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SRC: A Century of Science Brought to the Clinic

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Abstract

The SRC family kinases are the largest family of nonreceptor tyrosine kinases and one of the best-studied targets for cancer therapy. *SRC*, arguably the oldest oncogene, has been implicated in pathways regulating proliferation, angiogenesis, invasion and metastasis, and bone metabolism. More recently, researchers have proposed that the transforming ability of SRC is linked to its ability to activate key signaling molecules in these pathways, rather than through direct activity. It has been hypothesized that blocking SRC activation may inhibit these pathways, resulting in antitumor activity. However, successfully targeting SRC in a clinical setting remains a challenge, and SRC inhibitors have only recently begun to move through clinical development. Preclinical studies have identified specific molecular "subgroups" and histologies that may be more sensitive to SRC inhibition. In addition, other studies have demonstrated synergistic interactions between SRC inhibitors and other targeted therapies and cytotoxics. In this review, we summarize SRC biology and how it has been applied to the clinical development of SRC inhibitors. The status of SRC inhibitors, including dasatinib, saracatinib, and bosutinib, which are in phase 1, 2, and 3 trials, is highlighted.

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Introduction

The SRC family of tyrosine kinases (SFKs) has nine members: LYN, FYN, LCK, HCK, FGR, BLK, YRK, YES, and c-SRC. Of these, c-SRC is the best studied and most frequently implicated in oncogenesis [1].

Almost 100 years have elapsed since Peyton Rous first described a filterable agent (i.e., virus) that could induce solid tumors in birds. Arguably ahead of his time, Rous' discovery would linger on the fringes of the scientific establishment for more than 50 years. It took the advent of modern molecular biology techniques in the 1960s and 1970s for Rous' filterable agent, now renamed the Rous sarcoma virus, to ignite research that would help elucidate our current understanding of cancer biology. Studies into the molecular biology and genetics of Rous sarcoma virus identified *v-SRC* as the viral oncogene responsible for cellular transformation. Shortly thereafter, Bishop and Varmus demonstrated that *v-SRC* had a cellular counterpart, the proto-oncogene *c-SRC* [2].

c-SRC (henceforth referred to as SRC) encodes a nonreceptor tyrosine kinase that, when activated, is involved in cellular proliferation, survival, migration, and angiogenesis. When deregulated, these processes represent four of the six so-called "hallmarks of cancer" [1,3]. Furthermore, numerous human malignancies display increased SRC expression and activity, suggesting that SRC may be intimately involved in oncogenesis [4]. Despite this, SRC alone is insufficient in transforming human cells *in vitro*, and so far, only rare cases of activating SRC mutations have been identified in human cancers [5,6]. Although numerous questions regarding the role of SRC in cancer remain unanswered, SRC's involvement in intracellular signaling pathways and overexpression in many human malignancies has renewed interest in developing SRC inhibitors. In this review, we highlight

Abbreviations: AE, adverse event; CAS, CRK-associated substrate; c-FMS, macrophage colony-stimulating factor receptor; CRPC, castration-resistant prostate cancer; CSK, C-terminal SRC kinase; DLTs, dose-limiting toxicities; EGFR, epidermal growth factor receptor; ER, estrogen receptor; FAK, focal adhesion kinase; 5-FU, 5-fluorouracil; IC_{50}, 50% inhibitory concentration; IL-8, interleukin 8; MTD, maximum tolerated dose; PDGFR, platelet-derived growth factor receptor; PP2, 4-amino-5-(4-chlorophenyl)-7-(*t*-butyl)pyrazolo[3,4-*d*]pyrimidine; RTK, receptor tyrosine kinase; SFK, SRC family of tyrosine kinases; SH, SRC homology; VEGFR, vascular endothelial growth factor receptor

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the rationale for SRC as a therapeutic target in cancer medicine and examine the preclinical and clinical data relevant to SRC inhibitors in development.

SRC Structure and Function

Proteins in the SRC family have a conserved organization consisting of four SRC homology (SH) domains and a C-terminal segment containing a negative regulatory tyrosine residue (Tyr530) (Figure 1). SRC exists in both active and inactive conformations. Negative regulation occurs through phosphorylation of Tyr530, resulting in an intramolecular association between phosphorylated Tyr530 and the SH2 domain of SRC, thereby locking the protein in a closed conformation. Further stabilization of the inactive state occurs through interactions between the SH3 domain and a proline-rich stretch of residues within the kinase domain. Conversely, dephosphorylation of Tyr530 allows SRC to assume an open conformation. Full activity requires additional autophosphorylation of the Tyr419 residue within the catalytic domain. Loss of the negative-regulatory C-terminal segment, as occurs in v-Src, has been shown to result in increased activity and transforming potential [1,7]. However, similar activating mutations are rare in human tumors, with just one published report that found activating SRC mutations in approximately 12% of human colon cancers [5].

The intramolecular activity of SRC is regulated by a balance between kinases and phosphatases that act at the C-terminal Tyr530 residue. Phosphorylation by C-terminal SRC kinase (CSK) and CSK homology kinase results in increased intramolecular interactions and consequent SRC inactivation. Indeed, CSK overexpression suppresses metastasis in animal models of colon cancer, suggesting a possible tumor suppressor role [8]. By contrast, CSK levels are decreased in hepatocellular carcinoma compared with matched cirrhotic controls [9]. Less evidence exists relating to the involvement of specific phosphatases in SRC activation. Protein tyrosine phosphatase α (PTPα) and the SH-containing phosphatases SHP1/SHP2 are the most-studied examples, showing SRC-specific dephosphorylation activity *in vitro* and *in vivo* [1]. Furthermore, the SRC-specific PTP1β is upregulated in certain breast cancers [10].

SRC is also activated by direct binding of focal adhesion kinase (FAK) and CRK-associated substrate (CAS) to the SH2 domain [11]. When bound, these molecules activate SRC by disrupting inhibitory intramolecular interactions. Interestingly, both FAK and CAS are principal regulators of focal adhesion complex formation and actin

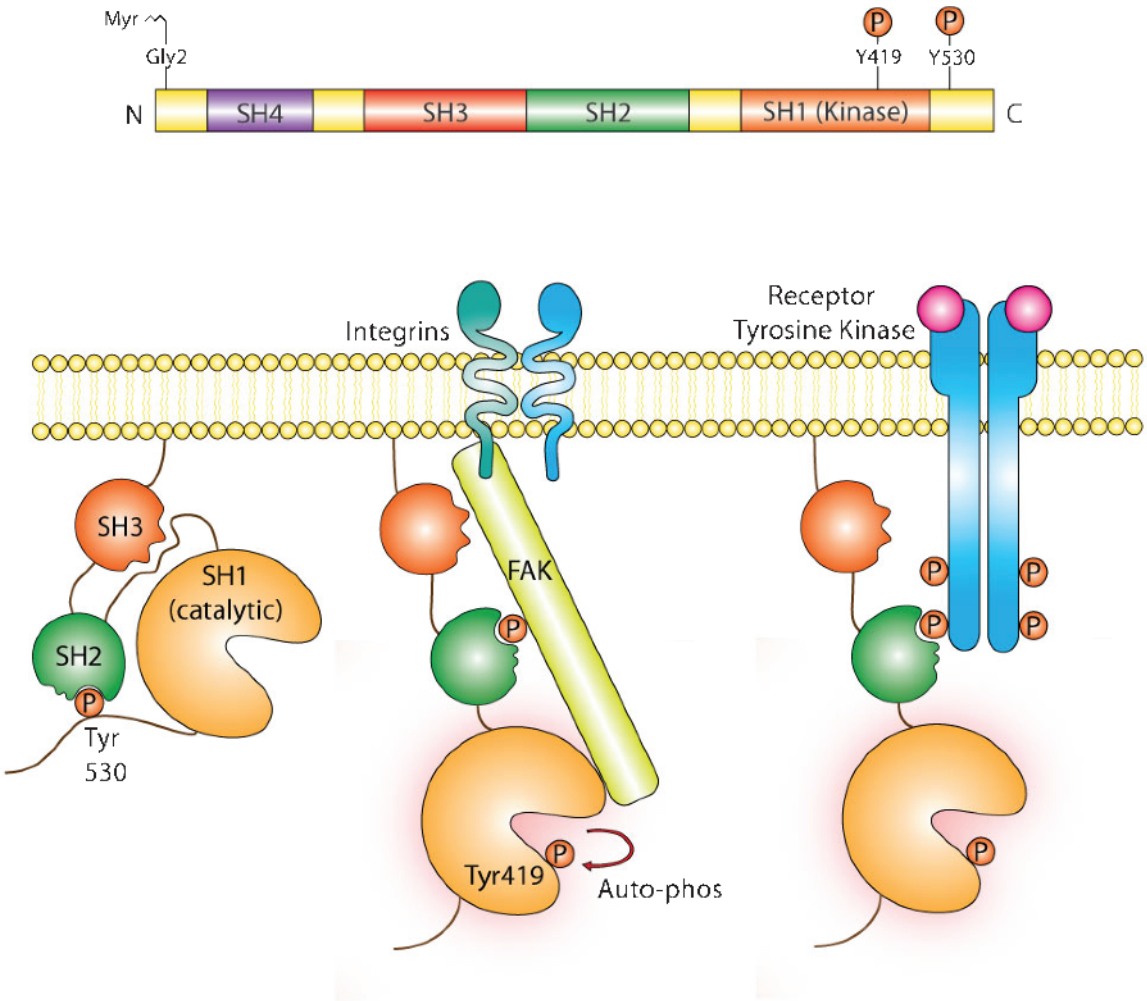

Figure 1. Methods of SRC activation and inactivation. Phosphorylation of Tyr530 at the C-terminus locks the protein in a closed, inactive conformation stabilized through interactions between the SH3 and kinase domains. Dephosphorylation of Tyr530 and autophosphorylation of Tyr419 within the catalytic domain allow SRC to assume an open, active conformation. SRC activity is also regulated by receptor tyrosine kinases and direct binding of FAK to the SH2 domain.

cytoskeleton dynamics, essential processes for cell adhesion and migration [12]. In addition, SRC activity can be regulated by numerous receptor tyrosine kinases (RTKs), such as epidermal growth factor receptor (EGFR), HER2, fibroblast growth factor receptor, platelet-derived growth factor receptor (PDGFR), and vascular endothelial growth factor receptor (VEGFR) [13].

SRC Activation in Normal and Malignant Cells

Cell Adhesion and Invasion

Dynamic turnover of cell-cell (adherens junctions) and cell-matrix (focal adhesions) junctions is crucial for normal cellular adhesion, migration, and division. SRC plays a key role in regulating the assembly and disassembly of these junctions [1]. The subcellular localization of SRC is critical to its function [14]. SRC associates with the plasma membrane through an N-terminal fatty acid moiety and when activated, translocates to sites of membrane-cytoskeletal interface where it acts to promote turnover of adherens junctions and focal adhesions [15].

Adherens junctions are maintained by homotypic interactions between E-cadherin molecules present on neighboring cells. Loss of E-cadherin is a key event in the epithelial-to-mesencymal transition and is associated with enhanced invasive and metastatic potential. Increased SRC signaling correlates with decreased E-cadherin expression and decreased cell-cell adhesion [16,17]. At the cell periphery, activated SRC forms complexes with cytoplasmic proteins such as FAK and CAS [15,18]. In association with FAK, SRC mediates signals from extracellular matrix–integrin complexes to the cell interior, thereby influencing cell motility, survival, and proliferation. The SRC-FAK complex interacts with a multitude of substrates, including CAS, paxillin, and p190RhoGAP, which play critical roles in promoting actin remodeling and cellular migration [19,20]. In cancer, dysregulated focal adhesion signaling has been implicated in increased invasion and metastasis, in addition to decreased patient survival [21].

Receptor-Mediated Activation

Growth factor signaling through RTKs can also activate SRC, most likely by disrupting inhibitory intramolecular forces. Many tumors that overexpress or have constitutively activated RTK signaling also have upregulated SRC expression or activity. Furthermore, experiments using epithelial and fibroblast cell lines suggest that SRC and EGFR act synergistically to increase cellular proliferation and invasion [22,23]. Direct phosphorylation of EGFR by SRC is required for efficient EGF-induced DNA synthesis and signal transducer and activator of transcription 5B (STAT5b) activation [24]. In addition, SRC overexpression increases ERBB2 (HER2) and ERBB3 (HER3) heterodimer formation and potentiates downstream signaling [25]. SRC also associates with PDGFR through its SH2 domain and is required for efficient PDGF-induced mitogenic signaling and DNA synthesis [26]. PDGFR seems to exert an activating effect on SRC through phosphotyrosines at Tyr579 and Tyr581 because replacement of these residues decreases SRC-mediated signaling [27].

Cell Proliferation and Mitogenesis

Increasing evidence suggests that SRC is intimately involved in regulating cell cycle progression and mitogenesis. For example, SRC overexpression abrogates MYC requirement for G_0/G_1, but not G_1/S, phase transition [28]. Furthermore, SRC inhibition is associated with decreased β-catenin binding to cyclin D1 and MYC promoters and decreased expression of these mediators [29]. SRC is transiently activated during G_2/M transition and is required for efficient cellular division [30]. Downstream substrates of SRC seem to act largely in parallel to increase cell proliferation and survival because simultaneous inhibition of PI3K and RAS signaling abrogates SRC-induced transformation, but inhibition of either pathway alone does not [2].

Regulation of Angiogenesis

Angiogenesis is frequently dysregulated in cancer, and antiangiogenics are approved for the treatment of several solid tumors. Angiogenesis is regulated by multiple cytokines that trigger a cellular cascade favoring endothelial cell migration and proliferation. SRC activation is associated with increased expression of proangiogenic cytokines such as VEGF and interleukin 8 (IL-8) [31]. In hypoxia-induced models of angiogenesis, SRC activation and antisense SRC inhibition positively and negatively regulate VEGF expression, respectively. Treatment with 4-amino-5-(4-chlorophenyl)-7-(*t*-butyl)pyrazolo[3,4-*d*]pyrimidine (PP2), a potent and selective inhibitor of SFKs, inhibits angiogenesis *in vivo* and blocks endothelial cell differentiation *in vitro* [32]. SRC is also involved in regulating IL-8 expression, with v-SRC–transformed cells showing enhanced IL-8 expression [33]. Conversely, inhibiting SRC blocks IL-8–mediated VEGFR2 activation and decreases vascular permeability [34]. Furthermore, SFKs are implicated in endothelial cell function, with inhibition of SRC, FYN, and YES decreasing VEGF-induced endothelial cell migration [35].

Metastasis and Bone Remodeling

Bone metastases often occur in patients with lung, prostate, colorectal, or breast carcinoma and often lead to pathologic fracture and bone pain. Metastatic cancer affects bone remodeling, which is normally regulated by the dynamic process of osteoblast-mediated bone formation and osteoclast-mediated bone resorption. SRC is implicated as a central regulator of bone remodeling, demonstrated by SRC−/− mice being highly prone to developing osteopetrosis, a disease characterized by decreased bone resorption [36]. In addition, SRC is increased in functioning osteoclasts, and disrupted SRC signaling prevents osteoclast migration and bone resorption activity [37,38].

Notably, nude mice injected with SRC-overexpressing MDA-231 breast cancer cells preferentially developed osteolytic bone metastases [39]. In a similar model of breast cancer, SRC inhibition decreased metastatic disease burden and overall lethality, reduced osteoclast bone resorption, and impaired function of osteoblasts *in vitro* [40].

Clinical Development of SRC Inhibitors

Given the critical role of SRC in promoting cell proliferation, invasion, and metastasis and in regulating bone remodeling, molecular inhibitors of SFKs are being developed and evaluated. Evidence discussed previously suggests that inhibiting SRC may slow disease progression and help control the formation of distant metastases, in addition to reducing concomitant lytic bone lesions.

Successful development of targeted therapeutics often depends on identifying reliable molecular and clinical markers associated with clinical benefit. Experience with oncologic agents such as trastuzumab, gefitinib, and cetuximab demonstrates that clinical efficacy may prove elusive if predictive markers of response and/or resistance are not identified. We now recognize that molecular heterogeneity exists even within a particular cancer type, and therefore targeted agents may only benefit select cohorts of patients. Consequently, biomarker identification is a focus of development for many new agents. Preclinical and

clinical data for the three most-studied SRC inhibitors (dasatinib, bosutinib, and saracatinib) are reviewed in the next sections.

SRC Inhibitors: Preclinical Data

Dasatinib

Dasatinib (Sprycel; Bristol Myers-Squibb) is an orally available, small-molecule SRC/ABL inhibitor that has robust antitumor and antiproliferative activity against numerous hematologic and solid tumor cell lines [41,42]. In addition to inhibiting SRC and BCR-ABL in the subnanomolar range, dasatinib also variably inhibits other SFKs, c-KIT, PDGFR, and ephrin A2 [41]. The mechanism of SRC inhibition results from a hydrogen bond–mediated association with the ATP binding site, resulting in competitive restriction of ATP binding by SRC [42].

In preclinical studies, dasatinib was active in numerous cancer cell lines and *in vivo* tumor models. Studies of dasatinib in prostate [43] and colon cancer cell lines [44] showed inhibition of cellular adhesion, migration, and invasion. Breast cancer cell lines belonging to the basal/"triple–negative" subtype were particularly sensitive to dasatinib. Breast cancers within this subgroup express basal cell cytokeratins (CK5 and CK17), do not express estrogen (ER) or progesterone receptors (PRs) or HER2 [45,46], and are notorious for poor prognosis [47]. Interestingly, in EGFR-overexpressing breast cancer cell lines, dasatinib inhibited cell growth, invasion, and angiogenesis, and stimulated apoptosis by activating caspase 8 and 9 [48]. Similarly, in lung cancer cells, dasatinib seemed to inhibit EGFR-dependent cell lines preferentially, whereas having a minimal effect on their wild-type EGFR-expressing counterparts. Moreover, dasatinib inhibited cell growth by promoting G_1/S cell cycle arrest, with associated changes in the levels of cyclin D and p27 [49].

Dasatinib can also reduce metastatic disease and osteoclast-mediated bone resorption. In animal models of pancreatic and prostate cancer, dasatinib significantly reduced tumor size and incidence of metastases [50,51]. In addition, recent data showed that dasatinib inhibits osteoclast activity *in vitro*, in part by inhibiting the macrophage colony-stimulating factor receptor (c-FMS), which may act in concert with SRC to potentiate osteoclast activation [52,53]. Signaling through c-FMS is critical for osteoclast survival and activity, with disruption resulting in an osteopetrotic phenotype, much like that observed in SRC–/– deficient mice [54]. In a recent study using osteoclast precursors obtained from ovarian tumor ascites, dasatinib inhibited osteoclast production at concentrations less than 1 nM; this effect may be mediated by the concerted inhibition of c-FMS and SRC because imatinib (a known c-FMS, but not SRC, inhibitor) produced inhibition, albeit at much higher concentrations [55].

Bosutinib

Bosutinib (previously SKI-606; Wyeth) is a dual SRC/ABL kinase inhibitor that inhibits SRC with an 50% inhibitory concentration (IC_{50}) of 1.2 nM and SRC-dependent fibroblasts in suspension with an IC_{50} of 100 nM. Bosutinib does not inhibit RTKs (KIT or PDGFR) at any appreciable level, but it does have activity against other SFKs [56,57].

In cellular assays, bosutinib treatment resulted in a dose-dependent reduction in proliferation, invasion, and migration of breast cancer cells [58,59]. Furthermore, in a murine model of breast carcinoma, bosutinib inhibited tumor growth and significantly reduced the number of liver, spleen, and lung metastases. These effects correlated with reduced phosphorylation of AKT, FAK, and MAPK and with an increase in apoptosis and E-cadherin expression [58]. In addition, bosutinib inhibited colorectal cancer cell adhesion and motility. Interestingly, this effect seemed to result from reduced SRC-dependent β-catenin activation, with small interfering RNA–driven knockdown of β-catenin abrogating the effects of bosutinib on cell-cell adhesion [60]. Furthermore, bosutinib showed modest activity in xenograft models of colon cancer and had an oral bioavailability of 18% and a plasma half-life of 8.6 hours [61].

Recent work has shown that SFKs are activated in 33% of non–small cell lung cancers (NSCLCs), with up-regulation correlating with male gender, active smoker status, and squamous cell histology. Treatment of NSCLC cell lines with bosutinib had an antiproliferative and proapoptotic effect, particularly in cell lines with increased Tyr419 SRC autophosphorylation at baseline [62]. Recent work has also showed that some human-derived pancreatic tumor xenografts were sensitive to bosutinib and sensitivity correlated with caveolin 1 expression, previously identified as a predictor of response to dasatinib in breast cancer cell lines [45,63].

Saracatinib

Saracatinib (formerly AZD0530; AstraZeneca) is another ATP-competitive inhibitor of SRC and SFKs, with activity against ABL and activated mutant forms of EGFR (L858R and L861Q) [64,65]. In a panel of 13 human cancer cell lines treated with saracatinib, there was submicromolar growth inhibition in four cell lines (derived from colon, prostate, and lung tumors) and inhibitory effects on migration and invasion [29,66]. *In vivo*, saracatinib inhibited the growth of 3 of 16 human-derived pancreatic cancer xenografts, with associated decreases in FAK, paxillin, and STAT3 activation. The authors also identified and validated a gene expression profile, based on the expression of *LRRC19* and *IGFBP2*, which achieved 100% sensitivity and 83% specificity at predicting growth inhibition in an independent sample of eight xenografts [67]. In addition, saracatinib showed activity in *in vitro* and *in vivo* models of castration-resistant prostate cancer (CRPC) [68].

SRC Inhibitors: Preclinical Data Evaluating Novel Combinations

Dysregulated SRC signaling has been implicated in the development of resistance to numerous anticancer agents, including cetuximab, oxaliplatin, and gemcitabine [69–71]. Given these findings, and the involvement of SRC in modulating multiple signaling pathways, there is considerable interest in studying SRC inhibitors in conjunction with chemotherapeutic and biologic agents.

Combination with Antiestrogen Therapies

Current antihormonal treatments for ER-positive breast cancer include selective ER modulators (e.g., tamoxifen) and aromatase inhibitors (e.g., anastrozole), which decrease ER signaling and estrogen production, respectively. SRC potentiates ER signaling by phosphorylating the ER on Tyr537, and when complexed with estrogen, the ER associates with SRC to promote cellular proliferation [72,73]. This cross talk suggests possible synergy between antiestrogens and SRC inhibitors, with recent data supporting this supposition. In ER-overexpressing breast cancer cell lines, saracatinib and tamoxifen synergistically inhibited cell growth [74] and prevented the development of tamoxifen resistance [75]. Similarly, saracatinib and anastrozole in combination reduced both the development of drug resistance and tumor growth *in vivo* [76]. Furthermore, treating tamoxifen-resistant cells with PP2 restores tamoxifen sensitivity [77].

Combination with Cytotoxic Therapies

In an *in vitro* study of 5-fluorouracil (5-FU)–resistant pancreatic cancer cells, PP2 reversed 5-FU chemoresistance and restored 5-FU–induced apoptosis. Furthermore, 5-FU and PP2 in combination decreased *in vivo* tumor growth and metastatic disease [78]. In pancreatic adenocarcinoma cell lines, the level of SRC expression correlated with increased resistance to gemcitabine, and small interfering RNA–mediated SRC inhibition potentiated gemcitabine-induced caspase-mediatedapoptosis [69]. In ovarian and colon carcinoma cells, dasatinib restored paclitaxel sensitivity and acted synergistically with oxaliplatin, respectively [70,79].

Combination with Anti-EGFR Therapies

Recent work using an *in vitro* model of colorectal cancer showed that combination of a monoclonal antibody to EGFR and a SRC inhibitor synergistically inhibited cell proliferation and colony formation [80]. Similarly, recent findings indicate that both dasatinib and saracatinib can restore the sensitivity of resistant head and neck squamous cell carcinoma cell lines to the EGFR inhibitors cetuximab and gefitinib [71,81].

SRC Inhibitors: Preliminary Clinical Activity

In light of promising preclinical studies, dasatinib, bosutinib, and saracatinib have entered clinical trials. Preliminary data suggest that the agents are well tolerated at doses that achieve clinically meaningful plasma drug concentrations. Clinical studies of SRC inhibitors as single agents or in combination are shown in Tables 1 and 2.

Single-Agent Studies with Dasatinib

Currently, dasatinib is approved for the second-line treatment of chronic myeloid leukemia and Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia. Dasatinib is currently being studied in numerous solid malignancies. In a phase 1 dose-escalation study, Demetri et al. [82] reported on the safety, tolerability, and pharmacologic profile of dasatinib in 67 patients with refractory solid tumors. Patients received oral dasatinib either every 12 hours for five consecutive days followed by two nontreatment days (5D2) or as continuous twice-daily dosing. Maximum tolerated dosages (MTDs) were established as 120 mg twice daily for dosing for five consecutive days followed by two nontreatment days and 70 mg for continuous twice-daily dosing. Dose-limiting toxicities (DLTs) included grade 2 rash, grade 3 lethargy, grade 3 proteinuria, and grade 3 hypocalcemia. Previous studies of dasatinib in Ph+ leukemias showed high rates of treatment-associated neutropenia (45%), thrombocytopenia (35%), and pleural effusion (35%) [83]. Interestingly, in solid tumors at least, most treatment-related toxicities were nonhematologic (nausea, fatigue, lethargy, anorexia, proteinuria, and diarrhea), suggesting that hematologic adverse effects may be related to antileukemic activity. Pleural effusions were infrequent (three patients), although subsequent phase 2 studies showed a higher incidence [84]. The reasons for these differences are unclear, although they may relate to patient selection and underlying malignancies. Whereas no objective responses were reported, 16% of patients had stable disease and 25% had metabolic partial response (as judged by positron emission tomography scan).

Recent phase 2 studies suggest that dasatinib is well tolerated with modest single-agent activity in breast cancer. In a phase 2 study, Finn et al. [84] enrolled 44 patients with recurrent or metastatic triple-negative

Table 1. Clinical Studies of SRC Inhibitors as Single Agents.

Drug	Tumor Type	ClinicalTrials.gov Identifier	Phase	Dose and Schedule	Completion Date	Enrolment Status	Expected Enrolment (*n*)
Dasatinib	Advanced solid tumors	NCT00099606	1	35-120 mg twice daily	Jul 2007	Completed	60
	Hormone-sensitive breast cancer	NCT00371345	2	70 mg twice daily	Mar 2009	Completed	70
	Triple-negative breast cancer	NCT00371254	2	70 mg twice daily	Sep 2008	Completed	44
	Head and neck squamous cell carcinoma	NCT00507767	2	100 mg twice daily	Jul 2010	Active, not recruiting	35
	Castration-resistant prostate cancer	NCT00385580	2	70 mg twice daily	Dec 2008	Active, not recruiting	100
	Multiple myeloma	NCT00429949	2	NR	NR	Completed	NR
	Pancreatic cancer	NCT00544908	2	(dose NR) twice daily	Dec 2009	Active, not recruiting	41
	Colorectal cancer	NCT00504153	2	(dose NR) twice daily	Nov 2008	Active, not recruiting	54
	Small cell lung cancer	NCT00470054	2	(dose NR) twice daily	Oct 2008	Active, not recruiting	56
	NSCLC	NCT00787267	2	70 mg twice daily	Sep 2011	Recruiting	100
	Breast cancer (patient selection by genomic status)	NCT00780676	2	100 mg once daily	Oct 2024	Recruiting	532
	Transitional cell carcinoma of the bladder (adjuvant treatment before surgery)	NCT00706641	Pilot study	100 mg once daily	Dec 2010	Recruiting	25
	Hepatocellular carcinoma	NCT00459108	2	(dose NR) twice daily	Jun 2009	Recruiting	41
	Sarcomas	NCT00464620	2	(dose NR) twice daily	Dec 2013	Recruiting	502
	Biomarker analysis of EGFR status	NCT00903734	1	NA	May 2013	Recruiting	102
Bosutinib	Advanced solid tumors	NCT00195260	1	50-600 mg once daily	Dec 2009	Active, not recruiting	151
	Breast cancer	NCT00319254	2	400 mg once daily	Jun 2008	Completed	75
Saracatinib	Advanced solid tumors	NCT00704366	1	(variable dose) once daily	Feb 2010	Active, not recruiting	24
	Osteosarcoma (localized to lung)	NCT00923286	2	175 mg once daily	Feb 2015	Recruiting	88
	Hormone receptor–negative breast cancer	NCT00559507	2	NR	Jul 2010	Recruiting	41
	Soft tissue sarcoma	NCT00659360	2	NR	Feb 2009	Active, not recruiting	37
	Melanoma	NCT00669019	2	NR	Feb 2008	Recruiting	40
	Castration-resistant prostate cancer	NCT00513071	2	(dose NR) once daily	Oct 2008	Completed	28
	Thymoma or thymic cancer	NCT00718809	2	(dose NR) once daily	Jan 2011	Recruiting	39
	Stomach or gastroesophageal junction cancer	NCT00607594	2	NR	Sep 2009	Recruiting	35
	Colorectal cancer	NCT00397878	2	(dose NR) once daily	Apr 2008	Active, not recruiting	35
	Head and neck cancer	NCT00513435	2	(dose NR) once daily	Sep 2010	Active, not recruiting	28
	Small cell lung cancer	NCT00528645	2	(dose NR) once daily	Apr 2009	Active, not recruiting	44

NA indicates not applicable; *NR*, not reported.

Table 2. Clinical Studies of SRC Inhibitors Combination with Other Agents.

Drug	Combination Agent(s)	Tumor Type	ClinicalTrials.gov Identifier	Phase	SRC Inhibitor Dose and Schedule	Completion Date	Enrolment Status	Expected Enrolment (*n*)
Dasatinib	Erlotinib	NSCLC	NCT00444015	1	NR	Jan 2010	Active, not recruiting	20
	Erlotinib	Glioma	NCT00609999	1	100 mg once daily	Jan 2009	Recruiting	48
	Capecitabine	Breast cancer	NCT00452673	1	50-100 mg twice daily	Dec 2009	Active, not recruiting	50
	Paclitaxel Carboplatin	Ovarian, peritoneal, or tubal cancer	NCT00672295	1	50-250 mg once daily	Mar 2010	Recruiting	24
	Capecitabine Oxaliplatin Bevacizumab	Colorectal cancer	NCT00920868	1	50 mg twice daily	May 2011	Recruiting	56
	Bevacizumab	Advanced solid tumors	NCT00792545	1	(dose NR) once daily	Jul 2010	Recruiting	48
	Paclitaxel	Breast cancer	NCT00820170	1/2	(dose NR) once daily	Jan 2012	Recruiting	60
	Docetaxel	Castration-resistant prostate cancer	NCT00439270	1/2	50-150 mg once daily	Oct 2009	Active, not recruiting	66
	Zoledronic acid	Breast cancer with bone metastasis	NCT00566618	1/2	100 mg once daily	Mar 2010	Recruiting	55
	Dacarbazine	Melanoma	NCT00597038	1/2	50-70 mg twice daily	Feb 2010	Recruiting	47
	Letrozole	Hormone receptor-positive/HER2-negative breast cancer	NCT00696072	2	100 mg once daily	Jun 2012	Recruiting	120
	Docetaxel Prednisone	Castration-resistant prostate cancer	NCT00744497	3	100 mg once daily	Sep 2012	Recruiting	1380
Bosutinib	Capecitabine	Solid tumors and HER2-advanced breast cancer	NCT00959946	1/2	NR	Dec 2011	Recruiting	152
	Letrozole	Hormone-sensitive breast cancer	NCT00880009	2	NR	Dec 2013	Recruiting	250
	Exemestane	Hormone-sensitive breast cancer	NCT00793546	2	NR	Jul 2011	Recruiting	224
Saracatinib	Carboplatin Paclitaxel	Advanced solid tumors	NCT00496028	1	NR	Oct 2009	Active, not recruiting	234
	Cediranib	Advanced solid tumors	NCT00475956	1	125 or 175 mg once daily	Mar 2009	Active, not recruiting	56
	Gemcitabine	Pancreatic cancer	NCT00265876	1/2	(dose NR) once daily	Jun 2009	Suspended	60
	Carboplatin Paclitaxel	Ovarian cancer	NCT00610714	2	(dose NR) once daily	May 2010	Active, not recruiting	241
	Zoledronic acid	Prostate or breast cancer with bone metastasis	NCT00558272	2	NR	Aug 2010	Recruiting	132

NR indicates not reported.

breast carcinomas. Initial dosing at 100 mg twice daily was modified to a 70-mg twice-daily protocol after serious adverse events (AEs) in 22% of patients at the higher dose. The lower dose was well tolerated, with partial responses confirmed in two patients and stable disease achieved in 11 patients (two for >16 weeks). In a phase 2 study of 68 patients with advanced hormone receptor–positive breast cancers (ER+ and/or PR+ and/or HER2 amplified), there were three partial responses and six instances of stable disease (range, 24-33 weeks) [85]. All nine of these patients had ER- and PR-positive tumors, with two tumors also having amplified HER2.

In an analysis of pretreatment and posttreatment prostate tumor samples from patients with CRPC, SRC activity was increased in 28% of patients and was associated with decreased survival and increased metastatic disease [86]. Two recent phase 2 studies have evaluated the efficacy of dasatinib in CRPC [87,88]. Both studies enrolled men who were chemotherapy naive and had progressive metastatic CRPC; the first study used dasatinib 100 mg or 70 mg twice daily and the second used 100 mg once daily. Response rates were similar for the two dosing regimens. However, once-daily dosing was better tolerated, with 13% of patients reporting grade 3/4 AEs compared with 32% on the twice-daily regimen. Of 48 patients treated with 100 mg once daily, 1 patient had a confirmed prostate-specific antigen response (>50% decrease from baseline), 1 patient had a partial tumor response, and 8 patients had stable disease after 12 weeks. Levels of urinary *N*-telopeptide (a marker of osteoclast activity and bone resorption) decreased by more than 40% in 21 of 43 evaluable patients. Similarly, serum bone-specific alkaline phosphatase (marker of osteoblast activity) was decreased in 25 of the 44 patients with data, suggesting that dasatinib is effective at stabilizing metastatic disease and decreasing bone turnover.

Single-Agent Studies with Bosutinib and Saracatinib

At this time, few clinical studies have assessed the safety and efficacy of saracatinib and bosutinib. A recent two-part, phase 1 study of 81 patients with advanced solid tumors sought to establish the MTD of saracatinib and its effect on downstream targets of SRC [89]. In the first part of the study, 30 patients received saracatinib at doses ranging from 50 to 250 mg daily. The MTD was established as 175 mg with once-daily dosing. DLTs were leukopenia (grade 3), asthenia (grade 3), febrile neutropenia (grade 3), and respiratory failure (grade 5). One patient had renal failure (grade 4) with concomitant septic shock (resulting in death), although the relationship of this event to saracatinib was unclear. Other AEs were relatively mild and included nausea, asthenia, anorexia, vomiting, and diarrhea. In the second part of the study, 51 patients were randomized to receive 50, 125, or 175 mg of saracatinib. Dose-dependent reductions in levels of phospho-FAK and phospho-paxillin were noted in posttreatment samples, and patients with high baseline levels had proportionally larger reductions in these substrates after treatment. A modulatory effect of saracatinib on bone turnover was also observed, with the authors reporting a dose-dependent decrease in C-terminal telopeptide (a bone resorption marker) levels after treatment. There were no objective tumor responses, although 16% of patients continued treatment for more than 12 weeks. Thus, at the doses tested, saracatinib seems to be well tolerated and able to inhibit SRC kinase activity. On the basis of these results, follow-up phase 2 studies used saracatinib as a monotherapy in patients with advanced CRPC ($n = 28$) and advanced colorectal cancer ($n = 10$). Although saracatinib was generally well tolerated, there was no meaningful single-agent clinical activity [90,91].

In a phase 1 dose-escalation study of bosutinib in 51 patients with advanced solid tumors, bosutinib was generally well tolerated with an

MTD of 400 mg for once-daily dosing. DLTs included grade 3 diarrhea (two patients) and grade 3 rash (one patient). Drug-related AEs were mainly gastrointestinal and included nausea, diarrhea, anorexia, vomiting, and asthenia, with diarrhea being the only grade 3 AE occurring in more than 5% of patients (14%). There were no objective responses, although six patients had stable disease lasting longer than 15 weeks and one patient had stable disease lasting longer than 52 weeks (pancreatic cancer) [92]. In a follow-up phase 2 study of women with stage IIIB, IIIC, or metastatic breast cancer, 73 women received bosutinib 400 mg daily. The drug was generally well tolerated, with only eight patients requiring dose reduction, mainly secondary to gastrointestinal adverse effects (diarrhea, nausea, and vomiting). Of 62 evaluable patients, four had partial responses and 13 and 25 had stable disease lasting 24 weeks or longer and less than 24 weeks, respectively [93].

Combination Studies

On the basis of promising results from preclinical studies, SRC inhibitors are being tested in combination with chemotherapies and other targeted agents. In a phase 1/2 study, dasatinib was administered with docetaxel to 46 patients with progressive CRPC. There was a prostate-specific antigen response in 13 of 32 patients and a Response Evaluation Criteria In Solid Tumors partial response in 12 of 21 patients. In addition, nine patients had stable disease (four at >21 weeks and five at >6 weeks). There was also indirect evidence of decreased bone resorption and formation. Of patients with measurable serum levels of urinary *N*-telopeptide and bone-specific alkaline phosphatase, there was more than 35% decrease in 12 of 26 and in 17 of 24 patients, respectively [94].

Dasatinib was also well tolerated in combination with 5-FU, leucovorin, oxaliplatin, and cetuximab in patients with metastatic colorectal cancer [95]. Of seven patients enrolled, two had radiographic evidence of response, including one confirmed partial response. This study is continuing at using higher dasatinib doses.

Additional studies are assessing SRC inhibitors in combination with anti-VEGF therapies. In a recent study, the effects of saracatinib (175 mg once daily) were examined in patients receiving daily oral cediranib (a small-molecule VEGFR inhibitor) at 20-, 30-, or 45-mg doses. All dose cohorts tolerated the treatment well, with no DLTs reported 28 days into the study. In the 11 patients for whom data were available, nine had stable disease (35-197 days in duration) [96].

Conclusions

There is no single oncogene better studied than *SRC*. Despite nearly a century of data suggesting a role in promoting malignancy, it is only recently, with the discovery of a class of highly selective and specific molecules, that we can effectively block SRC kinase activity. These clinical grade SRC inhibitors are currently being evaluated in the clinic. With such a central role in regulating so many cellular pathways, perhaps the most challenging task will be selecting the patients most likely to benefit from SRC inhibition. On the basis of SRC's role in tumor biology, these molecules may work best in early stage disease and in combination with other agents. Preliminary clinical data suggest a role for SRC inhibition in human disease. Ongoing studies evaluating the molecular effects of SRC inhibition in clinical tissue and combinations of SRC inhibitors with cytotoxics and other biologic agents are ongoing. Data from these studies are eagerly awaited, and these will help guide the next phase of development of this class of novel agents.

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