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Review

Advances in the structural biology, design and clinical development of Bcr-Abl kinase inhibitors for the treatment of chronic myeloid leukaemia

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Abstract

The constitutively activated Abl tyrosine kinase domain of the chimeric Bcr-Abl oncoprotein is responsible for the transformation of haematopoietic stem cells and the symptoms of chronic myeloid leukaemia (CML). Imatinib targets the tyrosine kinase activity of Bcr-Abl and is a first-line therapy for this malignancy. Although highly effective in chronic phase CML, patients who have progressed to the advanced phase of the disease frequently fail to respond to imatinib or develop resistance to therapy and relapse. This is often due to the emergence of clones expressing mutant forms of Bcr-Abl, which exhibit a decreased sensitivity towards inhibition by imatinib. Considerable progress has recently been made in understanding the structural biology of Abl and the molecular basis for resistance, facilitating the discovery and development of second generation drugs designed to combat mutant forms of Bcr-Abl. The first of these compounds to enter clinical development were BMS-354825 (BristolMyersSquibb) and AMN107 (Novartis Pharma) and, from Phase I results, both of these promise a breakthrough in the treatment of imatinib-resistant CML. Recent advances with these and other promising classes of new CML drugs are reviewed.

Keywords: Bcr-Abl; Chronic myeloid leukaemia; Protein kinase inhibitor; Crystal structure; AMN107; BMS-354825; AZD0530

1. Introduction

Chronic myeloid leukaemia (CML) is a malignant myeloproliferative disorder of self-renewing haematopoietic stem cells. The disease is caused by a reciprocal *t*(9;22) chromosome translocation, resulting in the short Philadelphia chromosome carrying the *BCR-ABL* (Breakpoint Cluster Region-Abelson Leukaemia) oncogene [1]. This oncogene encodes the chimeric Bcr-Abl protein, which incorporates an activated Abl tyrosine kinase domain and is responsible for the initial chronic phase of CML. This phase of the disease is characterised by a massive expansion of granulocyte/macrophage progenitor cells. Acquisition of subsequent genetic mutations then causes progression from the chronic phase to the terminal blast phase of the disease, characterised by an accumulation of either myeloid (66% of patients) or lymphoid blast cells (33% of patients). The majority of CML patients (95%) express the 210-kDa splice variant (p210) of this protein. Together with p210 Bcr-Abl, a 190-kDa (p190/p185) splice variant is expressed in the highly aggressive, but less common adult and paediatric acute lymphoblastic leukaemias [2]. Imatinib (STI571; Fig. 1) targets the tyrosine kinase activity of Bcr-Abl and is a generally well tolerated, first-line therapy for this malignancy. In chronic phase CML, imatinib is highly effective, with the majority of patients maintaining excellent and durable haematological and cytogenetic responses, although most still harbour many residual leukaemia cells, as detected by quantitative, reverse transcription polymerase chain reaction [3]. However, patients who have progressed to advanced phases of CML frequently fail to respond to imatinib or develop resistance to therapy and relapse. This is often due to the emergence of clones expressing mutant forms of Bcr-Abl, one source of which might be a leukaemic population of non-cycling G_o "quiescent stem cells", which are not eliminated by imatinib [4]. The Bcr-Abl mutants maintain the same degree of catalytic efficiency as the natural wild-type protein, but exhibit a decreased sensitivity towards inhibition by imatinib [5]. Following the initial observation of T315I Bcr-Abl, currently over 35 such mutant forms of the enzyme have been observed in CML patients [6,7]. Consequently, despite the excellent results obtained with

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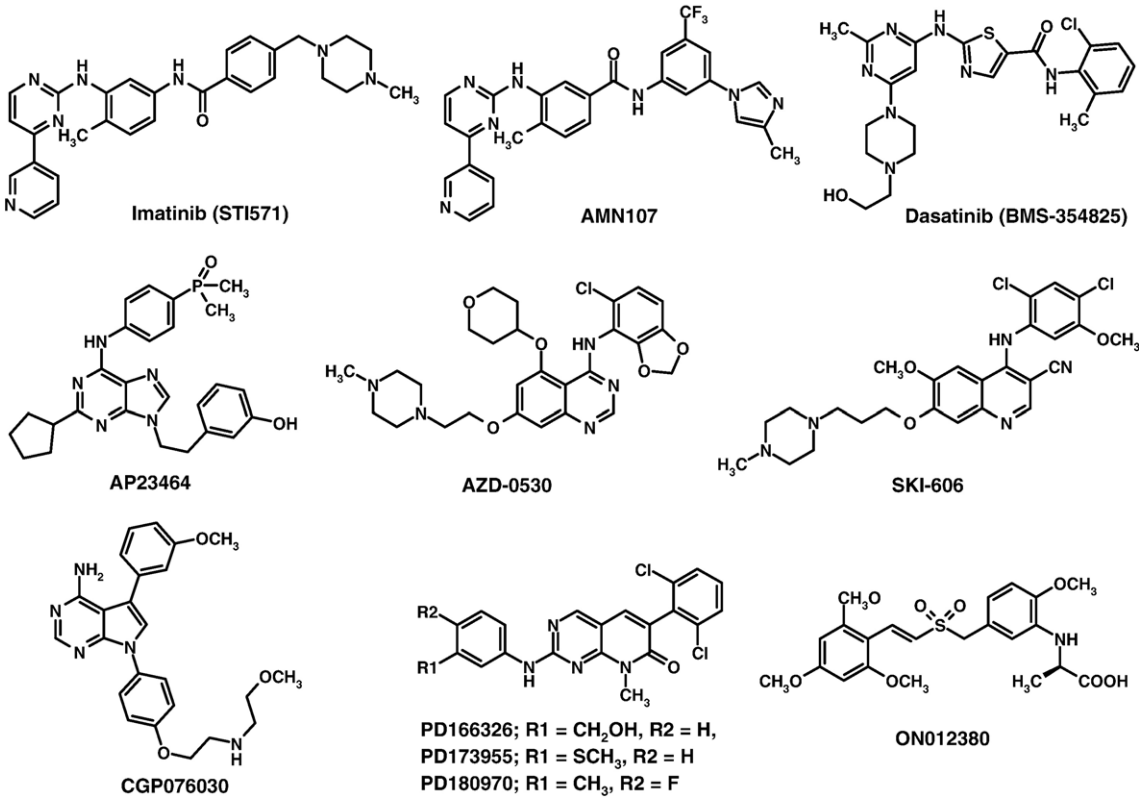

Fig. 1. Structures of selective Abl and dual Src-Abl kinase inhibitors.

imatinib in chronic phase CML, there is a need for improved therapies.

Several crystal structures of the Abl kinase domain in complex with inhibitors have been published. In most of these structures, the ligand binds to an inactive conformation of the enzyme, where the glycine-rich, P-loop folds over the ATP binding site and the activation-loop adopts a conformation in which it occludes the substrate binding site and disrupts the ATP-phosphate binding site to block the catalytic activity of the enzyme (Fig. 2) [5,8–10]. In addition, the shift of the AspPheGly (DFG)-triad, located at the *N*-terminal end of the activation-loop, results in the exposure of a supplementary binding pocket (often referred to as an allosteric-binding site) which can be utilised by inhibitors. A convincing argument that this binding mode is indeed physiologically relevant to the treatment of CML patients with imatinib is provided by the structural–biology-based rationalisation of why many Bcr-Abl mutants are less sensitive towards inhibition by imatinib [5,11,12]. This binding mode was initially considered to be an "Achilles heel" in the case of imatinib, because the leukaemic cells are capable of evolving to express mutant forms of Bcr-Abl kinase, which still bind the substrate ATP in the active conformation and phosphorylate substrate proteins, but hinder the formation of the inactive conformation to which imatinib binds. It was believed that such resistance-mutants would be less likely to arise under therapy with a drug targeting the active

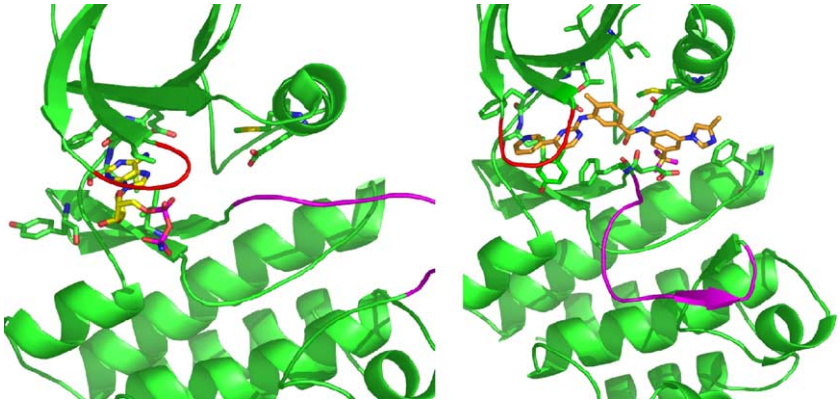

Fig. 2. Ribbon diagrams of the active conformation of the Abl kinase domain bound to ATP (left panel) and the inactive conformation bound to AMN107 (right panel), derived from x-ray crystal structures of Abl bound to tetrahydrostaurosporin and AMN107 respectively. The paths of the P-loop and the activation loop are depicted in red and magenta respectively.

conformation of the Abl kinase domain, since such modifications to the protein would have to reduce the binding affinity of the inhibitor, without affecting either ATP-binding or phosphate-transfer. However, it appears that such mutants can still arise, although they are less numerous (see Sections 2.2.1 and 2.2.4 below).

Compounds which target Bcr-Abl by binding to an active conformation, such as the pyridopyrimidine class of inhibitors (Fig. 1) [5,9], BMS-354825 [13] and staurosporin-derivatives [14] have been identified. However, since there is less sequence homology influencing the architecture of protein kinases outside of the kinase domain and a considerably greater scope for disparities between the inactive conformations between the 518 human protein kinases [15,16], many efforts to discover highly selective kinase inhibitors are being directed towards molecules which bind to the inactive conformation of the enzymes.

A crystal structure of the full length Abl kinase has revealed the presence of a regulatory myristoyl-binding site in the protein [17,18]. From a drug-discovery point of view, targeting this site is attractive since inhibitors would probably be quite selective with respect to other kinases and the potential for drug-resistance arising from mutations within the kinase domain could be circumvented. However, as yet, no inhibitors of Bcr-Abl utilising this binding site have been described.

2. New kinase-targeted therapies for CML

Currently, two classes of drug candidates are being evaluated as monotherapies in early clinical trials for the treatment of CML. These are the second generation selective Bcr-Abl kinase inhibitor, AMN107 and the dual Abl-Src inhibitors, BMS-354825, AZD05340 and SKI-606. These classes of agents are compared and discussed in the subsequent sections.

2.1. Selective Bcr-Abl inhibitors

The cellular efficacy of Bcr-Abl inhibitors can conveniently be assessed through their effects on the autophosphorylation of the full length kinase in transfected murine haematopoietic Ba/F3 cells. Whereas non-transfected murine haematopoietic Ba/F3 cells are dependent upon the interleukin-3 growth factor for survival, following transfection with Bcr-Abl they lose their interleukin-3 dependency and become Bcr-Abl dependent. Consequently, Bcr-Abl inhibition in these cells also reduces cell survival and proliferation, so that a cell proliferation assay can be employed as a measure of the efficacy of Bcr-Abl inhibition in this cell line. In the case of selective Bcr-Abl inhibitors, the effects of compounds on Bcr-Abl autophosphorylation and the proliferation of Ba/F3 cells is highly correlated, both for native Bcr-Abl and for the imatinib-resistant mutant forms of this enzyme (Fig. 3), and we have employed these assays to characterise investigational Bcr-Abl inhibitors (Table 1).

2.1.1. AMN107 (Novartis Pharma)

The phenylamino-pyrimidine derivative, AMN107 (Fig. 1) is in clinical development as a monohydrochloride salt. This molecule is structurally related to imatinib and was rationally designed based upon the crystal structure of imatinib–Abl complexes [5,9,19] together with medicinal chemistry paradigms for drug discovery. It was recognised that the shape and volume of the phenyl group and the orientation of the highly polar and basic *N*-methylpiperazine heterocycle of imatinib, which participates in H-bond interactions with both Ile360 and His361, were not optimal energetically. This promoted the search for alternatives to this pharmacophore element which relied upon lipophilic interactions within the binding site, whilst maintaining good solubility characteristics. The resulting compound, AMN107 has been characterised in vitro in cells expressing full length human kinases [20].

As a Bcr-Abl kinase inhibitor, AMN107 is in the range of 20- to 30-fold more potent than imatinib and is selective for Bcr-Abl (rank order of potency Bcr-Abl>PDGFR>Kit), whereas the latter is most potent against PDGFR kinases (rank order of potency PDGFR>Kit>Bcr-Abl; Table 1). It might have been predicted that a Bcr-Abl inhibitor, structurally-related to imatinib and interacting with the same inactive conformation of the protein with a similar buried surface area,

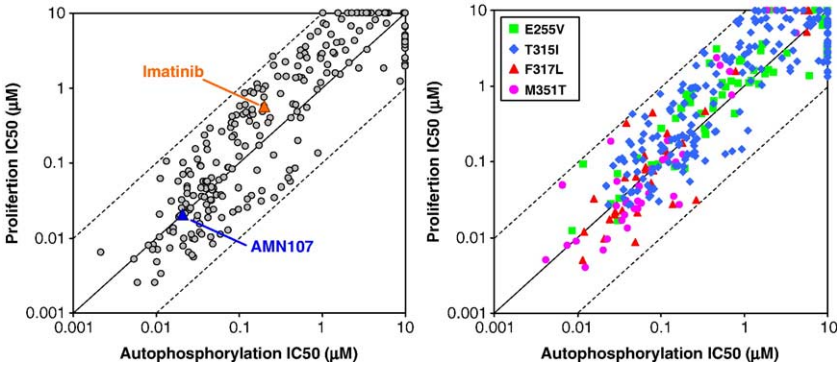

Fig. 3. Effects of Bcr-Abl inhibitors in vitro on Ba/F3 cell lines transfected to express a Bcr-Abl kinase, showing the correlations of activities against Bcr-Abl autophosphorylation and proliferation; solid lines represent line of identity, with the area within the dotted lines incorporating data pairs with up to 10-fold differences. (A) Left panel: activities of 252 compounds against p210 Bcr-Abl. (B) Right panel: comparison of effects of compounds on mutant Bcr-Abl: E255V (green squares), T315I (blue diamonds), F317L (red triangles) and M351T (magenta circles).

Table 1
Comparison of STI571, AMN107 and BMS-354825 for effects on autophosphorylation and proliferation in cells expressing native Bcr-Abl and some of the most prevalent imatinib-resistant mutant forms of the enzymes identified in patients

Kinase (Cell Type)	STI571		AMN107		BMS-354825	
	Autophosphorylation	Proliferation	Autophosphorylation	Proliferation	Autophosphorylation	Proliferation
(*wt*-32D+IL-3)	n.a	6109±393 *n*=16	n.a	6134±228 *n*=3	n.a	Not determined
(*wt*-Ba/F3+IL-3)	n.a	>7700 *n*=4	n.a	>10,000 *n*=15	n.a	>10,000 *n*=3
Bcr-Abl (p210 32D)	192±6 *n*=94	346±43 *n*=20	19±1 *n*=68	12±3 *n*=4	1.8±0.1 *n*=4	2.6±0.1 *n*=2
Bcr-Abl (p210 Ba/F3)	221±31 *n*=14	678±39 *n*=23	20±2 *n*=7	25±1 *n*=68	1.1±0.2 *n*=3	6.4±1.5 *n*=4
G250E Bcr-Abl (p185 Ba/F3)	2287±826 *n*=4	3329±1488 *n*=2	92±10 *n*=5	145±32 *n*=3	3.7±0.5 *n*=4	7.1±3.2 *n*=2
Q252H Bcr-Abl (p185 Ba/F3)	1080±119 *n*=2	851±436 *n*=2	117±25 *n=3*	67±22 *n*=4	4.3±1.2 *n*=2	1.4±0.9 *n=2*
Y253H Bcr-Abl (p185 Ba/F3)	>10,000 *n*=2	>7000 *n*=2	260±34 *n=6*	700±116 *n*=5	2.1±0.1 *n*=4	2.7±0.1 *n*=2
E255K Bcr-Abl (p210 Ba/F3)	2455±433 *n*=4	7161±970 *n*=3	153±9 *n*=4	548±72 *n*=6	6.3±0.2 *n*=3	83±20 *n*=3
E255V Bcr-Abl (p210 Ba/F3)	6353±636 n=14	6111±854 *n*=12	244±22 *n*=13	725±55 *n*=17	4.9±1.9 *n*=3	11±1 *n*=2
T315I Bcr-Abl (p210 Ba/F3)	>10,000 *n*=22	>7000 *n*=17	>10,000 *n*=48	>10,000 *n*=51	>10,000 *n*=4	>10,000 *n*=2
F317L Bcr-Abl (p210 Ba/F3)	797±92 *n*=11	1528±227 *n*=15	38±4 *n*=13	91±6.5 *n*=17	10±3 *n*=3	38±5 *n*=2
F317V Bcr-Abl (p185 Ba/F3)	544±47 *n*=3	756±38 *n*=2	95±28 *n*=3	25±1 *n*=2	95±22 *n*=3	200±2 *n*=2
M351T Bcr-Abl (p210 Ba/F3)	593±57 *n*=11	1682±233 *n*=18	29±3 *n*=13	38±4 *n*=18	3±1 *n*=2	8±1 *n*=4
F486S Bcr-Abl (p210 Ba/F3)	1238±110 *n*=11	3050±597 *n*=10	41±4 *n*=8	75±7 *n*=11	1.5±0.6 *n*=2	10±4 *n*=3
c-Kit exon 13 mut. (GIST882)	99±10 *n*=8	106±7 *n*=14	209±14 *n*=25	160±9 *n*=21	18±2 *n*=4	55±6 *n*=4
PDGFR-α/β (A31)	72±10 *n*=12	n.a.	75±6 *n*=9	n.a.	2.9±0.4 *n*=11	n.a.
PDGFR-β (Tel Ba/F3)	n.a	44±5 *n*=11	n.a	53±5 *n*=26	n.a	2.6±0.3 *n*=4

The influence of compounds on kinase autophosphorylation or cell viability was calculated as percentage-inhibition. Dose–response curves were used to calculate ED_{50} values, expressed as mean±S.E.M., *n*=number of experiments.
The influence of compounds on kinase autophosphorylation or cell viability was calculated as percentage-inhibition using the methods discussed in Section 2.1. Dose–response curves were used to calculate IC_{50} values, expressed as mean±S.E.M., *n*=number of experiments.

would be ineffective against most of the imatinib-resistant mutant forms of Bcr-Abl (Fig. 2). However, AMN107 has been found to maintain potency against both Bcr-Abl autophosphorylation and cell viability in cells expressing 32/33 mutations of Bcr-Abl [21,22]. These include the most prevalent mutants observed in relapsed patients, with the sole exception being the T315I Bcr-Abl (Table 1). This breadth of activity is probably due to an increased binding affinity to the inactive conformation of the Abl kinase domain through largely lipophilic interactions, and a less stringent requirement in the absolute shape and charge of the binding surface of the protein compared to imatinib. Imatinib relies greatly upon H-bonding interactions, which are highly directional and uses a larger surface area, which makes it very sensitive to changes in the protein surface [5]. Clearly, as in the case of imatinib, resistance against the T315I gatekeeper mutant is a consequence of the combined loss of an H-bond interaction between the aniline-NH and the threonine-O, together with a steric clash between the isoleucine-methyl group and the 2-methylphenyl phenyl group of AMN107.

The physicochemical properties of AMN107, such as basicity (pKa 5.1 and 3.9), lipophilicity (log $P_{octanol}$ 5.1) and polar surface area (97.6 $Å^2$), contribute to the molecule being well absorbed to give high plasma levels with a terminal elimination half-life of 2.1 h following oral administration to mice. Thus administration of either 20 or 75 mg/kg (10% *N*-methylpyrrolidinone–90% polyethyleneglycol 300 vehicle) to naïve mice gave mean plasma concentrations of 5.6, 5.4 and 0.4 μM and 29, 30 and 25 μM, at 2, 8 and 24 h following administration. This bioavailability is reflected in excellent efficacy in models of myeloproliferative disease [20]: In an acute model in which NOD-SCID mice were injected with murine 32D cells, harbouring the firefly luciferase gene and transfected to be dependent upon native Bcr-Abl, AMN107 (100 mg/kg q.d.) markedly reduced tumour burden, as assessed by non-invasive imaging. Furthermore, AMN107 (75 mg/kg p.o., q.d.) prolonged the survival and reduced tumour burden, as assessed by spleen weights, of Balb-c mice induced to have either parental 32D.p210 or imatinib-resistant 32D.E255V mutant Bcr-Abl driven myeloproliferative disease. AMN107 has also been evaluated in a disease model using primary haematopoietic cells, in which Balb-c mice were transplanted with bone-marrow cells transfected to express Bcr-Abl: Treated animals showed reduced morbidity and had spleen weights within the normal range. Similar, although slightly reduced efficacy was observed in mice receiving bone-marrow transplants after infection with either E255V or M351 Bcr-Abl.

AMN107 has also been shown to maintain efficacy against imatinib-resistant Bcr-Abl expressing cell lines (e.g. K562, KBM5), as well as against CML blast cells from imatinib-resistant patients, that do not express mutant forms of the kinase [23,24]. Treatment of immunodeficient mice bearing KBM5 cells with AMN107 (10, 20 or 30 mg/kg i.p.) for 20 days starting when the animals showed marked symptoms of disease (day 20), significantly increased mean animal survival times by 144%, 159% and 182%, respectively [23].

A phase I clinical trial with AMN107 has been carried out in advanced-stage CML and Ph+ ALL patients, who were either non-responsive or who had lost response to imatinib [25–27]. Dose escalation was carried out up to 1200 mg q.d., although a maximum tolerated dose was not identified with this dose regimen. From the once-daily dosing studies the apparent half-life was approximately 15 h and, with a treatment regimen of 400 mg b.i.d., mean trough drug levels were in the region of 2000 nM, representing >2-fold the IC_{50} concentration required

to inhibit all of the mutants evaluated with the exception of T315I (Table 1). In general the drug was well tolerated: Grade 3/4 adverse events included skin rash, thrombocytopenia and neutropenia. Objective haematological responses were seen in 19/30 accelerated-phase patients, eleven of whom achieved complete haematological response (CHR), one a cytogenetic bone-marrow response and seven patients returned to chronic phase. Of the 11 patients with CHR, six achieved a cytogenetic response. In 18 patients in myeloid or lymphoid blast phase disease, there were 10 objective responses with 4 patients reaching CHR, 6 patients reverting to chronic phase and 2 patients achieving a cytogenetic response. Mutational data available from 49 accelerated and myeloid blast crisis CML patients showed 12 mutations in 20 patients: G250E (5); G250E+F317L (1), M351T (4); E355G (2); M244V/T315I, Y253F, F311L, F317L, F359V, H396P, H396R and E459Q (1 patient each), and 15 of these 20 patients responded to treatment [26]. Phase II studies with this drug at a dose of 400 mg b.i.d. started in April 2005.

2.2. Dual Abl-Src inhibitors

Members of the Src family of kinases are important mediators of downstream signalling from cell-surface receptors and regulate cell migration, adhesion, growth differentiation and survival, all of which are important for tumour cells. Whereas Src is normally highly regulated, it is deregulated and activated in several human tumour types and this has been linked to both tumour progression and the formation of metastases [28]. Some relevance of Src family kinases to leukaemia is suggested by the observation that many of these kinases are expressed in haematopoietic cells (Blk, Fgr, Fyn, Hck, Lck, Lyn, c-Src and Yes). In addition, Bcr-Abl has been shown to be capable of activating Src kinases both through phosphorylation and merely by binding Src proteins. Furthermore, cell lysates from imatinib-resistant patients have been found to over-express Lyn kinase, and the proliferation of human CML K562 cells selected for resistance to imatinib, which also over-express Lyn, is inhibited by the Abl/Src inhibitor, PD180970 (see Section 2.2.4) [29]. However, despite the findings that Src family kinases probably contribute to the survival and proliferation of Bcr-Abl expressing immortalised myeloid cells in vitro, findings in animal models of leukaemia suggest that whereas they appear to play a role in ALL, they may not be important in CML [30]. Since Src family kinases regulate downstream elements of the Bcr-Abl signalling cascade, inhibition of these enzymes could therefore provide synergy with Bcr-Abl inhibition and potentially counteracting the availability of alternative survival pathways which CML cells could utilise in the face of Bcr-Abl inhibition. Consequently, it cannot be ruled out that the combined inhibition of the tyrosine kinase activity of both Abl and Src-family kinases might have an advantage over purely Abl inhibition for the treatment of CML, as well as ALL [31]. Therapy with combined Bcr-Abl and Src-family kinase inhibitors might also therefore counteract the oncogenic potential of drug-resistant mutant forms of Bcr-Abl in CML and/or ALL.

One of the first potent dual Abl/Src kinase inhibitors was the pyrrolopyrimidine CGP073060 (Fig. 1), which emerged from a Src kinase programme directed towards compounds for the treatment of osteoporosis [30–32], although this series of compounds was not optimised for use in leukaemia. However, the considerable sequence homology between the Abl and Src-family tyrosine kinases, readily enables molecules to possess cross-reactivity, particularly when they target the active conformation of the kinase domains of these enzymes [33] and a number of other structural classes of dual-inhibitors have been identified, the most advanced and thoroughly studied of these are discussed below.

2.2.1. BMS-354825 (Dasatinib; BristolMyersSquibb)

The most advanced dual Bcr-Abl/Src inhibitor is the thiazolylamino-pyrimidine, BMS-354825, developed as the hydrochloride salt, which is currently in Phase II clinical trials in patients with imatinib-resistant CML (Fig. 1) [13]. This drug emerged from a programme directed towards immunosuppressant drugs and, in addition to inhibiting the Src family kinases: Fyn (IC_{50} 0.2 nM), Lck (IC_{50} 1.1 nM), Src (IC_{50} 0.55 nM), Yes (IC_{50} 0.41 nM), it potently inhibits Abl (IC_{50} <1 nM), c-Kit (IC_{50} 13 nM), PDGFRβ (IC_{50} 28 nM), EPHA2 (IC_{50} 17 nM), HER1 (IC_{50} 180 nM) and p38 MAP (IC_{50} 100 nM) kinases [13,34].

In cellular assays, BMS-354825 inhibits the proliferation of Bcr-Abl transfected Ba/F3 cells and human Bcr-Abl expressing K562 cells with IC_{50} values of 1.3 and <1 nM, respectively, and the compound maintains high potency against a wide range of Bcr-Abl mutants [22,34,35], although, again like AMN107, with the exception of T315I (Table 1). This pattern of activity is explained by an x-ray crystal structure of a complex between BMS-354825 and the Abl kinase domain, in which the inhibitor binds to an active conformation of the enzyme [13]. This binding mode resembles that originally postulated for imatinib [36], where the aminothiazole pharmacophore element (corresponding to the aminopyrimidine in imatinib) makes a bidentate H-bonding interaction with the backbone C═O and NH of Met318 and the amide-NH makes an H-bond with the side-chain oxygen of Thr315. As can be seen from Fig. 1, BMS-354825 has some structural elements in common with AMN107, in particular the juxtaposition of the aminopyrimidine and the carboxamide groups. However, although it is theoretically possible that BMS-354825 can bind to the inactive as well as to the active conformations of Abl, which could contribute to the potency of the compound, as yet there is no experimental evidence to support this.

A consequence of the inhibition of the Src family kinases, is that BMS-354825 also inhibits the proliferation of a wide-range of human tumour cell lines which do not express Bcr-Abl, such as PC-3 (prostate; IC_{50} 5–9 nM), MDA-MB-211 (breast; IC_{50} 10–12 nM) and WiDr (colorectal; IC_{50} 38–52 nM) cells [13,34].

Assuming that human and murine Ba/F3 Src kinases respond similarly to Src kinase inhibitors, overall, the data suggest that Src family kinases might not be essential for the oncogenic activity of Bcr-Abl, otherwise BMS-354825 should be effective as an anti-proliferative agent in T315I Bcr-Abl Ba/

F3 cells. The role of Src family kinases as an important downstream Bcr-Abl signalling element also comes into question from the results of an experimental saturation mutagenesis study with BMS-354825 using the protocol employed to study imatinib-resistance. In the case of the original study, Azam and co-workers identified resistant clones expressing over 90 mutant forms of Bcr-Abl, which included most of those observed in patients [11]. For BMS-354825 (50 nM), resistant clones emerged expressing 10 mutant forms of Bcr-Abl, with the most frequently occurring mutants being F317V> T315A>T315I>F317L [37]. Although most of these amino-acid residues make contact with BMS-354825 in the crystal structure of the molecule bound to the active conformation of the Abl kinase domain, this does not unambiguously confirm the concept that this binding mode is physiologically relevant, since these residues would also be in contact with the inhibitor in the inactive conformation. The only mutants observed which did not represent contact residues were E255K and Q252H, both of which have been found in imatinib-resistant patients [7]. In the case of imatinib, mutants of the Glu255 residue have been rationalised on the basis of the destabilisation of the inactive conformation required for binding [5]. This residue also has a role in stabilising the active conformation of the P-loop, but other side chains, such as lysine or valine could also perform this task. It is difficult to explain why mutations of the Gln252 and Glu255 residues arose in the BMS-354825 resistance scan because the conformation of the P-loop, which is partially disordered in the crystal structure, does not appear to be important for the binding of this inhibitor [13,37]. It seems unlikely that these mutations could cause a change in the structure of the P-loop to impede binding of BMS-354825, without disturbing the binding of ATP. When considering the potential role of Src for the survival of Bcr-Abl expressing cells, it is surprising that these Bcr-Abl dependent sub-clones emerged. Compelling evidence for an important role of Src family kinase inhibition would be the emergence of BMS-354825-resistant Src family kinase mutants, but as yet no studies have been reported directed towards this question.

Following oral administration to mice, BMS-354825 (10 mg/kg) significantly prevented disease progression and prolonged survival in a murine myeloproliferative disease model driven by either native or M351T Bcr-Abl transformed haematopoietic cells [35]. Similarly, consistent with the its activity in cell lines, BMS-354825 (30 mg/kg b.i.d. 5/7 d) has been shown to inhibit the growth of human breast (KPL4 and HCC70), prostate (PC-3 and Pat-27), small cell lung cancer (H69 and H526) and colon (WiDr and LoVo) tumour xenograft models [34], which has supported the development of the compound for the treatment of solid tumours.

The results of phase I dose-escalating studies (15–180 mg administered as single or divided oral doses for 5 days followed by a 2-day break, every week) in chronic, accelerated and blast phase CML patients (36, 8 and 21, respectively), who had haematological progression or who were intolerant to imatinib have been reported [38–43]. Within 2 h of oral administration of the higher doses, the compound gave plasma concentrations in the range of 100–200 nM and a terminal half-life of about 5 h. BMS-354825 displayed significant efficacy in the chronic phase of the disease, with 31/36 patients achieving a CHR (86%, of which 31% were major cytogenetic responses) and 3 showing partial responses. Response rates were also good in the accelerated and blast phase groups of patients, with haematological responses achieved in 75% and 76% of patients, respectively, and of 28 blast phase patients for whom data was available, 8 demonstrated a major cytogenetic benefit. Of the patients expressing imatinib-resistant mutants, complete haematological remissions have been observed in most cases, with the exception of those expressing T315I (5, of which in 2 cases it emerged under treatment), F317L (1) and D276G (1) [42]. Grade 4 adverse events observed in these studies included thrombocytopoenia, gastrointestinal bleeding and tumour lysis syndrome, although no patients were reported to have discontinued treatment as a result of these. Additional side-effects included arthralgia (joint pain), pyrexia, fatigue, peripheral and pulmonary oedema, headache and diarrhoea, and mild prolongation of the QT interval. Despite the potent activity of BMS-354825 against Src family kinases, no effects were seen on the ability of activated T-cells, obtained from chronic phase CML patients during treatment, to synthesise IL-2, IL-10, Inf-γ or TNF-α cytokines [43].

BMS-354825 is currently being evaluated in Phase II trials with a standard dose of 70 mg b.i.d. in imatinib-resistant, chronic phase, accelerated phase and myeloid and lymphoid blast crisis.

2.2.2. AZD0530 (AstraZeneca)

Another structural type of dual Abl-Src inhibitors is represented by AZD0530, based upon the quinazoline scaffold (Fig. 1) [44]. Unlike other kinase inhibitors in this structural class, such as the HER inhibitor iressa (ZD1839) and the VEGFR inhibitor ZD6474, AZD0530 incorporates a 5-substituent in the quinazoline ring, which imparts excellent selectivity towards Src family kinases (IC_{50} values 1–5 nM), but maintains activity against Abl (IC_{50} value of 30 nM). The profile of this compound against Bcr-Abl kinase resistance mutants has not yet been reported. Efficacy related to Src activity has been shown for AZD0530 in inhibition of the migration of human breast MDA-MB231 cells and of osteoclast bone resorption in culture [45,46]. In rat models of both pancreatic (L3.6pl) and bladder NBT-II) cancer, AZD0530 (10–25 mg/kg) inhibited the formation of metastases without affecting the growth of the primary tumour and, prevented the growth of subcutaneously transplanted Y530F Src-transfected NIH-3T3 xenografts [44].

In Phase I dose-escalating clinical trials, carried out in healthy volunteers with the fumarate salt of AZD0530, the drug was well absorbed following oral administration to give mean trough plasma levels approaching 1 μM with a terminal half-life of ≈40 h [47]. The drug was generally well tolerated at doses up to 250 mg, with mild maculopapular facial/thoracic rash, influenza-like syndrome, diarrhoea and muscle pain were the most frequently reported adverse events, although at higher

doses elevated C-reactive protein and elevated plasma creatinine was reported [48].

2.2.3. SKI-606 (Wyeth)

A dual Bcr-Abl/Src inhibitor, SKI-606 (Fig. 1), based upon a quinoline scaffold, structurally related to the AstraZeneca quinazoline template, has been developed by Wyeth [49,50]. This compound inhibits c-Abl and c-Src transphosphorylation with IC_{50} values of <10 nM, and inhibits the proliferation of K562, KU812 and MEG-01 CML cell lines with IC_{50} values of 20, 5 and 20 nM, respectively. In imatinib-resistant K-562 cells [29], like PD180970 (see Section 2.2), SKI-606 has been shown to inhibit both Bcr-Abl and Lyn phosphorylation and, as a result of the latter activity also inhibited cell proliferation [51]. As well as possessing antiproliferative and cytostatic (accumulation in G1/S phase) effects in K562, MK2 and LAMA84 cells, associated with activation of caspase-9, SKI-606 (0.1–10 μM) induced G1 arrest and enhanced apoptosis in CD34+ cells isolated from blast crisis CML patients, including those harbouring Y253H, E255V, E255K or F359V mutant BCR-ABL [52]. Although this compound has not been evaluated in myeloproliferative disease models, it has been shown to inhibit the growth of K562 xenografts in nude mice, with oral doses above 50 mg/kg/day causing tumour regression and animals remaining tumour-free for 40 days following 75 mg/kg twice a day for 10 days [50]. A Clinical Phase I dose-escalation study to evaluate the safety and tolerability of oral SKI-606 in subjects with advanced malignant solid tumours started during 2004.

2.2.4. Pyrido[2,3]pyrimidines (PD166326, PD173955 and PD180970)

Among the first compounds to be described which potently inhibited both Bcr-Abl and the Src kinases were those based upon the pyrido[2,3-d]pyrimidine scaffold. To date the best characterised compounds from this class are PD166326, PD173955 and PD180970, which inhibit Abl, Src and Lck catalysed transphosphorylation in cell-free assays with IC_{50} values <50 nM (Fig. 1) [53–57]. This activity translates into activity in cellular assays, where the three compounds potently inhibit Bcr-Abl autophosphorylation [55–60]. In the case of PD180970, inhibition of Bcr-Abl autophosphorylation in K562 cells (IC_{50} 170 nM) has been shown to extend to the inhibition of Gab-2 (IC_{50} 80 nM) and Crkl (IC_{50} 80 nM) phosphorylation, both of which are downstream elements in the Bcr-Abl signalling pathway [55]. PD180970 has also been shown to inhibit Src-mediated phosphorylation of STAT3 (A431 cervical carcinoma cells) and paxillin (HT-29 colon carcinoma cells [54]. In terms of cell viability, the three pyridopyrimidines have been shown to potently inhibit the proliferation of Bcr-Abl dependent cell lines (K562, R10, RWLeu4 and transformed Ba/F3), to cause cell accumulation in G1 phase and to induce apoptosis [55–57,59–61]. The compounds were substantially less efficacious at concentrations below 200 nM in cell lines not expressing Bcr-Abl, from solid tumours (breast, colon, Ewing's sarcoma, glioblastoma, lung, neoblastoma, ovarian, prostate), as well as HL-60 leukaemia cells and untransformed parental Ba/F3 cells [54,58,59,61].

Although the compounds maintain potency against many imatinib-resistant mutant forms of Bcr-Abl in cells, they do not inhibit the refractory T315I Bcr-Abl [56–60]. In addition to the activity against imatinib-resistant cells referred to in Section 2.2 [29], PD180970 has also been shown to induce apoptosis in other Bcr-Abl expressing cell lines rendered resistant to imatinib by a continuous selection pressure of sub-lethal imatinib concentrations [62].

As rationalised by homology modelling and consistent with the x-ray crystal structures of PD173955 and PD180970 in complex with Abl, the activity of these compounds probably results from their interacting with the ATP-binding site of the active conformations of the kinase domains [5, 10]. This binding mode appears to permit this class of compounds to be quite indiscriminate in their enzymatic profiles, as the pyridopyrimidines also potently inhibit the EGF, EphB4, FGF, c-Kit, PDGF and VEGF receptor tyrosine kinases, as well as serine/threonine kinases possessing a threonine "gatekeeper" residue, such as p38, Raf, RICK and GAK [63].

A cell-based screen to identify drug-resistant mutations in Bcr-Abl, simpler to that employed for imatinib and BMS-354826 [11,37], has been developed by Duyster and co-workers, and applied to evaluate the mutation pattern for PD166326 [60]. In this study resistant colonies appeared with a lower frequency for PD166325 than for imatinib, and point mutations in the Abl kinase domain were more frequently detected (41% of colonies compared to 25% for imatinib). In all 14 point mutations were observed affecting 9 amino-acid residues, with the most common being G250E, Y253H, E255K(V), T315I, F317L and E355G, however, the kinase activity of these mutants could be inhibited by PD166326 at concentrations $\leq$500 nM, with the exception of T315I.

The most potent of these three Bcr-Abl inhibitors, PD166326, has recently been evaluated in a CML disease model, where mice were reconstituted with bone-marrow cells transfected to express Bcr-Abl [64]: In naïve mice, although oral administration of 50 mg/kg (suspension in 10% aqueous DMSO) only gave mean plasma levels of 98±15 nM at 2 h, with an estimated half-life of 8.4 h, this dose markedly suppressed Bcr-Abl autophosphorylation in peripheral blood cells taken 2–3 h after drug administration to animals having established disease. Following treatment with 50 mg/kg b.i.d. (maximum tolerated dose, initiated 10 days after inoculation) with p210 Bcr-Abl expressing cells, 10/10 animals survived for the duration of the experiment (33–37 days) and had a marked reduction in splenomegaly upon necropsy (mean weight 78 mg), compared to vehicle-treated mice who had a mean survival of 22 days and mean spleen weights of 670 mg. PD166326 (25 mg/kg b.i.d) also prolonged the survival of mice with disease induced by the H396P or M351T mutant forms of Bcr-Abl, although the compound was ineffective against T315I Bcr-Abl induced disease.

Whereas the pyrimidopyrimidines evaluated thus far have limited solubility, together with pharmacokinetic and tolerabil-

ity profiles which are unattractive for drug development, new compounds from this class designed to overcome these drawbacks could have clinical potential.

2.2.5. AP23464 (Ariad Pharmaceuticals)

Researchers at Ariad have elaborated a series of compounds based upon the purvanalol–purine template for combined Abl/Src inhibition [65]. A lead compound AP23464 (Fig. 1) inhibits the transphosphorylation activity of both enzymes with low nanomolar potency. In Ba/F3 cells transfected with either native, Q252H, Y253F, E255K, M351H or H396P Bcr-Abl, this compound inhibited both Bcr-Abl autophosphorylation and cell proliferation with IC_{50} values in the range of 8–94 nM [66]. AP23464 had no effect on either parental Ba/F3 cells or those expressing T315I Bcr-Abl at concentrations <5000 nM. X-ray crystallography of a complex between the kinase domain of Src and AP23464 revealed that it bound an active conformation of the enzyme, as has also been predicted for Abl using homology modelling [66]. These binding modes are consistent with the selectivity profile of the compound, as it potently inhibits a wide range of kinases, including HER-1, EphB4, Kit, PDGF, Ret and VEGF receptor kinases [67]. AP23464 also inhibits the D816V, D816Y and D816F activation-loop mutant forms of c-Kit expressed in human mastocytoma cells and a close analogue AP23848 has demonstrated anti-tumour activity in murine Kit-driven xenograft model following 100 mg/kg t.i.d. [68]. Compounds related to AP23464 and AP23848 with superior pharmacokinetic properties are being developed.

2.3. Other compounds

ON012380 (Fig. 1) has recently been reported as a non-ATP competitive inhibitor of Bcr-Abl, which potently inhibits the proliferation of cells expressing p210 and many imatinib-resistant forms of Bcr-Abl [69]. The authors postulate that the compound acts via binding the substrate binding-site of Bcr-Abl, although selectivity over other kinases has not yet been demonstrated for this compound.

3. Conclusions

The understanding of the structural biology of imatinib-resistant Bcr-Abl mutants has lead pharmaceutical research to the brink of a new advance in the treatment of Bcr-Abl dependent leukaemias. Only 3 years after the identification of imatinib-resistant Bcr-Abl mutants in patients, two new compounds which inhibit such mutants have already entered clinical studies. Although clinical candidates which address the currently intractable T315I mutant have not yet been identified, such drugs are likely to be discovered in the near future. With the advent of new drugs possessing different inhibitory profiles against the known spectrum of Bcr-Abl mutants, it will be important to identify which drug is most appropriate for each patient and therefore diagnostic methods to routinely genotype patient leukaemic cells will be required.

Although Bcr-Abl inhibitors are becoming increasingly more potent and more effective, eradicating CML from patients by drug therapy is still not on the immediate horizon, due to the need to eradicate 100% of a total tumour cell burden, typically in the range of 10^{12} cells, some of which might be quiescent or reside in tissues poorly accessible to the drugs [1,4]. As to whether combined inhibitors of the Bcr-Abl and Src kinases will possess advantages over selective Bcr-Abl inhibitors, this is still an open question. It is however likely that CML will continue to be a disease which can be well managed with chronic drug treatment. However, in the case of late stage disease, where additional abnormalities have developed resulting in genomic instability, as in the case of blast crisis CML and Philadelphia positive ALL the most effective therapy is likely to remain as stem cell transplantation, although combination regimens of Bcr-Abl inhibitors and, other treatment modalities, such as inhibitors of the phosphatidylinositol 3-kinase pathway [70], of farnesyl transferase [71], of histone deacetylase [72] or of Heat Shock Protein-90 [73], might emerge as being of benefit.

The new lessons learned from the treatment of CML will probably also be of value in refining the treatment of other malignancies such as imatinib-resistant hypereosinophilic syndrome [74] and in lung cancer, where the emergence of a mutant form of the EGFR kinase has recently been observed to lead to acquired resistance during gefitinib (iressa; AstraZeneca) and erlotinib (tarceva; Roche/OSI) therapy [75].

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References

[1] R. Ren, Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia, Nat. Rev., Cancer 5 (2005) 172–183.
[2] S. Faderl, G. Garcia-Manero, D.A. Thomas, H.M. Kantarjian, Philadelphia chromosome-positive acute lymphoblastic leukemia—current concepts and future perspectives, Rev. Clin. Exp. Haematol. 6 (2002) 142–160.
[3] F. Guilhot, Sustained durability of responses plus high rates of cytogenetic responses result in long-term benefit for newly diagnosed chronic-phase chronic myeloid leukemia (CML-CP) treated with imatinib (IM) therapy: update from the IRIS study, Blood 104 (2004) 10a, (Abstr.21).
[4] T.L. Holyoake, X. Jiang, H.G. Jorgensen, S. Graham, M.J. Alcorn, C. Laird, A.C. Eaves, C.J. Eaves, Primitive quiescent leukemic cells from patients with chronic myeloid leukemia spontaneously initiate factor-independent growth in vitro in association with up-regulation of expression of interleukin-3, Blood 97 (2001) 720–728.
[5] S.W. Cowan-Jacob, V. Guez, J.D. Griffin, D. Fabbro, G. Fendrich, P. Furet, J. Liebetanz, J. Mestan, P.W. Manley, Bcr-Abl kinase mutations and drug resistance to imatinib (STI571) in chronic myelogenous leukemia, Mini Rev. Med. Chem. 4 (2004) 285–299.
[6] M.E. Gorre, M. Mohammed, K. Ellwood, N. Hsu, R. Paquette, P.N. Rao, C.L. Sawyers, Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification, Science 293 (2001) 876–880.

[7] A. Hochhaus, P. La Rosée, Imatinib therapy in chronic myelogenous leukaemia: strategies to overcome and avoid resistance, Leukaemia 18 (2004) 1321–1331.
[8] T. Schindler, W. Bornmann, P. Pellicena, W.T. Miller, B. Clarkson, J. Kuriyan, Structural mechanism for STI-571 of Abelson tyrosine kinase, Science 289 (2000) 1938–1942.
[9] P.W. Manley, S.W. Cowan-Jacob, E. Buchdunger, D. Fabbro, G. Fendrich, P. Furet, T. Meyer, J. Zimmermann, Imatinib: a selective tyrosine kinase inhibitor, Eur. J. Cancer 38 (2002) S19–S27.
[10] B. Nagar, W.G. Bornmann, P. Pellicena, T. Schindler, D.R. Veach, W.T. Miller, B. Clarkson, J. Kuriyan, Crystal structures of the kinase domain of c-Abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571), Cancer Res. 62 (2002) 4236–4243.
[11] M. Azam, R.R. Latek, G.O. Daley, Mechanisms of autoinhibition and STI-571/imatinib resistance revealed by mutagenesis of BCR-ABL, Cell 112 (2003) 831–843.
[12] V. Nardi, M. Azam, G.O. Daley, Mechanisms and implications of imatinib resistance mutations in BCR-ABL, Curr. Opin. Hematol. 11 (2003) 35–43.
[13] L.J. Lombardo, F.Y. Lee, P. Chen, D. Norris, J.C. Barrish, K. Behnia, S. Castaneda, L.A.M. Cornelius, J. Das, A.M. Doweyko, C. Fairchild, J.T. Hunt, I. Inigo, K. Johnston, A. Kamath, D. Kan, H. Klei, P. Marathe, S. Pang, R. Peterson, S. Pitt, G.L. Schieven, R.J. Schmidt, J. Tokarski, M.-L. Wen, J. Wityak, R.M. Borzilleri, Discovery of *N*-(2-chloro-6-methylphenyl)-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 anti-tumor activity in preclinical assays, J. Med. Chem. 47 (2004) 6658–6661.
[14] A. Klon, manuscript in preparation.
[15] M. Huse, J. Kuriyan, The conformational plasticity of protein kinases, Cell 109 (2002) 275–282.
[16] G. Manning, D.B. Whyte, R. Martinez, T. Hunter, S. Sudarsanam, The protein kinase complement of the human genome, Science 298 (2002) 1912–1916.
[17] O. Hantschel, B. Nagar, S. Guettler, J. Kretzschmar, K. Dorey, J. Kuriyan, G. Superti-Furga, A myristoyl/phosphotyrosine switch regulates c-Abl, Cell 112 (2003) 845–857.
[18] B. Nagar, O. Hantschel, M.A. Young, K. Scheffzek, D. Veach, W. Bornmann, B. Clarkson, G. Superti-Furga, J. Kuriyan, Structural basis for the autoinhibition of c-Abl tyrosine kinase, Cell 112 (2003) 859–871.
[19] T. Schindler, W. Bornmann, P. Pellicena, W.T. Miller, B. Clarkson, J. Kuriyan, Structural mechanism for STI-571 inhibition of Abelson tyrosine kinase, Science 289 (2000) 1938–1942.
[20] E. Weisberg, P.W. Manley, W. Breitenstein, J. Brüggen, L. Callahan, S.W. Cowan-Jacob, D. Fabbro, G. Fendrich, E. Hall-Meyers, B. Huntley, A. Kung, J. Mestan, D. Neuberg, A. Ray, D.G. Gilliland, J.D. Griffin, AMN107: characterization of a novel inhibitor of both wild-type and imatinib-resistant mutant Bcr-Abl in vitro and in murine models of leukemia, Cancer Cell 7 (2005) 129–141.
[21] P.W. Manley, J. Mestan, S. Cowan-Jacob, M. Wartmann, E. Weisberg, D. Fabbro, D.G. Gillil, J.D. Griffin, AMN107: inhibitory profile against wild-type and mutant forms of the Bcr-Abl tyrosine kinase, Proc. Am. Assoc. Cancer Res. 46 (2005) 1408, (Abst. 5985).
[22] T. O'Hare, D.K. Walters, E.P. Stoffregen, T. Jia, P.W. Manley, J. Mestan, S. Cowan-Jacob, M.C. Heinrich, M.W.N. Deininger, B.J. Druker, In vitro activity of Bcr-Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants, Cancer Res. 65 (2005) 4500–4505.
[23] M. Golemovic, S. Verstovsek, F. Giles, J. Cortes, T. Manshouri, P.W. Manley, J. Mestan, M. Dugan, L. Alland, J.D. Griffin, R.B. Arlinghaus, T. Sun, H. Kantarjian, M. Beran, AMN107, a novel aminopyrimidine inhibitor of Bcr-Abl, has in vitro activity against imatinib-resistant chronic myeloid leukemia, Clin. Cancer Res. 11 (2005) 4941–4947.
[24] G. Martinelli, A.M. Martelli, G. Rosti, T. Grafone, I. Mantovani, A. Cappellini, P.L. Tazzari, E. Ottaviani, M. Renzulli, M. Amabile, A. Poerio, S. Soverini, C. Terragna, C. Taccioli, S. Bassi1, M. Bacccarani, A new Abl kinase inhibitor (AMN107) has in vitro activity on CML Ph+Blast cells resistant to imatinib, Blood 104 (2005) 255b, (Abstr.4687).
[25] F. Giles, H. Kantarjian, B. Wassmann, J. Cortes, S. O'Brien, C. Tanaka, P. Rae, W. Mietlowski, A. Romano, L. Alland, M. Dugan, M. Albitar, O. Ottmann, A phase I/II study of AMN107, a novel aminopyrimidine inhibitor of Bcr-Abl, on a continuous daily dosing schedule in adult patients (pts) with imatinib-resistant advanced phase chronic myeloid leukemia (CML) or relapsed/refractory Philadelphia chromosome (Ph+) acute lymphocytic leukemia (ALL), Blood 104 (2004) 10a, (Abstr.22).
[26] H. Kantarjian, O. Ottmann, J. Cortes, B. Wassmann, D. Jones, A. Hochhaus, L. Alland, M. Dugan, M. Albitar, F. Giles, AMN107, a novel aminopyrimidine inhibitor of Bcr-Abl, has significant activity in imatinib-resistant bcr-abl positive chronic myeloid leukemia (CML), J. Clin. Oncol. 23 (2005) 195s (Abstr. 3014).
[27] O. Ottmann, F. Giles, B. Wassmann, A. Hochhaus, P. Rae, M. Beran, M. Albitar, L. Alland, M. Dugan, H. Kantarjian, Activity of AMN107, a novel aminopyrimidine inhibitor of Bcr-Abl, in imatinib-resistant bcr-abl positive lymphoid malignancies, J. Clin. Oncol. 23 (2005) 195s (Abstr. 3015).
[28] M.V. Yezhelyev, G. Koehl, M. Guba, K.W. Jauch, A. Ryan, A. Barge, T. Green, M. Fennell, C.J. Bruns, Inhibition of Src tyrosine kinase as a treatment for human pancreatic cancer growing orthotopically in nude mice, Clin. Cancer Res. 10 (2004) 8028–8036.
[29] N.J. Donato, J.Y. Wu, J. Stapley, G. Gallick, H. Lin, R. Arlinghaus, M. Talpaz, BCR-ABL independence and LYN kinase overexpression in chronic myelogenous leukemia cells selected for resistance to STI571, Blood 101 (2000) 690–698.
[30] Y. Hu, Y. Liu, S. Pelletier, E. Buchdunger, M. Warmuth, D. Fabbro, M. Hallek, R.A. van Etten, S. Li, Requirement of Src kinases Lyn, Hck and Fgr for *BCR-ABL1*-induced B; lymphoblastic leukemia but not chronic myeloid leukemia, Nat. Genet. 36 (2004) 453–461.
[31] M. Warmuth, N. Simon, O. Mitina, R. Mathes, D. Fabbro, P.W. Manley, E. Buchdunger, K. Forster, I. Moarefi, M. Hallek, Dual-specific Src and Abl kinase inhibitors, PP1 and CGP76030, inhibit growth and survival of cells expressing STI571 resistant Bcr/Abl kinases, Blood 101 (2003) 664–672.
[32] M. Missbach, M. Jeschke, J. Feyen, K. Müller, M. Glatt, J. Green, M. Šuša, 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 24 (1999) 437–449.
[33] S.W. Cowan-Jacob, G. Fendrich, P.W. Manley, W. Jahnke, D. Fabbro, J. Liebetanz, T. Meyer, The crystal structure of a c-Src complex in an active conformation suggests possible steps in c-Src activation, Structure 13 (2005) 861–871.
[34] F.Y. Lee, L. Lombardo, A. Camuso, S. Castaneda, K. Fager, C. Flefleh, I. Inigo, K. Johnson, D. Kan, R. Luo, K. McGlinchey, S. Pang, R. Peterson, M. Wen, R. Wild, C. Fairchild, T. Wong, R. Borzilleri, R. Kramer, BMS-354825 potently inhibits multiple selected oncogenic tyrosine kinases and possesses broad spectrum anti-tumor activities in vitro and in vivo, Proc. Am. Assoc. Cancer Res. 46 (2005) 159 (Abst. 675).
[35] N.P. Shah, C. Tran, F.Y. Lee, P. Chen, D. Norris, C.L. Sawyers, Overriding imatinib resistance with a novel Abl kinase inhibitor, Science 305 (2004) 399–401.
[36] J. Zimmermann, P. Furet, E. Buchdunger, in: I. Ojima, G. Vite, K. Altmann (Eds.), Anticancer Agents: Frontiers in Cancer Chemotherapy, ACS Symposium Series, vol. 796, American Chemical Society, Washington, DC, 2001, pp. 245–259.
[37] M.R. Burgess, B.J. Skaggs, N.P. Shah, F.Y. Lee, C.L. Sawyers, Comparative analysis of two clinically active BCR-ABL kinase inhibitors reveals the role of conformation-specific binding in resistance, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 3395–3400.
[38] C.L. Sawyers, N.P. Shah, H.M. Kantarjian, N. Donato, J. Nicoll, S.A. Bai, F. Huang, E. Clark, A.P. DeCillis, M. Talpaz, 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 104 (2004) 4a, (Abstr.1).
[39] M. Talpaz, H. Kantarjian, N.P. Shah, N. Donato, J. Nicoll, S.A. Bai, F. Huang, E. Clark, A.P. DeCillis, C. Sawyers, 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 104 (2004) 10a, (Abstr.20).

[40] M. Talpaz, H.M. Kantarjian, R. Paquette, N. Shah, J. Cortes, J. Nicoll, S.A. Bai, F. Huang, A.P. Decillis, C.L. Sawyers, A Phase I Study of BMS-354825 in patients with imatinib-resistant and intolerant chronic phase chronic myeloid leukemia (CML): results from CA180002, J. Clin. Oncol. 23 (2005) 564s (Abstr. 6519).

[41] C.L. Sawyers, N.P. Shah, H.M. Kantarjian, J. Cortes, R. Paquette, J. Nicoll, S.A. Bai, E. Clark, A.P. Decillis, M. Talpaz, A Phase I Study of BMS-354825 in patients with imatinib-resistant and intolerant accelerated and blast phase chronic myeloid leukemia (CML): results from CA180002, J. Clin. Oncol. 23 (2005) 565s (Abstr. 6520).

[42] N. Shah, C.L. Sawyers, H.M. Kantarjian, N. Donato, J. Nicoll, J. Cortes, R. Paquette, F. Huang, E. Clark, M. Talpaz, Correlation of clinical response to BMS-354825 with BCR-ABL mutation status in imatinib-resistant patients with and chronic myeloid leukemia (CML) and philadelphia chromosome associated acute lymphoblastic leukemia (Ph+ALL), J. Clin. Oncol. 23 (2005) 565s (Abstr. 6521).

[43] H. Gao, M. Talpaz, B.N. Lee, N. Donato, J.E. Cortes, H.M. Kantarjian, J.M. Reuben, BMS-354825 induced complete hematologic remission in chronic phase CML patients without affecting T-cell cytokine production, J. Clin. Oncol. 23 (2005) 589s (Abstr. 6619).

[44] L.F. Hennequin, J. Allen, G.F. Costello, M. Fennell, T.P. Green, V. Jacobs, R. Mortengin, A. Olivier, P.A. Ple, The discovery of AZD0530: a novel, oral, highly selective and dual-specific inhibitor of the Src and Abl family kinases, Proc. Am. Assoc. Cancer Res. 46 (2005) 595 (Abst. 2537).

[45] S. Hiscox, D. Barrow, T. Green, R. Nicholson, Adhesion-independent focal adhesion kinase activation involves Src and promotes cell adhesion and motility in tamoxifen-resistant MCF-7 cells and is inhibited by the Src/Abl kinase inhibitor, AZD0530, Proc. Am. Assoc. Cancer Res. 46 (2005) 62–63 (Abst. 266).

[46] M.G. Mullender, V. Everts, T.P. Green, J. Klein-Nulend, Inhibition of osteoclastic bone resorption by the novel, potent and selective Src/Abl inhibitor AZD0530, Proc. Am. Assoc. Cancer Res. 46 (2005) 686 (Abst. 2923).

[47] J.A. Lockton, D. Smethurst, M. Macpherson, R. Tootell, A.L. Marshall, G. Clack, N.J. Gallagher, Phase I ascending single and multiple dose studies to assess the safety, tolerability and pharmacokinetics of AZD0530, a highly selective, dual specific Src-Abl inhibitor, J. Clin. Oncol. 23 (2005) 223s (Abstr. 3125).

[48] N.J. Gallagher, A.J. Lockton, M. Macpherson, A. Marshall, G. Clack, A phase I multiple ascending dose study to assess the safety, tolerability and pharmacokinetics of AZD0530, a highly selective, orally available, dual-specific Src-Abl kinase inhibitor, Proc. Am. Assoc. Cancer Res. 46 (2001) 935 (Abstr. 3972).

[49] D.H. Boschelli, Y.D. Wang, S. Johnson, B. Wu, F. Ye, A.C.B. Sosa, J.M. Golas, F. Boschelli, 7-Alkoxy-4-phenylamino-3-quinolinecarbonitriles as dual inhibitors of Src and Abl kinases, J. Med. Chem. 47 (2004) 1599–1601.

[50] J.M. Golas, K. Arndt, C. Etienne, J. Lucas, D. Nardin, J. Gibbons, P. Frost, F. Ye, D.H. Boschelli, F. Boschelli, 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. 63 (2003) 375–381.

[51] N.J. Donato, J.Y. Wu, M. Talpaz, M. Dutia, F. Ye, D. Boschelli, F. Boschelli, Novel tyrosine kinase inhibitors suppress BCR-ABL signaling and induce apoptosis in STI-571 sensitive and resistant CML cells, Blood 100 (2002) 370a, (Abstr.1434).

[52] T. Grafone, M. Mancini, E. Ottaviani, M. Renzulli, F. Boschelli, L. Ferraretti, M.A. Santucci, S. Soverini, M. Amabile, C. Terragna, A. Poerio, G. Rosti, C. Taccioli, E. Palmerini, M. Baccarani, G. Martinelli, A novel 4-anilino-3-quinolinecarbonitrile dual Src and Abl kinase inhibitor (SKI-606) has in vitro activity on CML Ph+ blast cells resistant to imatinib, Proc. Am. Assoc. Cancer Res. 46 (2005) 1408, (Abst. 5984).

[53] S.R. Klutchko, J.M. Hamby, D.H. Boschelli, Z. Wu, A.J. Kraker, A.M. Amar, B.G. Hartl, C. Shen, W.D. Klohs, R.W. Steinkampf, D.L. Driscoll, J.M. Nelson, W.L. Elliott, B.J. Roberts, C.L. Stoner, P.W. Vincent, D.J. Dykes, R.L. Panek, G.H. Lu, T.C. Major, T.K. Dahring, H. Hallak, L.A. Bradford, H.D.H. Showalter, A.M. Doherty, 2-substituted aminopyrido[2,3-d]pyrimidin-7(8H)-ones. Structure–activity relationships against selected tyrosine kinases and in vitro and in vivo anticancer activity, J. Med. Chem. 41 (1998) 3276–3292.

[54] A.J. Kraker, B.G. Hartl, A.M. Amar, M.R. Barvian, H.D.H. Showalter, C.W. Moore, Biochemical and cellular effects of c-Src kinase-selective pyrido[2,3-d]pyrimidine tyrosine kinase inhibitors, Biochem. Pharmacol. 60 (2000) 885–898.

[55] J.F. Dorsey, R. Jove, A.J. Kraker, J. Wu, The pyrido[2,3-d]pyrimidine derivative PD180970 inhibits p210Bcr-Abl tyrosine kinase and induces apoptosis of K562 leukemic cells, Cancer Res. 60 (2000) 3127–3131.

[56] P. La Rosée, A.S. Corbin, E.P. Stoffregen, M.W. Deininger, B.J. Druker, Activity of the Bcr-Abl kinase inhibitor PD180970 against clinically relevant bcr-abl isoforms that cause resistance to imatinib mesylate (Gleevec, STI571), Cancer Res. 62 (2002) 7149–7153.

[57] D.R. Huron, M.E. Gorre, A.J. Kraker, C.L. Sawyers, N. Rosen, M.M. Moasser, A Novel pyridopyrimidine inhibitor of Abl Kinase is a picomolar inhibitor of Bcr-abl-driven K562 cells and is effective against STI571-resistant Bcr-abl mutants, Clin. Cancer Res. 9 (2003) 1267–1273.

[58] A.J. Tipping, S. Baluch, D.J. Barnes, D.R. Veach, B.M. Clarkson, W.G. Bornmann, F.X. Mahon, J.M. Goldman, J.V. Melo, Efficacy of dual-specific Bcr-Abl and Src-family kinase inhibitors in cells sensitive and resistant to imatinib mesylate, Leukemia 18 (2004) 1352–1356.

[59] N. von Bubnoff, D.R. Veach, H. Van der Kuip, W.E. Aulitzky, J. Sänger, P. Seipel, W.G. Bornmann, C. Peschel, B. Clarkson, J. Duyster, A cell-based screen for resistance of Bcr-Abl-positive leukemia identifies the mutation pattern for PD166326, an alternative Abl kinase inhibitor, Blood 105 (2005) 1652–1659.

[60] N. von Bubnoff, D.R. Veach, W.T. Miller, W. Li, J. Sänger, C. Peschel, W.G. Bornmann, B. Clarkson, J. Duyster, Inhibition of wild-type and mutant Bcr-Abl by Pyrido-Pyrimidine-type small molecule kinase inhibitors, Cancer Res. 63 (2003) 6395–6404.

[61] D. Wisniewski, C.L. Lambek, C. Liu, A. Strife, D.R. Veach, B. Nagar, M.A. Young, T. Schindler, W.G. Bornmann, J.R. Bertino, J. Kuriyan, B. Clarkson, Characterization of potent inhibitors of the Bcr-Abl and the c-Kit receptor tyrosine kinases, Cancer Res. 62 (2002) 4244–4255.

[62] R. Nimmanapalli, E. O'Bryan, M. Huang, P. Bali, P.K. Burnette, T. Loughran, J. Tepperberg, R. Jove, K. Bhalla, Molecular characterization and sensitivity of STI-571 (imatinib mesylate, Gleevec)-resistant, Bcr-Abl-positive, human acute leukemia cells to SRC kinase inhibitor PD180970 and 17-allylamino-17-demethoxygeldanamycin, Cancer Res. 62 (2002) 5761–5769.

[63] J. Wissing, K. Godl, D. Brehmer, S. Blencke, M. Weber, P. Habenberger, M. Stein-Gerlach, A. Missio, M. Cotten, S. Mueller, H. Daub, Chemical proteomic analysis reveals alternative modes of action for pyrido[2,3-d]pyrimidine kinase inhibitors, Mol. Cell. Proteomics 3 (2004) 1181–1193.

[64] N.C. Wolff, D.R. Veach, W.P. Tong, W.G. Bornmann, B. Clarkson, R.L. Ilaria, PD166326, a novel tyrosine kinase inhibitor, has greater antileukemic activity than imatinib mesylate in a murine model of chronic myeloid leukemia, Blood 105 (2005) 3995–4003.

[65] N. Gray, L. Wodicka, A.W.H. Thunnissen, T.C. Norman, S. Kwon, F.H. Espinoza, D.O. Morgan, G. Barnes, S. LeClerc, L. Meijer, S. Kim, D.J. Lockhart, P.G. Schultz, Exploiting chemical libraries, structure, and genomics in the search for kinase inhibitors, Science 281 (1998) 533–538.

[66] T. O'Hare, R. Pollock, E.P. Stoffregen, J.A. Keats, O.M. Abdullah, E.M. Moseson, V.M. Rivera, H. Tang, C.A. Metcalf, R.S. Bohacek, Y. Wang, R. Sundaramoorthi, W.C. Shakespeare, D. Dalgarno, T. Clackson, T.K. Sawyer, M.W. Deininger, B.J. Druker, Inhibition of wild-type and mutant Bcr-Abl by AP23464, a potent ATP-based oncogenic protein kinase inhibitor: implications for CML, Blood 104 (2004) 2532–2539.

[67] J. Mestan, *unpublished results*.

[68] A.S. Corbin, S. Demehri, I.J. Griswold, Y. Wang, C.A. Metcalf, R.

Sundaramoorthi, W.C. Shakespeare, J. Snodgrass, S. Wardwell, D. Dalgarno, J. Iuliucci, T.K. Sawyer, M.C. Heinrich, B.J. Druker, M.W. Deininger, In vitro and in vivo activity of ATP-based kinase inhibitors AP23464 and AP23848 against activation loop mutants of Kit, Blood 106 (2005) 227–234.

[69] K. Gumireddy, S.J. Baker, S.C. Cosenza, J. Premila, A.D. Kang, K.A. Robell, M.V.R. Reddy, E.P. Reddy, A non-ATP-competitive inhibitor of BCR-ABL overrides imatinib resistance, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 1993–1997.

[70] P. Tseng, H. Lin, J. Zhu, K. Chen, E.M. Hade, D.C. Young, J.C. Byrd, M. Grever, K. Johnson, B.J. Druker, C. Chen, Synergistic interactions between imatinib mesylate and the novel phosphoinositide-dependent kinase-1 inhibitor OSU-03012 in overcoming imatinib mesylate resistance, Blood 105 (2005) 4021–4027.

[71] H. Daub, K. Specht, A. Ullrich, Strategies to overcome resistance to targeted protein kinase inhibitors, Nat. Rev., Drug Discov. 3 (2004) 1001–1010.

[72] A. Scuto, S. Annavarapu, P. Bali, M. McCullers, W. Fiskus, A. Mouttaki, F. Guo, C. Sigua, G. Sondarva, P. Atadja, P. Manley, K. Bhalla, Synergistic cytotoxic effects of a combination of a novel tyrosine kinase inhibitor AMN107 and histone deacetylase inhibitor LBH589 against wild type and mutant Bcr-Abl expressing human leukemia cells, Blood 104 (2004) 546a, (Abstr. 1977).

[73] P. Bali, W. Fiskus, F. Guo, S. Annavarapu, C. Sigua, G. Sondarva, A. Mouttaki, K. Bhalla, Molecular characterization of human CML cells with resistance to the heat shock protein (hsp) 90 inhibitor 17-allylamino-demethoxy geldanamycin (17-AAG), Blood 104 (2004) 546, (Abstr. 1980).

[74] J. Cools, P.D. Daniel, J. DeAngelo, J. Gotlib, E.H. Stover, M. Phil, R.D. Legare, J. Cortes, J. Lutok, J. Clark, I. Galinsky, J.D. Griffin, N. Cross, C.P. Nicholas, A. Tefferi, J. Malone, F. Alam, S.L. Schrier, J. Schmid, M. Rose, P. Vandenberghe, G. Verhoef, M. Boogaerts, I. Wlodarska, H. Kantarjian, P. Marynen, S.E. Coutre, R. Stone, D.G. Gilliland, A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome, N. Engl. J. Med. 348 (2003) 1201–1214.

[75] W. Pao, V.A. Miller, K.A. Politi, G.J. Riely, R. Somwar, M.F. Zakowski, M.G. Kris, H. Varmus, Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain, PLoS Med. 2 (2005) 225–235.