available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Review

Bosutinib: A review of preclinical studies in chronic myelogenous leukaemia

Frank Boschelli [a,*], *Kim Arndt* [a], *Carlo Gambacorti-Passerini* [b]

[a] *Department of Oncology, Wyeth Research (Wyeth Research is Now Pfizer Research), Pearl River, NY, USA*
[b] *Department of Clinical Medicine and Prevention, University of Milano-Bicocca, S Gerardo Hospital, Monza, Milan, Italy*

ARTICLE INFO

Article history:
Received 4 November 2009
Received in revised form 11 February 2010
Accepted 17 February 2010

Keywords:
Bosutinib
SKI-606
Chronic myelogenous leukaemia
Src
Bcr-Abl
Breast cancer
Tumour
Tyrosine kinase inhibitor
Imatinib resistance
Clinical trials

ABSTRACT

Bosutinib (SKI-606) is an orally active Src and Abl kinase inhibitor presently in Phase III trials for treatment of chronic myelogenous leukaemia (CML), and in Phase II trials for treatment of breast cancer. Bosutinib is a potent antiproliferative and proapoptotic agent in CML cells and inhibits Bcr-Abl mediated signalling at nanomolar concentrations. Short-term administration of bosutinib causes regression of K562 and KU812 CML tumour xenografts. BaF3 murine myeloid cells expressing wild-type Bcr-Abl are sensitive to bosutinib treatment, as are BaF3 cells expressing many imatinib-resistant forms of Bcr-Abl. Recent studies indicate that bosutinib is active against a broader spectrum of kinases than originally believed. These additional inhibitory activities have interesting possibilities for further clinical development. This review will focus on preclinical studies supporting the clinical development of bosutinib for treatment of CML, with a discussion on the broader potential of this agent in other oncology indications.

1. Introduction

The non-receptor protein tyrosine kinase c-Src (Src) has been a candidate for drug development for nearly two decades.[1] Oncology indications proposed for inhibitors of Src kinase activity include inhibition of primary tumour growth in several types of cancer, metastatic disease and lytic bone disease. Other indications, which have thus far been limited to preclinical investigations, include osteoporosis, ischaemic stroke, myocardial infarction and polycystic kidney disease. Interestingly, the mechanistic basis for use of Src inhibitors in these other indications is relevant to oncology applications.

Four pharmaceutical companies, AstraZeneca (saracatinib, AZD0530), Bristol Myers Squibb (dasatinib, BMS-354825), Wyeth (now Pfizer) (bosutinib, SKI-606) and Kinex (KX2-391, KX01) have Src inhibitors in various clinical trials for solid tumours.[2–5] The first three agents are ATP-binding site competitive inhibitors, while KX2-391 is a substrate-binding site inhibitor (see Fig. 1). Because the activated kinase domains of Src and the cytoplasmic tyrosine kinase Abl are structurally

* *Corresponding author:* Tel.: +1 11 845 602 5439; fax: +1 11 845 602 5557.
E-mail address: boschef@wyeth.com (F. Boschelli).
0959-8049/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2010.02.032

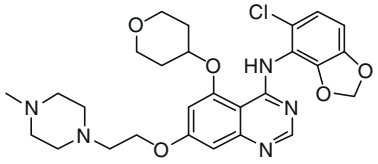
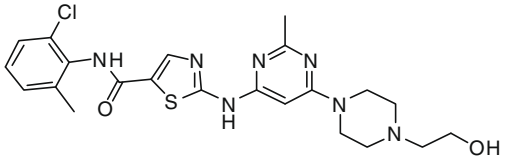
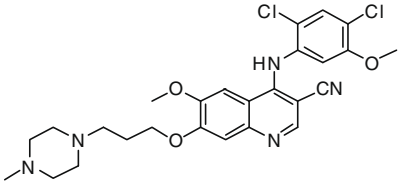
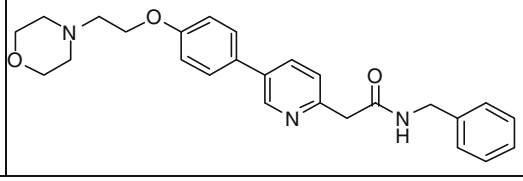
Compound	Structure	Pharmaceutical Company
Saracatinib AZD0530		AstraZeneca
Dasatinib BMS-354825 Sprycel		Bristol Myers Squibb
Bosutinib SKI-606		Wyeth
KX01 KX2-391		Kinex

Fig. 1 – Structures of Src inhibitors presently in clinical trials.

related, the three Src ATP-competitive inhibitors in clinical development are also potent Abl kinase inhibitors.[2,4,6] This serendipitous activity allowed clinical development of both dasatinib and bosutinib for treatment of chronic myelogenous leukaemia (CML). Dasatinib is approved for imatinib resistant or intolerant CML, while dasatinib and bosutinib are in Phase III trials for use in front-line treatment of CML.[7]

The first studies describing bosutinib activity were published in 2001.[5] At that time, a limited activity profile was disclosed, and the catch-phrase of 'selective' kinase inhibitor was used, since of the kinases examined, only Src and related family members were potently inhibited by bosutinib. Since that time, additional studies at Wyeth and elsewhere have indicated that the kinase inhibition profile of bosutinib is far less restricted than originally thought. Puttini and colleagues profiled a panel of 60 kinases and found that Csk, the kinase that phosphorylates c-Src directly on its negative regulatory tyrosine phosphorylation site (Y530), is inhibited by bosutinib.[8] Bantscheff and colleagues and more recently Rix and colleagues showed that bosutinib binds and inhibits a wider range of both tyrosine and serine–threonine kinases bearing little similarity in primary sequence.[9,10] Enzymatic assays conducted internally are in good agreement with these published studies. While lack of selectivity can be viewed as a potential drawback to clinical use of a kinase inhibitor, it can also provide opportunities for developing the inhibitor in appropriate clinical settings. For example, the c-Kit activity of the Abl kinase inhibitor imatinib allowed its development for treatment of gastrointestinal stromal tumour (GIST) patients.[11] On the other hand, the c-Kit and PDGF receptor inhibitory activity of imatinib are believed to contribute to side-effects in patients. At present, our ability to predict adverse effects on the basis of an inhibitory profile is limited. The liability of the additional inhibitory activities must therefore be determined empirically, while any benefit would ensue according to our understanding of the role of additional targets in human cancer.

The relative ease of developing Src/Abl inhibitors in a CML setting arises from the fact that early stage disease is driven by the kinase activity of Bcr-Abl, an oncogenic variant of Abl expressed in CML cells.[12] The clinical success of imatinib in treating CML firmly confirmed the direct relationship between disease and pharmacological target.[13] Moreover, clinical efficacy is directly related to well-understood biomarker responses. The same cannot be said for clinical development of these agents as Src inhibitors.

Elevated levels and activity, but not structural abnormalities of Src in breast, colorectal and sarcoma tumour samples were first reported in the 1980s.[14–17] These experiments demonstrated modest increases in Src levels in tumour cell lysates relative to lysates of normal tissue. Subsequent studies showed that Src activation was an early event in colorectal cancer, and that metastases of colorectal carcinomas had very high levels of Src and Src kinase activity.[18,19] Further-

more, modest elevation of Src levels is an indicator of poor prognosis in colorectal cancer.[20,21] Experimental evidence supports a role for Src in metastasis. Src-expressing D121 murine tumour xenografts implanted in Src-null mice exhibited impaired metastasis compared to the same tumours growing in mice with an active Src gene, consistent with numerous diverse observations indicating that Src activation promotes cell mobility and reduces cell–cell and cell–matrix interaction.[22–25] Dominant negative forms of Src inhibit tumour cell metastasis, and treatment with Src inhibitors, including bosutinib, also reduced metastatic burden in several tumour models.[24,26–29] It is reasonable to suppose then that tumour cells expressing high levels of Src are predisposed to metastasis, and that Src inhibitors would act as anti-metastatic agents. Clinical development of a Src inhibitor in this context would be a lengthy and high-risk venture.

In the past several years, preclinical data suggesting that Src inhibitors might be effective in the treatment of glioblastoma and pancreatic cancer were published.[30,31] Src is important in steroid hormone-dependent signalling in breast and prostate cancer, and in the acquisition of resistance to hormone ablation therapy.[32,33] In addition, metastatic bone disease has long been considered a potential application for Src inhibitors, and recent studies suggest that Src is required for growth of breast tumour cells in the bone marrow.[34] Hopefully, solid clinical data supporting these indications will appear in the not too distant future.

Bosutinib is presently in Phase III trials for CML. The preclinical studies supporting the clinical development of bosutinib in CML are described in this review.

2. Identification of 3-quinolinecarbonitriles as Src inhibitors

A Src kinase-dependent yeast screen led to characterisation of a 4-anilino-3-quinolinecarbonitrile as a Src inhibitor.[35] This class of compounds includes known kinase inhibitors, and Wyeth has developed related compounds, the epidermal growth factor receptor inhibitor pelitinib (EKB-569) and the Her2 receptor inhibitor neratinib (HKI-272) for oncology indications.[36,37] The hit from the yeast screen was a 30 nM inhibitor of Src kinase activity in an enzyme-linked immunosorbent (ELISA) assay. A focused enzyme assay screen of related compounds identified a compound with an IC_{50} (concentration required for 50% inhibition) of 15 nM in the same assay. Features of these two compounds were combined to yield a 4 nM Src inhibitor. Attachment of solubilising groups to improve cellular activity also increased enzyme activity and led to bosutinib, a 1.2 nM Src inhibitor the ELISA assay. In a homogeneous (Lance) assay, bosutinib exhibited an IC_{50} of 3.5 nM.[5] A more complete discussion of bosutinib activities is given below.

3. Bosutinib in CML cells

Clinical trials for bosutinib in CML are in Phase III, with clear evidence of efficacy. Bosutinib was disclosed as an Abl kinase inhibitor in 2003.[6] The Abl inhibitory activity was discovered by screening a panel of tumour cell lines, including the Philadelphia chromosome-positive cell lines K562, KU812 and Meg-01. Potent antiproliferative activity was observed in these cell lines, but not in other leukaemia cell lines, except HSB2, a T cell leukaemia line with mutationally activated Lck, or T cell acute lymphoblastic leukaemia cells with the Nup214-Abl fusion (data not shown, FB).[38,39] KU812 cells, for instance, were inhibited with an IC_{50} of 5 nM, a great leap from the micromolar antiproliferative activity of bosutinib observed in most human tumour cell lines. These results suggested that bosutinib was an Abl kinase inhibitor, and when tested in an Abl kinase assay, bosutinib had an IC_{50} of 1.4 nM, slightly more potent than the 3.5 nM Src inhibitory activity in a similar assay format. Bosutinib also inhibited the proliferation of v-Abl-transformed fibroblasts growing in suspension, much as was observed with Src-transformed fibroblasts, and the antiproliferative activity correlated with inhibition of phosphorylation of v-Abl, and of Bcr-Abl in CML cells. Puttini and colleagues confirmed these findings and demonstrated that this activity extended to mutated forms of Bcr-Abl (see below).[8] Bosutinib administered orally for 5 consecutive days caused regression of K562 CML tumours.[6] The lowest effective dose in this regimen was 15 mg/kg, corresponding to an AUC of 2054 ng h/mL, which compared favourably with clinical exposures of 2851 and 3660 ng h/mL at the once daily 400 and 500 mg doses, respectively[40,41] All of these results supported a therapeutic role of bosutinib for treatment of CML.

4. Bosutinib effects on signalling downstream of Bcr-Abl

Key elements of the Bcr-Abl signalling pathway important for CML cell proliferation and survival include phosphorylation of Bcr-Abl itself and phosphorylation of the docking protein CrkL on Y207. Src family kinases, including Lyn and Hck, also play a role in downstream signalling. In cultured CML cells, bosutinib treatment reduced Y245 Bcr-Abl phosphorylation, Y207 phosphorylation on CrkL, a clinical marker for biomarker efficacy, Y694 phosphorylation of the transcription factor STAT5 and Y397 phosphorylation of the Src family kinase Lyn.[6,40] These results are consistent with the Src and Abl kinase inhibitory activities of bosutinib. Mancini and collegues, in a study with murine myeloid lines transformed by Bcr-Abl, showed that bosutinib also reduced Cdk2 levels, behaviour not seen with imatinib treatment.[42]

5. Bosutinib effects on primary CML cells

Konig and colleagues found that bosutinib inhibited proliferation of primitive and committed CML progenitor cells from chronic phase CML patients. Under the conditions used, little effect was observed in normal cells. A small increase in apoptosis was observed in CML-committed progenitor cells ($CD34^+$ $CD38^+$) treated with bosutinib, but, as was the case with other Abl kinase inhibitors, bosutinib treatment did not eliminate primitive ($CD34^+$ $CD38^-$) CML cells at the highest concentration studied.[43,44]

6. Bosutinib activity against imatinib-resistant forms of Bcr-Abl

Puttini and colleagues reported that CML cell lines selected for imatinib-resistance were sensitive to bosutinib.[8] Bosutinib also inhibited the proliferation of BaF3 murine myeloid cells expressing Bcr-Abl with mutations Y253F, E255K and D276G at low concentrations, but was much less effective against cell expressing T315I Bcr-Abl. Bosutinib administered at 75 mg/kg po bid or 150 mg/kg po qd caused tumour regression and kept animals with subcutaneous KU812 xenografts tumour free out to 210 d. Tumours arising from the BaF3 Bcr-Abl transfectants were more refractory to treatment. Bosutinib caused regression of wt Bcr-Abl expressing BaF3 tumours, but tumours recovered a few weeks after dosing was stopped.[8] A summary of the responses of BaF3 tumours expressing wt or four different imatinib-resistant Bcr-Abl mutants is given in Table 1. Only the T315I tumours were completely resistant to treatment.

Table 1 – Bosutinib activity against Bcr-Abl BaF3 tumour xenografts.

Bcr-Abl	Dose	Effect
WT	150 mg/kg qd	Regression/relapse
E255K	150 mg/kg qd	Regression/relapse
Y253F	75 mg/kg bid	Slight regression/relapse
D276G	75 mg/kg bid	Slight regression /relapse
T315I	150 mg/kg qd	No inhibition

7. Bosutinib in comparison with dasatinib and the Abl kinase inhibitor nilotinib

A recent study by Redaelli and colleagues described relative activities of bosutinib, dasatinib and nilotinib against a panel of imatinib-resistant Bcr-Abl mutants in BaF3 murine myeloid cells.[45] Nilotinib is a second generation Abl kinase inhibitor of the same chemical class as imatinib, but considerably more potent in vitro. It is presently approved for second line treatment of CML and has exhibited superior efficacy to imatinib in a comparative Phase III trial.[46] The mutations in Bcr-Abl spanned the catalytic domain and represented many Bcr-Abl variants prevalent in the clinic. These data are reproduced in Fig. 2.

Dasatinib was the most potent of these inhibitors against wt Bcr-Abl and most of the mutants, and when examined in this simple context might seem greatly superior to nilotinib and bosutinib. Clinical observations belie this simple description, as noted by the authors in the case of the F317L mutation, which, while responding with an IC_{50} of 8 nM in the BaF3 assay, is insensitive to dasatinib in the clinic, while responses have been noted for bosutinib, which has an IC_{50} of 100 nM. For this reason, the authors chose the ratio of the mutant to wt IC_{50} as a better predictor of clinical efficacy. When

		IC50-fold increase (WT=1)			
		Imatinib	Bosutinib	Dasatinib	Nilotinib
	Parental	10.78	38.31	>50	38.43
	WT	1	1	1	1
P –LOOP	L248V	3.54	2.97	5.11	2.80
	G250E	6.86	4.31	4.45	4.56
	Q252H	1.39	0.81	3.05	2.64
	Y253F	3.58	0.96	1.58	3.23
	E255K	6.02	9.47	5.61	6.69
	E255V	16.99	5.53	3.44	10.31
	D276G	2.18	0.60	1.44	2.00
C-Helix	E279K	3.55	0.95	1.64	2.05
	V299L	1.54	26.10	8.65	1.34
Active site	T315I	17.50	45.42	75.03	39.41
	F317L	2.60	2.42	4.46	2.22
SH2-contact	M351T	1.76	0.70	0.88	0.44
Active site	F359V	2.86	0.93	1.49	5.16
A-LOOP	L384M	1.28	0.47	2.21	2.33
	H396P	2.43	0.43	1.07	2.41
	H396R	3.91	0.81	1.63	3.10
	G398R	0.35	1.16	0.69	0.49
C terminal lobe	F486S	8.10	2.31	3.04	1.85

Sensitive	≤2
Resistant	2.01 - 10
highly resistant	>10

Fig. 2 – Relative IC_{50} values for bosutinib, imatinib, dasatinib, and nilotinib against 18 mutated forms of BCR/ABL expressed in Ba/F3 transfected cells (reprinted from J Clin Oncol 2009; 27:469–471). The data in this table represent proliferation assay data performed with BaF3 murine myeloid cells expressing the indicated Bcr-Abl protein. Values in the table reflect the ratio of the IC_{50} of the compound in the given cell line relative to the IC_{50} of the compound in BaF3 cells expressing wild-type Bcr-Abl.

viewed in this context, bosutinib and dasatinib shared a similar inhibition profile in this assay with some differences in the P-loop region. Nilotinib was different in that it was less effective against the F359V and several of the P-loop mutants, but better than dasatinib against F317L. The utility of these measurements as a clinical predictor will become more apparent with observation of increasing numbers of patients presenting with a given mutation. In cases where tumour heterogeneity exists, these data may be unable to predict clinical outcome, such as might occur in case of rapid selection and overgrowth of a resistant minor clone, or in the presence of clones with multiple mutations. None of these agents look promising against the T315I mutation, but clear differences among them can be seen in several other cases. As larger data set with clinical comparators become available, such data may prove to be useful treatment guides for clinicians.

Multidrug resistance (MDR) transporters promote efflux of diverse foreign molecules from cells, including tyrosine kinase inhibitors such as imatinib. Upregulation of these transporters in tumour cells promotes imatinib resistance in CML cells in culture. A recent comparison of second generation Abl kinase inhibitors found that in contrast to nilotinib and dasatinib, bosutinib is not an efficient substrate for the MDR transporters.[47] Bosutinib also inhibits these transport proteins at concentrations greater than 1 μM, which may underlie, at least in part, the observed accumulation and retention of bosutinib in tumour tissue.[48]

8. Bosutinib as a multikinase inhibitor

The original study disclosing the structure of bosutinib provided limited selectivity information.[5] Little or low activity was observed against receptor tyrosine kinases such as IGF-1R, Her2, FGFR and PDGFR. Our earlier studies suggested that bosutinib inhibited EGFR in an enzymatic assay in the micromolar range and 1 μmol bosutinib did not inhibit EGFR in cells. Subsequent work indicated that bosutinib does inhibit EGFR enzyme activity with an IC_{50} of about 350 nM, and was able to reduce EGFR autophosphorylation (Y1068) or the levels of 'activated' EGFR in cells with an IC_{50} between 0.5 and 1 μmol. To gain a more complete understanding of bosutinib activity, Wyeth instituted broad-based screening of bosutinib against a panel of kinases (Invitrogen), which brought several unexpected activities to light. The protocol for the Z-Lyte screens used for these assays can be obtained at this web address http://www.invitrogen.com/etc/medialib/en/filelibrary/Drug-Discovery/PDFs.Par.5558.File.dat/SSBK%20Customer%20Protocol%20and%20Assay%20Conditions.pdf. Comparisons of bosutinib, dasatinib, nilotinib and imatinib as multikinase inhibitors were published independently.[9,10] In addition, the web page for Kinaxo depicts comparative binding affinities of bosutinib for kinases in PC3 cells (www.kinaxo.com). A compilation of the activities of bosutinib as described by these various studies is given in Table 2. The overall agreement amongst the various studies is excellent. Besides Src and Abl family kinases, five major kinase groups are inhibited by bosutinib: Eph receptors, Sterile 20 kinases, Trk family, Tec family and Axl family kinases. In addition, potent inhibition of some mutated EGFR kinases was observed. Other kinases include Ack1, Csk, some c-Kit mutants, calcium/calmodulin-dependent kinases CamK2G and K1D, FAK and c-Fms (CSF1R). GAK was also reported to bind tightly to bosutinib.

9. Additional activities of bosutinib as facilitators of clinical development

The most potent inhibition of the Sterile 20 family kinases occurred in the germinal centre kinase IV (GCK-IV) group, including GCK, KHS1 (GCKR and MAP4K5), HGK and MINK. These kinases have several reported functions, including linking Eph receptor with the actin cytoskeleton and activating JNK.[49,50] GCKR associates with the Bcr-Abl-CrkL complex in CML cells, and is itself activated, with subsequent activation of JNK.[51,52] Blocking this JNK pathway is deleterious to CML cells, suggesting that GCKR inhibition might potentiate the CML activity of bosutinib.[53,54] GCKR inhibition also reduces cytosolic β-catenin resulting from GSK3β phosphorylation on Ser9 in B lymphocytes.[55] In epithelial cells, HGK and MINK knockdown increased cell–cell adhesion, reduced cell migration, decreased cytosolic β-catenin and increased E-cadherin at the plasma membrane, all of which occur upon treatment with bosutinib.[56,57] Interestingly, inhibiting Src in colorectal tumour cells or Bcr-Abl in CML cells reduced the levels of tyrosine-phosphorylated β-catenin, with associated loss of its transcriptional activity.[58,59]

Data obtained in-house at Wyeth (now Pfizer) indicates that bosutinib inhibits Axl family kinases (Gas6 receptor). Zhang and colleagues reported that bosutinib inhibits Axl at similar concentrations to those required for inhibition of Src autophosphorylation in MDA-MB-231 cells.[60] Like Src and some of the Ste20 kinases, Axl promotes migration and invasiveness, and like Src, also has an angiogenic function. Axl knockdown inhibited MDA-MB-231 tumour growth, and reduced blood vessel formation. Jallal and colleagues showed that bosutinib inhibits MDA-MB-231 tumour growth and also reduces blood vessel formation, but Axl inhibition was not examined in this study.[28] Interestingly, Axl is expressed in a subset of oestrogen receptor (ER)-positive breast cancers, a patient group where clinical responses to bosutinib treatment occurred.[61,62] Co-expression of Axl and its ligand Gas6 is a poor prognostic indicator in acute myelogenous leukaemia (AML). Coincidentally or not, bosutinib treatment inhibited the growth of primary cells from 7 of 14 AML patients at submicromolar concentrations. Of the various 'off-target' kinase activities of bosutinib to emerge from these studies, Axl has emerged as a biomarker of great interest in ongoing clinical trials.

CAMK2G is activated in AML patient samples, and is also upregulated by Bcr-Abl in CML cells.[63] Knockdown of CAMK2G, dominant negative expression or treatment with chemical inhibitors reduces myeloid leukaemia proliferation, suggesting that bosutinib-mediated inhibition of CAMK2G might be clinically relevant in treating myeloid leukaemias.

Eph receptors, another class of kinases inhibited by bosutinib, are controversial oncology targets. Both tumour-promoting and suppressive effects have been reported, with context and expression level implicated in determining which property predominates.[64,65] There is some evidence suggest-

Table 2 – A comparison of reported bosutinib activities including contracted data from Invitrogen (values in nM).

	IC_{50} (nM)	Cell lysate-binding affinities[e]	% inhibition @ 0.2 μM (Invitrogen)[a]
Abl family			
Abl	1[d], 0.5[b], 2.4[c]	85	
Abl(T315I)	26[b]		56
ABL1 E255K			98
ABL1 G250E			97
ABL1 Y253F			99
ABL2 (Arg)	0.5[b]	102	96
Axl Family			
Tyro3			65
Axl	174[b]		
cMER		5011	70
EGFR family			
EGFR	53[b], 345[a]		
EGFR(T790M)	491[b], >1000[a]		
EGFR L858R	0.35[a]		
EGFR L861Q	3.51[a]		
EGFR(T790M, L858R)	655[a]		
Eph Family			
EphA2			100
EphA3			81
EphA4		>10,000	89
EphA5			83
EphA8			79
EphB1		707	84
EphB2	8.5[b]	>10,000	79
EphB3		422	50
EphB4		108	87
Fer/Fes family			
Fer	490[b]	1851	
Fes	358[b]		
c-Kit			
cKit	6313[b]	>10,000	
cKit(D816H)	32[b]		
cKit(D816V)	2772[b]		
cKit(V560G)	181[b]		
cKit(V654A)	132[b]		
Src family			
Src	3.5[d]	400	
Yes	0.4[b], 0.8[a]	784	
Fgr	1.1[b], 0.17[c]		
Fyn	1.8[b]	700	
Lck	1.3[b]		
Hck	3.2[b]	1178	
Lyn	0.85[c]	647	
Blk (murine)	7.3[b]		
Ste20 (Sterile 20)			
MAP4K2 (GCK)		1357	100
MAP4K4 (HGK)			103
MAP4K5 (KHS1)	0.5[a], 0.5[b]	1933	98
LOK	52[b]		
MINK	22[b]		
MST1	191[b]		40
MST3	3.9[b]		83
STK25 (YSK1)			79
Tec family			
Bmx	7.9[b]		66
Tec	282[b]	2964	
Btk	0.44[a]	213	
Trk Family			
TrkA	22[b]		

Table 2 (*continued*)

	IC_{50} (nM)	Cell lysate-binding affinities[e]	% inhibition @ 0.2 μM (Invitrogen)[a]
TrkB	27[b], 30[a]		
Others			
Ack1	2.7[b]	863	
c-Fms	44[b]		
Csk	34[a], 314[c], 62[b]	964	
FAK	538[b]	3187	
Pyk2	134[b]	4500	
FRK	2.2[b]	659	89
CaMKIIG	184[b]	>10,000	
CaMKID	92[b]	>10,000	
GAK		56	

[a] Invitrogen-contracted study.
[b] Rix et al., Ref. [10].
[c] Puttini et al., Ref. [8].
[d] Wyeth data.
[e] Bantscheff et al., Ref. [9].

ing that the tumour-promoting effects of Eph receptors is kinase activity dependent, whereas tumour suppressor activity is kinase independent.[66] Whether these observations are universally true is unclear at present. In breast cancer, EphB2 and Abl cooperate to stimulate cyclin D1 expression, providing an interesting target pair for an agent such as bosutinib.[66] Two independent studies with bosutinib in EphB2 enzyme assays suggest potent inhibition, but little binding was observed in the cell-based assay study (see Table 2). In contrast, potent inhibition of EphB4 was observed both in the cell-based binding assay and in an enzyme assay, and this Eph is reported to have tumour suppressive activity in breast cancer in a manner also involving Abl.[9,67] More cell-based as well as in vivo studies are required to clarify the activity of bosutinib against the various Eph receptors, and the consequences of inhibiting these receptors. It is worth emphasising that the variability of the observed effects of Eph/ephrin pathway activation calls into question the validity of tumour xenograft models from established tumour cell lines as predictors of clinical activity.

10. Summary

Preclinical development of bosutinib focused on improvement of Src inhibitory activity. This approach also yielded an agent with an additional activity as an Abl kinase inhibitor, activity in CML cells and xenografts, and advancement to Phase III clinical trials for use in front-line treatment of CML. Development as a 'Src' inhibitor is proceeding in Phase II in ER^+ positive breast cancer in combination with agents that interfere with ER function and in triple negative breast cancer with cytotoxic agents. The additional inhibitory activities discovered since the inception of the clinical trials suggest several additional development routes.

Conflict of interest statement

Frank Boschelli and Kim Arndt are employees of Pfizer (formerly Wyeth), which is conducting clinical trials of bosutinib. Carlo Gambacorti-Passerini has no conflicts. This article was written solely by these authors.

Acknowledgments

The authors thank Dr. Diane Boschelli for Fig. 1 and for critical editing of the manuscript.

REFERENCES

1. Kopetz S, Shah AN, Gallick GE. Src continues aging: current and future clinical directions. *Clin Cancer Res* 2007;**13**(24):7232–6.
2. Hennequin LF, Allen J, Breed J, et al. N-(5-Chloro-1, 3-benzodioxol-4-yl)-7-[2-(4-methylpiperazin-1-yl)ethoxy]-5-(tetrahydro-2H-pyran-4-yloxy)quinazolin-4-amine, a novel, highly selective, orally available, dual-specific c-Src/Abl kinase inhibitor. *J Med Chem* 2006;**49**(22):6465–88.
3. Lau GM, Lau GM, Yu GL, et al. Expression of Src and FAK in hepatocellular carcinoma and the effect of src inhibitors on hepatocellular carcinoma in vitro. *Dig Dis Sci* 2009;**54**(7):1465–74.
4. Lombardo LJ, Lee FY, Chen P, et al. Discovery of N-(2-chloro-6-methyl- phenyl)-2-(6-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumour activity in preclinical assays. *J Med Chem* 2004;**47**(27):6658–61.
5. Boschelli DH, Ye F, Wang YD, et al. Optimization of 4-phenylamino-3-quinolinecarbonitriles as potent inhibitors of Src kinase activity. *J Med Chem* 2001;**44**(23):3965–77.
6. Golas JM, Arndt K, Etienne C, 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**(2):375–81.
7. Brave M, Goodman V, Kaminskas E, et al. Sprycel for chronic myeloid leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia resistant to or intolerant of imatinib mesylate. *Clin Cancer Res* 2008;**14**(2):352–9.

8. Puttini M, Coluccia AM, Boschelli F, et al. In vitro and in vivo activity of SKI-606, a novel Src-Abl inhibitor, against imatinib-resistant Bcr-Abl+ neoplastic cells. *Cancer Res* 2006;**66**(23):11314–22.
9. Bantscheff M, Eberhard D, Abraham Y, et al. Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors. *Nat Biotechnol* 2007;**25**(9):1035–44.
10. Remsing Rix LL, Rix U, Colinge J, et al. Global target profile of the kinase inhibitor bosutinib in primary chronic myeloid leukemia cells. *Leukemia* 2009;**23**(3):477–85.
11. Dagher R, Cohen M, Williams G, et al. Approval summary: imatinib mesylate in the treatment of metastatic and/or unresectable malignant gastrointestinal stromal tumours. *Clin Cancer Res* 2002;**8**(10):3034–8.
12. Witte O. The role of Bcr-Abl in chronic myeloid leukemia and stem cell biology. *Semin Hematol* 2001;**38**(3 Suppl. 8):3–8.
13. Mauro MJ, O'Dwyer M, Heinrich MC, Druker BJ. STI571: a paradigm of new agents for cancer therapeutics. *J Clin Oncol* 2002;**20**(1):325–34.
14. Bolen JB, Veillette A, Schwartz AM, DeSeau V, Rosen N. Activation of pp60c-src protein kinase activity in human colon carcinoma. *Proc Natl Acad Sci USA* 1987;**84**(8):2251–5.
15. Cartwright CA, Kamps MP, Meisler AI, Pipas JM, Eckhart W. Pp60c-src activation in human colon carcinoma. *J Clin Invest* 1989;**83**(6):2025–33.
16. Rosen N, Bolen JB, Schwartz AM, et al. Analysis of pp60$^{c\text{-}src}$ protein kinase activity in human tumour cell lines and tissues. *J Biol Chem* 1986;**261**(29):13754–9.
17. Jacobs C, Rubsamen H. Expression of pp60c-src protein kinase in adult and fetal human tissue: high activities in some sarcomas and mammary carcinomas. *Cancer Res* 1983;**43**(4):1696–702.
18. 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**(1):53–60.
19. Termuhlen PM, Curley SA, Talamonti MS, Saboorian MH, Gallick GE. Site-specific differences in pp60$^{c\text{-}src}$ activity in human colorectal metastases. *J Surg Res* 1993;**54**(4):293–8.
20. Cartwright CA, Meisler AI, Eckhart W. Activation of the pp60c-src protein kinase is an early event in colonic carcinogenesis. *Proc Natl Acad Sci USA* 1990;**87**(2):558–62.
21. Aligayer H, Boyd DD, Heiss MM, et al. Activation of Src kinase in primary colorectal carcinoma: an indicator of poor clinical prognosis. *Cancer* 2002;**94**(2):344–51.
22. Irby RB, Yeatman TJ. Increased Src activity disrupts cadherin/catenin-mediated homotypic adhesion in human colon cancer and transformed rodent cells. *Cancer Res* 2002;**62**(9):2669–74.
23. Jones RJ, Avizienyte E, Wyke AW, et al. Elevated c-Src is linked to altered cell–matrix adhesion rather than proliferation in KM12C human colorectal cancer cells. *Br J Cancer* 2002;**87**(10):1128–35.
24. Weis S, Cui J, Barnes L, Cheresh D. Endothelial barrier disruption by VEGF-mediated Src activity potentiates tumour cell extravasation and metastasis. *J Cell Biol* 2004;**167**(2):223–9.
25. Criscuoli ML, Nguyen M, Eliceiri BP. Tumour metastasis but not tumour growth is dependent on Src-mediated vascular permeability. *Blood* 2005;**105**(4):1508–14.
26. 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**(7):2430–6.
27. Myoui A, Nishimura R, Williams PJ, et al. C-SRC tyrosine kinase activity is associated with tumour colonization in bone and lung in an animal model of human breast cancer metastasis. *Cancer Res* 2003;**63**(16):5028–33.
28. Jallal H, Valentino ML, Chen G, et al. A Src/Abl kinase inhibitor, SKI-606, blocks breast cancer invasion, growth, and metastasis in vitro and in vivo. *Cancer Res* 2007;**67**(4):1580–8.
29. Zheng R, Yano S, Matsumori Y, et al. SRC tyrosine kinase inhibitor, m475271, suppresses subcutaneous growth and production of lung metastasis via inhibition of proliferation, invasion, and vascularization of human lung adenocarcinoma cells. *Clin Exp Metastasis* 2005;**22**(3):195–204.
30. Du J, Bernasconi P, Clauser KR, et al. Bead-based profiling of tyrosine kinase phosphorylation identifies SRC as a potential target for glioblastoma therapy. *Nat Biotechnol* 2009;**27**(1):77–83.
31. Desgrosellier JS, Barnes LA, Shields DJ, et al. An integrin alpha(v)beta(3)-c-Src oncogenic unit promotes anchorage-independence and tumour progression. *Nat Med* 2009;**15**(10):1163–9.
32. Riggins RB, Thomas KS, Ta HQ, et al. Physical and functional interactions between Cas and c-Src induce tamoxifen resistance of breast cancer cells through pathways involving epidermal growth factor receptor and signal transducer and activator of transcription 5b. *Cancer Res* 2006;**66**(14):7007–15.
33. Tatarov O, Mitchell TJ, Seywright M, et al. SRC family kinase activity is up-regulated in hormone-refractory prostate cancer. *Clin Cancer Res* 2009;**15**(10):3540–9.
34. Zhang XH, Wang Q, Gerald W, et al. Latent bone metastasis in breast cancer tied to Src-dependent survival signals. *Cancer Cell* 2009;**16**(1):67–78.
35. Boschelli DH, Wang YD, Ye F, et al. Synthesis and Src kinase inhibitory activity of a series of 4-phenylamino-3-quinolinecarbonitriles. *J Med Chem* 2001;**44**(5):822–33.
36. Wissner A, Overbeek E, Reich MF, et al. Synthesis and structure-activity relationships of 6,7-disubstituted 4-anilinoquinoline-3-carbonitriles. The design of an orally active, irreversible inhibitor of the tyrosine kinase activity of the epidermal growth factor receptor (EGFR) and the human epidermal growth factor receptor-2 (HER-2). *J Med Chem* 2003;**46**(1):49–63.
37. Rabindran SK, Discafani CM, Rosfjord EC, et al. Antitumour activity of HKI-272, an orally active, irreversible inhibitor of the HER-2 tyrosine kinase. *Cancer Res* 2004;**64**(11):3958–65.
38. Wright DD, Sefton BM, Kamps MP. Oncogenic activation of the Lck protein accompanies translocation of the LCK gene in the human HSB2 T-cell leukemia. *Mol Cell Biol* 1994;**14**(4):2429–37.
39. Graux C, Cools J, Melotte C, et al. Fusion of NUP214 to ABL1 on amplified episomes in T-cell acute lymphoblastic leukemia. *Nat Genet* 2004;**36**(10):1084–9.
40. Boschelli DH, Boschelli F, Wu B, et al. SKI-606 and beyond. *Hematol Rep* 2005;**1**:28–31.
41. Gambacorti-Passerini C, Brummendorf H, Kantarjian H, et al. Bosutinib (SKI-606) exhibits clinical activity in patients with Philadelphia chromosome positive CML or ALL who failed imatinib. In: ASCO Annual Meeting Proceedings; 2007. J Clin Oncol; 2007. p. 18S: 7006.
42. Mancini M, Brusa G, Zuffa E, et al. Persistent Cdk2 inactivation drives growth arrest of BCR-ABL-expressing cells in response to dual inhibitor of SRC and ABL kinases SKI606. *Leuk Res* 2007;**31**(7):979–87.
43. Konig H, Holyoake TL, Bhatia R. Effective and selective inhibition of chronic myeloid leukemia primitive hematopoietic progenitors by the dual Src/Abl kinase inhibitor SKI-606. *Blood* 2008;**111**(4):2329–38.
44. Konig H, Copland M, Chu S. Effects of dasatinib on SRC kinase activity and downstream intracellular signaling in primitive chronic myelogenous leukemia hematopoietic cells. *Cancer Res* 2008;**68**(23):9624–33.
45. Redaelli S, Piazza R, Rostagno R, et al. Activity of bosutinib, dasatinib, and nilotinib against 18 imatinib-resistant BCR/ABL mutants. *J Clin Oncol* 2009;**27**(3):469–71.

46. Saglio G, Kim D-W, Issaragrisil S, et al. Nilotinib demonstrates superior efficacy compared with imatinib in patients with newly diagnosed chronic myeloid leukaemia in chronic phase: results from the International Randomized Phase III ENESTnd Trial. In: American Society for Hematology Annual Meeting, New Orleans; 2009.
47. Hegedus C, Ozvegy-Laczka C, Apati A, et al. Interaction of nilotinib, dasatinib and bosutinib with ABCB1 and ABCG2: implications for altered anti-cancer effects and pharmacological properties. *Br J Pharmacol* 2009;**158**(4):1153–64.
48. Golas JM, Lucas J, Etienne C, et al. SKI-606, a Src/Abl inhibitor with in vivo activity in colon tumour xenograft models. *Cancer Res* 2005;**65**(12):5358–64.
49. Dan I, Watanabe NM, Kusumi A. The Ste20 group kinases as regulators of MAP kinase cascades. *Trends Cell Biol* 2001;**11**(5):220–30.
50. Becker E, Huynh-Do U, Holland S, et al. Nck-interacting Ste20 kinase couples Eph receptors to c-Jun N-terminal kinase and integrin activation. *Mol Cell Biol* 2000;**20**(5):1537–45.
51. Shi CS, Tuscano JM, Witte ON, Kehrl JH. GCKR links the Bcr-Abl oncogene and Ras to the stress-activated protein kinase pathway. *Blood* 1999;**93**(4):1338–45.
52. Shi CS, Tuscano J, Kehrl JH. Adaptor proteins CRK and CRKL associate with the serine/threonine protein kinase GCKR promoting GCKR and SAPK activation. *Blood* 2000;**95**(3):776–82.
53. Raitano AB, Halpern JR, Hambuch TM, Sawyers CL. The Bcr-Abl leukemia oncogene activates Jun kinase and requires Jun for transformation. *Proc Natl Acad Sci USA* 1995;**92**(25):11746–50.
54. Dickens M, Rogers JS, Cavanagh J, et al. A cytoplasmic inhibitor of the JNK signal transduction pathway. *Science* 1997;**277**(5326):693–6.
55. Shi CS, Huang NN, Harrison K, Han SB, Kehrl JH. The mitogen-activated protein kinase kinase kinase kinase GCKR positively regulates canonical and noncanonical Wnt signaling in B lymphocytes. *Mol Cell Biol* 2006;**26**(17):6511–21.
56. Wright JH, Wang X, Manning G, et al. The STE20 kinase HGK is broadly expressed in human tumour cells and can modulate cellular transformation, invasion, and adhesion. *Mol Cell Biol* 2003;**23**(6):2068–82.
57. Hu Y, Leo C, Yu S, et al. Identification and functional characterization of a novel human misshapen/Nck interacting kinase-related kinase, hMINK beta. *J Biol Chem* 2004;**279**(52):54387–97.
58. Coluccia AM, Benati D, Dekhil H, et al. SKI-606 decreases growth and motility of colorectal cancer cells by preventing $pp60^{c\text{-}src}$-dependent tyrosine phosphorylation of beta-catenin and its nuclear signaling. *Cancer Res* 2006;**66**(4):2279–86.
59. Coluccia AM, Vacca A, Dunach M, et al. Bcr-Abl stabilizes beta-catenin in chronic myeloid leukemia through its tyrosine phosphorylation. *EMBO J* 2007;**26**(5):1456–66.
60. Zhang YX, Knyazev PG, Cheburkin YV, et al. AXL is a potential target for therapeutic intervention in breast cancer progression. *Cancer Res* 2008;**68**(6):1905–15.
61. Berclaz G, Altermatt HJ, Rohrbach V, et al. Estrogen dependent expression of the receptor tyrosine kinase axl in normal and malignant human breast. *Ann Oncol* 2001;**12**(6):819–24.
62. Campone M, Bondarenko I, Brincat S, et al. Preliminary results of a Phase 2 study of bosutinib (SKI-606), a dual Src/Abl kinase inhibitor, in patients with advanced breast cancer. In: 30th Annual San Antonio Breast Cancer symposium, 13–16 December 2007, San Antonio, TX; 2007.
63. Si J, Collins SJ. Activated Ca^{2+}/calmodulin-dependent protein kinase IIgamma is a critical regulator of myeloid leukemia cell proliferation. *Cancer Res* 2008;**68**(10):3733–42.
64. Pasquale EB. Eph-ephrin bidirectional signaling in physiology and disease. *Cell* 2008;**133**(1):38–52.
65. Noberini R, Pasquale EB. Proliferation and tumour suppression: not mutually exclusive for Eph receptors. *Cancer Cell* 2009;**16**(6):452–4.
66. Genander M, Halford MM, Xu NJ, et al. Dissociation of EphB2 signaling pathways mediating progenitor cell proliferation and tumour suppression. *Cell* 2009;**139**(4):679–92.
67. Noren NK, Foos G, Hauser CA, Pasquale EB. The EphB4 receptor suppresses breast cancer cell tumourigenicity through an Abl-Crk pathway. *Nat Cell Biol* 2006;**8**(8):815–25.