

Treatment for Advanced Tumors: Src Reclaims Center Stage

Justin M. Summy and Gary E. Gallick

Background

The non-receptor protein tyrosine, Src, is a 60-kDa protein that is the archetypal member of a nine-gene family, including Src, Yes, Fyn, Lyn, Lck, Hck, Fgr, Blk, and Yrk, that plays a critical role in regulation of proliferation, differentiation, migration, adhesion, invasion, angiogenesis, and immune function (for detailed reviews, see refs. 1–4). All Src family kinases are myristoylated at the NH₂ terminus, and several are additionally palmitoylated downstream of the myristoylation site. These fatty acid moieties are required for localization to cellular membranes. Each Src family kinase contains a poorly conserved (“unique”) domain and three conserved Src homology domains: SH2, SH3, and SH1 or protein tyrosine kinase domain. Critical to the regulation of Src is a COOH-terminal tyrosine (Y530) that, when phosphorylated by C-terminal Src kinase (Csk), leads to a more inactive Src conformation, as has been shown by numerous molecular studies and confirmed by crystal structures (5). An autophosphorylation site (Y418) is indicative of, but not entirely diagnostic for, Src activation. Biochemical and structural studies have discerned the general mechanism by which Src activity is regulated (6).

A myriad of cellular stimuli, including polypeptide growth factors, hormones, integrin aggregation, stress, cell cycle progression, and undoubtedly others, lead to the phosphorylation of signaling proteins for which the Src SH2 domain has higher affinity than for its own COOH-terminal phosphorylated site. Src thus associates with numerous proteins (depending on the input signal) and assumes an active conformation through phosphatase-mediated dephosphorylation of Y530 and autophosphorylation of Y418. Through its SH2 and SH3 domains, Src further associates with structural and signaling proteins, and the resulting complexes are critical to Src’s role in diverse cellular processes, as illustrated in Fig. 1. Specificity of Src signaling stems from the composition of these complexes as well as their duration and subcellular localization and may be additionally influenced by phosphorylation sites on Src mediated by protein kinase C, protein kinase A, cdc2 kinase, and growth factor receptors (7). In turn, Src substrates include signaling enzymes, cytoskeletal proteins, mitotic regulators, etc. (8).

Aberrantly high Src activity is seen in diverse cancers (9). Although a number of mechanisms may account for these increases, including increased transcription (10) and perhaps very rare activating mutations (4), activation is likely primarily a consequence of genetic and epigenetic alterations in tumor cells. As examples, overexpression and mutation of growth factor receptors and alterations in integrin regulation and downstream mediators, such as overexpression of focal adhesion kinase, frequently lead to activation of Src and deregulation of one or more of the pathways depicted in Fig. 1. As these epigenetic changes accumulate, Src activity increases during cancer progression, with the highest expression and/or activity of Src observed in metastases (11, 12). Src activity can be predictive of poor prognosis (13). Thus, current expectation is that Src inhibitors will hold the most promise for later stage neoplasms, and clinical trials are focusing on this potential. Src activation is not always confined to late-stage tumors, however, as it occurs in colon polyps of high malignant potential (14) as well as in ulcerative colitis (15) and can promote hyperproliferation in mouse models of skin carcinogenesis (16). Thus, in some malignancies, Src inhibitors may have efficacy in earlier-stage disease.

Recent Advances in Roles of Src in Tumor Progression

Recent studies using *in vitro* and *in vivo* models provide strong support for the use of Src inhibitors for cancer therapy. In models of epithelial cancer, Src activation promotes a more migratory and invasive phenotype, inducing morphologic and biochemical changes more commonly associated with fibroblasts and cells of mesenchymal origin (17), often termed epidermal to mesenchymal-like transition. This phenotype is associated with increased formation of integrin adhesion structures and decreased E-cadherin-mediated cell-cell contacts (18). Intact Src SH3 and SH2 domains are essential for formation of the focal adhesion-like integrin adhesion complexes (19), and Src catalytic activity is important for the dynamic modulation (including elongation and retraction) that occurs at these sites (20). A current area of intense investigation is proteomic analysis of whether specific alterations (such as phosphorylation) in proteins in these complexes will be predictive markers for patient response to Src inhibitors.

Another key role of Src that may be exploited therapeutically is its regulation of critical angiogenic factors that promote tumor progression. Src activation is required for hypoxia-mediated expression of vascular endothelial growth factor (VEGF; ref. 21), and in mouse models using colon cancer cells, inhibition of Src inhibits VEGF expression and limits tumor growth (22). More recently, we have shown that Src regulates both constitutive and growth factor-induced VEGF and interleukin-8 expression (23, 24) and that Src activation up-regulates VEGF mRNA

Authors’ Affiliation: Department of Cancer Biology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas

Received 12/7/05; revised 1/15/06; accepted 1/17/06.

Grant support: National Cancer Institute grant CA-16672, Lockton Foundation, and Gillson Longenbaugh Foundation (G.E. Gallick).

Requests for reprints: Gary E. Gallick, Department of Cancer Biology, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030-4009. Phone: 713-563-4919; Fax: 713-563-5489; E-mail: ggallick@mdanderson.org.

©2006 American Association for Cancer Research.
doi:10.1158/1078-0432.CCR-05-2692

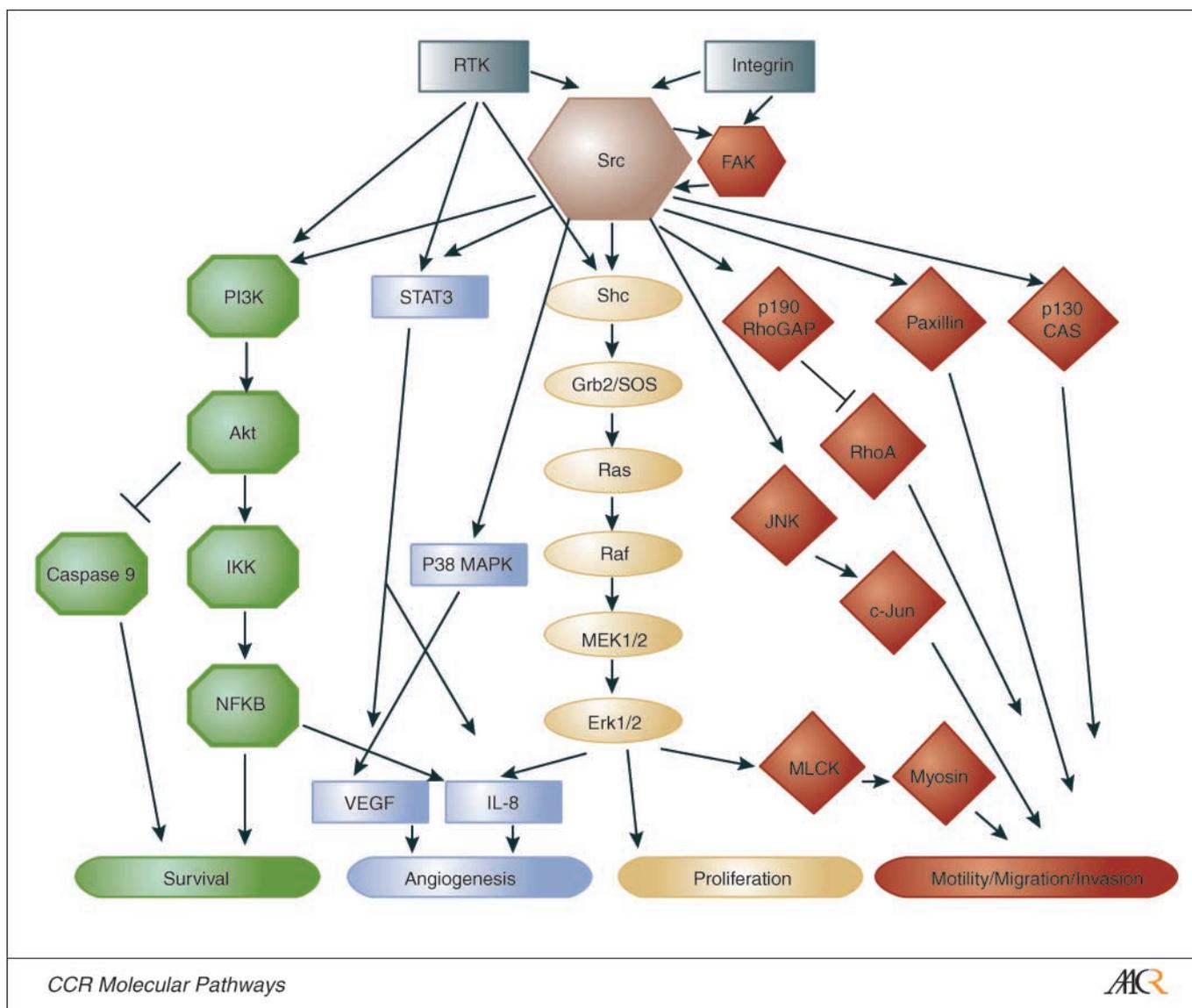


Fig. 1. Src-mediated pathways contributing to tumor progression. As discussed in the text, Src family members are obligate intermediates in numerous signaling pathways governing cell proliferation and homeostasis. In tumor cells, association of Src with overexpressed and mutated receptor tyrosine kinases leads to constitutively increased enzymatic activity of Src, thereby activating pro-survival pathways (green) and angiogenic pathways (teal). Aberrant integrin function and/or overexpression of focal adhesion kinase (FAK) result in Src activation in focal adhesion complexes, contributing to cell survival, and activating pathways, leading to increased motility and invasion (red). Although activation of Src by both receptor tyrosine kinases (RTK) and focal adhesion kinase is known to contribute to proliferation of some cell types (primarily through the Ras pathways, yellow), most Src inhibitors have little effect on tumor cell proliferation *in vitro* or *in vivo*. It should be noted also that there is considerable "crosstalk" among the pathways, with several of the intermediates key to multiple biologic effects of Src activation. PI3K, phosphatidylinositol 3-kinase; STAT3, signal transducers and activators of transcription 3; IKK, I κ B kinase; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; IL-8, interleukin-8; ERK, extracellular signal-regulated kinase; NF- κ B, nuclear factor- κ B.

transcription by activation of signal transducers and activators of transcription 3, which forms a complex with hypoxia-inducible factor-1 and other factors on the VEGF promoter (25). Using *in vivo* models of angiogenesis, we have shown that Src inhibition effectively blocks neovascularization (26), suggesting an anti-angiogenic role for Src inhibitors in the clinic.

Equally important to the role of Src in promoting expression of proangiogenic molecules in tumor cells is its function in endothelial cells. Src family members are obligatory intermediates in promoting biological functions of VEGF through VEGF receptors on endothelial cells (27). Src activation leads to increased permeability of endothelial cells, and recent studies using *src*^{-/-} mice and Src inhibitors in mouse colon cancer

models show that abolishing or reducing Src expression/activity decreases tumor cell extravasation, thereby decreasing experimental metastases (28). As in tumor cells, activated Src promotes a mesenchymal-like phenotype in endothelial cells, increasing migratory potential of these cells as well (29). Current results therefore suggest Src activation in tumor cells indirectly regulates Src activity in endothelial cells. Increased Src activity in the tumor cells increases VEGF expression, resulting in increased binding to VEGF receptors on endothelial cells. This process then leads to association of Src with these receptors, increasing Src activity in endothelial cells as well. This continuing cycle promotes Src-mediated increases in migratory potential and permeability of endothelial cells and

facilitates tumor cell extravasation. Inhibitors of Src thus have the potential to interfere with this cycle by affecting biological functions in both tumor and tumor-associated endothelial cells that contribute to metastasis. The importance of targeting tumor-associated endothelial cells for therapeutic efficacy has been highlighted from recent work on inhibitors of receptor tyrosine kinases in clinical trial (30–35); similar principles may apply to Src inhibitors.

Another example of the potential efficacy of Src inhibitors on both tumor and normal cells is in treating bone metastases. In cultured prostate tumor cells, the Src inhibitor, dasatinib, has the expected effects on suppressing tumorigenic properties (36). Src also plays an essential role in normal osteoclast function that is absolutely required for bone resorption, however (37). Src inhibitors, by affecting Src-mediated functions in both tumor cells and osteoclasts, have the potential to interfere with the well-described vicious cycle of tumor cells promoting bone destruction that in turn yields growth factors that promote further tumor growth. Bone-targeted Src inhibitors are being developed to further test this possibility.¹ The Src-selective inhibitor, AZD 0530, does indeed affect osteoclast function in humans, inhibiting osteoclastic bone resorption.²

Clinical/Translational Advances

Currently, three inhibitors, dasatinib (BMS354825; Bristol Meyers Squibb Oncology, Princeton, NY), AZD-0530 (AstraZeneca, Macclesfield, United Kingdom), and SKI-606 (Wyeth Research, Pearl River, NY), have reached phase 1 clinical trials. These small molecule inhibitors are directed at the ATP-binding site of Src family kinases and thus are also inhibitors of kinases with closely related structure, such as Abl. Each inhibitor has shown activity against Src in preclinical studies, however. *In vitro*, dasatinib suppresses invasion and induces cell cycle arrest in head and neck squamous cell carcinomas (38), affects properties of prostate tumor progression (36) and in orthotopic mouse models of pancreatic carcinoma, greatly inhibits the development of liver metastases (39). AZD0530 shows activity at the nanomolar level against v-Src-transformed cells in models of invasion and migration, supporting the hypothesis that Src is an anti-invasive target rather than a direct antiproliferative target. Dramatic inhibition of human breast cancer cell (MDA-MB231) migration by the Src inhibitor AZD0530 has been shown *in vitro* (40). SKI606 shows activity against colon tumors in mouse models (41).

Given that Src is involved in so many fundamental cellular processes, it might be expected that Src-selective inhibitors

would be toxic. Knockouts of Src and other family members are viable, however, (42), and current studies on Src/Abl inhibitors in chronic myelogenous leukemia patients (used for their effectiveness against Bcr-Abl) show only limited toxicity. Thus, numerous clinical trials on each of the previously described inhibitors are ongoing or soon to be initiated. A number of different strategies are being used, including trials with a Src inhibitor as single agent in metastatic colon cancer, and in various combinations in other solid tumors. Recent evidence suggests logical combinations of chemotherapeutic agents with Src inhibitors in specific tumors. Some gleevec-resistant chronic myelogenous leukemia patients (43), gemcitabine-resistant pancreatic tumor cells (44), and taxol-resistant ovarian tumor cells (45, 46) have all been associated with increased Src activation, and Src inhibitors sensitize cells to these standard chemotherapeutic agents. As yet, ongoing clinical trials are in too early a stage to determine potential effectiveness of any of the Src inhibitor trials, but in phase I trials, dasatinib seems generally well tolerated³ as is AZD0530.²

Although the successes of the ongoing clinical trials using these inhibitors remain to be determined, the definition of success needs to be considered carefully. Given the roles for Src in tumor progression and metastasis, the expectations from ongoing and potential future phase II/III trials are likely to differ from many other signal transduction-targeted inhibitors and the efficacy end points for the clinical trials will need to be chosen carefully. End points, such as disease stabilization and time to recurrence, may be more important to assess than more classic measures, such as partial and complete remission. Old lessons will undoubtedly be relearned, including the fallibility of single-agent therapy for most solid tumors. Toxicities of these agents, which still lack complete selectivity, are likely to be problematic in some patients, and drug resistance will almost assuredly arise. Nevertheless, Src-selective agents may advance new concepts in cancer therapy, including how to better limit metastatic spread without eradicating tumor growth, and the importance of targeting the host stromal cells as well as the tumor cells themselves to achieve efficacy. Because of the current flurry of studies with Src inhibitors, these predictions will be tested rapidly. The success of both ongoing laboratory work and clinical trials will determine if Src remains on center stage, stimulates more imaginative approaches to therapy of advanced stage disease, or returns to the wings with respect to its relevance as a therapeutic target for solid tumors.

Acknowledgments

We thank Victor A. Levin, MD; James L. Abbruzzese, MD; Jan C. Liang, PhD; and Isaiah J. Fidler, DVM, PhD for their critical reading and insightful suggestions with respect to the article. The authors apologize to the many workers in the field whose important work was not cited due to space limitations.

¹ Tomi K. Sawyer, personal communication.

² Tim P. Green, personal communication.

³ Faye M. Johnson, personal communication.

References

1. Taylor SJ, Shalloway D. Src and the control of cell division. *BioEssays* 1996;18:9–11.
2. Thomas SM, Brugge JS. Cellular functions regulated by Src family kinases. *Annu Rev Cell Dev Biol* 1997;13:513–609.
3. Frame MC. Newest findings on the oldest oncogene: how activated src does it. *J Cell Sci* 2004;117:989–98.
4. Yeatman TJ. A renaissance for SRC. *Nat Rev Cancer* 2004;4:470–80.
5. Sicheri F, Kuriyan J. Structures of Src-family tyrosine kinases. *Curr Opin Struct Biol* 1997;7:777–85.
6. Roskoski R, Jr. Src kinase regulation by phosphorylation and dephosphorylation. *Biochem Biophys Res Commun* 2005;331:1–14.
7. Bromann PA, Korkaya H, Courtneidge SA. The interplay between Src family kinases and receptor tyrosine kinases. *Oncogene* 2004;23:7957–68.
8. Levin VA. Basis and importance of Src as a target in cancer. *Cancer Treat Res* 2004;119:89–119.
9. Summy JM, Gallick GE. Src family kinases in tumor progression and metastasis. *Cancer Metastasis Rev* 2003;22:337–58.
10. Dehm SM, Bonham K. SRC gene expression in

- human cancer: the role of transcriptional activation. *Biochem Cell Biol* 2004;82:263–74.
11. Talamonti MS, Roh MS, Curley SA, Gallick GE. Increase in activity and level of pp60c-src in progressive stages of human colorectal cancer. *J Clin Invest* 1993;91:53–60.
 12. Termuhlen PM, Curley SA, Talamonti MS, Saboorian MH, Gallick GE. Site-specific differences in pp60c-src activity in human colorectal metastases. *J Surg Res* 1993;54:293–8.
 13. Allgayer H, Boyd DD, Heiss MM, Abdalla EK, Curley SA, Gallick GE. Activation of Src kinase in primary colorectal carcinoma: an indicator of poor clinical prognosis. *Cancer* 2002;94:344–51.
 14. Cartwright CA, Meisler AI, Eckhart W. Activation of the pp60c-src protein kinase is an early event in colonic carcinogenesis. *Proc Natl Acad Sci U S A* 1990;87:558–62.
 15. Cartwright CA, Coad CA, Egbert BM. Elevated c-Src tyrosine kinase activity in premalignant epithelia of ulcerative colitis. *J Clin Invest* 1994;93:509–15.
 16. Matsumoto T, Kiguchi K, Jiang J, et al. Development of transgenic mice that inducibly express an active form of c-Src in the epidermis. *Mol Carcinog* 2004; 40:189–200.
 17. Avizienyte E, Frame MC. Src and FAK signalling controls adhesion fate and the epithelial-to-mesenchymal transition. *Curr Opin Cell Biol* 2005;17:542–7.
 18. Avizienyte E, Fincham VJ, Brunton VG, Frame MC. Src SH3/2 domain-mediated peripheral accumulation of Src and phospho-myosin is linked to deregulation of E-cadherin and the epithelial-mesenchymal transition. *Mol Biol Cell* 2004;15:2794–803.
 19. Avizienyte E, Wyke AW, Jones RJ, et al. Src-induced de-regulation of E-cadherin in colon cancer cells requires integrin signalling. *Nat Cell Biol* 2002;4: 632–8.
 20. Brunton VG, Avizienyte E, Fincham VJ, et al. Identification of Src-specific phosphorylation site on focal adhesion kinase: dissection of the role of Src SH2 and catalytic functions and their consequences for tumor cell behavior. *Cancer Res* 2005;65:1335–42.
 21. Mukhopadhyay D, Tsiokas L, Zhou XM, Foster D, Brugge JS, Sukhatme VP. Hypoxic induction of human vascular endothelial growth factor expression through c-Src activation. *Nature* 1995;375:577–81.
 22. Ellis LM, Staley CA, Liu W, et al. Down-regulation of vascular endothelial growth factor in a human colon carcinoma cell line transfected with an antisense expression vector specific for c-src. *J Biol Chem* 1998; 273:1052–7.
 23. Trevino JG, Summy JM, Gray MJ, et al. Expression and activity of SRC regulate interleukin-8 expression in pancreatic adenocarcinoma cells: implications for angiogenesis. *Cancer Res* 2005;65:7214–22.
 24. Summy JM, Trevino JG, Baker CH, Gallick GE. c-Src regulates constitutive and EGF-mediated VEGF expression in pancreatic tumor cells through activation of phosphatidylinositol-3 kinase and p38 MAPK. *Pancreas* 2005;31:263–74.
 25. Gray MJ, Wey JS, Belcheva A, et al. Neuropilin-1 suppresses tumorigenic properties in a human pancreatic adenocarcinoma cell line lacking neuropilin-1 coreceptors. *Cancer Res* 2005;65:3664–70.
 26. Summy JM, Trevino JG, Lesslie DP, et al. AP23846, a novel and highly potent Src family kinase inhibitor, reduces vascular endothelial growth factor and interleukin-8 expression in human solid tumor cell lines and abrogates downstream angiogenic processes. *Mol Cancer Ther* 2005;4:1900–11.
 27. Weis SM, Cheresh DA. Pathophysiological consequences of VEGF-induced vascular permeability. *Nature* 2005;437:497–504.
 28. Weis S, Cui J, Barnes L, Cheresh D. Endothelial barrier disruption by VEGF-mediated Src activity potentiates tumor cell extravasation and metastasis. *J Cell Biol* 2004;167:223–9.
 29. Potter MD, Barbero S, Cheresh DA. Tyrosine phosphorylation of VE-cadherin prevents binding of p120 and beta-catenin and maintains cellular mesenchymal state. *J Biol Chem* 2005;280:31906–12.
 30. Yokoi K, Sasaki T, Bucana CD, et al. Simultaneous inhibition of EGFR, VEGFR, and platelet-derived growth factor receptor signaling combined with gemcitabine produces therapy of human pancreatic carcinoma and prolongs survival in an orthotopic nude mouse model. *Cancer Res* 2005;65:10371–80.
 31. Thaker PH, Yazici S, Nilsson MB, et al. Antivascular therapy for orthotopic human ovarian carcinoma through blockade of the vascular endothelial growth factor and epidermal growth factor receptors. *Clin Cancer Res* 2005;11:4923–33.
 32. Yazici S, Kim SJ, Busby JE, et al. Dual inhibition of the epidermal growth factor and vascular endothelial growth factor phosphorylation for antivascular therapy of human prostate cancer in the prostate of nude mice. *Prostate* 2005;65:203–15.
 33. Yokoi K, Thaker PH, Yazici S, Hamilton SR, Fidler IJ, et al. Dual inhibition of epidermal growth factor receptor and vascular endothelial growth factor receptor phosphorylation by AEE788 reduces growth and metastasis of human colon carcinoma in an orthotopic nude mouse model. *Cancer Res* 2005;65:3716–25.
 34. Lev DC, Kim SJ, Onn A, et al. Inhibition of platelet-derived growth factor receptor signaling restricts the growth of human breast cancer in the bone of nude mice. *Clin Cancer Res* 2005;11:306–14.
 35. Yigitbasi OG, Younes MN, Doan D, et al. Tumor cell and endothelial cell therapy of oral cancer by dual tyrosine kinase receptor blockade. *Cancer Res* 2004; 64:7977–84.
 36. Nam S, Kim D, Cheng JQ, et al. Action of the Src family kinase inhibitor, dasatinib (BMS-354825), on human prostate cancer cells. *Cancer Res* 2005;65: 9185–9.
 37. Shakespeare WC, Metcalf CA III, Wang Y, et al. Novel bone-targeted Src tyrosine kinase inhibitor drug discovery. *Curr Opin Drug Discov Devel* 2003; 6:729–41.
 38. Johnson FM, Saigal B, Talpaz M, Donato NJ. Dasatinib (BMS-354825) tyrosine kinase inhibitor suppresses invasion and induces cell cycle arrest and apoptosis of head and neck squamous cell carcinoma and non-small cell lung cancer cells. *Clin Cancer Res* 2005;11:6924–32.
 39. Trevino JG, Summy JM, Lesslie DP, et al. Inhibition of Src expression and activity inhibits tumor progression and metastasis of human pancreatic adenocarcinoma cells in an orthotopic nude mouse model. *Am J Pathol*. In Press.
 40. Henniquin LF, Allen J, Costello GF, et al. The discovery of AZD0530: a novel, oral, highly selective and dual-specific inhibitor of the Src and Abl family kinases. *Proc Am Assoc Cancer Res* 2005;46:A2537.
 41. Golas JM, Lucas J, Etienne C, et al. Related SKI-606, a Src/Abl inhibitor with *in vivo* activity in colon tumor xenograft models. *Cancer Res* 2005;65:5358–64.
 42. Lowell CA, Soriano P. Knockouts of Src-family kinases: stiff bones, wimpy T cells, and bad memories. *Genes Dev* 1996;10:1845–57.
 43. Donato NJ, Wu JY, Stapley J, et al. BCR-ABL independence and LYN kinase overexpression in chronic myelogenous leukemia cells selected for resistance to ST1571. *Blood* 2003;101:690–8.
 44. Duxbury MS, Ito H, Zinner MJ, Ashley SW, Whang EE. Inhibition of SRC tyrosine kinase impairs inherent and acquired gemcitabine resistance in human pancreatic adenocarcinoma cells. *Clin Cancer Res* 2004;10: 2307–18.
 45. George JA, Chen T, Taylor CC. SRC tyrosine kinase and multidrug resistance protein-1 inhibitions act independently but cooperatively to restore paclitaxel sensitivity to paclitaxel-resistant ovarian cancer cells. *Cancer Res* 2005;65:10381–8.
 46. Chen T, Pengetnze Y, Taylor CC. Src inhibition enhances paclitaxel cytotoxicity in ovarian cancer cells by caspase-9-independent activation of caspase-3. *Mol Cancer Ther* 2005;4:217–24.

Clinical Cancer Research

Treatment for Advanced Tumors: Src Reclaims Center Stage

Justin M. Summy and Gary E. Gallick

Clin Cancer Res 2006;12:1398-1401.

Updated version Access the most recent version of this article at:
<http://clincancerres.aacrjournals.org/content/12/5/1398>

Cited articles This article cites 45 articles, 23 of which you can access for free at:
<http://clincancerres.aacrjournals.org/content/12/5/1398.full#ref-list-1>

Citing articles This article has been cited by 35 HighWire-hosted articles. Access the articles at:
<http://clincancerres.aacrjournals.org/content/12/5/1398.full#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, use this link
<http://clincancerres.aacrjournals.org/content/12/5/1398>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.