Targeting Src in breast cancer

R. S. Finn*

Department of Medicine, Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA

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The clinical benefit of blocking oncogenic pathways in breast cancer and other malignancies has validated this approach and ushered in the era of molecularly targeted therapeutics. Src and its family members make up the largest group of nonreceptor tyrosine kinases. In laboratory models, these proteins have been shown to play a critical role in cellular growth and proliferation, angiogenesis, and invasion and metastasis. In addition, Src plays an important role in osteoclast activation and bone resorption, which are often aberrantly activated in the setting of bone metastases. Given its role in these functions, blocking Src kinase would be predicted to have a broad therapeutic benefit in patients with Src-dependent cancers. In this review, we highlight the rationale for targeting Src in breast cancer, including laboratory and clinical data implicating it in these signaling pathways, and review small-molecule tyrosine kinase inhibitors currently in clinical development. Identifying which patients should be selected for Src-directed therapies will be important to the clinical success of these agents. Importantly, recent preclinical data support a role for this class of inhibitors in basal-type/triple-negative breast cancer, which represents a group of patients with limited effective treatment options.

Key words: breast cancer, cancer drug development, growth factors and receptors, oncology—metastasis, Src, translational oncology—signal transduction

introduction

Unlike many solid tumors, we have long had an understanding of some of the key molecular drivers of breast cancer, using them both as prognostic factors and as predictive markers for specific therapies. We know that women with hormone receptor-positive disease generally have a better prognosis than hormone-negative disease and respond to estrogen deprivation [1]. Similarly, women with human epidermal growth factor receptor 2 (HER2)-amplified disease have a more aggressive phenotype but are candidates for trastuzumab, which has changed the natural history of this type of breast cancer [2, 3]. There are, however, women who have so-called ‘triple-negative’ breast cancer that is defined by the absence of estrogen receptor (ER), progesterone receptor, and HER2 who carry a poor prognosis and for whom there is no ‘specific’ therapy [4]. The molecular alterations driving this subtype of disease are still to be defined. We know from molecular profiling studies that all three of these clinical ‘subtypes’ have distinct gene expression patterns that correlate with clinical outcome [5–7]. These data are now being used to drive therapeutic development in breast cancer by guiding the use of existing therapies and identifying new targets.

Many well-known genes have been identified as valid targets for treatment in breast cancer and other solid tumors. Of these, Src is arguably the oldest known oncogene, initially identified by Peyton Rous in 1911 as the transforming agent in chicken sarcomas. Over the past century, large amounts of data have been generated supporting the role of Src as a key messenger in many important cellular pathways, including those involved in regulating proliferation, differentiation, survival, motility, and angiogenesis [8, 9]. Despite this, c-Src by itself has not been shown to be a dominant transforming oncogene in human cancers [10]. Because of its essential role in many intracellular signaling pathways, however, interrupting Src signaling may disrupt oncogenic pathways. In this article, we will review data supporting the targeting of Src in breast cancer and Src inhibitors in clinical development.

Src: structure and function

Src is the most widely studied member of the largest family of nonreceptor protein tyrosine kinases, known as the Src family kinases (SFKs). Other SFK members include Lyn, Fyn, Lck, Hck, Fgr, Blk, York, and Yes [8, 9]. The structure of Src is shown in Figure 1.

Src can be activated by cytoplasmic proteins, e.g. focal adhesion kinase (FAK) or its molecular partner Crk-associated substrate (CAS), which play a prominent role in integrin signaling, and by ligand activation of cell surface receptors, e.g. epidermal growth factor receptor (EGFR) [11]. These interactions disrupt intramolecular interactions within Src, leading to an open conformation that enables the protein to
interact with potential substrates and downstream signaling molecules. Src can also be activated by dephosphorylation of tyrosine residue Y530. Full Src activation requires the autophosphorylation of another tyrosine residue (Y419 in the human protein) present within the catalytic domain [12].

Elevated Src activity may be caused by increased transcription or by deregulation due to overexpression of upstream growth factor receptors such as EGFR, HER2, platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), vascular endothelial growth factor receptor, ephrins, integrin, or FAK [11, 13, 14]. Alternatively, some human tumors show reduced expression of the negative Src regulator, Csk [8, 9].

**rationale for targeting SFKs and Src in breast cancer tumorigenesis**

**cell growth and survival**

Elevated Src expression has been seen in multiple solid tumors including breast cancer [8, 9, 15]. Src kinase activity is greatly increased in breast cancer tissue compared with normal breast tissue [15–18], yet transfection of Src alone does not have transforming ability [10]. Src, however, plays a role in signaling and cross talk between growth-promoting pathways, such as the ER and EGFR family signaling pathways, known to be active in breast cancer.

Steroid hormone receptors play a key role in the pathogenesis and treatment of many breast cancers. Many mitogenic effects of estrogen are mitigated through SFKs, both directly and indirectly [19]. In support of these hypotheses, mitogen-activated protein kinase (MAPK) is not activated by estrogen in Src-deficient cell lines and mice [20]. In addition, steroid hormones can directly activate SFKs and other signaling molecules in the cytoplasm such as Shc, phosphoinositol-3-kinase, and p130CAS, without first binding their nuclear receptors [21]. In a study of breast cancer cells expressing either wild-type or a hypersensitive mutant ER, wild-type cells responded to estrogen stimulation by increasing Src kinase activity [22]. In hypersensitive mutants, basal Src activity was much higher than that in wild type, and addition of estrogen had no further effect.

Src is an important mediator of many downstream effects of receptor tyrosine kinases (RTKs) including the EGFR family, whose prototype receptor EGFR and its sister molecule HER2 are therapeutic targets [3, 23]. Activation of the tyrosine kinase domain leads to auto- and transphosphorylation of the intracellular domain of the receptor. Phosphorylated tyrosine residues serve as docking sites for proteins including Src, which subsequently turn on complex networks of cell-signaling pathways (Figure 2). c-Src transfection potentiates epidermal growth factor (EGF)-induced oncogenesis [24]. Also, breast cancer cell lines expressing higher levels of HER1 and Src have higher levels of phosphorylated Shc, increased activation of MAPK, and increased tumorigenicity compared with those that do not ‘overexpress’ EGFR or overexpress only Src [17]. Also, EGF-dependent DNA synthesis is dependent on a functional Src kinase domain [25, 26], and SFK activity is necessary for growth factor-induced MAPK activation in various breast cancer cell lines [27]. Src mediates activation of signal transducer and activator of transcription (STAT) by RTKs [28] and inhibition of the Src pathway reduced STAT3 activity in breast cancer cell lines with elevated levels of EGFR [29]. Finally, in preclinical models of acquired tamoxifen resistance, elevated levels of EGFR activity are accompanied by an increase in Src activity and sensitivity to the Src inhibitor AZD0530 [30].

In addition to a role for Src downstream of HER2 activation, data have indicated a possible role in HER2–HER3 interactions. Overexpression of c-Src increases HER2–HER3 dimerization and subsequent receptor and downstream intracellular target activation [31]. These studies also showed that c-Src is required for cellular motility and anchorage-independent growth promoted by the HER2–HER3 heterocomplex.

**SFKs in angiogenesis, detachment, motility, spread, and invasiveness of breast cancer**

The acquired ability of a localized tumor to metastasize is a multistep process involving many pathways, including those involved in angiogenesis, focal adhesion, invasion, and eventually colonization of a distant site [32]. This ability is accompanied by an epithelial-to-mesenchymal transition (EMT), involving changes in gene expression patterns [33]. Studies using clinical material and laboratory models have implicated Src signaling in these processes.

Vascular endothelial growth factor (VEGF) is a pivotal component of both normal and malignant angiogenesis. It has been validated as a clinical target in many tumors, including breast cancer [34, 35]. In vitro, hypoxia activates Src leading to up-regulation of VEGF messenger RNA. Hypoxia induction of VEGF expression is impaired in Src knockouts and in breast cancer cell lines in the presence of Src antisense RNA [36, 37].

Src activity is increased in invasive compared with noninvasive breast cancer cell lines. Following treatment with an SFK inhibitor, breast cancer cell lines showed decreased motility and invasiveness [38]. Similarly, studies using an inducible dominant-negative Src demonstrated that Src suppression significantly reduced cell migration, attachment, and spread in MCF-7 cells through changes in FAK activation and p130CAS interactions [39]. In addition, SFK inhibition blocked cell rounding and detachment through interference with integrin-mediated cell migration and adhesion [40]. Finally, a recent study using clinical breast cancer tissue...
showed that levels of activated phospho-STAT3 and phospho-Src were significantly higher in invasive carcinoma than in non-neoplastic tissue [41].

**Src and bone metastases**

Bone is the primary metastatic site of patients with breast cancer. Because of its role in osteoclast function, Src is a novel target for this common complication in breast cancer and other malignancies.

Under normal conditions, bone is a dynamic organ undergoing remodeling in response to weight-bearing forces. There is a delicate balance between bone resorption (osteoclast function) and bone production (osteoblast function). Src’s role in bone formation was first demonstrated when engineered Src null mice were found to be deficient in bone remodeling and developed osteoporosis [42]. Disruption of Src signaling in osteoclasts results in decreased migrational ability and prevents the formation of ruffled membranes, a key step during bone resorption [43, 44]. Mice injected with MDA 231 human breast cancer cells transfected with a constitutively active Src developed increased osteolytic bone metastases compared with control animals, indicating a role for Src not only in osteoclast function but also in tumor colonization of bone [45]. Expression of parathyroid hormone-related peptide, an activator of osteoclasts and a cause of malignant hypercalcemia, is a downstream target of Src activation [46]. Conversely, Src inhibition can affect osteoblast function by stimulating osteoblast differentiation and bone formation [47] and Src inhibition in osteoblasts can also impair osteoclast bone resorption by affecting osteoblast cytokine expression [48].

Recently, gene expression profiling of node-negative breast cancers that developed bone metastasis versus those that experienced relapses elsewhere identified differentially expressed genes [49]. While Src was not identified as being differentially expressed, genes in the FGFR/MAPK pathway were up-regulated in those tumors that developed bone metastasis. Genes in this pathway also overlapped with differentially expressed genes described previously in a human xenograft model of breast cancer [50]. These observations are of interest to Src inhibition strategies as Src activation is downstream from FGFR signaling and can also activate MAPK [11, 27].

**Src as a therapeutic target for several breast cancer subtypes**

Given the role of Src in growth and proliferation, invasion, angiogenesis and metastasis, and physiology of bone turn over and metastasis, data reviewed here and elsewhere support the development of Src inhibitors in breast cancer (Figure 3). Blocking Src activation may slow disease progression and potentially play an important role in the adjuvant setting to prevent disease recurrence and the development of metastases from residual disease. Src inhibition may also decrease the development of destructive bone metastases and the pain associated with these lesions.
The successful development of targeted therapeutics in oncology has been hindered by the lack of reliable predictive markers for response to any given agent perhaps with the exception of hormone receptor measures and tests for the presence of the HER2 alteration [51]. It is also apparent that simple measurement of target expression is unlikely to be associated with response to an inhibitor of the target [23]. Recent studies using gene expression profiling have provided insights into the molecular complexity and heterogeneity of clinical breast cancer [5–7] and may provide a technique for selecting patients for therapy with the hypothesis that there may be a 'signature' for target-'driven' disease (in this case Src) and not just target-'expressing' disease.

Studies have identified unique subtypes of breast cancer on the basis of gene expression profiles and Src inhibition may be an option for patients in various groups. ‘Luminal’ breast cancer represents the majority of clinical breast cancer and expresses specific cytokeratins (CK8 and CK18) associated with the luminal layer in the normal breast epithelium. This subtype is often hormone receptor positive and is associated with the best prognosis. It has been postulated that luminal breast cancer may be divided into further subtypes that are associated with different patient outcomes [52], although this topic remains under debate. The ‘basal’ group is differentiated by the expression of cytokeratins (CK5 and CK17) found in the basal layer of the breast epithelium. Triple-negative breast cancers (ER−, PR−, and HER2−) are generally of the basal subtype. This latter subtype is of clinical importance as there are limited approved treatment options other than chemotherapy, which has only had a minimal impact in this setting compared with ER+ or HER2+ disease, for which there are hormonal manipulations and trastuzumab, respectively [4]. In addition, the basal subtype has recently been identified as having an increased incidence in young African-American women [53]. Recent preclinical studies indicate that Src inhibition may be a therapeutic option for basal/triple-negative breast cancers [54, 55].

There are several Src inhibitors in clinical development (Table 1). Of these, three are being actively developed in breast cancer.

**dasatinib**

Dasatinib (Bristol-Myers Squibb, Princeton, New Jersey) is an oral, small-molecule multikinase inhibitor of several SFKs as well as c-kit, PDGFR, Bcr-Abl, and ephrin receptor kinases [56]. Dasatinib is approved for second-line treatment of chronic myelogenous leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL). Preclinical studies with dasatinib in pancreatic, head and neck, and lung cancer models have demonstrated its ability to inhibit metastasis, cause cell cycle arrest, block proliferation, and induce apoptosis [57–59]. Preclinical studies in breast cancer carried out independently using two different microarray platforms (Agilent and Affymetrix, Santa Clara, California) have identified that breast cancer cell lines representing the basal/triple-negative group are uniquely sensitive to growth inhibition by dasatinib [54, 55]. These data indicate that cancers in this subgroup may be ‘Src dependent’. Dasatinib inhibited the growth of cell lines of luminal, basal and post-EMT origin and was effective (to varying degrees) independent of ER or HER2 status [54, 55]. In addition, gene marker sets were identified with high sensitivity and specificity for predicting response to dasatinib in vitro. This observation is of importance since it identifies a unique subgroup of breast cancers that may be more sensitive to Src inhibition than other subtypes. Interestingly, and somewhat surprisingly, breast cancer cell lines with HER2 amplification, higher EGFR levels, or hormone dependence were less sensitive to inhibition of proliferation in vitro compared with the basal subgroup, despite the evidence pointing to a key role for Src in these signaling pathways [54, 55]. Dasatinib, however, did inhibit the growth of HER2-amplified xenografts in vivo [60].
**Table 1. Src inhibitors in clinical development**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular targets</th>
<th>Stage of development in breast cancer</th>
<th>Stage of development in other malignancies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dasatinib</td>
<td>SFKs, Bcr-Abl, Kit, Eph, PDGFR receptors</td>
<td>Phase II</td>
<td>Approved: CML, Ph+ ALL; Phase II: other hematologic malignancies, colorectal cancer, GIST, head and neck cancer, liver cancer, melanoma/skin cancer, mesothelioa, NSCLC, pancreatic cancer, prostate cancer, solid tumors; Phase I: solid tumors</td>
</tr>
<tr>
<td>SKI-606 (bosutinib)</td>
<td>SFKs, Bcr-Abl, Src, Src-Abl</td>
<td>Phase II</td>
<td>Phase III: CML; Phase II: CML and Ph+ ALL; Phase I: solid tumors</td>
</tr>
<tr>
<td>AZD0530</td>
<td>SFKs, Bcr-Abl</td>
<td>Phase II</td>
<td>Phase II: colorectal cancer, gastric cancer, head and neck cancer, NSCLC, ovarian cancer, pancreatic cancer, prostate cancer; Phase I: solid tumors</td>
</tr>
<tr>
<td>XL999</td>
<td>Src, FGER1, Kit, PDGFR, VEGFR2</td>
<td>Phase II</td>
<td>Phase II: NSCLC*</td>
</tr>
<tr>
<td>AP22161</td>
<td>Src</td>
<td>Preclinical</td>
<td></td>
</tr>
<tr>
<td>AP22408, AP23451, AP23588</td>
<td>Src (in bone)</td>
<td>Preclinical</td>
<td></td>
</tr>
<tr>
<td>CGP76030</td>
<td>SFKs, Abl, EGFR, VEGFR</td>
<td>Preclinical</td>
<td></td>
</tr>
<tr>
<td>CGP77675</td>
<td>SFKs, EGFR, FAK, VEGFR</td>
<td>Preclinical</td>
<td></td>
</tr>
<tr>
<td>PD173955</td>
<td>Src, Abl, Kit, Yes</td>
<td>Preclinical</td>
<td></td>
</tr>
<tr>
<td>UC15A</td>
<td>Src</td>
<td>Preclinical</td>
<td></td>
</tr>
<tr>
<td>PP2</td>
<td>Src</td>
<td>Research agent</td>
<td></td>
</tr>
</tbody>
</table>

*Clinical development of XL999 was suspended in all tumor types following concerns over cardiovascular toxicity; approval was recently granted by the Food and Drug Administration to resume development in NSCLC.

SFK: Src family kinases; PDGFR: platelet-derived growth factor receptor; CML: chronic myeloid leukemia; Ph+: ALL, Philadelphia chromosome-positive acute lymphoblastic leukemia; GIST: gastrointestinal stromal tumor; NSCLC: non-small-cell lung cancer; FGFR1: fibroblast growth factor receptor-1; VEGFR: vascular endothelial growth factor receptor; EGFR: epidermal growth factor receptor; FAK: focal adhesion kinase.

Phase I and II clinical studies to validate the role of dasatinib in various subtypes of breast cancer—either as a single agent or in combination with capcitabine—are planned or ongoing (NCT00546104, NCT00452673, NCT00371345 and NCT00371254). A specific phase II study aimed at evaluating dasatinib’s potential in breast cancer bone metastasis is also ongoing (NCT00410813). Studies of dasatinib in pretreated patients with other solid tumors are ongoing. Initial pharmacokinetic data show that levels required to inhibit SFK activity are clinically achievable and that the drug is well tolerated [61].

**SKI-606**

Bosutinib (SKI-606; Wyeth, Madison, New Jersey) is an orally active inhibitor of Abl and SFKs that inhibits Src-dependent tyrosine phosphorylation. Treatment of human breast cancer MDA-MB-231 basal cells with SKI-606 resulted in a dose-dependent inhibition of cellular proliferation and invasion [62]. Additionally, SKI-606-treated cells showed reduced migration and increased apoptosis. Inhibition of migration was also observed in the minimally invasive MCF-7 and BT-474 breast cancer cell lines [62]. Preclinical studies have also shown activity in colon cancer xenografts [63]. SKI-606 is currently in clinical development in breast cancer and other solid tumors (NCT00319254). This compound has also reached phase II/III development in CML and Ph+ ALL.

**AZD0530**

AZD0530 (AstraZeneca, Wilmington, Delaware) is a potent, oral, selective inhibitor of Src and Abl kinases currently in phase I studies [64]. In MCF-7 human breast cancer cells expressing wild-type ER or a mutant ER hypersensitive to low levels of estrogen, AZD0530 blocked Src phosphorylation, resulting in reduced Src kinase activity [22]. The presence of estrogen did not significantly affect Src inhibition. AZD0530 also inhibited anchorage-dependent and -independent growth in several cell lines. In estrogen-responsive cell lines, higher levels of the inhibitor were required in the presence of estrogen, supporting the hypothesis that mitogenic effects of estrogen signaling are at least partly associated with higher Src kinase activity [22]. Additionally, AZD0530 increased cell sensitivity to growth inhibition by tamoxifen, although the addition of estrogen reversed these synergistic effects [22].

In a tamoxifen-resistant breast cancer cell line, AZD0530 significantly reduced basal activation of FAK and paxillin, suppressed cellular invasion, and had an additive effect on blocking invasion, when used in combination with an EGFR tyrosine kinase inhibitor in vitro [30]. This inhibitory activity is linked to FAK expression as treatment of the tamoxifen-resistant cells with FAK small interfering RNA (siRNA) reduced the effects of AZD0530 on cell adhesion and migration [65]. AZD0530 has also been shown to suppress tumor growth and proliferation in a range of tumor types and to inhibit osteoclast-mediated bone resorption [66–68]. A phase II trial of AZD0530 in patients with nonresectable metastatic or locally advanced breast cancer is under way (NCT00559507). A phase II study in patients with breast or prostate cancer with bone metastasis is also ongoing (NCT00558272).

**Src inhibitor-based combination treatment**

Although Src is widely implicated in cancer development, most evidence indicates that Src activity alone is not sufficient to cause oncogenic transformation. Because Src represents a novel target for anticancer therapies, however, the possibility exists that Src-targeting agents could be used in combination with...
other classes of agent with a potential for synergistic activity. Targeted agents currently available and in development for breast cancer that might be suitable for combination include EGFR/HER family inhibitors (e.g. trastuzumab, lapatinib, gefitinib and erlotinib), VEGF inhibitors (e.g. bevacizumab and sunitinib), and PDGFR inhibitors (e.g. sunitinib) [69]. In addition, given the role of Src in ER signaling, Src inhibitors might also be useful in combination with endocrine therapy. This is supported by the observation that estrogen reduced the effectiveness of AZD0530 in restricting the growth of ER-positive cells [22]. Src inhibition has also been shown to augment the therapeutic effects of cytotoxic chemotherapy drugs in vitro [70, 71]. Studies on the clinical effects of combination treatment including Src inhibition are awaited.

conclusions

Src is one of the best studied oncogenes, yet it has not been validated as an effective therapeutic target to treat human cancers. As we have seen, it is an important messenger in many signal transduction pathways that have been implicated in oncogenesis—from proliferation to invasion and metastatic spread. Moreover, SFKs appear to be important in the growth of breast cancer cells of luminal, basal and post-EMT origin, and independently of hormonal receptor or HER2 status [22, 54, 55, 62]. Now that pharmacologic inhibitors of Src are moving into the clinic, the challenge will be identifying those patients most likely to benefit from one of these agents. Preclinical studies with the only currently approved Src inhibitor, dasatinib, provide a platform to help guide its clinical development in solid tumors. When examining luminal, basal and HER2-amplified breast cancer cell lines, it was the basal/triple-negative subtype that seemed more likely to respond in vitro than the ER+ or HER2-amplified groups. Clinical studies of dasatinib in breast cancer are planned, including one for either HER2-amplified or ER+ disease and one for the triple-negative group.

In addition to identifying the best ‘molecular’ type of cancer, rather than ‘histological’ type, in which to pursue the development of an Src inhibitor, the optimal clinical setting must also be determined. Src inhibitors may have a role in advanced disease, but even if no activity is observed in this setting, Src’s role in invasion and metastasis might still enable a potential role for Src inhibitors in the adjuvant setting to reduce the risk of recurrence after definitive therapy. Further studies are necessary to identify potential combinations that will be of benefit including combinations with cytotoxic chemotherapy and antihormonal agents in the frontline setting, and after the development of resistance. Arguably the most exciting prospect will be to identify a rational combination of novel agents such as an Src inhibitor with another signal transduction inhibitor that provides improved patient outcomes with manageable toxic effects. The large amount of knowledge surrounding Src and its cellular interactions supports this approach. Finally, as we translate the decades of research into Src biology into clinical practice, we will need to successfully integrate the scientific triumphs of the past with the technological tools of the present and future.

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references


