

### The Role of Src in Solid Tumors

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#### ABSTRACT

The proto-oncogene *c-Src* (*Src*) encodes a nonreceptor tyrosine kinase whose expression and activity are correlated with advanced malignancy and poor prognosis in a variety of human cancers. Nine additional enzymes with homology to Src have been identified and collectively are referred to as Src family kinases (SFKs). Together, SFKs represent the largest family of nonreceptor tyrosine kinases and interact directly with receptor tyrosine kinases, G-protein-coupled receptors, steroid receptors, signal transducers and activators of transcription, and molecules involved in cell adhesion and migration. These interactions lead to a diverse array of biological functions including proliferation, cell growth, differentiation, cell shape, motility, migration, angiogenesis, and survival. Studies investigating

mutational activation of Src in human cancers suggest that this may be a rare event and that wild-type Src is weakly oncogenic. Thus, the role of Src in the development and progression of human cancer remains unclear. Recently, it was suggested that increased SFK protein levels and, more importantly, SFK tyrosine kinase activity are linked to cancer progression and metastatic disease by facilitating the action of other signaling proteins. This accumulating body of evidence indicates that SFKs may represent a promising therapeutic target for the treatment of solid tumors. This review discusses the role of SFKs in solid tumors and the recent therapeutic advances aimed at targeting this family of tyrosine kinases in cancer. *The Oncologist* 2009;14:667–678

#### SRC AND THE SRC FAMILY KINASES

Src was first identified as the cellular form of v-Src, the transforming gene product of the avian Rous sarcoma virus [1, 2]. Src has been strongly implicated in the development, maintenance, progression, and metastatic spread of several human cancers, such as prostate, lung, breast, and colorectal cancer. Since the discovery of the *Src* proto-oncogene in

1976, nine additional variants closely related to Src have been identified in the human genome and collectively termed Src family kinases (SFKs) [3, 4]. In general, SFKs are subdivided into three distinct groups primarily based upon their general pattern of expression (Table 1). The first group (Src, Fyn, and Yes) is ubiquitously expressed. The second group (Hck, Lck, Lyn, Blk, Yrk, and Fgr) is found

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**Table 1.** The Src family kinases

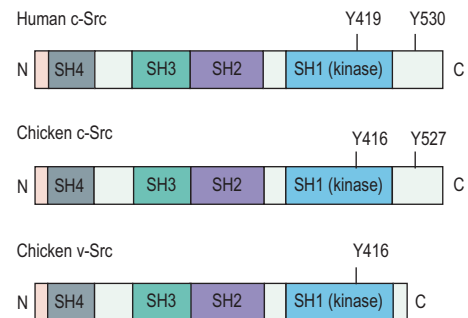
Gene	Expressing tissues	Subfamily	Chromosomal locus
<i>Src</i>	Ubiquitous	SrcA	20q11
<i>Fyn</i>	Ubiquitous	SrcA	6q21
<i>Yes</i>	Ubiquitous	SrcA	18q2
<i>Hck</i>	Myeloid, lymphoid	SrcB	20q11
<i>Lck</i>	Lymphoid	SrcB	1p35
<i>Lyn</i>	Myeloid, prostate, pancreatic	SrcB	8q13
<i>Blk</i>	Myeloid	Other	8p22
<i>Yrk</i>	Neural, hematopoietic tissues	Other	1p36
<i>Fgr</i>	Leukocytic	Other	1p36
<i>Frk</i>	Pancreatic, kidney, breast	Other	6q21

primarily in hematopoietic cells, and the third group (Frk-related kinases) is expressed predominantly in epithelial-derived tissues [5–8]. Although these proteins are classified based upon their expression in various tissues, it is also widely recognized that alternatively spliced isoforms, as well as the level of expression and activity, play a role in their cellular function.

### SFK STRUCTURE AND ACTIVATION

Structurally, SFKs are highly related to one another and contain conserved structural elements among family members (Fig. 1). These elements include the N-terminal Src homology 4 (SH4) domain, the SH3 domain, the SH2 domain, a linker sequence, the tyrosine kinase domain, and the C-terminal tail [9]. The N-terminal domain, SH4, serves as a site for myristoylation and thus targets SFKs to the cytoplasmic membrane. The SH3 domain binds amino acid sequences rich in proline residues [10]. This domain is critical for Src activity, intracellular localization, and the recruitment and binding of Src substrates. The SH2 domain binds to short motifs containing phosphotyrosines. Together, the SH2 and SH3 domains cooperate in regulating SFK catalytic activity (Fig. 2).

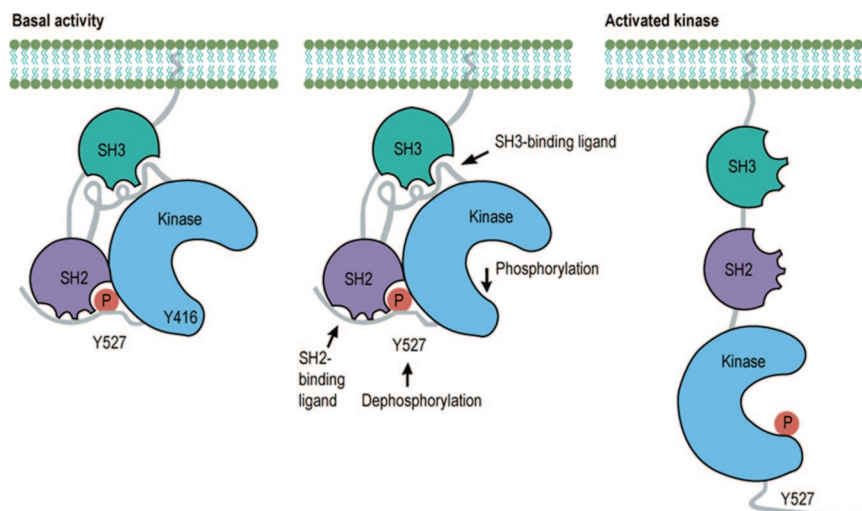
In the inactive conformation, Src contains a phosphorylated tyrosine at position 530 in humans, which interacts with its own SH2 domain. This positions the SH3 domain to interact with the proline-rich linker domain and keeps Src in a tightly bound inactive state. Upon dephosphorylation of tyrosine 530, intramolecular interactions are destabilized, ultimately resulting in the autophosphorylation of tyrosine 419 [11–13]. This series of events then allows the opening of the molecule and frees the SH2 and SH3 domains to in-



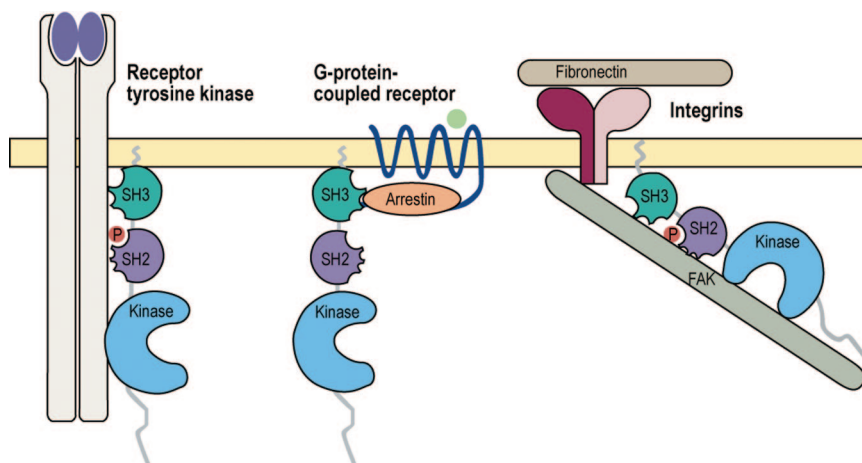
**Figure 1.** Comparison of the molecular structures of human c-Src, chicken c-Src, and chicken v-Src. All three proteins contain four Src homology (SH) domains and a unique amino-terminal domain of unknown function. The SH1 domain contains the kinase domain and a conserved tyrosine residue involved in autophosphorylation (Tyr419 in human c-Src, Tyr416 in chicken). Chicken v-Src lacks the carboxy-terminal negative-regulatory domain and contains 12 substituted carboxy-terminal amino acids, as well as numerous point mutations throughout the molecule, explaining the high level of activity of this protein. Reprinted from Yeatman TJ. A renaissance for Src. *Nat Rev Cancer* 2004;4:470–480, with permission from Macmillan Publishers Ltd.

teract with receptor tyrosine kinases, G-protein-coupled receptors, and focal adhesion kinase (FAK) (Fig. 3).

Once activated, Src is involved in the regulation of normal and oncogenic processes, including proliferation, differentiation, survival, motility, and angiogenesis (Fig. 4). However, it is well established that overexpression of wild-type *Src* is weakly oncogenic on its own [14]. Furthermore, a number of investigators have shown that mutations leading to the constitutive activation of Src in human cancers are rare [15–19]. Taken together, the poor transformation potential of Src coupled with the lack of mutational activation in human cancers has clouded our understanding of Src in the development, maintenance, and progression of cancer. Recent evidence has suggested that overexpression of wild-type Src may promote the activity of other signaling molecules in contrast to being a lone dominant transforming agent [20]. Indeed, Src has been shown to interact with several proteins, including receptor tyrosine kinases—colony-stimulating factor 1 receptor, platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR), epidermal growth factor receptor (EGFR), human epidermal growth factor receptor (HER)-2, and HER-3 [2]. Other interaction partners include signal transducers and activators of transcription (STATs), heterotrimeric G proteins, the mitogen-activated protein kinase extracellular signal-regulated kinase-2, cyclins D and E, and FAK [21, 22]. In antigen-presenting cells, the SFK Fyn has been shown to be recruited and activated by signaling lymphocyte activation molecule family proteins via interactions with its SH3 domain [23].



**Figure 2.** Activation of c-Src. The left panel represents the inactive conformation of Src in which Tyr527 (chicken c-Src) interacts with the Src homology 2 (SH2) domain, positioning the SH3 domain to interact with the linker between the SH2 and catalytic domains. The middle panel illustrates different mechanisms involved in the activation of Src, and the right panel represents the open or active conformation. Reprinted from Martin GS. The hunting of the Src. *Nat Rev Mol Cell Biol* 2001;2:467–475 with permission from Macmillan Publishers Ltd.



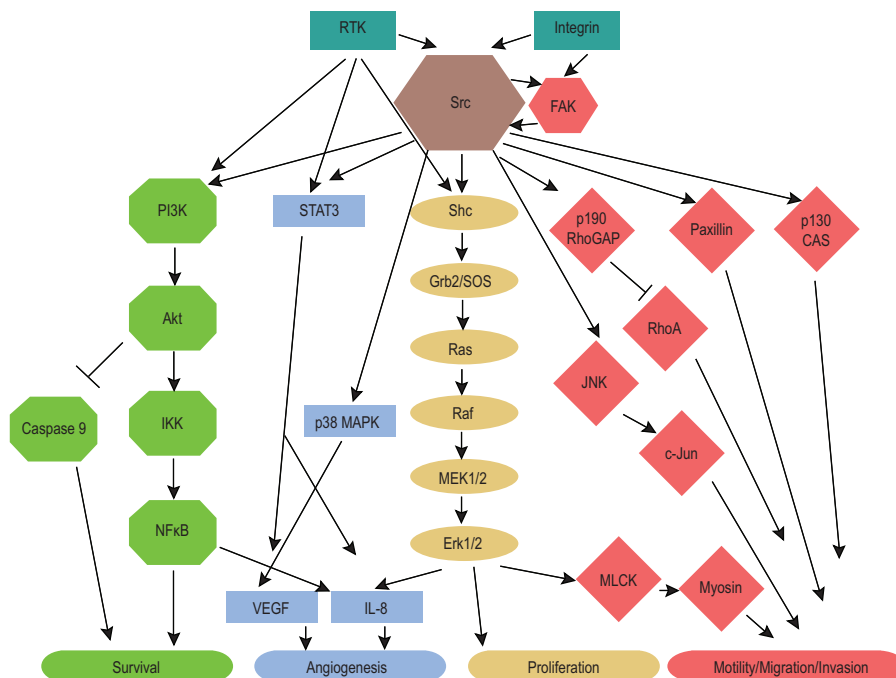
**Figure 3.** Postulated regulation of c-Src by surface receptors. Src can be activated by several types of cell surface or cytoplasmic receptors. Shown here is, from left to right, a receptor tyrosine kinase such as the platelet-derived growth factor receptor, a G-protein-coupled receptor such as the  $\beta$ -adrenergic receptor, and an integrin bound to extracellular matrix. In each case, Src is activated by binding of a ligand to the Src homology 2 (SH2) and/or SH3 domains. In the case of a receptor tyrosine kinase, the SH2 ligand is a phosphotyrosine residue of the autophosphorylated receptor. In the case of the  $\beta$ -adrenergic receptor, the SH3 ligand is a proline motif of  $\beta$ -arrestin bound to the receptor. In the case of an integrin, the SH2 ligand is a phosphorylated tyrosine residue of autophosphorylated focal adhesion kinase (FAK). Reprinted from Martin GS. The hunting of the Src. *Nat Rev Mol Cell Biol* 2001;2:467–475 with permission from Macmillan Publishers Ltd.

With the re-emergence of SFKs in cancer, intense efforts have been made to identify and characterize agents that possess inhibitory activity to SFKs. These small molecules function by interfering with the kinase domain or blocking the SH2 and SH3 domains from entering the conformation necessary for activation. Most notable of these agents are dasatinib (BMS-354825), bosutinib (SKI-606), AZD-0530, XL-999, INNO-406 (NS-187), KX01, and XL-228. These agents exhibit a variety of mechanisms of action

and have often shown marked efficacy in preclinical and clinical settings (Table 2). This review addresses the latest studies that strengthen the role of Src and SFKs in solid tumor types and their potential as therapeutic targets.

#### SRC AND SFK MOLECULAR TARGETING AGENTS IN DEVELOPMENT

Dasatinib (Sprycel®; Bristol-Myers Squibb, Princeton, NJ), also known as BMS-354825, is the only U.S. Food and



**Figure 4.** Selected examples of Src signal transduction pathways.

Abbreviations: CAS, Crk-associated substrate; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; IKK, IκB kinase; IL-8, interleukin 8; JNK, Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase MAPK/ERK kinase; MLCK, myosin light chain kinase; NFκB, nuclear factor κB; PI3K, phosphatidylinositol 3' kinase; RhoGAP, Rho GTPase-activating protein; RTK, receptor tyrosine kinase; SOS, son of sevenless; STAT3, signal transducer and activator of transcription 3; VEGF, vascular endothelial growth factor. Reprinted with permission from American Association for Cancer Research, from Summy JM, Gallick GE. Treatment for advanced tumors: Src reclaims center stage. *Clin Cancer Res* 2006;12:1398–1401; permission conveyed through Copyright Clearance Center, Inc.

Drug Administration (FDA) approved SFK inhibitor for use in chronic myeloid leukemia (CML) or Philadelphia chromosome-positive (Ph<sup>+</sup>) acute lymphocytic leukemia (ALL). Several phase II and III clinical trials regarding its use in CML and ALL have been reported, and others are ongoing. Phase I and II trials regarding dasatinib's use in non-Hodgkin's lymphoma, metastatic breast and prostate cancer, refractory leukemia in adolescents, and other metastatic cancers are also ongoing. Dasatinib has the potential for becoming a beneficial treatment for solid tumors.

Bosutinib (SKI-6606; Wyeth Pharmaceuticals, Inc., Madison, NJ) is a dual kinase inhibitor of both SFKs and Abl. There are currently clinical trials studying bosutinib's effect in imatinib-resistant CML (phase I, II, and III) as well as two closed clinical trials using bosutinib in breast cancer (phase II) and advanced malignant solid tumors (phase I).

AZD-0530 (AstraZeneca Pharmaceuticals, Wilmington, DE) is a dual tyrosine kinase inhibitor of both SFKs and Abl. There are several phase II studies ongoing using AZD-0530, all of which include metastatic cancer or cancer refractory to standard chemotherapies (i.e., breast cancer, non-small cell lung cancer [NSCLC], sarcoma, head and

neck squamous cell carcinoma [HNSCC], prostate cancer, thymoma, thymic carcinoma, pancreatic cancer, ovarian cancer, and adenocarcinoma of the stomach or gastroesophageal junction).

XL-999 (Exelixis, South San Francisco, CA) is a receptor tyrosine kinase inhibitor with several targets, including VEGFR, PDGFR, fibroblast growth factor receptor, Src, and FMS-like tyrosine kinase 3. A phase II trial was initiated using XL-999 in four solid tumors: renal cell carcinoma, colon cancer, ovarian cancer, and NSCLC. The FDA suspended the trial in 2006 because of adverse cardiovascular events. However, recently, a phase I trial in NSCLC was reinitiated in 2007.

INNO-406 (CytRx, Los Angeles, CA), also known as NS-187, is also a dual kinase inhibitor of Abl and Lyn (SFK) and is structurally similar to nilotinib. It was intended for imatinib-resistant CML patients with Lyn overexpression.

KX01 (KX2-391; Kinex, Buffalo, NY) is a recent addition to the Src inhibitor class. This drug targets the peptide-binding domain of SFKs and has been shown to suppress oncogenic proliferation *in vitro* and *in vivo*. It is currently being tested in phase I clinical trials [24, 25].

**Table 2.** Src and SFK molecular targeting agents in development

Agent	Sponsor	Target	Target site	Solid tumor setting <sup>a</sup>
Dasatinib	Bristol-Myers Squibb	Bcr-Abl, SFKs, Abl, Kit, PDGFR- $\beta$ , Eph receptors	ATP binding	SCLC, NSCLC, breast cancer, CRC, HNSCC, liver cancer, melanoma, ovarian cancer, pancreatic cancer, sarcomas
Bosutinib (SKI-606)	Wyeth	Abl, SFKs	ATP binding	Breast cancer
AZD-0530	AstraZeneca	SFKs, Abl	ATP binding	SCLC, NSCLC, breast cancer, CRC, HNSCC, osteosarcoma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer
XL-999	Exelixis	SFKs, VEGFR-2, PDGFR, Kit, and FGFR-1	ATP binding	NSCLC, CRC, kidney cancer, ovarian cancer <sup>b</sup>
INNO-406	CytRx	Abl, SFKs	ATP binding	No current relevant trials
KX01	Kinex	Src	Peptide binding	Phase I
XL-228	Exelixis	Bcr-Abl, Abl, SFKs, IGF-R1	ATP binding	Phase I

<sup>a</sup> Information from <http://www.clinicaltrials.gov>. Accessed May 14, 2009.

<sup>b</sup> Studies were terminated because of safety concerns.

Abbreviations: CRC, colorectal cancer; FGFR, fibroblast growth factor receptor; HNSCC, head and neck squamous cell carcinoma; IGF-1R, insulin-like growth factor 1 receptor; NSCLC, non-small cell lung cancer; PDGFR- $\beta$ , platelet-derived growth factor receptor  $\beta$ ; SCLC, small cell lung cancer; SFK, Src family kinase.

XL-228 (Exelixis, South San Francisco, CA) is a molecule that blocks several tyrosine kinases receptors, such as insulin-like growth factor 1 receptor (IGF-1R), SFKs, and Bcr-Abl. Bcr-Abl blockade includes the mutant form of *Abl* (T315I), which has been correlated with imatinib-resistant CML and Ph<sup>+</sup> ALL. Currently XL-228 is in phase I trials with ALL, CML, and advanced malignancies.

### SRC AND SFKS IN PROSTATE CANCER

SFKs and Src perform important functions in the oncogenesis of prostate cancer. Src and the SFKs Lyn and Fgr are expressed at high levels in malignant tissues and primary cell cultures derived from the prostate [26, 27]. Treating primary prostate cells with the Lyn inhibitor KRX-123 resulted in a reduction in cell proliferation, migration, and invasive potential in vitro [22, 27]. Furthermore, the activity of SFKs has been implicated in androgen-induced proliferation of malignant cells derived from the prostate. These data extend to in vivo models, such that tumor growth in mice resulted in reduced disease progression and metastasis when treated with a Src inhibitor [28–30].

The development of therapies to address unregulated Src signaling in the prostate is already in progress, and pre-clinical evidence for effective treatment with dasatinib is enticing. Dasatinib has been shown to suppress proliferation of PC-3 human prostate cancer cells [31], as well as inhibit the poor adhesion, greater migration, and potential invasiveness of the DU145 human prostate cancer cell line

[26]. Signals originating from Src and Lyn were also mitigated, as measured by the diminished activity of FAK and secreted proteases in DU145 cells. In addition, dasatinib treatment of mice injected with PC-3 cells resulted in diminished tumor development [29].

Recently, a phase II study was initiated to test the efficacy of dasatinib in hormone-refractory prostate cancer patients. Patients with progressive metastatic prostate cancer, a rising prostate-specific antigen level, testosterone <50 ng/dl, and no prior chemotherapy were recruited for this study. Preliminary results indicate that 10 of 15 Response Evaluation Criteria in Solid Tumors evaluable patients exhibited disease control (67%) [32]. A  $\geq 35\%$  decrease in urinary N-telopeptide excretion (a marker of bone resorption) was noted among 57% of evaluable patients. These early clinical results are the first and only efficacy data for SFK inhibition in a solid tumor setting and appear promising for the potential application of SFK inhibitors in prostate cancer treatment.

Phase II trials of AZD-0530 are also currently in progress. One study is evaluating AZD-0530 in patients with hormone-refractory prostate cancer, and another is comparing the efficacy and safety of AZD-0530 in combination with zoledronic acid in prostate cancer patients with metastatic bone disease.

### SRC AND SFKS IN COLORECTAL CANCER

The study of colon cancer has yielded some of the most compelling evidence of the central role of SFKs in cancer

progression. Bolen and colleagues showed that Src expression levels are five- to eight-fold higher in premalignant polyps than in normal mucosa, with more elevated concentrations identified in adenocarcinoma tissue [33–35]. These expression levels have been found to correlate not only with tumor stage, size, and metastatic potential but also with the likelihood of progression-free survival and overall survival [36, 37]. Further investigation also identified Src kinase activity in premalignant colitis lesions and determined that the greatest amount of dysplasia in these injuries often resulted in the most potential for progression to advanced stages [38].

In addition to increased Src activity and expression levels, the activity of Yes has been reported in premalignant tissues in the colon. This activity correlates with disease progression [39, 40]. Preclinical investigation supports a role for Yes, in that both Src and Yes have been shown to become activated after estradiol treatment of cells derived from colon carcinoma [41]. The expression of Lck was identified in colon carcinoma cell lines, which is particularly intriguing because of the typical hematopoietic origin of cells expressing this SFK [42]. However, few additional data on the role of Lck in colon cancer have been obtained, and further investigation in this area should prove informative.

Current treatment modalities for human colorectal cancer often favorably combine targeted inhibitors of EGFR with cytotoxic agents. However, the development of resistance to these agents is a perpetual challenge, and a role for Src in this process has been identified [43–46]. Kopetz and colleagues were able to restore sensitivity to cetuximab-resistant cell lines when treated with dasatinib [47]. There appeared to be a synergistic effect between these two agents, which resulted in the enhanced modulation of Src with this combination. In addition, preclinical studies suggest that Src blockade can restore sensitivity to cetuximab in cetuximab-resistant cells [48]. A phase I study evaluating dasatinib in combination with 5-fluorouracil, leucovorin, and oxaliplatin plus cetuximab treatment [49] is in progress.

A phase II trial studying how well AZD-0530 performs in patients with previously treated metastatic colon cancer or rectal cancer is also under way. There is also a phase II study of XL-999 administered i.v. to patients with metastatic colorectal cancer that was recently completed, but the results for that trial have not yet been reported. These trials represent an exciting new undertaking for incorporating Src inhibition in combination with other targeted agents or chemotherapies for a promising approach to novel colorectal cancer treatment.

## SRC AND SFKS IN BREAST CANCER

Breast cancer exhibits altered signal transduction pathways involving Src [50]. Evidence of greater SFK activity and higher protein expression levels was found in human breast cancer tissue compared with normal tissue [51–53]. Two transgenic mouse models first highlighted the prominent role of Src in breast cancer. Mammary tumors were induced by the oncogenic expression of polyoma virus middle T antigen. The transforming ability of the middle T antigen, in part, is a result of its ability to bind and activate several SFKs (Src, Yes, and Fyn) [54]. Another model by Muthuswamy et al. [55] discovered six- to eight-fold higher Src signaling in transgenic HER-2/neu mice, a molecule related to the EGF receptor tyrosine kinase and found to be overexpressed in 20%–25% of human breast cancer cases. The higher amounts of Src signaling were confined to malignant tissue only and were not accounted for by elevated expression levels alone.

Collectively, these data present a clinical rationale for targeted inhibition of SFKs in a breast cancer setting. Recent data showed that dasatinib in conjunction with the nucleoside analog gemcitabine in patients with breast tumors was tolerable [56]. Response and survival data were not reported in that phase I study, but the safety profile among these patients is encouraging, and further investigation is ongoing.

The interaction between estrogen signaling and SFK inhibition is also being investigated. Overexpression of the estrogen receptor (ER) in the nucleus of breast cancer cells has an established role in cell-cycle regulation while conferring tumor growth responsiveness to steroid hormones [57]. 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. In hypersensitive mutants, the basal Src activity was much higher than in wild-type cells, and the artificial addition of estrogen had no further effect [58]. However, steroid hormone receptors like ER do not require binding of ligand to invoke signaling cascades, and ER-mediated changes in gene expression and activation of SFKs have occurred independently of estrogen exposure [59].

A randomized, open-label, phase II study of bosutinib in combination with exemestane as second-line treatment for locally advanced or metastatic breast cancer is currently under way. This trial will be key in the evaluation of the combined inhibition of ER signaling and Src inhibition in the clinical setting. Lastly, a phase II trial of AZD-0530 in patients with metastatic or locally advanced breast cancer that is inoperable is also in progress.

There is also evidence that Src inhibitors may have a role in treating HER-2-expressing breast cancer. Preclinical

evidence has shown that Src binds to HER-2 and is activated in HER-2-expressing breast cancer cells, permitting phosphatidylinositol 3' kinase (PI3K) signaling via phosphatase and tensin homolog inactivation [60]. Furthermore, the anti-HER-2 antibody trastuzumab causes dissociation of Src from HER-2, inactivating Src and consequently inhibiting HER-2-mediated PI3K pathway signaling. This evidence supports the rational combination of Src inhibitors and trastuzumab in HER-2-expressing breast cancer.

Breast cancers that are "triple-negative" (estrogen, progesterone, and HER-2 receptor negative) have few treatment options and have a poor prognosis. Preliminary data from a phase II study of single-agent dasatinib in women with locally advanced or metastatic triple-negative breast cancer suggest a tumor response rate of 5% and a clinical benefit rate of 9% [61]. This modest, but encouraging, activity supports further studies to address optimal dasatinib dosing and combination with chemotherapy in this disease.

### SRC AND SFKS IN LUNG CANCER

The activities of Src, SFKs, and their downstream effectors have strong implications in the etiology of NSCLC. The inhibition of Src in EGFR-dependent NSCLC cell lines was shown to result in growth signaling shutdown and the induction of apoptosis [62]. Expression of the Src substrates STAT3 and FAK is often found in NSCLC tissues and in immortalized cell lines derived from these tissues [63]. STAT3 activity is modulated by the presence of a variety of growth factors, and stimulation by each of these requires functional Src [64].

The relationship between receptor tyrosine kinase signaling and effector molecules downstream of Src was highlighted by Sordella et al. [65]. Mutations in *EGFR* that activate the STAT pathway were found to confer resistance to chemotherapy and a heightened sensitivity to inhibitors of EGFR, like gefitinib and erlotinib [65]. Other receptor tyrosine kinases like VEGFR may also function similarly, as evidenced by the activation of STAT3 that is observed in human lung adenocarcinoma during periods of hypoxia, resulting in increased blood flow to nascent tumors deprived of oxygen [66, 67]. It appears likely that Src-mediated signaling in the progression of malignancy extends beyond growth factor-induced phenomena to include additional homeostatic regulatory mechanisms in the cell. This potential further emphasizes the application of targeted inhibition of SFKs in the effective treatment of nascent tumors of the lung.

Given the clear rationale for targeting SFKs and EGFR, many of the latest therapies that are effective in the treatment of NSCLC involve inhibition of these molecules [68–70] (Fig. 4). For instance, two therapies effective for

patients with NSCLC are gefitinib and erlotinib, which are small molecules that inhibit EGFR and consequently reduce activation of Src and its substrates, including STAT3 [68, 71]. These therapies underscore the role of tyrosine kinase signaling and implicate Src as a prominent molecular target in the treatment of lung cancer [72].

Evaluating the use of these agents in combination is in progress. A phase I trial has commenced that evaluates the potential for simultaneous EGFR and SFK inhibition. Chiappori and colleagues demonstrated that combination therapy with erlotinib and dasatinib in patients with advanced NSCLC is tolerable [73]. The safety profile for both of these agents was consistent with known adverse events (rash and pleural effusion). The efficacy of this therapy is currently being ascertained and preliminary results will be of interest.

Currently, a phase II trial of AZD-0530 in patients with recurrent, stage IIIB or stage IV NSCLC who were previously treated with chemotherapy combination regimens is recruiting patients. There is a placebo-controlled study of AZD-0530 in patients with recurrent osteosarcoma localized to the lung under way as well. A dose-finding and tolerability study XL-999 in patients with NSCLC is also in progress.

### SRC AND SFKS IN HEAD AND NECK CANCER

Less is known of the effect of Src inhibition in other aerodigestive settings such as HNSCC. HNSCC has shown evidence of SFK overexpression (Src, Yes, Fyn, and Lyn) and signaling through STAT3 and STAT5 proteins [74–76]. Inhibition of SFKs led to reduced STAT activation and decreased growth in cell lines derived from HNSCC malignancies [76]. Investigation by Grandis and colleagues revealed that STAT3 activation was able to increase cell survival via abrogation of apoptosis in tumors of the head and neck in vivo [77]. Additionally, STAT3 activity has been shown to eliminate growth factor dependence and contribute to HNSCC tumor growth [78].

Src- and SFK-targeted compounds are currently being evaluated for efficacy and safety in patients with HNSCC. Investigation of dasatinib in preclinical models of HNSCC yielded an increase in apoptosis and decrease in cell division as well as inhibition of the migratory and invasive potential of cancer cells [79]. AZD-0530 is also being evaluated, and preliminary results show decreased Src levels accompanied by reduced cell proliferation and invasion in treated cell lines derived from HNSCC [80]. AZD-0530 is also being tested in combination with gefitinib in preclinical breast cancer models, but preliminary results have not yet been reported.

### SRC AND SFKS IN PANCREATIC CANCER

Src and SFKs play a prominent role in pancreatic cancer [81]. High levels of Src have been detected in tumor tissues and cell cultures derived from pancreatic malignancies [82]. The SFK Lyn has also been found to be expressed above basal levels in the PANC-1 immortalized cell line [83]. In addition, signal modulation by Src has been identified for a number of proteins that are found to be overexpressed in pancreatic tumors. These include growth factor receptors, carcinoembryonic antigen-related adhesion molecule 6, and cholecystokinin-2 [84–86]. Furthermore, an increase in the expression of IGF-1 results from activation of Akt signaling downstream of Src (Fig. 4) [87]. This causes an increase in the cell's proliferative ability and provides an additional mechanism for SFKs to contribute to tumorigenesis in the pancreas.

The nucleoside analog gemcitabine has been used successfully to treat pancreatic cancer for >10 years. However, resistance to this agent is also a significant therapeutic challenge. Abrogating Src signaling has been shown to restore sensitivity to gemcitabine in tumor and tissue transplant cell lines derived from the pancreas [45, 46, 88]. A similar result was obtained when FAK expression was silenced in PANC-1 cells treated with gemcitabine. Recent preclinical evidence suggests that inhibition of Src also reverts chemoresistance against 5-fluorouracil in human pancreatic carcinoma cells [89].

The targeted inhibition of EGFR has proven to be an effective therapy in treating pancreatic cancer. The tyrosine kinase inhibitor (TKI) erlotinib was approved for patients with pancreatic cancer, and favorable outcomes were obtained when it was used in combination with gemcitabine [90]. The overall survival rate was higher with the combination regimen after 1 year (24% versus 17%) and the event-free survival duration was longer, at 6.4 months versus 5.9 months. These data are modest by clinical assessment, but represent an important proof of concept for the role of targeted therapy in mitigating tumorigenesis of the pancreas. This was further supported by additional studies investigating the role of TKIs in pancreatic cancer that were conducted recently. A small study investigated a combination of cediranib with AZD-0530 in treating patients with pancreatic tumors. The available data are limited, but this combination appears to have a tolerable safety profile [91].

### SRC AND SFKS IN CANCERS OF THE NERVOUS SYSTEM

The role of SFK in the brain and neuronal tumors is not as extensively studied as solid tumors derived from the breast, lung, colon, prostate, or pancreas. However, neuronal tissues do exhibit greater expression of Src and Fyn, and it is

likely that these proteins play a role in the deregulated proliferation and uninhibited growth of tumors arising in the nervous system [92]. Indeed, one study correlated FAK expression with the development of astrocytomas in the brains of mice [93]. Furthermore, neuroblastoma cell lines have been shown to express Src at levels much higher than those observed in primary cultures from noncancerous tissues of the central nervous system (CNS) [94]. Neuroendocrine tumors have also exhibited elevated Src expression, which correlates with the differentiation state of the tumor [95]. Further preclinical modeling is necessary, but preliminary clinical evidence indicates that dasatinib can cross the blood–brain barrier and consequently reduce the burden of CNS Ph<sup>+</sup> ALL [96]. This suggests that oral dasatinib may be effective against a number of CNS malignancies.

One CNS malignancy that dasatinib may have clinical activity against is glioblastoma multiforme. Preclinical evidence indicates that dasatinib inhibits cell viability and migration in vitro and tumor growth in vivo, and exerts these actions through inhibition of Src [97]. Safety results of a phase II study of dasatinib following treatment with temozolomide and radiotherapy for patients with recurrent glioblastoma multiforme were recently reported [98]. The toxicity observed was relatively mild despite a 100-mg twice-daily dose. There were no grade 4 or 5 adverse events reported. These data are encouraging, but further research is necessary to uncover the full role of SFK inhibition in cancers developing in the CNS.

### OTHER TUMOR TYPES

There is substantial evidence implicating the Src pathway in melanoma and other tumor types [99]. Preclinical studies have shown dasatinib to have antiproliferative and anti-invasive effects against melanoma cell lines, and to induce apoptosis in sarcoma cells [100–102]. Src inhibition also has been shown to have antiangiogenic effects and to significantly reduce tumor burden in ovarian cancer models [103]. Clinical studies of dasatinib in solid tumors, including these tumor types, are currently under way. Investigations of AZD-0530 in osteosarcoma, melanoma, and ovarian tumors are in progress. XL-999 is currently being evaluated in the kidney and ovarian disease settings.

### CONCLUSIONS AND FUTURE DIRECTIONS

Accumulating evidence suggests an important role for SFKs in solid tumors from a variety of tissues. The array of receptors that trigger SFK activity and the subsequent activation of downstream effectors present a compelling therapeutic target for treatment modalities in cancer management. SFK-directed TKIs have potential use as sin-



gle-agent therapy in some disease states. However, in other forms of cancer, like malignancies of the breast, there is only a limited amount of evidence obtained that implicates single-agent SFK inhibition as a potentially effective intervention. Src's involvement with numerous growth and cell metabolism functions suggests that combination with chemotherapeutics is worth additional evaluation. Preclinical and early phase trial data suggest that compelling combination regimens with targeted therapies, such as an Src/SFK inhibitor and an EGFR inhibitor, warrant further investigation.

The array of TKIs with activity against Src and other SFKs is noteworthy (Table 2) and presents a considerable possibility for future investigation. These agents also feature different affinities for Src/SFK inhibition, as well as separate inhibitory activity against other molecules, suggesting variations in binding potential as well as mechanism of action within the class. Dasatinib is FDA approved for the treatment of imatinib-resistant CML and Ph<sup>+</sup> ALL. Bosutinib is currently in phase II/III clinical trials and will likely be available for clinical use in the near future. AZD-0530 and XL-99 are currently being investigated in phase II trials.

These drugs coupled with existing molecular targeting agents or other molecules, cytotoxins, radiotherapy, and surgical interventions present a wide array of new therapeutic approaches to evaluate. Ongoing work continues to highlight the most effective of these that will control the aberrant intracellular signaling that is the hallmark of oncogenesis.

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