Advances in Targeting Src in the Treatment of Breast Cancer and Other Solid Malignancies

Erica L. Mayer and Ian E. Krop

Abstract

Src, a membrane-associated nonreceptor tyrosine kinase, plays a crucial role in the coordination and facilitation of cell-signaling pathways controlling a wide range of cellular functions, including growth, survival, invasion, adhesion, and migration. Deregulation and increased activity of Src has been observed in multiple human malignancies, prompting the development of specific inhibitors of Src. In preclinical studies, Src inhibitors show antitumor effects in multiple solid tumor types. Recently completed early-phase trials using the inhibitors dasatinib and bosutinib have suggested modest activity as monotherapy in breast and prostate cancer, with potentially greater activity in combination regimens. Given the interaction between Src and the estrogen receptor, ongoing trials are exploring combinations with endocrine therapy. The relationship between Src and the vascular endothelial growth factor receptor also justifies investigation of combinations with angiogenesis inhibitors. Future trials will continue to explore the contribution of Src inhibition with both chemotherapy and targeted agents.

Background

Src, a membrane-associated nonreceptor tyrosine kinase, belongs to the Src family kinase group (SFK), which includes the proteins Lyn, Fyn, Lck, Yes, Hck, Blk, Fgr, and Yrk (1, 2). SFKs are involved in a number of important signal transduction pathways and have pleiotropic effects on cellular function. SFKs integrate and regulate signaling from multiple transmembrane receptor-associated tyrosine kinases, such as the epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), insulin-like growth factor-1 receptor (IGF-1R), vascular endothelial growth factor receptor (VEGFR), HER2, and others (2–4), leading to activation of intracellular target proteins including PI3-kinase, focal adhesion kinase (FAK), Ras, and signal transducers and activators of transcription 3 (Stat3). Together, these actions modulate cell survival, proliferation, differentiation, and angiogenesis (5–7). SFKs, through interactions with integrins, FAK, the catenin-cadherin complex, and RhoA, also play a prominent role in the regulation of cell motility, adhesion, and invasion. Figure 1 summarizes the intracellular functions of Src. Src typically is expressed ubiquitously, with predominance in platelets, neuronal tissue, and bone. Activity of Src is regulated by several mechanisms, including phosphorylation of key tyrosine residues in its regulatory domain and interaction with binding proteins (2).

Src activation is relevant for activation of osteoclasts (8), and Src-deficient osteoclasts show aberrant cellular function (9). Src-deficient mice display a phenotype of osteopetrosis, secondary to decreased bone resorption by osteoclasts (10). Interestingly, Src-deficient mice do not display any other detectable abnormalities in tissues with high Src expression, potentially because of redundancy of function with other signal transduction molecules.

Given its central role in many of the cellular functions involved in tumor progression, it is not surprising that dysregulation of Src can lead to malignant transformation, and notably, Src was the first proto-oncogene identified. Over the past 30 years, multiple studies have shown increased activity of Src in a variety of neoplastic tissues (11–13). Directed gene mutations leading to constitutive Src activation can result in cellular transformation (14). However, in human cancers, the majority of Src dysregulation seems to occur via nongenetic events, including maintenance of an activated phosphorylated status, association with stimulatory binding proteins, and increased signaling from receptor tyrosine kinases (1, 2). Src activation also seems to facilitate development of the epidermal-to-mesenchymal transformation characteristic of the malignant phenotype (15), and increased Src activity has been associated with increased propensity for metastases in animal models of breast and prostate cancer (16, 17).

Inhibitors of Src

Together, these data linking overexpression and/or increased activity of Src with a malignant phenotype led to...
the recognition that Src may potentially be a useful therapeutic target in a number of cancer types. Subsequently, several inhibitors of Src have been developed. One of the best studied is dasatinib (Sprycel, BMS354825; Bristol-Myers Squibb Oncology). Dasatinib is a potent oral small molecule inhibitor of the Src tyrosine kinase ($IC_{50} = 0.55$ nmol/L) and related SFKs, in addition to c-kit, PDGFR, and Bcr-Abl (18, 19). In addition to targeting Src, dasatinib has activity against BCR-ABL mutants resistant to imatinib, and is currently approved for the treatment of imatinib-resistant chronic myelogenous leukemia and Philadelphia chromosome positive (Ph-positive) acute lymphoblastic leukemia (20). Another agent, bosutinib (SKI-606, Wyeth), is an oral dual selective competitive inhibitor of both Src ($IC_{50} = 1.0$ nmol/L) and Abl tyrosine kinases, with moderate inhibition of the Axl tyrosine kinase, Eph receptors, and Ste20 family kinases (21). Multiple other agents with activity against Src, including Saracatinib (AZD0530; AstraZeneca) and XL999 (Exelixis) are in preclinical or early-phase clinical development.

Preclinical data supporting Src inhibition

*In vitro*, inhibitors of Src inhibit growth and migration in a variety of neoplastic solid tumor cell lines, including breast, prostate, colon, pancreatic, and lung (17, 22–27). Use of Src inhibitors leads to decreased phosphorylation of multiple Src substrates including mitogen-activated protein kinase (MAPK), FAK, Akt, and subsequent growth inhibition (24, 28). Additionally, in tumor xenograft models, treatment with Src inhibitors leads not only to growth inhibition, but also to reduction in the presence of tumor metastases (24, 29).

To improve the chance of successful clinical development of Src inhibitors in breast cancer, efforts have been made to identify molecular subtypes of breast cancer that are most likely to respond to Src-targeted agents. One candidate subtype is the “basal-like” subgroup, molecularly characterized by a basal-like gene expression signature and defined clinically as “triple negative,” lacking expression of estrogen receptor, progesterone receptor, and HER2. Screening a spectrum of breast cancer cell lines with dasatinib highlighted preferential sensitivity to Src inhibition in basal-like breast cancers, with overexpression of moesin, caveolin-1, and yes-associated protein-1 identified as markers of dasatinib sensitivity (23). Triple negative tumors also tend to overexpress EGFR, and dose-dependent inhibition of cell lines overexpressing EGFR has been observed with dasatinib (25). Of note, decreased EGFR phosphorylation in Src inhibitor-treated cells was also observed, further supporting a functional interaction between Src and EGFR.

Src is also involved in coordinating signaling from the steroid receptors, including the estrogen receptor (ER) and androgen receptor (AR), and plays a role in the non-classical (nongenomic) effects of these receptors. For example, multiple studies have shown crosstalk between ER/AR and Src, with ER/AR activation leading to activation of Src, and subsequent Src-mediated cell proliferation (30, 31). Blocking the interaction between ER/AR and Src leads to inhibition of downstream cellular pathways, and cessation of cell growth (31). Several studies have shown associations between resistance to endocrine therapy and both increased levels of Src activity and an increasingly invasive and aggressive tumor phenotype (32–34). Most recently, genomic profiling of prostate cancer cell lines has identified an AR signature predictive...
of AR activity; levels of this signature were inversely correlated with Src activity, suggesting a role for Src in the setting of endocrine resistance (35).

Given this data, specifically targeting Src may overcome endocrine resistance in hormonally driven cancers. Use of a Src inhibitor in endocrine-resistant breast cancer cell lines results in decreases in activated Src, as well as reductions in invasive ability (32). Similarly, the Src inhibitor AZD0530 has been found to inhibit growth of androgen-independent prostate cancer cell lines and to prevent nuclear translocation of the AR (36). Combined approaches, using Src inhibitors in combination with endocrine or growth factor inhibitors, have also suggested benefit. For example, combination therapy with a Src inhibitor and the EGFR inhibitor gefitinib produced additive growth inhibition of tamoxifen-resistant breast cancer cell lines (32). Combination strategies with endocrine agents and Src inhibitors have shown not only synergistic inhibition of Src activity, but also suppression of emergence of a resistant phenotype, and eventual cell death (37, 38).

Src is known to directly associate with activated HER2, and this interaction results in increased Src kinase activity (39, 40). Src activity plays a role in HER2-mediated invasion and metastasis (41). Several recent studies suggest that Src also plays a role in resistance of HER2+ breast cancer to the HER2-targeted antibody trastuzumab (42, 43). Consistent with these results, the combination of dasatinib and trastuzumab in breast cancer cell lines overexpressing HER2 leads to synergistic growth inhibition (44).

Clinical-Translational Advances

Phase I data in human solid tumors have shown both dasatinib and bosutinib monotherapy to be generally well tolerated, with pleural effusion as a prominent toxicity (45–47). Other toxicities seen with Src inhibitor treatment in these studies included gastrointestinal (nausea, vomiting, diarrhea), anorexia, and fatigue. Stable disease was observed in multiple tumor types, and pharmacokinetic data showed rapid absorption and lack of accumulation using once daily dosing for both agents. Although no objective radiologic responses were seen in one of the studies of dasatinib in refractory patients, preliminary pharmacodynamic analysis using PET imaging showed a metabolic partial response (PR) in 25% of patients at the end of cycles 1 and 2 (45). Inhibition of phospho-Src in peripheral blood mononuclear cells seemed to be dose dependent and directly correlated with dasatinib plasma concentration (48). Multiple subsequent studies, described below and in Table 1, have investigated the role of Src inhibitors in several solid tumor types.

Breast Cancer

Monotherapy

Several phase II studies have evaluated Src inhibitor monotherapy in breast cancer. In one study, 73 patients with pretreated metastatic breast cancer received bosutinib at 400 mg daily. As in the phase I experience, gastrointestinal toxicity and fatigue were prominent. Six percent of patients achieved a PR, whereas an additional 21% showed stable disease ≥24 weeks. The 16-week progression-free survival (PFS) rate was 45%, and the median PFS was 15 weeks (49). Dasatinib monotherapy has been evaluated in two parallel phase II breast cancer trials, each focusing on a distinct breast cancer subtype. In CA180059, 44 patients with predominantly pretreated metastatic triple negative breast cancer received dasatinib monotherapy. A response rate (RR) of 5% was observed, with a clinical benefit rate (PR + stable disease ≥26 weeks) of 9%. Although toxicity was prominent at the initial dose of 100 mg twice daily, the drug was well tolerated after the dose was reduced to 70 mg twice daily (50). A second study, CA180088, was open to patients with both ER+ and/or HER2+ disease. A total of 70 patients were treated: 24 HER2+ and 46 ER+. Of the response-evaluable population from both subtypes, a RR of 4% was seen, with a clinical benefit rate of 8% in the HER2+ cohort, and 16% in the ER+ cohort. Interestingly, all benefit was seen in patients with ER+ any tumors. As with CA180059, toxicity was manageable once dosing was adjusted (51).

Efforts are underway to analyze correlative data from these trials to better identify tissue-based and circulating biomarkers predictive of benefit from Src inhibition. Markers under investigation include downstream effectors of Src pathway signaling, including caveolin, EphA2, insulin-like growth factor binding protein 2, VEGFR2, and Coll IV (52). Additionally, analyses using proteomic and RNA microarray technology are being pursued.

Combination therapy

There has been particular interest in combining Src inhibitors with endocrine agents for hormone receptor-positive breast cancer pretreated with endocrine therapy. Several ongoing trials are evaluating the Src inhibitors dasatinib and bosutinib in combination with endocrine agents in patients with evidence of endocrine resistance.

Src inhibitors are also being studied in combination with chemotherapy in breast cancer. Src inhibition has been shown in vitro to augment the chemosensitivity of cancer cells to 5-FU (53), leading to exploration of Src inhibitors in combination with capecitabine. CA180-004 was a phase I study evaluating escalating doses of capecitabine and dasatinib. Thirty-one patients with metastatic breast cancer were treated in a dose escalation phase, with an additional 21 patients treated in an expansion cohort at dasatinib 100 mg daily and capecitabine 1,000 mg twice a day, days 1 through 14 of a 21-day cycle. A total of 7 patients (13.5%) experienced a PR, with an additional 7 having stable disease ≥6 months (54). An ongoing phase I-II trial will evaluate a similar combination of bosutinib and capecitabine. Dasatinib has also been explored in a phase I dose escalation trial in combination with weekly paclitaxel at 80 mg/m². In this trial of 13 patients, therapy was well tolerated with dose limiting toxicity observed only in the top dose level of dasatinib, 150 mg daily. Responses and/or
Table 1. Src inhibitors in clinical trials

<table>
<thead>
<tr>
<th>Name</th>
<th>Study status</th>
<th>Disease</th>
<th>Phase</th>
<th>Type</th>
<th>No. of treated patients</th>
<th>RECIST RR-CBR (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dasatinib</td>
<td>Completed</td>
<td>Breast</td>
<td>II</td>
<td>Monotherapy HR+ and/or HER2+ negative</td>
<td>70</td>
<td>4-13</td>
<td>Mayer et al. (51)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Breast</td>
<td>II</td>
<td>Monotherapy triple negative</td>
<td>44</td>
<td>5-9</td>
<td>Finn et al. (50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Breast</td>
<td>I</td>
<td>Combination with capecitabine</td>
<td>52</td>
<td>14-27</td>
<td>Cortes et al. (54)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Breast</td>
<td>I</td>
<td>Combination with paclitaxel</td>
<td>13</td>
<td>30-55</td>
<td>Morris et al. (55)</td>
</tr>
<tr>
<td>Prostate</td>
<td>II</td>
<td>Monotherapy</td>
<td></td>
<td></td>
<td>47</td>
<td>9-26</td>
<td>Yu et al. (56)</td>
</tr>
<tr>
<td>Prostate</td>
<td>I-II</td>
<td>Combination with docetaxel</td>
<td></td>
<td></td>
<td>46</td>
<td>42-68</td>
<td>Araujo et al. (57)</td>
</tr>
<tr>
<td>SCLC</td>
<td>II</td>
<td>CALGB 30602; Monotherapy</td>
<td></td>
<td></td>
<td>43</td>
<td>0-0</td>
<td>Miller et al. (58)</td>
</tr>
<tr>
<td>NSCLC</td>
<td>I-II</td>
<td>Combination with erlotinib</td>
<td></td>
<td></td>
<td>34</td>
<td>7-63</td>
<td>Haura et al. (60)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>II</td>
<td>Monotherapy</td>
<td></td>
<td></td>
<td>37</td>
<td>6-14</td>
<td>Kluger et al. (59)</td>
</tr>
<tr>
<td>Colon</td>
<td>I</td>
<td>Combination with FOLFOX6 and cetuximab</td>
<td></td>
<td></td>
<td>30</td>
<td>20-56</td>
<td>Kopetz et al. (61)</td>
</tr>
<tr>
<td>Bosutinib</td>
<td></td>
<td>Breast</td>
<td>II</td>
<td>Monotherapy</td>
<td>73</td>
<td>6-27</td>
<td>Campone et al. (49)</td>
</tr>
<tr>
<td>Saracatinib</td>
<td></td>
<td>Prostate</td>
<td>II</td>
<td>Monotherapy; AIPC</td>
<td>28</td>
<td>0-0</td>
<td>Lara et al. (69)</td>
</tr>
<tr>
<td>Saracatinib</td>
<td></td>
<td>Pancreatic</td>
<td>II</td>
<td>Monotherapy</td>
<td>19</td>
<td>0-0</td>
<td>Nallapareddy et al. (70)</td>
</tr>
<tr>
<td>Dasatinib</td>
<td>Ongoing (selected)</td>
<td>Prostate</td>
<td>III</td>
<td>Docetaxel 75 mg/m² Q21D ± dasatinib 100 mg QD</td>
<td>82</td>
<td>20-24</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ovarian</td>
<td>I</td>
<td>Dasatinib, paclitaxel, carboplatin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solid tumor</td>
<td>I</td>
<td>Dasatinib with bevacizumab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Breast</td>
<td>I</td>
<td>Dasatinib with weekly paclitaxel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colon</td>
<td>II</td>
<td>FOLFOX6 + cetuximab ± dasatinib</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bosutinib</td>
<td></td>
<td>Breast</td>
<td>II</td>
<td>Randomized exemestane ± bosutinib</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Breast</td>
<td>II</td>
<td>Randomized letrozole ± bosutinib</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Breast</td>
<td>I-II</td>
<td>Capecitabine with bosutinib</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saracatinib</td>
<td></td>
<td>SCLC</td>
<td>II</td>
<td>Monotherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saracatinib</td>
<td></td>
<td>Pancreatic</td>
<td>I-II</td>
<td>AZM475271 + gemcitabine</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CBR, clinical benefit rate (RR + stable disease ≥ 16-24 weeks); HR+, hormone receptor positive; HER2+, human epidermal receptor 2 positive; AIPC, androgen-independent prostate cancer; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; FOLFOX, oxaliplatin, leucovorin, and 5-FU; QD, daily.
prolonged stable disease were observed, including in patients with prior taxane exposure (55).

**Prostate Cancer**

On the basis of the preclinical data suggesting Src inhibition can overcome resistance to androgen deprivation in prostate cancer, several phase I-II studies have evaluated Src inhibitors in castration-resistant prostate cancer (CRPC). A phase II study of dasatinib monotherapy in 47 patients with chemotherapy-naïve CRPC and increasing prostate-specific antigen (PSA) showed 43% of patients with lack of progression at week 12, and 19% at week 24. Two of 19 patients had a Response Evaluation Criteria In Solid Tumors (RECIST) response, with a disease control rate at 24 weeks of 26%. Three patients experienced a PSA decline of ≥50%, and 79% showed prolongation in PSA doubling time (56). Markers of bone turnover were also examined; 51% achieved a ≥40% reduction in urinary N-telopeptide at 3 months, a marker of the extent of bone metastases (56), regardless of concurrent bisphosphonate use (56). Toxicity in this study was in line with prior experience, including diarrhea (62%), nausea (47%), and fatigue (45%). Dosing was decreased midtrial from 100 mg to 70 mg twice daily, because of a higher than expected incidence of pleural effusions. A total of 42% of patients experienced a PR, with 68% having PR or stable disease ≥16 weeks. A PSA response (PSA decrease of ≥50% from baseline for at least 6 weeks) was observed in almost half of the patients. Half of all patients were also noted to have a ≥35% reduction in the uNTX markers of bone turnover (57). On the basis of the results of this study, an ongoing phase III trial randomizing patients to docetaxel 100 mg/m² every 21 days alone, or with dasatinib 100 mg daily by mouth, is ongoing and will examine the contribution of the Src inhibitor to chemotherapy in prostate cancer.

**Other Tumor Types**

Early stage evaluation of Src inhibitor monotherapy in other tumor types has shown limited clinical activity. CALGB 30602 was a phase II trial of dasatinib monotherapy in chemosensitive relapsed small cell lung cancer. A total of 43 patients were treated; no responses were seen, and only 13 instances of PFS ≥6 weeks were observed, leading to early termination of the study for futility (58). A phase II trial in advanced melanoma showed a RR of 6% with a clinical benefit rate of 14%; toxicity was notable with 37% of patients developing a pleural effusion (59).

Results of recent clinical trials have suggested potential promise from the combination of Src and EGFR inhibitors. In a phase I-II study of dasatinib and erlotinib in advanced non-small cell lung cancer, 19 patients received escalating doses of combination therapy, with a phase 2 dose of erlotinib 150 mg daily and dasatinib 140 mg daily, confirmed in a subsequent cohort of 15 patients. Toxicity seemed nonsynergistic, and activity was observed, with two PRs and a clinical benefit rate of 62% (60). In metastatic colon cancer, a phase I trial combining dasatinib with FOLFOX6 chemotherapy and cetuximab found dasatinib dosed at 150 mg daily to be tolerable, with activity observed in the 30-patient heavily pretreated cohort (61). Ongoing phase II trials will discern the contribution of the Src inhibitor to these regimens.

**Src Inhibition and Angiogenesis**

Evidence supports a role for Src signaling in angiogenesis. Expression of vascular endothelial growth factor (VEGF) is promoted by hypoxic conditions. Cell lines overexpressing Src show increased production of VEGF when exposed to hypoxic conditions, suggesting a role for Src in linking the hypoxia signal and VEGF expression (62). Inhibition of Src with an antisense expression vector leads to reduced levels of VEGF (63). Other observations have shown the requirement for Src activity for VEGF-mediated angiogenesis, vascular permeability, and tumor cell extravasation (29, 64). Recent work has suggested a role for dual VEGFR/Src blockade in the treatment of neovascular-related retinopathy (65). In the parallel breast cancer phase II studies of dasatinib monotherapy (CA180059/88), biomarker analysis showed rapid increases in circulating VEGFR with exposure to dasatinib (52). Increases in VEGF have previously been observed with exposure to the anti-VEGF antibody bevacizumab (66). This observation further supports the link between Src and VEGF signaling, and suggests that combination therapy with Src inhibitors and VEGF or VEGFR inhibitors warrants exploration.

**Future Developments**

Src plays a central role in cell signaling, leading to growth and metastatic potential. Multiple inhibitors of Src have been developed and are in trials for solid tumors. To date, results have been modest, with some variation in activity among the agents and potentially improved activity in combination with chemotherapy. Ongoing trials are investigating new tumor types and, in breast cancer, evaluating the activity of Src inhibitors in combination with endocrine therapy. Future directions of investigation will determine the clinical relevance of Src inhibition for solid tumors. As redundancy in cellular pathways may limit the efficacy of single receptor blockade, multitargeted therapy, perhaps including agents against EGFR or VEGFR, might improve observed activity. 
Additionally, enhanced identification of markers predictive of response to anti-Src therapy would enhance patient selection for treatment. Already, early results from contemporary genomics show promise in identifying such molecular and genomic predictors of therapeutic response to Src inhibitors (67, 68). Ultimately, despite our rich understanding of the biology of Src in human malignancy, more work is needed to determine whether this knowledge can be translated into an effective clinical tool.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.


Advances in Targeting Src in the Treatment of Breast Cancer and Other Solid Malignancies

Erica L. Mayer and Ian E. Krop


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/16/14/3526

Cited articles
This article cites 69 articles, 28 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/16/14/3526.full.html#ref-list-1

Citing articles
This article has been cited by 18 HighWire-hosted articles. Access the articles at:
/content/16/14/3526.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.