Src Continues Aging: Current and Future Clinical Directions
Scott Kopetz,1,2 Ami N. Shah,2,3 and Gary E. Gallick2

Abstract
Aberrant activation of members of the Src family of nonreceptor protein tyrosine kinases is common in solid tumor malignancies and may contribute to the development and/or progression of these tumors. As a result, four Src inhibitors are now in more than 50 clinical trials for at least 14 different types of solid tumors. In this review, we briefly discuss the preclinical rationale for Src inhibitors, the development strategies most likely to be successful in the clinic, and the rationale for Src inhibitors in combination with other agents as part of a more comprehensive therapeutic strategy. As the use of Src family inhibitors in clinical trials on solid tumors is in its infancy, further studies on the roles of Src family kinases in tumor progression, chemoresistance, epidermal-to-mesenchymal transition, and other properties of tumor progression will be important in designing the most effective clinical trials using these inhibitors.

Very few molecular targets in oncology have the pedigree of Src: the first oncogene discovered, the first shown to have intrinsic tyrosine kinase activity, and the subject of two Nobel prizes. The virus harboring v-Src, Rous sarcoma virus, was discovered early in the 20th century as a transmissible agent that induced sarcomas in fowl (1). V-Src was then used to show that viral oncogenes originated from normal cellular proto-oncogenes (2). Thus, many of the concepts of proto-oncogenes and their roles in signal transduction and cancer emanated from studies on Src. Nevertheless, until recently, Src was not seriously considered as a target for development of anticancer drugs. With a maturing understanding of the complexities of Src function and the burgeoning number of Src inhibitors entering the clinic, however, Src and its family members have indeed come of age as a potential target for cancer therapy.

Src is the prototypical member of a nine-gene family that includes Yes, Fyn, Lyn, Lck, Hck, Blk, and Yrk (3). Src and Src family kinases cooperate in several cellular processes including migration, adhesion, invasion, angiogenesis, proliferation, differentiation, and immune function (for detailed reviews, see refs. 4–6). Based on several lines of promising preclinical research, Src is now being extensively studied in the clinic. This review will touch only briefly on Src functions, focusing instead on the areas of greatest promise and greatest difficulties for moving Src inhibitors from the laboratory to success in the clinic.

Src Structure and Function
Src family kinases consist of a unique NH2-terminal region, two Src homology domains (SH2 and SH3), a highly conserved kinase domain, and a COOH-terminal tail containing a negative regulatory tyrosine residue. The phosphorylation of the COOH-terminal tail by COOH-terminal Src kinase (Csk) results in a closed, less active protein conformation. Autophosphorylation in the kinase domain alters the conformation to increase the intrinsic kinase activity. This relative simplicity of regulation belies the fact that Src can be activated by a host of interacting proteins including growth factor receptors, integrins, and G protein–coupled receptors.

Because of their central role in multiple signaling pathways, aberrant Src activity promotes dysregulation of numerous processes, including invasion, migration, proliferation, angiogenesis, and apoptosis (for reviews, see refs. 4, 7). Ironically, these processes are associated primarily with tumor progression and metastasis despite early observations that v-Src was a tumor-initiating oncogene. Biological functions also mediated by Src activity in tumor cells include epithelial-to-mesenchymal transition, which is implicated in cancer progression and development of chemotherapy resistance (8, 9). Further, Src functions in endothelial cells and stromal cells, and elegant recent experiments have shown that Src activation in these cells contributes to dissemination of metastatic tumor cells (10).

Part of the slow acceptance of Src as a drug development target was due to the lack of mutation or gene amplification in the vast majority of tumors. Instead, enzymatic activity increases in the majority of primary tumors, with further increases in synchronous metastases (11). In colorectal carcinoma, increased Src activity correlates with patient survival (12, 13). Although Src activation is prominent in colorectal and breast cancers, overexpression or activation is also seen in most other tumor types (7, 14–17).

Several mechanisms lead to increased Src activity in tumors. Src is downstream in signaling from a number of growth factor receptors including epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor, and vascular...
endothelial growth factor receptor (3, 18). In many tumor types, overexpression of these receptors, their ligands, or both is common (19). Overexpression of other Src binding partners, including focal adhesion kinase, also activate Src (20), as do physiologic processes such as cellular stress, including that induced by chemotherapy.

The Transition of Src Inhibitors to the Clinic

Currently, four compounds have sufficient potency and acceptable toxicity for development as Src family inhibitors. Three compounds, dasatinib, bosutinib, and AZD0530, are small-molecule competitive inhibitors of ATP binding and are also inhibitors of Abl and BCR-Abl, and a fourth, KXO1, inhibits binding of selective Src substrates. Although initially selected for its ability to inhibit Src, dasatinib has been approved by the Food and Drug Administration for the treatment of imatinib-refractory chronic myelogenous leukemia based, in part, on a 95% complete hematologic response rate for chronic-phase chronic myelogenous leukemia (21). As a result of this approval, the side effect profile of dasatinib has been the best described. Pleural effusions occur in 35% of chronic myelogenous leukemia patients in one study and were a prominent toxicity in one phase I study in patients with solid tumors (22). Despite the fact that grade 3 or 4 neutropenia and thrombocytopenia were seen in 45% and 35% of chronic myelogenous leukemia patients, respectively, myelosuppression has not been prominent in studies of dasatinib in patients with solid tumors (21, 22). Mild peripheral edema and hypocalcemia have also been reported (21).

There is not a convincing overlap in the toxicity profiles seen with the various agents, suggesting that many of the side effects may be agent specific. Phase I trials of other Src inhibitors have also been fairly well tolerated (23, 24). Dose-limiting toxicities for these Src family kinase inhibitors have included diarrhea and fatigue.

Guided by preclinical investigations, clinical development has proceeded with trials with inhibitors as single agents and with multiple combinations, including growth factor receptor inhibitors, traditional cytotoxic chemotherapy, and hormonal therapies (Fig. 1). More than 50 trials of Src inhibitors are currently ongoing or soon to open (Table 1; for all publicly available trials at the time of this review, see Supplementary Table S1).

Single-agent trials and preclinical rationale. As single agents, Src inhibitors are undergoing investigation in most major tumor types, including breast, lung (non–small-cell and small-cell), colorectal, pancreatic, prostate, and renal cell carcinomas, as well as in less common tumor types such as mesothelioma, melanoma, and sarcoma based on preclinical evidence of Src activation in these tumors (25–27). For example, in a breast cancer murine model, treatment with bosutinib reduced the volume of mammary fat pad tumors compared with untreated controls (28). A prostate murine model showed reduction in the growth of primary prostatic tumors after treatment with AZD0530 (29). In some cell lines, however, the effects of Src inhibitors on cell proliferation seem to be independent of Src inhibition and, instead, may be the result of inhibition of other tyrosine kinases (30, 31).

Despite this initial broad approach to exploring the action of Src as a single agent, most preclinical reports suggest that Src inhibitors affect proliferation in only a small subset of cell lines or animal tumors (32–34). For these reasons, as single agents, Src inhibitors would be predicted to have modest benefit in most tumors, a prediction supported by the initial results from single-agent phase I studies, in which radiographic tumor regression has not been seen. Instead, the best reported responses have been stable disease in a variety of tumor types.
can activate Src independently of ligand binding; conversely, based on several lines of research (reviewed in ref. 46). EGFR inhibitors and Src inhibitors are likely best used in combination with other chemotherapy. Similarly, the antiangiogenic properties of Src agents are effective only in combination with cytotoxic agents with an anti-invasive and antimetastatic phenotype in breast cancer.

**Conclusion**

Several questions remain in the development of Src inhibitors. Can we identify a subgroup of tumors that will be uniquely susceptible to treatment with Src inhibitors? When administered alone or in combination regimens, are Src functions too ubiquitous to be inhibited without unacceptable toxicity in patients? Are there easily assayed and validated biomarkers that will predict success for Src inhibitors? Given that subclinical metastases may already be established at the time of diagnosis, is there a role for an agent that prevents tumor migration and invasion. As yet, the ability of Src inhibitors to suppress outgrowth of these established subclinical metastases has not been tested.

**Table 1. Strategies for use of Src inhibitors in clinical trials**

| Single agents | Breast, prostate, breast, pancreatic, prostate, melanoma |
| Targeting established bone metastases | Breast, prostate |
| Combination with Her family | Breast, prostate, ovarian, breast, pancreatic, prostate, melanoma |
| Combination with cytotoxics | Breast, prostate, ovarian, breast, pancreatic, prostate, melanoma |
| Combination with hormonal agents | Breast |

**NOTE:** Details on all public trials using Src inhibitors available at the time of this review are provided in Supplementary Table S1.
myelogenous leukemia patients provide hope that Src inhibitors in biologically relevant combination with other anticancer agents will find their way as standard treatments for at least some tumors. The next few years will provide many answers on whether Src inhibitors will find a role in cancer treatment, just in time for the centennial anniversary of the discovery of Rous sarcoma virus. This coming of age of Src has indeed been a long time in coming.

References


Src Continues Aging: Current and Future Clinical Directions
Scott Kopetz, Ami N. Shah and Gary E. Gallick


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/13/24/7232

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2007/12/14/13.24.7232.DC1

Cited articles
This article cites 50 articles, 18 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/13/24/7232.full.html#ref-list-1

Citing articles
This article has been cited by 19 HighWire-hosted articles. Access the articles at:
/content/13/24/7232.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.