Src tyrosine kinase as a chemotherapeutic target: is there a clinical case?

Ting Chen[a], Jessica A. George[a] and Christopher C. Taylor[a]

Src tyrosine kinase was the first protooncogene described. It has been found to be overexpressed and activated in a large number of different cancers. Cellular Src has been shown to activate a number of different effectors that are involved in different aspects of cancer biology such as metastasis, cell cycle regulation and cell survival. Despite this, Src inhibitors have not entered the regular arsenal of chemotherapeutics. This article reviews some of the biology, rationale, *in vitro* and *in vivo* preclinical evidence, and some very early clinical trials demonstrating efficacy of Src inhibitors. *Anti-Cancer Drugs* 17:123–131 © 2006 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2006, **17**:123–131

Keywords: cancer, chemotherapy, small molecule, Src

[a]Department of Cell Biology, Vincent T. Lombardi Comprehensive Cancer Center, Georgetown University School of Medicine, Washington, District of Columbia, USA.

Correspondence to C. Taylor, Department of Cell Biology, Georgetown University School of Medicine, 3900 Reservoir Road, Washington, D.C. 20007, USA.
Tel: +1 202 687-2552; fax: +1 202 687-1823;
e-mail: cct5@georgetown.edu

Sponsorship: The authors gratefully acknowledge the Department of Defense (OC990038, C.C.T.), the Ovarian Cancer Research Fund (C.C.T.) and AstraZeneca (C.C.T.) for financial support.

Received 19 August 2005 **Accepted** 16 September 2005

Introduction

Src tyrosine kinase was the first characterized proto-oncogene. Pioneering work on the Rous sarcoma virus, for which Src is named, and the viral derivative (v-*src*) of the cellular *src* gene (c-*src*) has yielded two Nobel prizes [1].

Like its viral counterpart, c-*src* encodes a non-receptor tyrosine kinase, c-Src [2,3]. In normal cells c-Src kinase activity is tightly regulated. For the most part, c-Src exists in an inactive state and becomes transiently activated during certain cellular events, including mitosis and neural development [4,5]. Cellular Src is a key element in various signaling pathways (Fig. 1) involved in proliferation, maintenance of normal intercellular contacts and cell motility [6]. The c-Src protein consists of a C-terminal tail, four Src homology (SH) domains and a unique N-terminal domain. Positive regulation of c-Src is accomplished through autophosphorylation of Tyr419 (Tyr416 in chickens), which is necessary for optimal activity. The negative regulation of c-Src involves intramolecular interactions between the C-terminal domain and SH2/SH3 domains. When Tyr530 in the human c-Src protein (Tyr527 in chicken) is phosphorylated it binds to its own SH2 domain and the protein assumes a closed, inactive conformation (Fig. 2). When this tyrosine is displaced from the SH2 domain and dephosphorylated the protein assumes an open configuration, exposing the kinase domain, SH1 [7]. This active configuration also allows association with other signaling molecules through the SH2 and SH3 domains [8]. The SH4 domain is required for myristylation of the protein, allowing membrane localization, which is considered essential for cellular transformation [9,10].

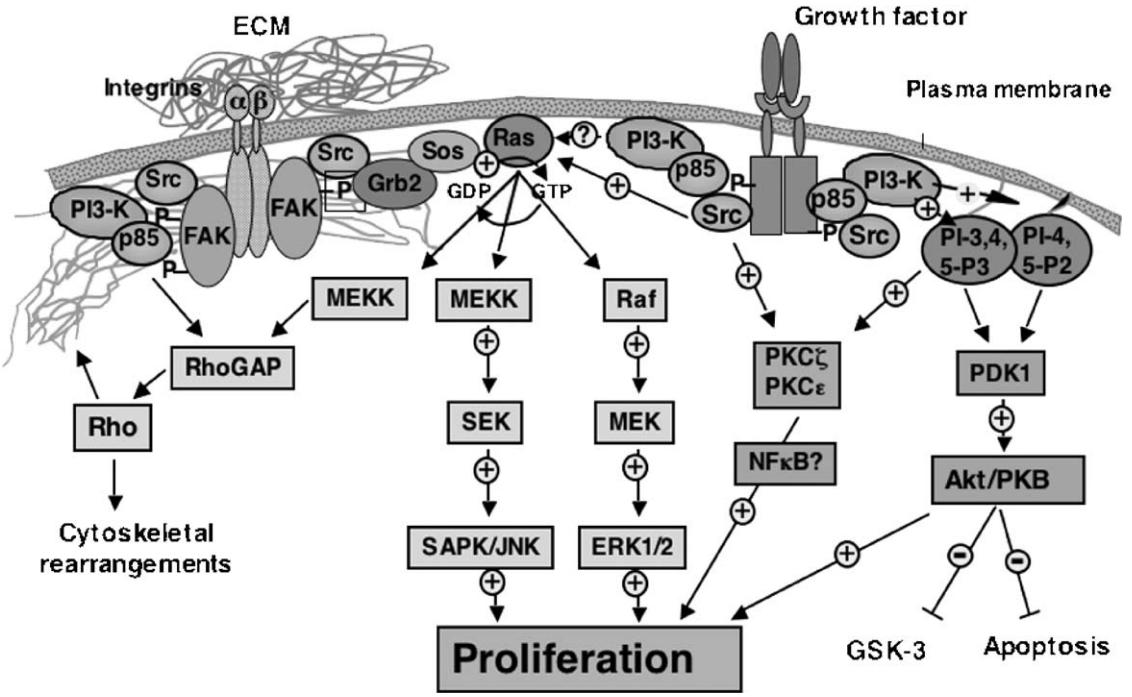
0959-4973

Src in cancer

Src family kinases, in general, and c-Src, specifically, act at points of integration, relaying signals from cell surface receptors to the nucleus. As such, c-Src mediates many different cell fate decisions. c-Src activation has been associated with proliferation, survival, differentiation and motility (Fig. 1) in both normal and transformed cells [6,11,12].

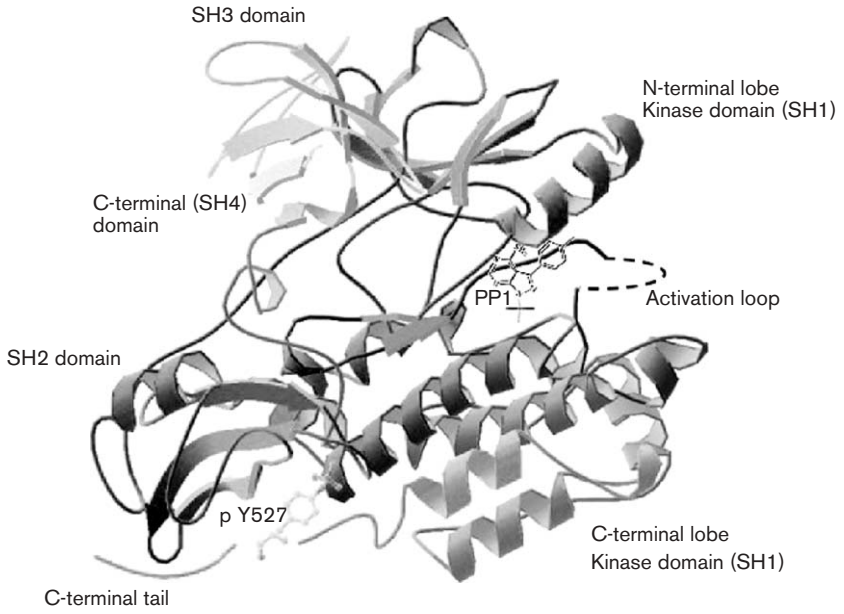
Activating c-Src mutations have been described in a small subset of colon [13] and endometrial [14] cancers; however, these seem to be relatively rare. Rather, a far more common observation is the overexpression and increased activity of c-Src. Increased c-Src expression and/or activity has been described for many different cancers, including colorectal [15,16], breast [17,18], hepatocellular [19], pancreatic [20], prostate [21,22], lung [23] and ovarian [24] carcinomas. Several possible explanations have been proposed for the increased activity of c-Src in human cancers. These include activation by receptor tyrosine kinases, including epidermal growth factor (EGF) receptor [25], platelet-derived growth factor (PDGF) receptor [26], ErbB2 [27], fibroblast growth factor (FGF) receptor [28], colony-stimulating factor-1 [29] and hepatocyte growth factor receptor [30]; insufficient activity of Csk or Csk homologous kinase (Chk), which phosphorylate c-Src at Tyr530, negatively regulating c-Src activity [31]; and increased phosphatase activity, such as protein tyrosine phosphatase (PTP)-α [32] and PTP-1B [33], leading to removal of the c-Src C-terminal negative regulating phosphate group.

Fig. 1



Selected signaling pathways involving Src tyrosine kinase.

Fig. 2



Ribbon structure of Tyr527 phosphorylated inactive Src tyrosine kinase [129] in complex with the Src selective inhibitor PP1.

Studies using v-Src or activated c-Src to model c-Src overexpression and activation have demonstrated that increased c-Src activity is associated with various oncogenic characteristics, including disruption of the cell cycle, protection from apoptotic stimuli, increased cellular motility, and invasive capacity and stimulation

of angiogenesis. Increased c-Src activity is associated with decreased expression of the cyclin-dependent kinase (CDK) inhibitor, p27 [34], and a concomitant increase in expression of cyclins A, D and E, and CDK2, as well as a hyperphosphorylation of the tumor suppressor retinoblastoma (pRb) [8]. The end result is rapid, unchecked progression through G_1 phase to the S phase of the cell cycle [35].

Epithelial cells transformed by v-*src* are protected from death due to detachment from the extracellular matrix (ECM) (or anoikis), through activation of the phosphotidylinositol-3-kinase/Akt pathway [36]. Increased c-Src activity also results in focal adhesion kinase (FAK) activation, inducing increased focal adhesion disassembly and disruption of the associated actin filaments, ultimately increasing cell motility [37] and protection from anoikis [38]. Additionally, c-Src activation induces phosphorylation of β_3 integrin and a decrease of $\alpha_v\beta_3$ adhesion to fibronectin [39] as well as p190 RhoGAP, p120 RasGAP and cortactin, all of which play roles to increase cellular motility [40]. c-Src activation affects cellular adhesion to the ECM by inducing phosphorylation of R-Ras, a member of the Ras GTPase superfamily. R-Ras and c-Src form a complex that suppresses integrin activity and reduces cell–matrix adhesion [41]. c-Src has been shown to suppress E-cadherin localization and function at adherens junctions [42], perhaps by phosphorylation and ubiquitylation of E-cadherin complexes [43] resulting in decreased homotypic adhesion and increased invasion [44]. Finally, there is evidence that c-Src may regulate matrix metalloproteinases (MMPs) and inhibitors of MMPs [45,46], increasing the degradation of the ECM, and further enhancing cell motility and invasive capacity.

In addition to cellular motility and invasion, c-Src also regulates molecules that are associated with angiogenesis, an important component of tumor growth. For example, c-Src has been shown to activate STAT3, which results in increased vascular endothelial growth factor (VEGF) expression [47,48]. It has also been demonstrated that c-Src is required for hypoxia-induced VEGF production [49]. Independent of angiogenesis, activation of STAT-3 by c-Src has other consequences important for cancer progression, including cell growth and survival, as well as tumor cell immune evasion [50].

Given that c-Src plays such a pivotal role in so many aspects of the oncogenic process, it may seem surprising that c-Src has not been seen as a primary chemotherapeutic target. The lack of enthusiasm may stem from the ubiquitous expression of c-Src leading to the supposition that c-Src inhibition would result in major side-effects. However, the relatively mild phenotype of *src* knockout mice argues against this [51]. In fact, the impaired bone resorption associated with c-Src inhibition may be beneficial [52,53]. The apparent dearth of documentation of activating mutations may have also led investigators to discount or underestimate the role of c-Src in tumor progression. The realization of significant c-Src activation in many types of cancers has prompted a resurgence in the interest of c-Src as a target [54,55]. A major challenge is the design of small molecules with suitable specificity and bioavailability.

Src inhibitors

Src inhibitors may be categorized into three major classes, including ATP-competitive inhibitors, SH2/SH3-blocking inhibitors and c-Src-destabilizing agents (Table 1).

ATP-competitive Src kinase inhibitors

ATP-competitive inhibitors bind to the ATP-binding pocket, thus blocking ATP binding and phosphotransferase activity. However, due to significant homology in the primary sequence and three-dimensional structure in the ATP-binding pockets of many different kinases [54,56], few ATP-competitive inhibitors have suitable specificity [57,58].

PP1 and PP2

4-Amino-5-(4-methylphenyl)-7-(*t*-butyl)pyrazolo[3,4-*d*]pyrimidine (PP1) and 4-amino-5-(4-chlorophenyl)-7-(*t*-butyl)pyrazolo[3,4-*d*]pyrimidine (PP2) were originally identified as selective, ATP-competitive inhibitors of Src family kinases [59]. The selectivity of PP1 and PP2 for Src family kinases over ZAP-70 and Jak2 has been reported [59], while some reports showed that PP1 also directly inhibited PDGF receptor tyrosine kinase [60] and Ret tyrosine kinase activity [61]. PP2 has been widely used to study the role of c-Src kinase in various

Table 1 Selected Src tyrosine kinase inhibitors

	Src kinase IC_{50} (nmol/l)	Inhibition of other kinases (nmol/l)
ATP-competitive Src kinase inhibitors		
PP1	170	Lck 5, Abl 250, Jak2 >1000
PP2		Lck 4, EGF receptor 480, Jak2 >50
SU6656	280	Lyn 130, Lck 6880, Abl1740, PDGF receptor >1000
CPG76030	47	Lyn 121, Abl 101, EGF receptor 160
PD173955	22	PDGF receptor 1660, FGF receptor 1250
AZM475271	10	Lck 30, Yes 80, KDR 700
SKI-606	1.2	Abl 1, ErbB2 2600
SH2/SH3 inhibitors		
AP22408	300	NR
UCS15A	3000 (bone resorption assay)	NR
Src-destabilizing agents		
geldanamycin	8 (bone resorption assay)	
herbimycin A	70 (bone resorption assay)	

cellular events. PP2 has been demonstrated to completely block tyrosine phosphorylation of Raf-1 [62], a direct substrate of c-Src kinase. It has also been reported that c-Src inhibition with PP2 prevented constitutive ERK activity and abolished Akt phosphorylation [63]. Another study demonstrated that treatment of ovarian cancer cells with PP2 resulted in loss of FAK, Akt and FOXO1 phosphorylation and decreased the amount of GTP-bound Ras [64]. In the same study, small interfering RNA (siRNA) knockdown of c-Src protein expression demonstrated that the effects of PP2 could largely be attributed to Src inhibition.

SU6656

2-Oxo-3-(4,5,6,7-tetrahydro-1*H*-indol-2-ylmethylene)-2,3-dihydro-1*H*-indole-5-sulfonic acid dimethylamide (SU6656) is a small-molecular inhibitor that displays selective activity against Src kinase family members and does not appear to have significant PDGF receptor inhibitory activity [65]. SU6656 acts as a competitive inhibitor with respect to ATP and inhibits tyrosine phosphorylation of the c-Src substrates Cbl and protein kinase C (PKC) δ.

CGP77675 and CGP76030

The CGP compounds are two substituted 5,7-diphenylpyrrolo[2,3-*d*]pyrimidines [66,67]. CGP77675 shows significant selectivity against c-Src activity in comparison to the Src family members Lck and Yes [66]. Most of the effects of CGP76030 have been attributed to inhibition of Src family members and not inhibition of the closely related Abl kinase [68].

PD173955

The pyrido[2,3-*d*]pyrimidine PD173955 acts as an ATP-competitive inhibitor. Selectivity for both c-Src and c-Yes inhibition has been reported with an IC_{50} of 22 nmol/l [69]. Inhibition of αl-FGF receptor and PDGF receptors was reported to be 1.6 μmol/l and no activity against the insulin receptor or PKC was observed.

AZM475271

AZM475271 is of the anilinoquinazoline class of compounds. AZM475271 has been reported to be selective for c-Src inhibition as compared to kinase insert domain receptor (KDR), which has a similar ATP-binding pocket structure [70]. AZM475271 dose-dependently inhibits c-Src tyrosine kinase activity in human pancreatic carcinoma cells [71]. A particular advantage of this compound is that it is orally deliverable.

SKI-606

4-Anilino-3-quinolinecarbonitrile (SKI-606) is another orally available compound with inhibitory activity against Src family kinases and Abl tyrosine kinase [72]. This dual inhibitory activity may be especially valuable for chronic myeloid leukemia (CML; see below).

SH2/SH3 inhibitors

The SH2 and SH3 are protein recognition domains. The SH2 domain recognizes specific sequences containing phosphotyrosine and the SH3 domain binds to specific proline-rich sequences [6]. These domains guide Src to its substrates; thus, drugs designed to sterically block SH2- or SH3-mediated interactions will inhibit specific subsets of Src protein–protein interactions.

The SH2 and SH3 inhibitors include both peptidomimetic and non-peptide inhibitors [73]. SH2 peptidomimetics contain a phosphotyrosine or a phosphotyrosine mimic. Unfortunately, phosphotyrosine-containing inhibitors demonstrate poor transport and uptake properties, and are susceptible to phosphatase activity [74,75]. Phosphotyrosine mimics, however, tend to have lower affinity and decreased specificity [76]. These limitations have led to the search for non-peptide SH2 and SH3 inhibitors. Shakespeare *et al.* have described a non-peptide inhibitor AP22408, which inhibited c-Src SH2 ligand binding with an IC_{50} of 0.30 μmol/l [77]. However, AP22408 was designed to have bone-targeting properties and thus may not be suitable for cancer chemotherapy.

A non-peptide small molecule that inhibits SH3-mediated interactions has also been described [78,79]. UCS15A has been reported to inhibit the Src specific tyrosine phosphorylation of numerous proteins in v-*src*-transformed cells without inhibiting Src tyrosine kinase activity [78]. It was subsequently found that UCS15A disrupted SH3-mediated protein–protein interactions, but not SH2-mediated interactions, probably by targeting the proline-rich sequences of the substrate proteins rather than the SH3 domain itself [79]. The inhibition of only a subset of c-Src protein–protein interactions may limit the oncological clinical efficacy of this class of inhibitor.

Src-destabilizing agents

Destabilizing inhibitors interfere with the association between c-Src and its associated molecular chaperone, heat shock protein (Hsp) 90. Geldanamycin and herbimycin A are benzenoid ansamycin antibiotics [80]. It has been reported that geldanamycin reverted the transformed morphology of v-*src*-transformed fibroblasts without inhibiting Src kinase activity [80,81]. The same study also found that both geldanamycin and herbimycin A could bind Hsp90 and inhibited the formation of Src–Hsp90 heteroprotein complexes, leading to the increased degradation of v-Src protein. This mechanism of action of the benzoquinone ansamycins is relatively non-specific, and results in the disruption of many HSP interactions and increased degradation of client proteins [82]. This feature may therefore result in significant side-effects [83].

Src inhibition: in-vitro effects

Many of the reported roles of c-Src tyrosine kinase in regulating cell growth, motility, invasive potential and sensitivity to stressors have been discerned from in-vitro pharmacologic Src inhibition using various different cancer cell lines.

Inhibiting cell growth

The effect of c-Src inhibition on cell growth has been demonstrated in many reports using various different inhibitors. Herbimycin A (50 ng/ml) decreased growth of human pancreatic carcinoma cells overexpressing c-Src, but had little effect on cells with low levels of c-Src expression [84]. Similarly, the ATP competitive c-Src inhibitor PD173955 has been reported to have significant antiproliferative activity due to an arrest of mitotic progression [85]. MDA-MB-468 breast cancer cells treated with PD173955 (5 μmol/l) resulted in a dramatic accumulation of cells in the G_2/M phase of the cell cycle [85]. Treatment of leukemic cell lines with SU6656 (2.5 μmol/l) resulted in rapid terminal differentiation and cessation of cell division [86]. SU6656 induced polyploidization, morphologic changes indicative of megakaryocytic maturation, and expression of the specific differentiation markers CD41 and CD61.

Apoptotic effect

On the flip side of mitosis is apoptosis (programmed cell death). For tumor regression to occur cells must die. In-vitro Src inhibition in various cancer cell lines has been shown to enhance apoptosis in various cancer cell lines under various conditions. In the Caki-2 renal carcinoma cell line the Src inhibitor PP1 induced greater cytotoxicity with connexin 32 (Cx32) expression than without Cx32. This was accompanied by a decrease in the anti-apoptotic Bcl-2 and Bcl-X_L molecules [87]. In the 70Z/3 murine B cell leukemia cell line c-Src inhibition with PP2 was associated with suppressed proliferation and increased apoptosis [88]. It has also been reported that PP2 reduced DNA synthesis, decreased Akt phosphorylation and increased apoptosis in medullary thyroid cancer cells [89]. In ovarian cancer cells, c-Src activation has been associated with Akt phosphorylation [90] – a pro-survival signal. c-Src inhibition, however, decreased Akt phosphorylation [64].

Impairing chemoresistance

Chemoresistance is a major clinical challenge. Interestingly, c-Src inhibition appears to be associated with resensitization of cancer cells to different classes of chemotherapeutics. Inhibition of c-Src with PP2 resensitized pancreatic adenocarcinoma cells with both inherent and acquired resistance to gemcitabine – a deoxycytidine analog [91]. The effects of PP2 are most likely the result of Src inhibition as expression of a Src dominant-negative and knockdown of Src expression by siRNA had a similar effect [91,92]. In paclitaxel-resistant ovarian cancer cells, c-Src inhibition with either PP2 or SU6656 has been reported to restore sensitivity to paclitaxel and cisplatin to which the cells were cross-resistant [64,93]. Expression of a Src dominant-negative also restored sensitivity, again suggesting the effects of the inhibitors can be attributed to c-Src or Src family member inhibition [93]. These studies suggest that Src family kinase inhibitors may be useful agents in the treatment of drug-resistant cancers.

Preclinical and clinical studies

CML

CML is manifested by the malignant expansion of bone marrow stem cells. Approximately 90% of CML patients carry a t(9;22)(q34;q11) reciprocal chromosomal translocation, producing a $9q^+$ and small $22q^-$, the so-called Philadelphia chromosome, Ph^+ [94]. The result of this translocation is a fusion gene encoding the Bcr–Abl protein, a misregulated tyrosine kinase that has been shown to be sufficient and necessary for the CML phenotype [95,96]. The Bcr–Abl small-molecule inhibitor, imatinib mesylate (Gleevec, STI-1571), has shown great clinical efficacy in chronic phase CML [97]. However, imatinib is relatively ineffective against B cell acute lymphoblastic leukemia (B-ALL) and the blast phase crisis of CML [98]. The lack of effect is due to acquired resistance to imatinib resulting from gene amplification, upregulation and overexpression of Bcr–Abl [99,100] or through the accumulation of mutations in the kinase domain [101–103].

Bcr–Abl activates several downstream kinases including Src family kinases [68,104]. This has prompted investigation into the use of dual Src–Abl inhibitors. SKI-606 has both c-Src and Abl inhibitory activity [72]. *In vitro* studies with various CML cell lines demonstrated that SKI-606 was an order of magnitude more potent than imatinib at inhibiting CML cell proliferation [72]. Furthermore, oral administration of the compound for 5 days led to complete regression of large K562 CML xenografts in nude mice [72]. SKI-606 has since been shown to have *in vitro* activity against CML Ph^+ imatinib-resistant cells from patients in blast crisis [105].

Another Src–Abl dual inhibitor, BMS-354825, has entered phase I trials [106,107]. BMS-354825 is orally available and was well tolerated at doses up to 180 mg/day for 5–7 days/week for up to 9 months. Preliminary assessment of clinical activity of 26 Ph^+ CML patients (22 with imatinib resistance, four with intolerance and average CML duration of 6.1 years) followed for greater than 4 weeks has been reported [106]. Bcr–Abl kinase domain mutations were detected in 22 patients prior to start of treatment. All 26 patients have had clinical benefit, including 19 (73%) with complete hematologic responses. Of the seven partial responders, two have had disease

progression, one of whom had expansion of a CML subclone harboring a T315I mutation in Bcr–Abl, a mutation that confers resistance to imatinib as well as multiple Src–Abl inhibitors [108,109]. A second phase I trial with BMS-354825 has been reported in accelerated phase and blast phase CML patients [107]. Of the 11 blast phase patients, seven have had hematologic response, including three with complete response, two 'no evidence of leukemia' and two 'return to chronic phase'. In the accelerated phase cohort, three of six patients showed hematologic response including two complete hematologic responses and two 'no evidence of leukemia'. Thus Src–Abl dual inhibitors appear to have a good safety profile and early clinical evaluation demonstrate efficacy for imatinib-resistant, blast phase CML.

A mouse model of Ph^+ B-ALL has provided preclinical evidence that the Src family inhibitor CGP76030 prolonged survival of mice with B-ALL, but not CML. Combination treatment with CGP76030 and imatinib showed even greater prolongation of survival than either agent alone. Interestingly, the study did not examine combination therapy in the mouse model of CML [68].

Overall, these studies suggest that dual inhibition of Src family kinases and Bcr–Abl may be a useful strategy for treatment of Ph^+ acute leukemia.

Colon cancer

Increased c-Src activity in colon cancer has been associated with disease progression and poor patient survival [110,111], thus raising the possibility that c-Src may be a potential therapeutic target.

In a preclinical evaluation, the orally available Src–Abl inhibitor SKI-606 was shown to decrease c-Src autophosphorylation, an indicator of c-Src activation, in human HT29 and Colo205 tumor xenografts. Once daily oral administration of SKI-606 decreased HT29 tumor growth. Twice daily administration was required to produce a similar decrease of Colo205, HCT116 and DLD1 tumor growth [112]. Additionally, the Src inhibitor PP2 has been demonstrated to decrease HT29 tumor growth and liver metastasis in a SCID mouse model [113]. There appear to be no published reports of clinical trials in colon cancer patients.

Pancreatic cancer

Pancreatic cancer presents a particularly difficult clinical challenge with 5-year survival rates at roughly 4% [114]. Though few studies have been carried out, one published report demonstrated c-Src overexpression in 13 of 13 pancreatic carcinoma tissues and 14 of 17 cell lines [84]. Two studies report that c-Src inhibition enhances gemcitabine chemosensitivity in mouse xenograft models of human pancreatic cancer [91,115]. In an orthotopic xenograft model with Panc1 gemcitabine-resistant pancreatic carcinoma cells, i.p. injection of the Src inhibitor PP2 (2 mg/kg, 3 times per week) in combination with gemcitabine (100 mg/kg, 3 times per week) produced a tumor growth inhibition of 98% compared with 25% for PP2 alone and 5% for gemcitabine alone. Furthermore, hepatic metastasis was completely blocked in the combination treated mice [91]. The orally available Src inhibitor, AZM475271, when administered daily at 50 mg/kg in combination with twice weekly administration of gemcitabine (100 mg/kg) reduced tumor growth by 91%. As with PP2, hepatic metastasis was completely inhibited [115]. These results provide compelling evidence that c-Src may be a viable target in pancreatic cancer, especially when used in combination with current chemotherapeutics.

Prostate cancer

There are no reported trials with Src inhibitors for prostate cancer. In-vitro studies have demonstrated that c-Src inhibition with either CGP77675 or CPG76030 decreased proliferation and invasive capacity of PC-3 cells [116]. PP2 has been demonstrated to decrease migration is PC-3 and DU145 prostate cancer cell lines [22]. Given the propensity of prostate cancers to metastasize to bone, it has been proposed that the anti-osteoclastic activity of Src inhibitors may decrease bone metastases in a clinical setting [116].

Future directions

While newer generation small-molecule inhibitors show great promise with better specificity and potency, the experience with Imatinib/Gleevec/STI-571 demonstrates that cancers can develop multiple mechanisms of resistance to even the most effective small-molecule inhibitors [117–120]. Furthermore, overexpressed kinases such as c-Src may have oncogenic activities that are independent of kinase activity [121]. Thus, new methods to interfere with c-Src or other oncogenic kinases need to be explored.

Antisense phosphorodiamidate morpholino oligomers (AS-PMO) are a newer generation RNA antisense technology that can be designed and relatively easily synthesized for virtually any message [122]. AS-PMOs have been through phase I clinical trials and they do not appear to cause any untoward toxicity. In addition, they do not produce the hematological side-effects seen with the phosphorothiote-based antisense agents [123]. Stability and good bioavailability by multiple routes of administration, including oral [124] and i.p. [125,126], have been reported. Finally, there do not appear to be any reports that AS-PMOs are substrates for MDR proteins, thus AS-PMOs may provide a new means of silencing individual proteins with great specificity, few side effects and in a drug resistant context.

siRNA technology, much like AS-PMO, provides a means to target specific mRNA for silencing. A very recent publication reported the use of cholesterol-conjugated siRNAs to knockdown apolipoprotein B by systemic i.v. injection in mice [127]. The modified siRNAs showed significant in-vitro cellular uptake in the absence of transfection reagent and wide bioavailability *in vivo*. This report provides compelling evidence that siRNA may be a valuable tool for gene knockdown in a therapeutic setting.

Conclusion

As c-Src overexpression itself is not transforming and does not appear to be a primary lesion in any particular tumor type, it is unlikely that c-Src inhibition on its own will be an effective therapeutic option. Rather, it is more likely that c-Src inhibition will be used in conjunction with standard chemotherapy or as part of a small-molecule cocktail targeting multiple oncogenic kinases. With the recent observation that non-small cell lung cancers carrying EGF receptor-activating mutations are more sensitive to the EGF receptor inhibitor Iressa than cancers without the mutations [128], it is likely that therapy in the future will be much more individualized, based upon the tumor profile. One of the many bullets in the anti-tumor arsenal should be aimed at Src tyrosine kinase.

References

1 Martin GS. The Road to Src. *Oncogene* 2004; **23**:7910–7917.
2 Eckhart W, Hutchinson MA, Hunter T. An activity phosphorylating tyrosine in polyoma T antigen immunoprecipitates. *Cell* 1979; **18**:925–933.
3 Oppermann H, Levinson AD, Varmus HE, Levintow L, Bishop JM. Uninfected vertebrate cells contain a protein that is closely related to the product of the avian sarcoma virus transforming gene (*src*). *Proc Natl Acad Sci USA* 1979; **76**:1804–1808.
4 Bjorge JD, Jakymiw A, Fujita DJ. Selected glimpses into the activation and function of Src kinase. *Oncogene* 2000; **19**:5620–5635.
5 Bjelfman C, Meyerson G, Cartwright CA, Mellstrom K, Hammerling U, Phlman S. Early activation of endogenous pp60^{src} kinase activity during neuronal differentiation of cultured human neuroblastoma cells. *Mol Cell Biol* 1990; **10**:361–370.
6 Brown M, Cooper J. Regulation, substrates and functions of *src*. *Biochem Biophys Acta* 1996; **1287**:121–149.
7 Yeatman TJ. A Renaissance for Src. *Nat Rev Cancer* 2004; **4**:470–480.
8 Frame MC. Src in cancer: deregulation and consequences for cell behaviour. *Biochim Biophys Acta* 2002; **1602**:114–130.
9 Buss JE, Sefton BM. Myristic acid, a rare fatty acid, is the lipid attached to the transforming protein of Rous sarcoma virus and its cellular homolog. *J Virol* 1985; **53**:7–12.
10 Kaplan JM, Marden G, Bishop JM, Varmus HE. The first seven amino acids encoded by the v-*src* oncogene act as a myristylation signal: lysine 7 is a critical determinant. *Mol Cell Biol* 1988; **8**:2435–2441.
11 Courtneidge SA. Isolation of novel Src substrates. *Biochem Soc Trans* 2003; **31**:25–28.
12 Thomas SM, Brugge JS. Cellular functions regulated by Src family kinases. *Annu Rev Cell Dev Biol* 1997; **13**:513–609.
13 Irby RB, Mao W, Coppola D, Kang J, Loubeau JM, Trudeau W, *et al.* Activating SRC mutation in a subset of advanced human colon cancers. *Nat Genet* 1999; **21**:187–190.
14 Sugimura M, Kobayashi K, Sagae S, Nishioka Y, Ishioka S, Terasawa K, *et al.* Mutation of the SRC gene in endometrial carcinoma. *Jpn J Cancer Res* 2000; **91**:395–398.
15 Cartwright CA, Kamps MP, Meisler AI, Pipas J, Eckhart W. pp60$^{c\text{-}src}$ activation in human colon carcinoma. *J Clin Invest* 1989; **3**:2025–2033.
16 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.
17 Jacobs C, Rubsamen H. Expression of pp60$^{c\text{-}src}$ protein kinase in adult and fetal human tissue: High activities in some sarcomas and mammary carcinomas. *Cancer Res* 1983; **43**:1696–1702.
18 Ottenhalff-Kalff AE, Rijksen G, van Beurden EACM, Hennipman A, Michels AA, Staal GEJ. Characterization of protein tyrosine kinases from human breast cancer: Involvement of the c-*src* oncogene product. *Cancer Res* 1992; **52**:4773–4778.
19 Masaki T, Masaki T, Okada M, Shiratori Y, Rengifo W, Matsumoto K, *et al.* pp60$^{c\text{-}src}$ activation in hepatocellular carcinoma of humans and LEC rats. *Hepatology* 1998; **27**:1257–1264.
20 Lutz MP, Esser IB, Flossmann-Kast BB, Vogelmann R, Luhrs H, Friess H, *et al.* Overexpression and activation of the tyrosine kinase Src in human pancreatic carcinoma. *Biochem Biophys Res Commun* 1998; **243**:503–508.
21 Lee L-F, Guan Y, Qiu Y, Kung H-J. Neuropeptide-induced androgen independence in prostate cancer cells: roles of nonreceptor tyrosine kinase Etk/Bmx, Src, and focal adhesion kinase. *Mol Cell Biol* 2001; **21**:8385–8397.
22 Slack JK, Adams RB, Rovin JD, Bissonette EA, Stoker CE, Parsons JT. Alterations in the focal adhesion kinase/Src signal transduction pathway correlate with increased migratory capacity of prostate carcinoma cells. *Oncogene* 2001; **20**:1152–1163.
23 Mazurenko NN, Kogan EA, Zborovskaya IB, Kisseljov FL. Expression of pp60$^{c\text{-}src}$ in human small cell and non-small cell lung carcinomas. *Eur J Cancer* 1992; **28**:372–377.
24 Wiener JR, Windham TC, Estrella VC, Parikh NU, Thall PF, Deavers MT, *et al.* Activated SRC protein tyrosine kinase is overexpressed in late-stage human ovarian cancers. *Gynecol Oncol* 2003; **88**:73–79.
25 Tice DA, Biscardi JS, Nickles AL, Parsons SJ. Mechanism of biological synergy between cellular Src and epidermal growth factor receptor. *Proc Natl Acad Sci USA* 1999; **96**:1415–1420.
26 DeMali KA, Godwin SL, Soltoff SP, Kazlauskas A. Multiple roles for Src in a PDGF-stimulated cell. *Exp Cell Res* 1999; **253**:271–279.
27 Muthuswamy SK, Siegel PM, Dankort DL, Webster MA, Muller WJ. Mammary tumors expressing the *neu* protooncogene possess elevated c-Src tyrosine kinase activity. *Mol Cell Biol* 1994; **14**:735–743.
28 Landgren E, Blume-Jensen P, Courtneidge SA, Claesson-Welsh L. Fibroblast growth factor receptor-1 regulation of Src family kinases. *Oncogene* 1995; **10**:2027–2035.
29 Courtneidge SA, Dhand R, Pilat D, Twamley GM, Waterfield MD, Roussel MF. Activation of Src family kinases by colony stimulating factor-1, and their association with its receptor. *EMBO J* 1993; **12**:943–950.
30 Mao W, Irby R, Coppola D, Fu L, Wloch M, Turner J, *et al.* Activation of c-Src by receptor tyrosine kinases in human colon cancer cells with high metastatic potential. *Oncogene* 1997; **15**:3083–3090.
31 Masaki T, Okada M, Tokuda M, Shiratori Y, Hatase O, Shirai M, *et al.* Reduced C-terminal Src kinase (Csk) activities in hepatocellular carcinoma. *Hepatology* 1999; **29**:379–384.
32 Tabiti K, Smith DR, Goh HS, Pallen CJ. Increased mRNA expression of the receptor-like protein tyrosine phosphatase alpha in late stage colon carcinomas. *Cancer Lett* 1995; **93**:239–248.
33 Bjorge JD, Pang A, Fujita DJ. Identification of protein tyrosine phosphatase 1B as the major tyrosine phosphatase activity capable of dephosphorylating and activating c-Src in several human breast cancer cell lines. *J Biol Chem* 2000; **275**:41439–41446.
34 Johnson D, Frame MC, Wyke JA. Expression of the v-Src oncoprotein in fibroblasts disrupts normal regulation of the CDK inhibitor p27 and inhibits quiescence. *Oncogene* 1998; **16**:2017–2028.
35 Riley D, Carragher NO, Frame MC, Wyke JA. The mechanism of cell cycle regulation by v-Src. *Oncogene* 2001; **20**:5941–5950.
36 Khwaja A, Rodriguez-Viciana P, Wennstrom SPH, Warne PH, Downward J. Matrix adhesion and Ras transformation both activate a phosphoinositide 3-OH kinase and protein kinase B/Akt cellular survival pathway. *EMBO J* 1997; **16**:2783–2793.
37 Fincham VJ, Frame MC. The catalytic activity of Src is dispensable for translocation to focal adhesions but controls the turnover of these structures during cell motility. *EMBO J* 1998; **17**:81–92.
38 Frisch SM, Vuori K, Ruoslahti E, Chan-Hui PY. Control of adhesion-dependent cell survival by focal adhesion kinase. *J Cell Biol* 1996; **134**:793–799.
39 Datta A, Huber F, Boettiger D. Phosphorylation of beta3 integrin controls ligand binding strength. *J Biol Chem* 2002; **277**:3943–3949.

40 Chang JH, Gill S, Settleman J, Parsons SJ. c-Src regulates the simultaneous rearrangement of actin cytoskeleton, p190RhoGAP, and p120RasGAP following epidermal growth factor stimulation. *J Cell Biol* 1995; **130**:355–368.

41 Zou JX, Liu Y, Pasquale EB, Ruoslahti E. Activated *SRC* oncogene phosphorylates R-ras and suppresses integrin activity. *J Biol Chem* 2002; **277**:1824–1827.

42 Owens DW, McLean GW, Wyke AW, Paraskeva C, Parkinson EK, Frame MC, *et al.* The catalytic activity of the Src family kinases is required to disrupt cadherin-dependent cell-cell contacts. *Mol Biol Cell* 2000; **11**:51–64.

43 Fujita Y, Krause G, Scheffner M, Zechner D, Leddy HE, Behrens J, *et al.* Hakai, a c-Cbl-like protein, ubiquitinates and induces endocytosis of the E-cadherin complex. *Nature Cell Biol* 2002; **4**:222–231.

44 Frixen UH, Behrens J, Sachs M, Eberle G, Voss B, Warda A, *et al.* E-cadherin-mediated cell–cell adhesion prevents invasiveness of human carcinoma cells. *J Cell Biol* 1991; **113**:173–185.

45 Hauck CR, Hsia DA, Schlaepfer DD. The focal adhesion kinase: a regulator of cell migration and invasion. *IUBMB Life* 2002; **53**:115–119.

46 Hsia DA, Mitra SK, Hauck CR, Streblow DN, Nelson JA, Ilic D, *et al.* Differential regulation of cell motility and invasion by FAK. *J Cell Biol* 2003; **160**:753–767.

47 Yu CL, Meyer DJ, Campbell GS, Larner AC, Carter-Su C, Schwartz J, *et al.* Enhanced DNA-binding activity of a Stat3-related protein in cells transformed by the Src oncoprotein. *Science* 1995; **269**:81–83.

48 Niu G, Niu G, Wright KL, Huang M, Song L, Haura E, *et al.* Constitutive Stat3 activity up-regulates VEGF expression and tumor angiogenesis. *Oncogene* 2002; **21**:2000–2008.

49 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**:577–581.

50 Yu H, Jove R. The STATs of cancer – new molecular targets come of age. *Nat Rev Cancer* 2004; **4**:97–105.

51 Lowe C, Yoneda T, Boyce BF, Chen H, Mundy GR, Soriano P. Osteopetrosis in Src-deficient mice is due to an autonomous defect of osteoclasts. *Proc Natl Acad Sci USA.* 1993; **90**:4485–4489.

52 Violette SM, Guan W, Bartlett C, Smith JA, Bardelay C, Antoine E, *et al.* Bone-targeted Src SH2 inhibitors block Src cellular activity and osteoclast-mediated resorption. *Bone* 2001; **28**:54–64.

53 Missbach M, Jeschke M, Feyen J, Muller K, Glatt M, Green J, *et al.* A novel inhibitor of the tyrosine kinase Src suppresses phosphorylation of its major cellular substrates and reduces bone resorption *in vitro* and in rodent models *in vivo*. *Bone* 1999; **24**:437–449.

54 Warmuth M, Damoiseaux R, Liu Y, Fabbro D, Gray N. SRC family kinases: potential targets for the treatment of human cancer and leukemia. *Curr Pharm Des* 2003; **9**:2043–2059. Review.

55 Ishizawar R, Parsons SJ. c-Src and cooperating partners in human cancer. *Cancer Cell* 2004; **6**:209–214.

56 Hubbard SR, Till JH. Protein tyrosine kinase structure and function. *Annu Rev Biochem* 2000; **69**:373–398.

57 Davies SP, Reddy H, Caivano M, Cohen P. Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem J* 2000; **351**:95–105.

58 Bain J, McLauchlan H, Elliot M, Cohen P. The specificities of protein kinase inhibitors, an update. *Biochem J* 2003; **371**:199–204.

59 Hanke JH, Gardner JP, Dow RL, Changelian PS, Brissette WH, Weringer EJ, *et al.* Discovery of a novel, potent, and Src family selective tyrosine kinase inhibitor. *J Biol Chem* 1996; **271**:695–701.

60 Waltenberger J, Uecker A, Kroll J, Frank H, Mayr U, Bjorge JD, *et al.* A dual inhibitor of platelet-derived growth factor beta-receptor and Src kinase activity potently interferes with motogenic and mitogenic responses to PDGF in vascular smooth muscle cells. A novel candidate for prevention of vascular remodeling. *Circ Res* 1999; **85**:12–22.

61 Carlomagno F, Vitagliano D, Guida T, Napolitano M, Vecchio G, Fusco A, *et al.* The kinase inhibitor PP1 blocks tumorigenesis induced by RET oncogenes. *Cancer Res* 2002; **62**:1077–1082.

62 Lee M, Kim JY, Koh WS. Apoptotic effect of PP2 a Src tyrosine kinase inhibitor, in murine B cell leukemia. *J Cell Biochem* 2004; **93**:629–638.

63 Liu Z, Falola J, Zhu X, Gu Y, Kim LT, Sarosi GA, *et al.* Antiproliferative effects of Src inhibition on medullary thyroid cancer. *J Clin Endocrinol Metab* 2004; **89**:3503–3509.

64 Pengetnze Y, Steed M, Rody KF, Terranova PF, Taylor CC. Src tyrosine kinase promotes survival and resistance to chemotherapeutics in a mouse ovarian cancer cell line. *Biochem Biophys Res Commun* 2003; **309**:377–383.

65 Blake RA, Broome MA, Liu X, Wu J, Gishizky M, Sun L, *et al.* SU6656, a selective src family kinase inhibitor, used to probe growth factor signaling. *Mol Cell Biol* 2000; **20**:9018–9027.

66 Missbach M, Jeschke M, Feyen J, Muller K, Glatt M, Green J, *et al.* A novel inhibitor of the tyrosine kinase Src suppresses phosphorylation of its major cellular substrates and reduces bone resorption *in vitro* and in rodent models *in vivo*. *Bone* 1999; **24**:437–449.

67 Recchia I, Rucci N, Festuccia C, Bologna M, Mackay AR, Migliaccio S, *et al.* Pyrrolopyrimidine c-Src inhibitors reduce growth, adhesion, motility and invasion of prostate cancer cells *in vitro*. *Eur J Cancer* 2003; **39**:1927–1935.

68 Hu Y, Liu Y, Pelletier S, Buchdunger E, Warmuth M, Fabbro D, *et al.* Requirement of Src kinases Lyn, Hck and Fgr for BCR–ABL1-induced B-lymphoblastic leukemia but not chronic myeloid leukemia. *Nat Genet* 2004; **36**:453–461.

69 Moasser MM, Srethapakdi M, Sachar KS, Kraker AJ, Rosen N. Inhibition of Src kinases by a selective tyrosine kinase inhibitor causes mitotic arrest. *Cancer Res* 1999; **59**:6145–6152.

70 Ple P, Green TP, Hennequin LF, Curwen J, Fennell M, Allen J, *et al.* Discovery of a new class of anilinoquinazoline inhibitors with high affinity and specificity for the tyrosine kinase domain of c-Src. *J Med Chem* 2004; **47**:871–887.

71 Yezhelyev MV, Koehl G, Guba M, Brabletz T, Jauch KW, Ryan A, *et al.* Inhibition of SRC tyrosine kinase as treatment for human pancreatic cancer growing orthotopically in nude mice. *Clin Cancer Res* 2004; **10**:8028–8036.

72 Golas JM, Arndt K, Etienne C, Lucas J, Nardin D, Gibbons J, *et al.* SKI-606, a 4-anilino-3-quinolinecarbonitrile dual inhibitor of Src and Abl kinases, is a potent antiproliferative agent against chronic myelogenous leukemia cells in culture and causes regression of K562 xenografts in nude mice. *Cancer Res* 2003; **63**:375–381.

73 Sawyer TK, Bohacek RS, Dalgarno DC, Eyermann CJ, Kawahata N, Metcalf CA 3rd, *et al.* SRC homology-2 inhibitors: peptidomimetic and nonpeptide. *Mini Rev Med Chem* 2002; **2**:475–488.

74 Bradshaw JM, Waksman G. Calorimetric investigation of proton linkage by monitoring both the enthalpy and association constant of binding: application to the interaction of the Src SH2 domain with a high-affinity tyrosyl phosphopeptide. *Biochemistry* 1998; **37**:15400–15407.

75 Gilmer T, Rodriguez M, Jordan S, Crosby R, Alligood K, Green M, *et al.* Peptide inhibitors of src SH3–SH2–phosphoprotein interactions. *J Biol Chem* 1994; **269**:31711–31719.

76 Machida K, Mayer BJ. The SH2 domain: versatile signaling module and pharmaceutical target. *Biochim Biophys Acta* 2005; **1747**:1–25.

77 Shakespeare W, Yang M, Bohacek R, Cerasoli F, Stebbins K, Sundaramoorthi R, *et al.* Structure-based design of an osteoclast-selective, nonpeptide src homology 2 inhibitor with *in vivo* antiresorptive activity. *Proc Natl Acad Sci USA* 2000; **97**:9373–9378.

78 Sharma SV, Oneyama C, Yamashita Y, Nakano H, Sugawara K, Hamada M, *et al.* UCS15A, a non-kinase inhibitor of Src signal transduction. *Oncogene* 2001; **20**:2068–2079.

79 Oneyama C, Nakano H, Sharma SV. UCS15A, a novel small molecule, SH3 domain-mediated protein–protein interaction blocking drug. *Oncogene* 2002; **21**:2037–2050.

80 Uehara Y, Hori M, Takeuchi T, Umezawa H. Phenotypic change from transformed to normal induced by benzoquinonoid ansamycins accompanies inactivation of $p60^{src}$ in rat kidney cells infected with Rous sarcoma virus. *Mol Cell Biol* 1986; **6**:2198–2206.

81 Whitesell L, Mimnaugh EG, Costa BE, Myers CE, Neckers LM. Inhibition of heat shock protein HSP90–pp60v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. *Proc Natl Acad Sci USA* 1994; **91**:8324–8328.

82 Uehara Y. Natural product origins of Hsp90 inhibitors. *Curr Cancer Drug Targets* 2003; **3**:325–330.

83 Banerji U, Judson I, Workman P. The clinical applications of heat shock protein inhibitors in cancer – present and future. *Curr Cancer Drug Targets* 2003; **3**:385–390.

84 Lutz MP, Eber IBS, Flossmann-Kast BBM, Vogelmann R, Luhrs H, Friess H, *et al.* Overexpression and activation of the tyrosine kinase Src in human pancreatic carcinoma. *Biochem Biophys Res Commun* 1998; **243**:503–508.

85 Moasser MM, Srethapakdi M, Sachar KS, Kraker AJ, Rosen N. Inhibition of Src kinases by a selective tyrosine kinase inhibitor causes mitotic arrest. *Cancer Res* 1999; **59**:6145–6152.

86 Lannutti BJ, Blake N, Gandhi MJ, Reems JA, Drachman JG. Induction of polyploidization in leukemic cell lines and primary bone marrow by Src kinase inhibitor SU6656. *Blood* 2005; **105**:3875–3878.

87 Fujimoto E, Sato H, Nagashima Y, Negishi E, Shirai S, Fukumoto K, *et al.* A Src family inhibitor (PP1) potentiates tumor-suppressive effect of connexin 32 gene in renal cancer cells. *Life Sci* 2005; **76**:2711–2720.

88 Lee M, Kim JY, Koh WS. Apoptotic effect of PP2 a Src tyrosine kinase inhibitor, in murine B cell leukemia. *J Cell Biochem* 2004; **93**:629–638.

89 Liu Z, Falola J, Zhu X, Gu Y, Kim LT, Sarosi GA, *et al.* Antiproliferative effects of Src inhibition on medullary thyroid cancer. *J Clin Endocrinol Metab* 2004; **89**:3503–3509.

90 Liu AX, Testa JR, Hamilton TC, Jove R, Nicosia SV, Cheng JQ. AKT2, a member of the protein kinase B family, is activated by growth factors, v-Ha-ras, and v-src through phosphatidylinositol 3-kinase in human ovarian epithelial cancer cells. *Cancer Res* 1998; **58**:2973–2977.

91 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**:2307–2318.

92 Duxbury MS, Ito H, Zinner MJ, Ashley SW, Whang EE. siRNA directed against c-Src enhances pancreatic adenocarcinoma cell gemcitabine chemosensitivity. *J Am Coll Surg* 2004; **198**:953–959.

93 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**:217–224.

94 Bartram CR, de Klein A, Hagemeijer A, van Agthoven T, Geurts van Kessel A, Bootsma D, *et al.* Translocation of c-*abl* oncogene correlates with the presence of a Philadelphia chromosome in chronic myelocytic leukaemia. *Nature* 1983; **306**:277–280.

95 Lugo TG, Pendergast AM, Muller AJ, Witte ON. Tyrosine kinase activity and transformation potency of bcr–abl oncogene products. *Science* 1990; **247**:1079–1082.

96 Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the $P210^{bcr/abl}$ gene of the Philadelphia chromosome. *Science* 1990; **247**:824–830.

97 Kantarjian H, Sawyers C, Hochhaus A, Guilhot F, Schiffer C, Gambacorti-Passerini C, *et al.* Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N Engl J Med* 2002; **346**: 645–652.

98 Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, *et al.* Activity of a specific inhibitor of the BCR–ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N Engl J Med* 2001; **344**:1038–1042.

99 Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, *et al.* Clinical resistance to STI-571 cancer therapy caused by BCR–ABL gene mutation or amplification. *Science* 2001; **293**:876–880.

100 Hochhaus A, Kreil S, Corbin AS, La Rosee P, Muller MC, Lahaye T, *et al.* Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. *Leukemia* 2002; **16**:2190–2196.

101 Branford S, Rudzki Z, Walsh S, Grigg A, Arthur C, Taylor K, *et al.* High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. *Blood* 2002; **99**:3472–3475.

102 Roumiantsev S, Shah NP, Gorre ME, Nicoll J, Brasher BB, Sawyers CL, *et al.* Clinical resistance to the kinase inhibitor STI-571 in chronic myeloid leukemia by mutation of Tyr-253 in the Abl kinase domain P-loop. *Proc Natl Acad Sci USA* 2002; **99**:10700–10705.

103 Shah NP, Nicoll JM, Nagar B, Gorre ME, Paquette RL, Kuriyan J, *et al.* Multiple BCR–ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell* 2002; **2**:117–125.

104 Danhauser-Riedl S, Warmuth M, Druker BJ, Emmerich B, Hallek M. Activation of Src kinases $p53/56^{lyn}$ and $p59^{hck}$ by $p210^{bcr/abl}$ in myeloid cells. *Cancer Res* 1996; **56**:3589–3596.

105 Grafone T, Mancini M, Ottaviani E, Renzulli M, Boschelli F, Ferraretti L, *et al.* A novel 4-anilo-3-quinolinecarbonitrile dual Src and Abl kinase inhibitor (SKI-606) has *in vitro* activity on CML Ph^+ blast cells resistant to imatinib. *Blood* 2004; **104**(**10A**): Abstract 1991.

106 Sawyers CL, Shah NP, Hagop M, Kantarjian HM, Donato N, Nicoll J, *et al.* Hematologic and cytogenetic responses in imatinib-resistant chronic phase chronic myeloid leukemia patients treated with the dual SRC/ABL kinase inhibitor BMS-354825: results from a phase I dose escalation study. *Blood* 2004; **104**(**10A**):abstr 1.

107 Talpaz M, Kantarjian HM, Shah NP, Donato N, Nicoll J, Bai SA, *et al.* Hematologic and cytogenetic responses in imatinib-resistant accelerated and blast phase chronic myeloid leukemia (CML) patients treated with the dual SRC/ABL kinase inhibitor BMS-354825: results from a phase I dose escalation study. *Blood* 2004; **104**(**10A**):abstr 20.

108 Tipping AJ, Baluch S, Barnes DJ, Veach DR, Clarkson BM, Bornmann WG, *et al.* Efficacy of dual-specific Bcr–Abl and Src-family kinase inhibitors in cells sensitive and resistant to imatinib mesylate. *Leukemia* 2004; **18**:1352–1356.

109 Martinelli G, Soverini S, Rosti G, Cilloni D, Baccarani M. New tyrosine kinase inhibitors in chronic myeloid leukemia. *Haematologica* 2005; **90**:534–541.

110 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.

111 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**:344–351.

112 Golas JM, Lucas J, Etienne C, Golas J, Discafani C, Sridharan L, *et al.* SKI-606, a Src/Abl inhibitor with *in vivo* activity in colon tumor xenograft models. *Cancer Res* 2005; **65**:5358–5364.

113 Nam JS, Ino Y, Sakamoto M, Hirohashi S. Src family kinase inhibitor PP2 restores the E-cadherin/catenin cell adhesion system in human cancer cells and reduces cancer metastasis. *Clin Cancer Res* 2002; **8**:2430–2436.

114 American Cancer Society. *Cancer facts and figures 2005*. Washington, DC: American Cancer Society; 2005. http://www.cancer.org/docroot/STT/stt_0.asp

115 Yezhelyev MV, Koehl G, Guba M, Brabletz T, Jauch KW, Ryan A, *et al.* Inhibition of SRC tyrosine kinase as treatment for human pancreatic cancer growing orthotopically in nude mice. *Clin Cancer Res* 2004; **10**:8028–8036.

116 Recchia I, Rucci N, Festuccia C, Bologna M, Mackay AR, Migliaccio S, *et al.* Pyrrolopyrimidine c-Src inhibitors reduce growth, adhesion, motility and invasion of prostate cancer cells *in vitro*. *Eur J Cancer* 2003; **39**:1927–1935.

117 Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, *et al.* Clinical resistance to STI-571 cancer therapy caused by BCR–ABL gene mutation or amplification. *Science* 2001; **293**:876–880.

118 Shah NP, Nicoll JM, Nagar B, Gorre ME, Paquette RL, Kuriyan J, *et al.* Multiple BCR–ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell* 2002; **2**:117–1125.

119 Weisberg E, Griffin JD. Resistance to imatinib (Glivec): update on clinical mechanisms. *Drug Resist Update* 2003; **6**:231–238.

120 Mahon FX, Belloc F, Lagarde V, Chollet C, Moreau-Gaudry F, Reiffers J, *et al.* MDR1 gene overexpression confers resistance to imatinib mesylate in leukemia cell line models. *Blood* 2003; **101**:2368–2373.

121 Brunton VG, Avizienyte E, Fincham VJ, Serrels B, Metcalf 3rd CA, Sawyer TK, *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**:1335–1342.

122 Summerton J. Morpholino antisense oligomers: the case for an RNase H-independent structural type. *Biochim Biophys Acta* 1999; **1489**:141–158.

123 Iversen PL, Arora V, Acker AJ, Mason DH, Devi GR. Efficacy of antisense morpholino oligomer targeted to c-*myc* in prostate cancer xenograft murine model and a Phase I safety study in humans. *Clin Cancer Res* 2003; **9**:2510–2519.

124 Arora V, Knapp DC, Reddy MT, Weller DD, Iversen PL. Bioavailability and efficacy of antisense morpholino oligomers targeted to c-*myc* and cytochrome P-450 3A2 following oral administration in rats. *J Pharm Sci* 2002; **91**:1009–1018.

125 Devi GR, Oldenkamp JR, London CA, Iversen PL. Inhibition of human chorionic gonadotropin beta-subunit modulates the mitogenic effect of c-*myc* in human prostate cancer cells. *Prostate* 2002; **53**:200–210.

126 Knapp DC, Mata JE, Reddy MT, Devi GR, Iversen PL. Resistance to chemotherapeutic drugs overcome by c-Myc inhibition in a Lewis lung carcinoma murine model. *Anticancer Drugs* 2003; **14**:39–47.

127 Soutschek J, Akinc A, Bramlage B, Charisse K, Constien R, Donoghue M, *et al.* Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. *Nature* 2004; **432**:173–178.

128 Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, *et al.* EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004; **304**:1497–1500.

129 Berman HM, Westbrook J, Feng ZG, Gilliland G, Bhat TN, Weissig H, *et al.* The Protein Data Bank. *Nucleic Acids Res* 2000; **28**:235–242.