Therapeutic Perspective

Src tyrosine kinase inhibitors in the treatment of lung cancer: rationale and clinical data


Src-family kinases have an important role in many oncologic functions in human cancers, including proliferation, motility, migration, survival and angiogenesis. Several Src inhibitors have been developed, of which dasatinib has been most explored in the clinic. Preclinical studies in a wide variety of solid tumor cell lines have shown that dasatinib acts as a cytostatic agent, inhibiting the processes of cell proliferation, invasion and metastasis. In particular, in preclinical studies, an interaction between Src-family kinases inhibition and cell survival has been noted in EGFR-dependent non-small-cell lung cancer cell lines. Src tyrosine kinase inhibitors in clinical trials have shown a favorable safety profile and moderate anticancer activity in unselected patient populations. Future trials will continue to explore the contribution of Src inhibition in combination with chemotherapy and other targeted agents.

Keywords: bosutinib • dasatinib • EGF receptor • saracatinib • Src-family kinases

Based on the early work of Peyton Rous on the transmission of sarcoma in fowl, the Rous sarcoma virus, which carries the \( v\text{-}src \) oncogene, was eventually discovered [1]. The \( v\text{-}src \) oncogene is a truncated, constitutively active form of the wild-type proto-oncogene \( c\text{-}SRC \) (or ‘Src’). Since the discovery of the \( Src \) proto-oncogene as a tyrosine kinase in 1976, nine additional variants closely related to Src have been identified in the human genome. Together these ten Src-related kinases are grouped as ‘Src-family kinases’ (SFKs) and mainly regulate cell adhesion and motility [2,3]. According to their pattern of expression in different tissues, SFKs are subdivided into three distinct groups. The first group (SRC, FYN and YES) is ubiquitously expressed. The second group (HCK, LCK, LYN, BLK, YRK and FGR) is expressed primarily in hematopoietic cells, and the third group (FRK-related kinases) is expressed predominantly in epithelial-derived tissues [4–7].

Structurally, SFKs are highly related to one another. They share a conserved domain structure consisting of consecutive SH3, SH2 and tyrosine kinase (SH1) domains (Figure 1). All SFKs also contain a SH4 membrane-targeting region at their N-terminus, which is always myristoylated and sometimes palmitoylated [8,9]. The SH4 region is followed by a unique domain of 50–70 residues, which is divergent among family members [10,11]. The SH3 domain is critical for Src activity, intracellular localization, and the recruitment and binding of Src substrates. Src kinases can be activated by binding of cognate ligands to their SH2 and/or SH3 domains [12]. Src can be activated by the PDGF receptor by its interaction with SH2 [13] or through interaction of SH2 and SH3 domain with FAK [14,15].

SFKs cooperate with multiple-receptor tyrosine kinases (RTKs), such as EGFR, to modulate intracellular signaling, transform cells and promote tumor growth, invasion and metastasis [2,16–21] (Figure 2). SFKs activate downstream
signaling pathways, such as RAS/RAF/MAPK, PI3K/AKT and STATs, which control tumor growth [32–34]. Angiogenic growth factors, such as VEGF and IL-8, are downstream targets of c-Src, and small-molecule inhibitors of SFK have been shown to inhibit angiogenesis [25–28]. However, there are different results in the literature regarding effects on angiogenesis through Src inhibition. The pleiotropic functions of Src and other SFKs underscore the importance of these kinases and explain why many of the SFKs have been involved in carcinogenesis [29]. The regulation of cell adhesion, invasion and motility is one of the key functions of Src by interaction with E-cadherin [30] and FAK [31]. This effect is consistently described in different cancer cell lines. Except for rare cases of colorectal and endometrial cancers, the constitutive activation of Src by oncogenic Src mutations or by increased gene copy number has not been detected in most cancer types, including lung cancer [32–37]. However, the aberrant expression of activated Src has been found in most types of cancer, including lung, prostate, breast, ovarian, pancreatic, hepatocellular, gastric and colorectal [24]. The increased Src kinase activity in these tumors has been proposed as a consequence of several alterations, including tyrosine phosphatase-mediated dephosphorylation of the carboxy-terminal negative regulatory element, an increase in Src protein levels and/or altered protein stability, an increase in upstream RTK activity, or loss of key regulatory proteins [18,38–42]. Interestingly, increased levels of activated Src have been detected in advanced, metastatic disease compared with early disease. This finding suggests that Src may regulate tumor invasion and metastasis, rather than tumor formation [43–46].

The interaction of Src and RTKs, notably with the HER family of RTKs, including EGFR, HER2, HER3 and HER4, is of special interest. This interaction of SFKs with RTKs can result in enhanced or synergistic SFK activation and has been demonstrated in different tumor types, most notably in head and neck squamous-cell carcinoma (SCC), non-small-cell lung cancer (NSCLC) and colorectal cancer (CRC) [38,47–49]. In breast cancer, increased Src activity conferred considerable resistance to the HER2 antibody trastuzumab. Moreover, combining Src inhibitors with trastuzumab restored the sensitivity of trastuzumab-resistant cell lines to trastuzumab [50]. Although EGFR is activated through ligand binding and autophosphorylation of its cytoplasmic tail, it is well established that Src, or SFKs, are necessary for full activation of the EGFR [20]. Investigations into the molecular interactions between SFKs and EGFR have revealed that SFKs can physically associate with activated EGFR [18,51,52].

In lung cancer cells, the interaction and cooperation between mutant EGFR and Src play critical roles in constitutive engagement of the downstream signaling pathways, and mediate oncogenic potential [53]. In EGFR-dependent lung adenocarcinomas, protein levels of SFKs are increased [57]. Src signaling is also involved in normal bone remodeling and in the formation of bone metastases [54–58].

**Src & SFKs in lung cancer**

Several research groups have examined Src expression and activation in human lung cancer [43,59–62]. An increased level of Src protein and kinase activity have been reported in 50–80% of patients with lung cancer [60]. Several preclinical studies have suggested that SFK inhibitors are active against lung cancer cell lines in vitro [63,64]. Preclinical studies with small-molecule Src inhibitors have provided evidence to support the role for Src as a potential therapeutic target in lung cancer [65]. Our own experiments showed that the interaction of Src with Id1 is one potential mechanism that regulates migration and invasion in lung cancer cells [66,67]. We also reported that Src and Id1 are frequently co-expressed in primary lung cancers and early malignant lesions. Recently, our group demonstrated that specific miRNAs are involved in Src–Id1 signaling and cancer cell resistance towards SFK inhibitors, providing a potential explanation of why the activity of SFK inhibitors might be limited in lung cancer [68].

**Clinical data of SFK inhibitors**

In the 1990s, the first SFK inhibitors such as PP1...
and PP2 were used in vitro. In the meantime, several small-molecule inhibitors for SFKs have been tested in clinical trials. Among others, dasatinib, saracatinib, bosutinib, XL228, KX2-391 and XL999, have been tested in patients with different solid tumors, including lung cancer.

### Dasatinib

Dasatinib (BMS-354825; Bristol-Myers Squibb, NY, USA) is a potent, orally available multikinase-inhibitor against BCR-ABL, c-KIT, SFKs, PDGFR, BTK and EPFA2 [69,70]. Dasatinib has clinical targets in Philadelphia-chromosome-positive leukemias (BCR-ABL) and gastrointestinal stromal tumors (PDGFR). Dasatinib inhibits the kinases Src and ABL with IC₅₀ values of 0.55 and 3.0 nM, respectively. Dasatinib also inhibits other Src-family members such as FYN (IC₅₀ of 0.2 nM), LCK (IC₅₀ of 1.1 nM) and YES (IC₅₀ of 0.4 nM) [69].

Dasatinib is currently approved for imatinib-resistant chronic myelogenous leukemia (CML) and is being studied in numerous clinical trials for solid tumors. Preclinical data suggested that SFK inhibitors could inhibit tumor growth and induce tumor cell death. Dasatinib inhibited migration and invasion in different NSCLC and head and neck SCC cell lines and induced cell cycle arrest and apoptosis in some cell lines [64]. The first clinical data with dasatinib in NSCLC were reported by Johnson et al. In their Phase II study, 34 patients received dasatinib as first-line therapy for previously untreated advanced NSCLC. The primary objective was overall disease control rate (partial responses plus stable disease), which was 43% including one case with a long-lasting remission. Major toxicities reported from this trial were fatigue and dyspnea due to pleural effusion, which led to a reduced starting dose in subsequent patients. EGFR and KRAS mutational analyses were performed successfully in 31 tumors. Baseline EGFR mutational status, KRAS mutational status, EGFR amplification, phosphorylated SRC score, phosphorylated STAT3 level, and histology were not significant predictors of progression-free survival (PFS) [71]. Based on preclinical data showing cooperation between EGFR and Src in lung cancer cell lines, Haura et al. ran a trial investigating the combination of dasatinib with erlotinib in previously treated patients with advanced NSCLC [72]. A total of 34 patients were enrolled, the recommended dose from the Phase I part of the trial was erlotinib 150 mg once daily and dasatinib 70 mg twice daily. The main adverse events included gastrointestinal side effects (diarrhea, anorexia and nausea), skin rash, cytopenias, pleural effusions and fatigue. More than half of patients had previously received two or more lines of chemotherapy. The disease control rate was 63% with two partial responses and one bone response. To investigate plasma markers, plasma was collected before treatment and on days 15 and 29. There was no correlation between pretreatment levels of VEGF, IL-8 and basic FGF. However, reductions in plasma VEGF on day 29 correlated with disease control. The authors concluded that plasma biomarkers of Src-dependent angiogenic factors should be further explored to

![Src-family kinases signaling pathways and function](image)

**Figure 2.** Src-family kinases signaling pathways and function. Src-family kinases (SFK) can interact with RTK and cooperate in activation of downstream signaling, leading to cell proliferation, survival, invasion and angiogenesis. Through the interaction with the RAS/ERK pathway, DNA synthesis and cell proliferation is enhanced. By activating the PI3K signaling, cell survival is promoted. By activating transcriptional factors, such as STAT3, SFKs promote the transcription of proangiogenic growth factors and cytokines. Furthermore, SFKs can phosphorylate p120 catenin and therefore disrupt adherens junctions stabilized by E-cadherin. The interaction of Src with FAK activates the downstream targets p130Cas, paxillin and RhoA that form complexes with integrin molecules and are responsible for the interaction with the extracellular matrix.

RTK: Receptor tyrosine kinase.

Adapted with permission from [65,74].
Therapeutic Perspective

Rothschild & Gautschi

monitor the effect of treatment [72].

KRAS mutation is a predictive biomarker for resistance to cetuximab in metastatic CRC. Although approximately 30% of NSCLC harbor mutations in KRAS, these mutations are not predictive for cetuximab or EGFR TKIs. In a preclinical study, dasatinib sensitized KRAS-mutant CRC tumors to cetuximab [73]. In NSCLC cell lines with acquired resistance to cetuximab, SFK activation was increased relative to the parental cell line. Following dasatinib treatment, cetuximab-resistant cells exhibited a decrease in total EGFR phosphorylation and SFK activity, resulting in inhibition of cell proliferation. Combination treatment augmented growth inhibition, indicating that dual targeting of EGFR and SFKs might have a greater clinical impact than either agent alone [74]. A Phase I clinical trial investigated combination therapy with dasatinib and cetuximab in patients with advanced solid tumors. Dose-limiting toxicities were headache and nausea. The recommended dose for further trials was dasatinib 150 mg once daily and cetuximab 250 mg/m² weekly. In 23 patients evaluable for response, there were no objective responses; ten patients had stable disease as best response (one patient with NSCLC). Median duration of stable disease was 4.3 months (range 2–22) [75].

Owing to the functional associations between SFKs and EGFR, SFKs have been proposed as a target to overcome acquired resistance in EGFR-mutant tumors. Preclinical models demonstrate that EGFR-mutant cell lines containing either exon 21 mutation (H3255) or exon 19 deletions (PC9 or HCC827) undergo apoptosis when treated with dasatinib [69]. Furthermore, gefitinib-resistant adenocarcinoma cells with T790M (PC9/ZD) or MET amplification (HCC827 GR5) also undergo cell death when treated with dasatinib [70]. Based on these data, a Phase II study of dasatinib in patients with EGFR-mutant lung adenocarcinomas and acquired resistance to the EGFR-TKIs erlotinib and gefitinib was conducted. The trial led to a negative result, with no objective response in 21 enrolled patients [77]. In advanced solid tumors, the combination of dasatinib with gemcitabine was safe, but pleural effusion was a relevant toxicity [78]. The CALGB 30602 trial investigated dasatinib 70 mg twice daily in patients with chemo-sensitive relapsed small-cell lung cancer. A total of 45 patients were enrolled. No objective response was recorded, only 13 cases with PFS ≥ 6 weeks were observed. Therefore, dasatinib did not reach the specified efficacy criteria in this clinical setting [79]. Ongoing clinical trials with dasatinib alone and in combination in patients with NSCLC are listed in Table 1.
trials, in patients with CML who had failed to improve with imatinib, and in patients with solid tumors and HER2-negative metastatic breast cancer in combination with capecitabine, are ongoing.

**Other SFKs inhibitors**

Other ATP-competitive tyrosine kinase inhibitors aimed at multiple targets, including SFKs, are being evaluated. XL999 was an oral inhibitor of SFKs, VEGFR, PDGFR, FGFR and FLT3. However, Exelixis Inc. (CA, USA) discontinued its development program. XL228 targets IGF1 receptor (and Src, but also BCR-ABL, including the T315I mutant form, which is resistant to approved BCR-ABL inhibitors. In a Phase I trial, XL288 was given as an intravenous infusion weekly. One patient with NSCLC had a partial response, and one patient with small-cell lung cancer had long-lasting disease stabilization [90]. M475271 is an oral inhibitor of Src and VEGFR. It has shown pre-clinical activity in lung adenocarcinoma cell lines [91].

KX2-391 is a synthetic, orally bioavailable small molecule Src tyrosine kinase signaling inhibitor. KX2-391 is distinct from all other Src kinase inhibitors in that it targets the substrate-binding site, and not the ATP-binding site. In addition, KX2-391 also inhibits microtubule polymerization. In a Phase I trial, 32 patients were enrolled. Dose-limiting toxicities occurred in four patients and included elevated ALT and AST, neutropenia and fatigue. The maximum tolerated dose was 40 mg twice daily. Seven patients had prolonged stable disease for 4 months or longer [92].

**Future perspective**

SFKs play a critical role in cell signaling, leading to tumor growth, invasion, metastasis and angiogenesis. Multiple tyrosine kinase inhibitors of SFK have been developed and are in clinical trials for different indications. Until today, there are no trials supporting the use of Src TKIs in lung cancer. *In vitro* apoptotic effects of Src TKIs have been minimal; however, these...
Drugs have consistently shown to inhibit cell adhesion, migration and invasion. As such, the effects of Src TKIs could be described as cytostatic rather than cytotoxic. As cytostatic agents are likely to inhibit tumor growth but are unlikely to induce tumor shrinkage, the end points of clinical trials should be chosen accordingly. One perspective for Src TKIs could be to use these agents in early-stage disease or in the adjuvant setting after tumor or metastasis resection where tumor cell migration and invasion is of greater importance than in more advanced disease where cytotoxic drugs play a crucial role. Furthermore, Src TKIs could be used to increase the cytotoxic effects of other agents.

Chemotherapy resistance may be mediated in part by the activation of SFKs. Inhibition of c-Src has been shown to enhance the effects of chemotherapeutic agents such as platinums, taxanes, and gemcitabine, in ovarian, pancreatic and CRCs [95–98]. Therefore, combination of SFK inhibition with chemotherapy is an encouraging therapeutic strategy that is currently investigated in several clinical trials in different tumor types. As the redundancy in cellular pathways may limit the efficacy of single-receptor blockade, multitargeted therapies, including inhibition of EGFR and VEGFR, might improve SFK inhibition. Up to 15% of caucasian patients with lung adenocarcinoma, exhibit activating-EGFR mutations. Discovering the link between Src and EGFR in human lung cancer cell lines was therefore an important step forward [97]. Meanwhile, several groups confirmed that lung cancer cells harboring activating EGFR mutations are highly sensitive to the combination of Src and EGFR inhibition, which leads to increased apoptosis in these cells [49,53,63]. Recently, further oncogenic mutations and amplifications have been discovered in lung cancer, including SCC. Approximately 10–20% of lung SCC have genomic amplification of the tyrosine kinase FGFR1 (CD331) [98]. Furthermore, mutations in the tyrosine kinase DDR2 have been identified in 3–4% of lung SCC [99]. The multikinase inhibitor dasatinib did efficiently block DDR2 in preclinical models [100]. Therefore, dasatinib is now tested in patients with advanced SCC of the lung (Table 1).

Future directions of investigation need to address the identification of predictive markers for anti-Src-directed therapy. Already, early results from contemporary genomic analyses show promise in identifying such molecular and genomic predictors of therapeutic response to SFK inhibitors [101,102]. Our own preclinical data suggest that ID1 and miRNA-29b could be potential predictive markers for the use of Src TKIs. Anti-miR-29b enhanced ID1 mRNA and protein levels, and significantly increased lung cancer cell migration and invasion, a hallmark of the Src-ID1 pathway. miR-29b suppressed the level of ID1 and significantly reduced migration and invasion. Anti-miR-29b and ID1 overexpression both diminished the effects of the Src inhibitors saracatinib and dasatinib on migration and invasion. miR-29b was significantly downregulated in primary lung adenocarcinoma samples compared with matched alveolar lung tissue, and miR-29b expression was a significant prognostic factor for patient outcome. These results suggest that miR-29b is involved in the Src-ID1 signaling pathway, is dysregulated in lung adenocarcinoma, and is a potential predictive marker for Src kinase inhibitors [68]. To establish these factors as predictive biomarkers for the use of Src TKIs in the clinic, prospective evaluation in clinical trials would be needed.

Conclusion
Preliminary results of trials with Src inhibitors demonstrated low single-agent activity in patients with advanced lung cancer. It is unclear if Src inhibitors are of use against tumors that have already metastasized, and clinical data with tumors at early stages are lacking. Future preclinical and clinical investigations will have to focus on potential predictive molecular markers for accurate selection of patients who may benefit from Src inhibitors, and studies in tumors with mutations of EGFR or DDR2 will be particularly interesting. Furthermore, ongoing clinical trials investigate combinations of SFK TKIs and other targeted agents, including EGFR inhibitors and antiangiogenic drugs. Although this strategy is promising, development of Src inhibitors in lung cancer therapy remains challenging. New results from the laboratory will help us to better understand the Src pathway and to develop rational therapeutic concepts.

Personnel review
Based on the reported clinical trials on Src tyrosine kinase inhibitors in lung cancer, we speculated that there will be no place for this substance in advanced/metastatic disease as single treatment. There are few reports on combinatory treatment of Src inhibitors with other targeted agents. These data are too preliminary to draw any conclusions but we think that this approach could be of relevance in the future. On the other hand, use of this substance in early disease to prevent tumor invasion and metastasis could be of interest. Based on the main characteristics of Src tyrosine kinase inhibitors to reduce cell migration and invasion, the use of these inhibitors in early-stage disease could be promising.
O Gautschi and SI Rothschild are supported by the Swiss Cancer League (Grant KLS 02164–02–2008) and the Bernese Cancer League. SI Rothschild works as an Advisor for Sanofi-Aventis, Pfizer and Merck (Switzerland). O Gautschi works as an Advisor for AstraZeneca, Aventis, Pfizer and Merck (Switzerland). O Gautschi and SI Rothschild are supported by the Swiss Cancer League (Grant KLS 02164–02–2008) and the Bernese Cancer League. SI Rothschild is a member of the management committee of the Bernese Cancer League. O Gautschi and SI Rothschild are supported by the Swiss Cancer League (Grant KLS 02164–02–2008) and the Bernese Cancer League. SI Rothschild is a member of the management committee of the Bernese Cancer League.

References
25 Ellis LM, Staley CA, Liu W et al. Down-


59 Mellstrom K, Bjelfman C, Hammerling U, Pahlman S. Expression of c-src in cultured human neuroblastoma and small-cell lung carcinoma cell lines correlates with
Src tyrosine kinase inhibitors in the treatment of lung cancer

Therapeutic Perspective


future science group


395


**Website**