatment using suboptimal concentrations of saracatinib and tamoxifen included synergistic inhibition of MCF-7 anchorage-independent growth[74] and inhibition of FAK phosphorylation, abrogating the invasive behaviour of two ER+ cell lines.[48] Importantly, combination therapy has also been shown to prevent the emergence of tamoxifen resistance.[67]

Similar synergistic effects have been observed during combined treatment with a Src inhibitor and AI. *In vitro*, the addition of 1 μmol/L saracatinib, which had limited antiproliferative activity on its own, decreased by 10-fold the concentration of anastrozole required to induce cell cycle arrest in ER+ breast cancer cells. Similarly, dual therapy in xenograft models significantly suppressed tumour growth and reduced the occurrence of drug resistance.[50]

## 6. Clinical trials combining AIs with Src inhibitors

Four ongoing phase II trials are investigating combination therapy with AIs and Src inhibitors in patients with HR+ breast cancer (Table 2). CA180-261 (NCT00767520) is a randomised, placebo-controlled phase II trial comparing the

**Table 2 – Combination studies of Src inhibitors and aromatase inhibitors in hormone receptor-positive breast cancer. Clinical trials of dasatinib, bosutinib and saracatinib as monotherapies, or in combination with other chemotherapeutic and biologic agents, are also ongoing in patients with advanced breast cancer.**

| Src inhibitor | Aromatase inhibitor | ClinicalTrials.gov identifier | Trial description | Estimated completion date | Primary outcome measures | Secondary outcome measures |
|---|---|---|---|---|---|---|
| Dasatinib | Letrozole | NCT00696072 | Phase 2, randomised study of letrozole with or without dasatinib as first and second-line treatment in postmenopausal women with unresectable, locally advanced or metastatic ER+/PgR+/HER2-breast cancer | December 2010 | Clinical benefit rate (complete response, partial response or stable disease for $\geqslant$6 months) | Overall response rate; median progression-free survival; time to treatment failure; changes in bone markers, bone pain and bone mineral density; safety |
| Dasatinib | Exemestane | NCT00767520 | Phase 2, randomised, double-blind, multicentre study of dasatinib plus exemestane versus exemestane plus placebo in advanced ER+ breast cancer after disease progression on a non-steroidal aromatase inhibitor | May 2011 | Progression-free survival at 8-week intervals | Clinical benefit rate; progression-free survival; objective response rate, time to response; response duration; safety and tolerability; changes in bone pain and markers of bone lysis |
| Bosutinib | Letrozole | NCT00880009 | Phase 2, randomised, open-label study of bosutinib plus letrozole versus letrozole alone as first-line therapy in postmenopausal women with locally advanced or metastatic ER+/PgR+/HER2- breast cancer | December 2013 | Progression-free survival at 36 months | Safety (reporting of adverse events) |
| Bosutinib | Exemestane | NCT00793546 | Phase 2, randomised, open-label study of bosutinib plus exemestane versus exemestane alone as second-line therapy in postmenopausal women with locally advanced or metastatic ER+/PgR+/HER2- breast cancer | July 2011 | Progression-free survival at 6 months | Safety; pharmacokinetics; overall response rate; overall survival at 2 years; response duration; progression-free survival and health-related quality of life |

*Abbreviations:* ER, oestrogen receptor; HR, hormone receptor; PgR, progesterone receptor.

safety and efficacy of exemestane plus dasatinib to exemestane alone in patients with HR+ MBC. Changes in markers of bone resorption are to be evaluated in patients with bone metastases. CA180-185 (NCT00696072) is a randomised trial comparing letrozole plus dasatinib to letrozole alone in the first- and second-line treatment of unresectable, locally recurrent or metastatic HR+ breast cancer. Effects on bone pain and bone mineral density will be assessed as secondary endpoints. Studies 3160A6-2206 (NCT00793546) and 3160A6-2207 (NCT00880009) are comparing bosutinib in combination with either letrozole (first-line) or exemestane (second-line) to AI treatment alone in women with locally advanced or metastatic HR+ breast cancer. In both studies, progression-free survival is the primary endpoint.

## 7. Future directions

Breast cancer is a heterogeneous disease; therefore, any benefit associated with Src inhibitor therapy is likely to vary between patients. For example, breast cancer cell lines representing the basal/triple-negative subgroup of patients show increased sensitivity to growth inhibition by dasatinib. In contrast, although Src has a suggested role in HER2 signalling, most cells with amplified HER2 appear resistant to dasatinib treatment.[52] ER+ cell lines, however, have shown moderate sensitivity to Src inhibitors, which is increased by the concurrent reduction of oestrogen levels.[52,67,74] A challenge of future clinical trials will be to identify the molecular and histological phenotype of patients that show benefit from treatment with Src inhibitors to subsequently enable a more tailored therapeutic approach.

High numbers of regulatory T (T-reg) cells in breast cancer are associated with risk of late relapse[75], potentially through the ability of T-reg cells to suppress T-cell proliferation and cytokine production, thus allowing tumours to elude host antitumour immune responses. Interestingly, recent data suggests that AIs are able to reduce levels of T-reg cells in breast cancer patients[76] and Src inhibition has been demonstrated to suppress T-reg proliferation and function at physiologically achievable concentrations.[77] It is therefore possible that treatment regimens consisting of AIs in combination with Src inhibitors may provide additional benefit in breast cancer through their potentially synergistic effects on T-reg cell function.

Because Src is a key modulator of bone metabolism and deregulated Src promotes increases in osteoclast-mediated bone resorption, changes in bone biomarker levels and bone mineral density are being assessed as endpoints in early clinical studies of Src inhibitors. However, to demonstrate a true clinical benefit in terms of bone effects, Src inhibitors will need to show a reduction in the rate of bone fractures and/or SREs, as has been demonstrated with established bone-targeting agents such as bisphosphonates.

Finally, although the combination of Src inhibitors and AIs have potential in the treatment of advanced disease, the role of Src in tumour invasion/metastasis and osteoclast function suggests Src inhibitors should also be assessed in the adjuvant setting, where combination with inhibitors of oestrogen signalling could improve patient outcome by preventing metastasis and skeletal morbidity.

## 8. Conclusions

MBC is incurable, but long-term or beneficial short-term responses can be achieved using appropriate treatments. AIs are indicated as the first-line treatment for postmenopausal women with HR+ breast cancer, but are associated with clinical resistance and adverse bone effects. Because Src is involved in multiple processes relevant to MBC, including oncogenic activities, endocrine resistance and osteoclast function, Src is an attractive target for anticancer therapy. Clinical studies with Src inhibitors as single agents have so far shown relatively modest response rates. However, preclinical data suggests that combination therapy with Src inhibitors and endocrine agents inhibits the proliferation and metastasis of both endocrine-responsive and endocrine-resistant breast cancer cell lines more effectively than either of the agent alone. Furthermore, Src inhibition inhibits osteoclast formation, differentiation and activity *in vitro* and reduces tumour growth in bone *in vivo*. By providing antitumour, antimetastatic and antiosteoclast activities, combination therapy with Src inhibitors and AIs is a promising treatment strategy for patients with HR+ MBC. Results from ongoing clinical studies are needed to determine whether this combination has potential in a clinical setting.

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