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BCR ABL Kinase Inhibitors for Cancer Therapy

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ABSTRACT
BCR-ABL tyrosine kinase inhibitors have started era of molecular targeted therapy and marked a greatest milestone in cancer drug discovery. Despite of impressive cytogenetic response rates achieved with several agents in patients with chronic myelogenous leukemia (CML) in chronic phase, those with advanced stage CML frequently obtain more modest responses that are in many instances of short duration. Several mechanisms of resistance to imatinib are also observed among patients that develop clinical resistance to imatinib. Some approaches are being pursued to overcome these mutations. Deregulated tyrosine kinase activity of the BCR-ABL fusion protein has been established as the causative molecular event in CML. BCR-ABL tyrosine kinase is an ideal target for pharmacological inhibition. Following the initial success of imatinib as frontline therapy for CML, several second generation therapeutic inhibitors have been developed with increased potency and the ability to inhibit the majority of imatinib resistant mutations. In addition, many other protein kinases implicated in signaling transduction downstream BCR-ABL play critical roles in the pathogenesis of CML, thus representing potential therapeutic targets revealed from several clinical studies. The objective of present article is on current developments of potential existing and new innovative BCR-ABL kinase inhibitors for tumor therapy and specificity of several new inhibitors.

Keywords: BCR-ABL, Kinase Inhibitors, Cancer, Chronic Myelogenous Leukemia, Imatinib.

INTRODUCTION

The ABL leukemia virus (v-Abl) was initially described in 1970 and led to the cloning of its normal cellular homolog, c-Abl, which was found to map to the long arm of chromosome 9. As further investigation transpired to unravel the abnormality known as the Ph chromosome, it became apparent that c-Abl had been translocated to chromosome 22 into a region known as the breakpoint cluster region, or Bcr. [1] In 1960, Nowell and Hungerford described a consistent chromosomal abnormality (an acrocentric chromosome thought to be a chromosomal deletion) in CML patients. With improvements in chromosomal banding techniques, it became apparent that the observed abnormality was a shortened chromosome 22. In time, Rowley determined that the shortened chromosome, the so-called ‘‘Philadelphia (Ph) chromosome,’’ was in fact the product of a reciprocal translocation between the long arms of chromosomes 9 and 22, t (9:22)(q34;q11).

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Several important experimental works established the ability of Bcr-Abl, as a singular oncogenic abnormality, to cause leukemia. Transgenic mice that express the p190 Bcr-Abl fusion protein were shown to develop a rapidly fatal acute leukemia. [2] Transduction of p210 Bcr-Abl into murine hematopoetic stem cells, followed by transplantation into syngeneic mice, causes a CML-like syndrome. [3] Although convincing, there was and remains controversy about the genesis of CML, particularly surrounding the possibility of clonal stem cell events antecedent to 9:22 translocation and Bcr-Abl fusion. More recent work has bolstered the transforming potential of Bcr-Abl by demonstrating that mice expressing a Bcr-Abl transgene, under the control of a tetracycline-repressible promoter, develop a reversible leukemia entirely dependent on the presence or absence of tetracycline. [4]

Using classic karyotyping, the Ph chromosome abnormality can be detected in approximately 90 % of patients with CML. An additional 10 % of patients will have a cryptic’ translocation and will be detected only by molecular testing for Bcr-Abl [fluorescent in situ hybridization (FISH) and/or quantitative reverse transcriptase polymerase chain reaction (qPCR)]. Different Bcr-Abl fusion proteins are produced, depending on the site of the breakpoint in Bcr: p185

(185kDa), p210 (210kDa), or rarely p230. The different fusion proteins, all variants of Bcr-Abl, segregate to a high degree among the different Ph (+) leukemias. The p210 protein is seen in 95% of patients with CML and up to 20 % of adult patients with de novo acute lymphoblastic leukemia (ALL); the p185 form is seen in approximately 10 % of patients with ALL and in the majority of pediatric patients with Ph (+) ALL (5 % of all pediatric ALL cases). If the Ph chromosome is suspected or possible while working up the diagnosis of leukemia, several assays may be used to query for the Bcr-Abl fusion: classical karyotyping, FISH analysis for Bcr-Abl gene rearrangements, qPCR for Bcr-Abl mRNA, or less often immunoblotting for Bcr-Abl protein.

Pathways involved with malignant transformation and proliferation

A well-known aspect of BCR–ABL transformation is its ability to activate multiple signaling pathways that lead to proliferation, reduced growth factor-dependence and apoptosis, and abnormal interaction with extra-cellular matrix and stroma. Accumulating evidence suggests that the suppression of apoptosis constitutes an important mechanism by which BCR– ABL drives the expansion of myeloid cells. Notably, the primary consequence of tyrosine kinase inhibition with imatinib in BCR–ABL-transformed cells is the induction of apoptosis. [5-6] In growth factor-dependent hematopoietic cells, BCR– ABL induces the survival and proliferation of cells that would otherwise undergo apoptotic cell death in response to growth factor withdrawal. Furthermore, antisense oligonucleotide mediated inhibition of BCR–ABL in these growth factor independent transformed cells results in apoptosis without altering their cell cycle. [7]

A. BCR–ABL activation of STAT

The signal transduction and activators of transcription (STAT) transcription factors have been extensively studied for their potential role in leukemogenesis. The STAT family of transcription factors participates in diverse processes, including cell growth, differentiation, apoptosis, fetal development, inflammation, and immune response. Ligand binding to cytokine or growth factor receptors initiates a series of signaling events that result in STAT phosphorylation, dimerization, and subsequent translocation to the nucleus. Some STAT target genes include Bcl-xL and Mcl-1, substantiating an anti-apoptotic role for the activity of STAT transcription factors. [8]. BCR–ABL-positive CML cell lines display constitutive phosphorylation and activation of STAT-1 and STAT-5. STAT-5 activation induces upregulation of the serine/threonine kinase Pim-1 and the anti-apoptotic genes of the Bcl-2 family, A1 and Bcl-xL. [9]

B. BCR–ABL activation of NF-Kb

The Nuclear Factor-κB (NF-κB) families of pleiotropic transcription factors function as dimers and are activated by a broad range of stimuli including cytokines, physical and oxidative stresses, viruses and viral products. The IκB (inhibitor of κB) proteins negatively regulate NF-κB by sequestering it to the cytoplasm. Phosphorylation and subsequent degradation of IκB relieves NF-κB to translocate to the nucleus. Upon their activation, NF-κB proteins promote the transcription of numerous genes involved in diverse cellular processes, including inflammation, cell cycle, survival/anti-apoptosis, and angiogenesis. [10] The constitutive activation of NF-κB is frequently observed in various cancers, and correlates with resistance of tumor cells to apoptosis. The NF-κB anti-apoptotic target genes include those from the Bcl-2 family (Bcl-xL, BFL1) and the inhibitors of apoptosis proteins, IAP1, IAP2, and XIAP.

C. BCR–ABL activation of the Ras pathway

Within the Ras superfamily of low molecular weight GTP binding proteins is the Ras subfamily, consisting of H-Ras, N-Ras and K-Ras. The Ras pathway regulates various aspects of cellular growth and has been one of the most extensively studied both in the context of normal and cancer cells. [11] Activating mutations in Ras, or changes in molecular components that comprise Ras signaling, are found in most human cancers including leukemias, and result in increased cellular proliferation and survival. [12]

D. BCR–ABL activation of the PI3-K/Akt pathway

BCR–ABL activation of the PI3-K/Akt pathway Signal transduction pathways that emanate from the activation of phosphoinositide-3 kinase (PI3-K) have been intensively investigated in mammalian systems, and play a central role in survival, proliferation, differentiation, adhesion, metabolism, and motility. [13] While there are several families and classes of phosphoinositide kinases (PIKs), the class IA PI3-Ks within the PI3-K family, become activated when recruited to the cell surface by growth factor receptor tyrosine kinases. PI3-K is a lipid kinase that functions as a heterodimer consisting of a p110 catalytic subunit and a p85 regulatory subunit. Upon its activation by growth factor tyrosine kinase receptors, PI3-K phosphorylates phosphatidylinositol bisphosphate (PIP2) to form phosphatidylinositol triphosphate (PIP3). The formation of PIP3 can be reversed by the phosphatase and tensin homolog deleted on chromosome 10 (PTEN). On the other hand, PIP3 provides a platform for the recruitment of kinases, such as the serine/threonine kinases Akt, 3-phosphoinositide- dependent protein kinase-1 (PDK1), and perhaps others via their pleckstrin homology (PH) domains. In this setting, Akt is phosphorylated at distinct residues, namely at threonine 308 within the activation loop, most probably by PDK1, and at serine 473 within the hydrophobic motif by a mechanism that involves PDK2 (mTORC2). Activated Akt regulates numerous cellular substrates, resulting in cell growth, survival, and suppression of apoptosis, among other effects. [14]

Pharmacological inhibitors of PI3-K (LY294002 and Wortmannin) synergize with imatinib in inducing apoptosis of both chronic and blast crisis C L cells. [15] Combination of a PDK-1 inhibitor (OSU-03012), which essentially inhibits Akt activation, with imatinib resulted in apoptosis even in cells expressing the BCR–ABL T315I imatinib-resistant mutant. [16] Besides substantiating a role for PI3-K/Akt signaling in BCR–ABL-mediated transformation and leukemogenesis, some of these observations also indicate that PI3-K/Akt activation is potentially a crucial event in BCR–ABL-mediated resistance to imatinib. On the other hand, BCR–ABL-induced tyrosine autophosphorylation, and/or phosphorylation of its substrates, can provide the platform for PI3-K activation. As mentioned earlier, recent evidence indicates a strong role for tyrosine 177 of BCR– ABL in PI3-K/Akt activation. Central to the effects of signaling via this site in BCR–ABL is the adaptor protein Gab2, which is indirectly recruited to BCR–ABL tyrosine 177 through a Grb2/ Gab2 complex. Consequent Gab2 tyrosine phosphorylation provides binding sites for the SH2 domain of the p85 regulatory subunit of PI3-K, thereby causing

activation of PI3-K. BCR– ABL Y177F-expressing BaF3 cells show reduced activation of PI3-K and Akt and slightly decreased proliferation in the absence of cytokines compared to wild type BCR–ABL. In a related manner, BCR–ABL-expressing Gab2-deficient myeloid cells and lymphoblasts also display drastic reductions in PI3-K and Akt activation, and remain refractory to BCR–ABL transformation. [17] Collectively, in vitro and in vivo studies have substantiated a role for PI3-K/Akt activation in BCR–ABL transformation and leukemogenesis. Furthermore, multiple mechanisms exist for PI3-K and subsequent Akt activation in BCR–ABL-transformed Cells.

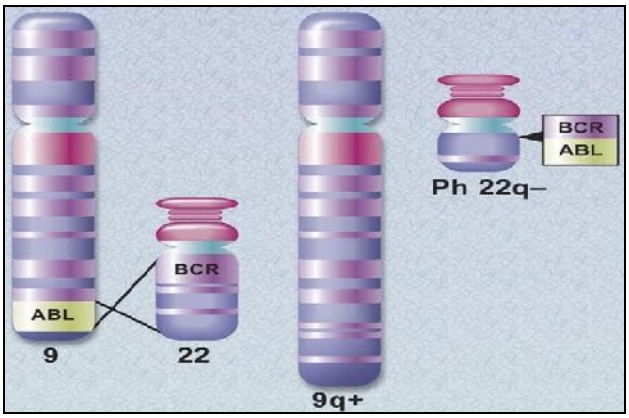


Fig. 1: Schematic diagram of the translocation that creates the Philadelphia chromosome. The *ABL* and *BCR* genes reside on the long arms of chromosomes 9 and 22, respectively. As a result of the (9;22) translocation, a *BCR-ABL* gene is formed on the derivative chromosome 22 'Philadelphia chromosome'

Table: 1 List of BCR ABL tyrosine kinase inhibitors

Compound	Target	Indication	Status	Sponsor
First generation BCR ABL kinase inhibitors				
Imatinib mesylate	Bcr, abl	CML, GIST	Approved	Novartis
Second generation bcr abl kinase inhibitor				
Nilotinib	Bcr, abl	CML, Ph+ALL	Approved	Novartis
Dasatinib	Bcr, abl	CML, Ph+ALL	Approved	Sprycel, Bristol-Myers Squibb
Bosutinib	Bcr, abl	CML	Phase1/2	Pfizer
AZD05340	Bcr, abl, src	CML, Ph(+)ALL	Phase II	AstraZeneca
Other Bcr abl kinase inhibitor				
MK-0457	ABL1,JAK2, FLT3	CML	Phase II	Merck & Vertex Co.
PHA-739358	ABL1, RET, TRK-A, FGFR1	CML	Phase II	Nerviano Medical Sciences
AP23464	Abl/ Src	CML	Phase1/2	Ariad Pharmaceuticals
PD166326	Bcr, abl,fyn	CML, Ph(+)ALL	preclinical	-
PD173955	Src, abl	Blast crisis, CML	Phase II	-
PD180970	Bcr ,abl	Blast crisis, CML	Phase II	-
AZD1152	Aurora B kinase	AML	Phase I	AstraZeneca
KW-2449	FGFR1,FLT3, VEGFR	CML	Phase I	Kyowa Hakko Kogyo Co. Ltd.
XL228	ABL1,IGF1R, SRC	CML	Phase I	Exelixis Inc.

IMATINIB MESYLATE - First generation BCR ABL kinase inhibitor

Tyrosine kinases regulate many cellular processes, including growth and survival, and deregulated activity of these enzymes has been implicated in malignant transformation in various neoplasms. Therefore, specific inhibitors of tyrosine kinases are attractive therapeutic agents. BCR-ABL functions as a constitutively activated tyrosine kinase and mutagenic analysis has shown that this activity is essential for the transforming function of the protein. Imatinib mesylate binds to the amino acids of the BCR/ABL tyrosine kinase ATP binding site and stabilizes the inactive, non-ATP-binding form of BCR/ABL, thereby preventing tyrosine auto phosphorylation and, in turn, phosphorylation of its substrates. This process ultimately results in "switching-off" the downstream signaling pathways that promote leukemogenesis. An agent that specifically blocked ABL tyrosine kinase activity would be an ideal targeted therapy for CML. STI571 (formerly CGP57148B), a specific inhibitor of ABL tyrosine kinase, is an inhibitor of low molecular mass that serves as a useful model for the development of a specifically targeted agent.

Starting in the late 1980s, scientists at Ciba Geigy (now Novartis), under the direction of N. Lydon and A. Matter, initiated projects on the identification of compounds with inhibitory activity against protein kinases. In one medicinal chemistry project focusing on protein kinase C (PKC) as a target, a 2-phenylaminopyrimidine derivative was identified as a lead compound. [18-19] This compound had low potency and poor specificity, inhibiting both serine/threonine and tyrosine kinases, but from this starting point, a series of derivatives were synthesized. The addition of a 3' pyridyl group at the 3'-position of the pyrimidine enhanced the cellular activity of the derivatives. Activity against tyrosine kinases was enhanced by introduction of a benzamide group at the phenyl ring. A key observation from analysis of structure activity relationships was that substitutions at the 6-position of the anilino phenyl ring led to loss of PKC inhibition. However, the introduction of a "flag-methyl" group at this position retained or enhanced activity against tyrosine kinases. Profiling of the compounds showed that they also inhibited the ABL tyrosine kinase. The first series of compounds had poor oral bioavailability, with low water solubility. The attachment of a highly polar side chain, N-methylpiperazine, markedly improved solubility and oral bioavailability. STI571 (formerly CGP57148B, now imatinib mesylate; Gleevec or Glivec, Novartis, Basel, Switzerland) emerged as the most promising compound for clinical development, since it had the highest selectivity for growth inhibition of BCR-ABL–expressing cells.

STI-571 is an effective inhibitor of c-Abl and Bcr-Abl protein-tyrosine kinase activity. [20] John Kuriyan and coworkers, using X-ray crystallography, found that STI-571 binds to an inactive conformation of c-Abl. [21] The drug binds in the cleft between the amino- and carboxy-terminal lobes of the kinase domain.Only the leftmost portion of STI-571 is found where the adenine base of ATP normally binds. The rest of the compound penetrates further into the hydrophobic core of the kinase, inserted between the activation loop and helix αC, thereby keeping the kinase in an inactive conformation. Recent steady-state kinetic studies show that STI-571 is a competitive inhibitor with respect to ATP, which is consistent with the X-ray studies showing that

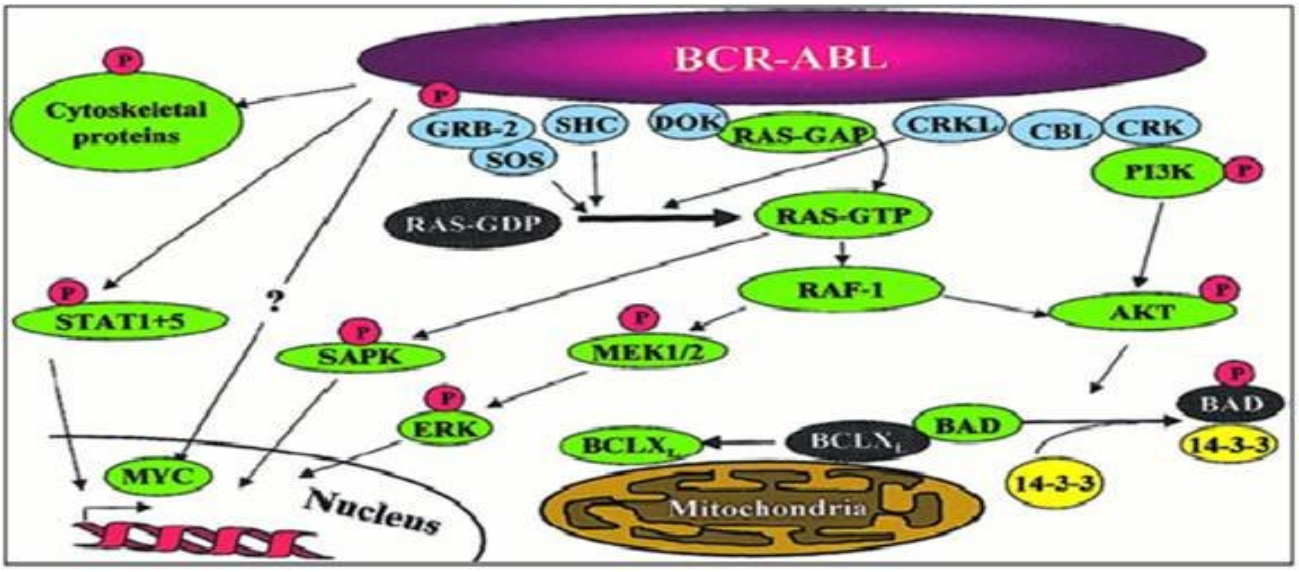


Fig. 2: Signaling pathways impacted by BCR-ABL expression.
Simplified diagram with many more associations between BCR-ABL and signaling proteins have been reported.

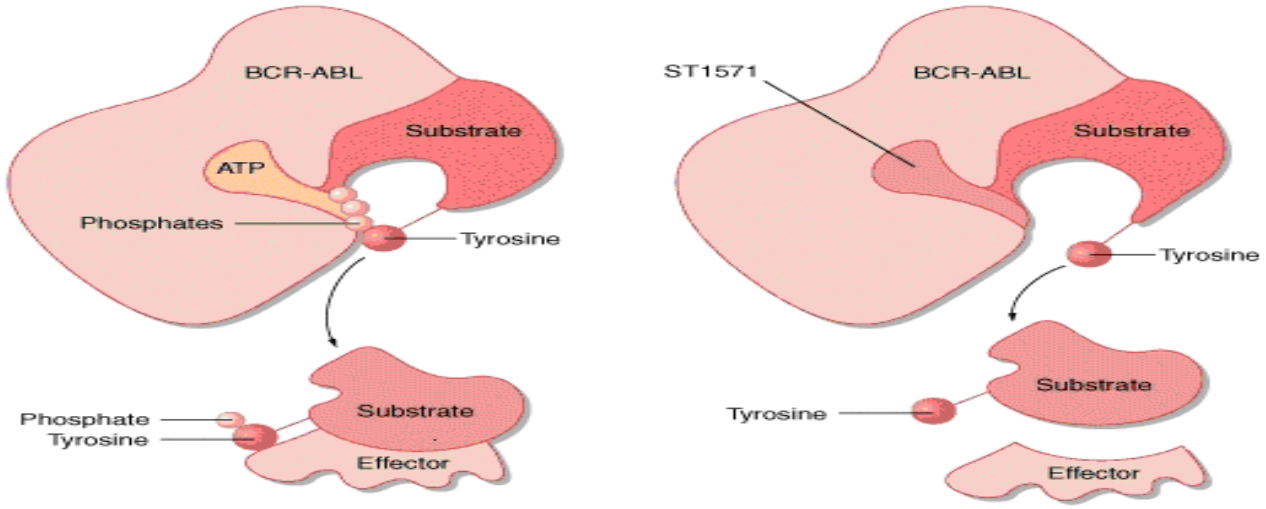


Fig. 3: Mechanism of action of imatinib. (A)
The constitutively active BCR-ABL tyrosine kinase functions by transferring phosphate from ATP to tyrosine residues on various substrates to cause excess proliferation of myeloid cells characteristic of CML. (B) Imatinib blocks the binding of ATP to the BCR-ABL tyrosine kinase, thus inhibiting kinase activity.

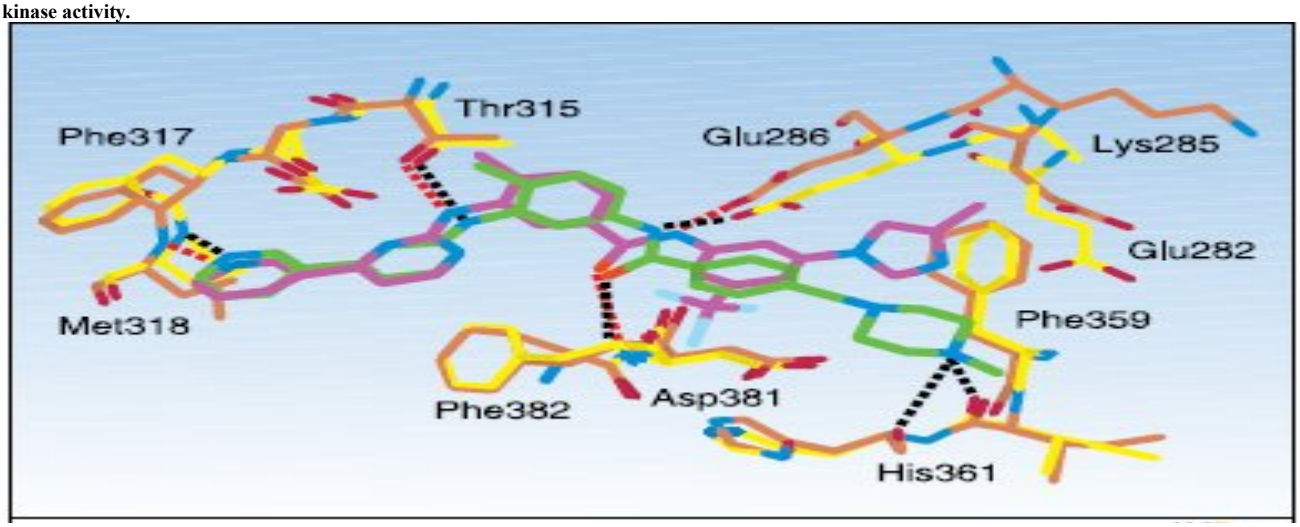
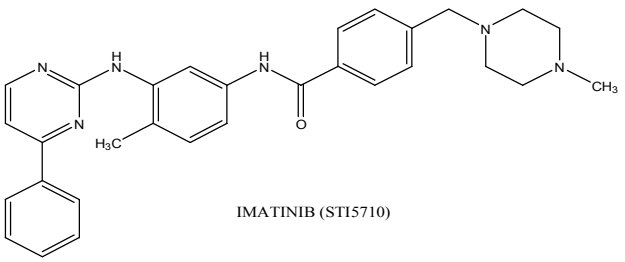
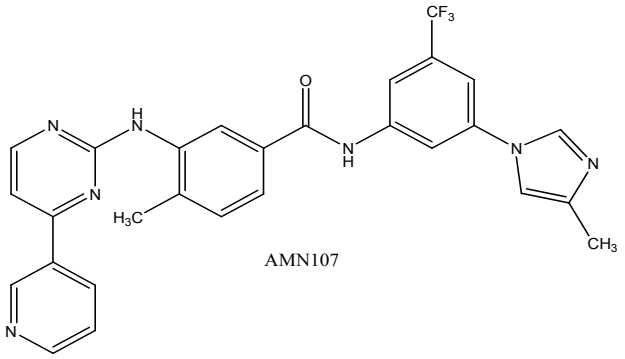


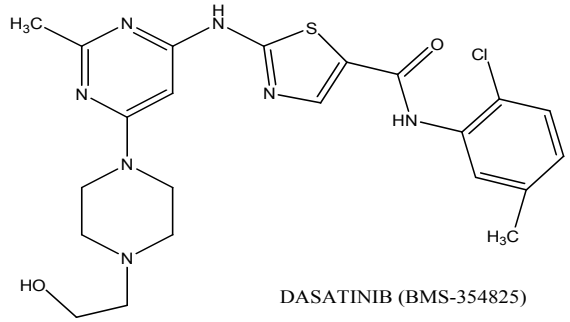
Fig. 4: Superposition of nilotinib (magenta) bound to ABLM351T (orange), and imatinib (green) bound to ABL (yellow). Hydrogen bonds within the nilotinib-ABLM351T complex are depicted as dashed red lines, whereas those in the imatinib complex are shown in black.Note that the hydrogen bonds between the piperazine ring of imatinib and Ile360 and His361are absent from the nilotinib-ABL complex. The methyl-imidazole group of nilotinib packs in a hydrophobic pocket. (Weisberg E, Manley PW, Breitenstein W. Characterization of AMN107, a selective inhibitor of native andmutant BCR-ABL. Cancer Cell. 2005;7:129-41.)



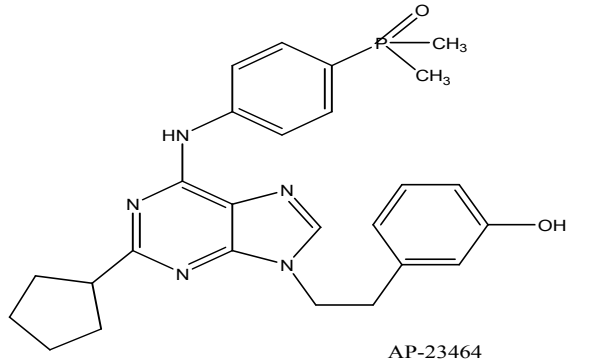
IMATINIB (STI5710)



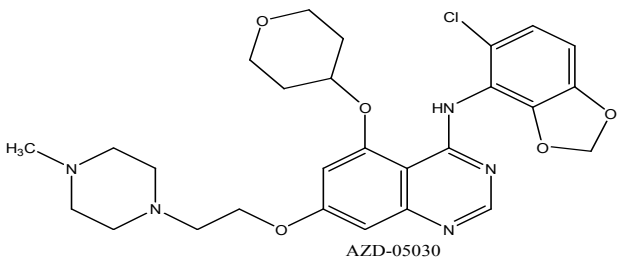
AMN107



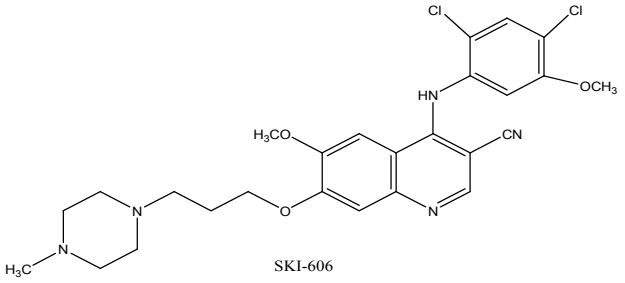
DASATINIB (BMS-354825)



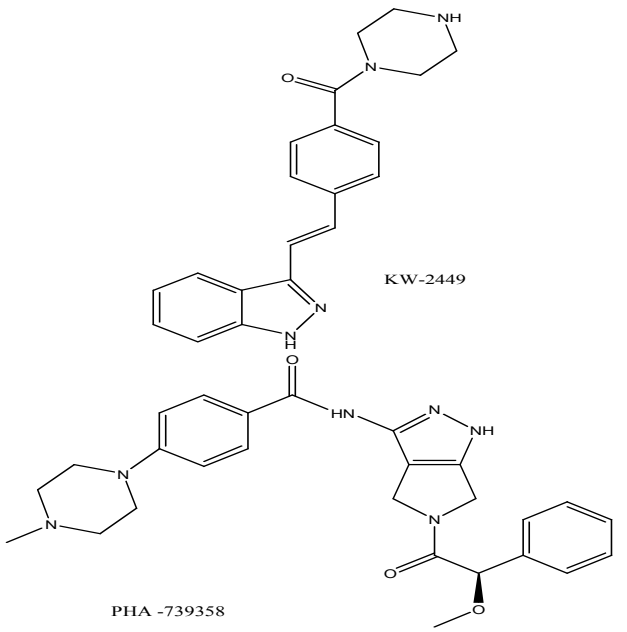
AP-23464



AZD-05030

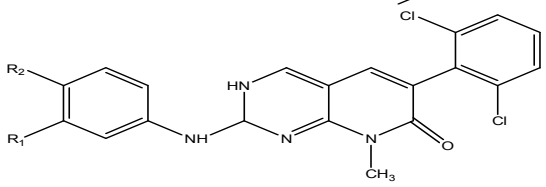


SKI-606

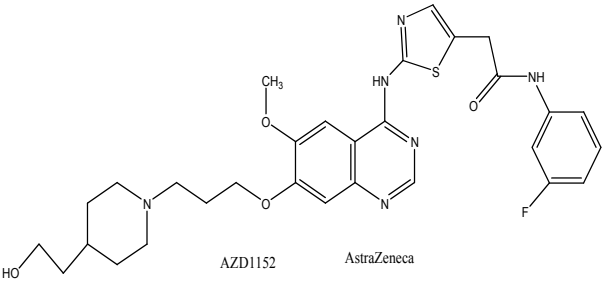


KW-2449

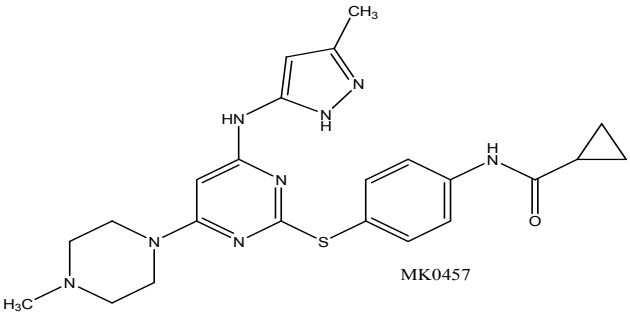
PHA -739358



PD166326: R1 = CH_2 OH R2 =H
PD173955: R1 = SCH_3 R2 =H
PD180970: R1 = CH_3 R2 =F



AZD1152 AstraZeneca



MK0457

Fig. 5: Structures of BCR ABL kinase inhibitor

the drug binds to the ATP-binding site. [22] In chronic myeloid leukemia, c-Abl is fused with Bcr causing it to be constitutively active. Thus, the activation loop in Bcr-Abl would be in the open conformation and phosphorylated. When the activation loop is transiently dephosphorylated, STI-571 can bind and inactivate the kinase. The determination of rates of phosphorylation and dephosphorylation of Bcr-Abl and c-Abl in cells is a procedure that warrants development.

***In vitro* profile of imatinib - Inhibition of kinase activity**

Studies using purified enzymes expressed as bacterial fusion proteins or using immunoprecipitations of intact proteins showed that imatinib potently inhibits all of the ABL tyrosine kinases. This includes cellular ABL, viral ABL (v-ABL), and BCR-ABL. [23] In contrast, the compound was inactive against serine/ threonine kinases, did not inhibit the epidermal growth factor (EGF) receptor intracellular domain, and showed weak or no inhibition of the kinase activity of the receptors for vascular endothelial growth factor (VEGF-R1 and VEGF-R2), fibroblast growth factor receptor 1 (FGF-R1), tyrosine kinase with immunoglobulin and EGF homology-2 (TIE-2 [TEK]), c-MET, and nonreceptor tyrosine kinases of the SRC family (FGR, LYN, and LCK). The results of the kinase assays were confirmed in cell lines expressing constitutively active forms of ABL such as v-ABL, p210BCR-ABL, p185 BCR-ABL, and translocated ets leukemia (TEL)–ABL, 20 where imatinib was found to inhibit ABL kinase activity with 50 % inhibitory concentration (IC50) values ranging between 0.1 and 0.35 μM. Numerous Ph+ cell lines derived from patients with CML or acute lymphoblastic leukemia (ALL) have subsequently been tested. In most of these lines, the IC50 values were also in the range of 0.1 to 0.5 μM, indicating that the compound effectively penetrates the cell membrane. [24]

In contrast, signal transduction mediated by EGF, insulin, insulin like growth factor I (IGF-I), FGF, and phorbol ester was insensitive to imatinib. [5] Furthermore, imatinib did not affect FLT-3 or the receptor for colony-stimulating factor 1 (CSF-1, FMS), or the nonreceptor tyrosine kinases SRC and JAK-2. The latter mediates signaling from a number of cytokine receptors, including the receptors for IL-3, G-CSF, and erythropoietin. To test the effects of imatinib on tumor growth, BCR-ABL– transformed 32D cells were injected into syngeneic mice. [5] Once daily intraperitoneal treatment using doses of imatinib from 2.5 to 50 mg/kg, starting 1 week after cell injection, caused dose dependent inhibition of tumor growth, while 50 mg/kg intraperitoneal treatment was inactive against tumors derived from v-SRC–transformed 32D cells, consistent with the lack of inhibition of SRC kinase activity by imatinib. Similar experiments in nude mice using KU812 cells, a BCR-ABL+ human cell line, demonstrated the need for continuous inhibition of BCR-ABL kinase activity to achieve maximal antitumor effects. [25] Early pharmacokinetic studies at Novartis had demonstrated that imatinib is rapidly absorbed following oral administration to mice and pharmacologically relevant concentrations are achieved in the plasma, with a half-life of approximately 1.3 hours. Imatinib was also tested in the transduction-transplantation model of CML. In this system, lethally irradiated syngeneic mice receive marrow infected with a BCR-ABL retrovirus and consistently die within 3 weeks from an aggressive CML. [26] Treatment with imatinib (50 mg/kg in the morning, 100 mg/kg in the evening) led to prolonged survival. Responses were quite variable, with 25 % of animals having refractory disease. Imatinib was not capable of preventing CML, even if started as early as 48 hours after transplantation, but none of the responders progressed on therapy. No consistent association between response and BCR-ABL mRNA and protein levels was seen, and no other cause underlying refractoriness could be determined. Notably, there was a trend toward "clonal depletion" in responding animals, suggesting that imatinib was able to successfully target some, but not all leukemic clones.

Resistance to Imatinib Mesylate

During disease progression, CML progenitor cells acquire a number of genetic alterations, most probably because of increased genomic instability, that may explain the aggressive phenotype, chemotherapeutic drug resistance, and poor prognosis of CML in BP. Despite the exciting results obtained with imatinib mesylate noted above, CML patients eventually show resistance at a rate of 80 % in BP, 40–50 % in AP, and 10 % in CP post-IFNα failure, at 2 years. [27] Identification of the molecular basis of resistance is important, because it could provide insight into disease progression and into the design of novel therapeutic strategies to prevent and overcome treatment resistance.

CML patients with imatinib mesylate resistance can be stratified into those with primary refractory disease, most frequently in accelerated or BPs, and those who relapse after initial response, who are most frequently in. On the basis of the presence or absence of BCR/ABL tyrosine kinase activity in leukemia cells, it is also possible to discriminate between cases with BCR/ABL-dependent and -independent mechanisms of imatinib mesylate resistance. Notably, because the BCR/ABL enzymatic activity cannot be easily measured in blood or BM patient samples, levels of phosphorylation of the BCR/ABL substrate CRKL have been used as a surrogate end point for the tyrosine kinase activity. [28] In patients with higher levels of CRKL phosphorylation despite treatment with imatinib mesylate, resistance has been found to result from at least three different BCR/ABL-dependent mechanisms: BCR/ABL gene amplification, BCR/ABL mutations, and high plasma levels of AGP. [29]

Other mechanisms of imatinib mesylate resistance involve mutations in the BCR/ABL gene itself. Several different mutations have been detected in at least 13 amino acids of the ATP-binding site or other regions of the tyrosine kinase domain, and the list is growing. [30] These mutations usually prevent imatinib mesylate from binding to BCR/ABL, thereby resulting in lack of inhibition of the tyrosine kinase activity. Among these mutations, substitution of a threonine to isoleucine at position 315 of ABL that prevents imatinib mesylate from binding to the ATP-binding domain, is the first described and one of the most frequent. A third mechanism of resistance relies on plasma levels of AGP. It has been shown that AGP binds imatinib mesylate at physiological concentrations in vitro and in vivo, and blocks the ability of imatinib mesylate to inhibit BCR/ABL kinase activity in a dose-dependent manner. Finally, in patients with primary refractoriness to imatinib mesylate, resistance more often occurs in absence of significant CRKL phosphorylation, suggesting activation of BCR/ABL-independent leukemogenic pathways.

Second generation Selective Bcr-Abl inhibitors

Currently, two classes of drug candidates are being evaluated as monotherapies 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. The cellular efficacy of Bcr-Abl inhibitors can conveniently be accessed 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.

NILOTINIB (AMN107)

The phenylamino-pyrimidine derivative, AMN107 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 together with medicinal chemistry paradigms for drug discovery. [22] 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 characterized in vitro in cells expressing full length human kinases.

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. 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, would be ineffective against most of the imatinib-resistant mutant forms of Bcr-Abl. 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. [31] 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. [32]

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. [33] 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. However, despite the findings that Src family kinases probably contribute to the survival and proliferation of Bcr-Abl expressing immortalized 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. Since Src family kinases regulate downstream elements of the Bcr-Abl signaling 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. 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. [34] One of the first potent dual Abl/Src kinase inhibitors was the pyrrolopyrimidine CGP073060, which emerged from a Src kinase programme directed towards compounds for the treatment of osteoporosis, although this series of compounds was not optimised for use in leukaemia. However, the considerable sequence homology between the Abl and Srcfamily tyrosine kinases readily enables molecules to possess cross-reactivity, particularly when they target the active conformation of the kinase domains of these enzymes and a number of other structural classes of dual-inhibitors have been identified. [35]

DASATINIB (BMS-354825)

The most advanced dual Bcr-Abl/Src inhibitor is the thiazolylamino-pyrimidine, BMS-354825, developed as the hydrochloride salt, which is used in patients with imatinib-resistant CML. This drug emerged from a programme directed towards immunosuppressant drugs and, in addition to inhibiting the Src family kinases: Fyn (IC50 0.2 nM), Lck (IC50 1.1 nM), Src (IC50 0.55 nM), Yes (IC50 0.41 nM), it potently inhibits Abl (IC50<1 nM), c-Kit (IC50 13 nM), PDGFRh (IC50 28 nM), EPHA2 (IC50 17 nM), HER1 (IC50 180 nM) and p38 MAP (IC50 100 nM) kinases. [36] In cellular assays, BMS-354825 inhibits the proliferation of Bcr-Abl transfected Ba/F3 cells and human Bcr-Abl expressing K562 cells with IC50 values of 1.3 and <1 nM, respectively, and the compound maintains high potency against a wide range of Bcr-Abl mutants, although, again like AMN107, with the exception of T315I. 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. [37] A consequence of the inhibition of the Src family kinases, is that BMS-354825 also inhibits the proliferation of a widerange of human tumour cell lines which do not express Bcr- Abl, such as PC-3 (prostate; IC50 5–9 nM), MDA-MB-211 (breast; IC50 10–12 nM) and WiDr (colorectal; IC50 38–52 nM) cells. [37]

BOSUTINIB (SKI-606)

A dual Bcr-Abl/Src inhibitor, SKI-606, based upon a quinoline scaffold, structurally related to the AstraZeneca quinazoline template, has been developed by Wyeth. [38] This compound inhibits c-Abl and c-Src transphosphorylation with IC50 values of <10 nM, and inhibits the proliferation of K562, KU812 and MEG-01 CML cell lines with IC50 values of 20, 5 and 20 nM, respectively. In imatinib-resistant K-562 cells, like PD180970, 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. [39] 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. 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. [40] A Clinical Phase I dose escalation study to evaluate the safety and tolerability of oral SKI-606 in subjects with advanced malignant solid tumors started during 2004.

AZD0530 (AstraZeneca)

Another structural type of dual Abl-Src inhibitors is represented by AZD0530, based upon the quinazoline scaffold. [41] 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. [42] 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.

Other tyrosine kinase inhibitor

MK-0457

The Aurora kinase inhibitor MK-0457 (formerly L-001281814; VX-680) is a potent inhibitor of both wild type (IC50, 10 nmol/L) and T315I BCR-ABL1 kinases (IC50, 30nmol/L). [43] In cell-based assays in which the pre-B Ba/F3 cell line is engineered to express different BCR-ABL1 mutant isoforms, MK-0457 inhibited the proliferation of cells expressing unmutated, Y253F, or T315I BCR-ABL1 kinase with IC50 values of f300 nmol/L. A high-resolution crystal structure of Aurora-A in complex with MK-0457 has been recently compared with that of imatinib bound to ABL1 kinase, revealing that both drugs exhibit nonoverlapping interactions with their respective kinases. [44] MK-0457, however, anchors at the hinge region engaging Asp381 but does not reach as deep into the kinase domain as does imatinib, which allows MK-0457 to avoid the steric constraints imposed by the T315I mutations. [45] In a recent phase I study that included 9 patients with BCR-ABL1 T315I-positive refractory CML in either AP (n = 4) or BP (n = 5), therapy with MK-0457 at 12 to 32 mg/m2/hour given as a 5-day continuous i.v. infusion at 2- to 3-week intervals, rendered 4 CCyRs (1 CCyR, 2 partial, and 1 minor). The main toxicities consisted of myelosuppression, alopecia, and mucositis. Significant inhibition of CrKL phosphorylation was observed in responders. MK-0457 steady-state plasma concentrations were ≥1 μmol/L at a dose over 20 mg/m^2/hour, which are higher than those necessary to inhibit T315I kinase.

Dasatinib has also been shown to synergize with MK-0457. Treatment of BCR-ABL1 T315–positive Ba/F3 cells with MK- 0457 (1 μmol/L) and dasatinib (50 nmol/L) resulted in higher attenuation of STAT5 phosphorylation and increased apoptosis compared with treatment with either agent separately, and prolonged survival in athymic nude mice i.v. injected with BCRABL1T315I-positive Ba/F3 cells, compared with either agent alone . These results provide the rationale for combination trials of MK-0457 and dasatinib in patients with BCR-ABL1 T315I-positive CML. [46]

PHA-739358

PHA-739358 is a pan–Aurora kinase inhibitor with activity against T315 BCR-ABL1 kinase. Treatment with PHA-739358 of CD34+ cells carrying T315Iobtained from imatinib-resistant patients with BP CML significantly decreased phosphorylation of histone H3 Ser10, a marker of Aurora B activity, and CrKL, indicating that this compound inhibits simultaneously Aurora and BCR-ABL1. [47] The cocrystal structure of BCR-ABL1 T315I with PHA- 739358 reveals that the compound binds to the active conformation of the mutant kinase in a mode that accommodates the substitution of isoleucine for threonine, thus avoiding steric clash. In an ongoing multicenter phase II study for patients with CML who failed TKI therapy, seven patients (one CP, one AP, and five BP) have been enrolled, including six carrying the T315I mutation. PHA-739358 was administered at 250 or 330 mg/m2/day as a weekly 6-hour infusion for 3 consecutive weeks, every 4 weeks. [48] Two BCRABL1 T315I-positive patients achieved a complete hematologic response, including 1 in AP who also had a CCyR durable after >6 months and a complete molecular response on the 330 mg/m2 dose level. The second patient was treated in CP and achieved a minor CyR at the 330 mg/m2 dose level. At 330 mg/m2/day, the Cmax was 4 to 6 Amol/L/h. PHA-739358was well-tolerated, with only one patient having grade 4 neutropenia and an infusion-related reaction. [48] Dose escalation in patients with advanced-phase CML is ongoing.

AP23464

Researchers at Ariad have elaborated a series of compounds based upon the purvanalol–purine template for combined Abl/ Src inhibition. [49] A lead compound AP23464 inhibits the transphosphorylation activity of both enzymes withlow 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. 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 modeling. [50] 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. 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. [51] Compounds

related to AP23464 and AP23848 with superior pharmacokinetic properties are being developed.

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 IC50 values <50 nM. [52-53] This activity translates into activity in cellular assays, where the three compounds potently inhibit Bcr-Abl autophosphorylation. In the case of PD180970, inhibition of Bcr-Abl autophosphorylation in K562 cells (IC50 170 nM) has been shown to extend to the inhibition of Gab-2 (IC50 80 nM) and Crkl (IC50 80 nM) phosphorylation, both of which are downstream elements in the Bcr-Abl signalling pathway. [54] PD180970 has also been shown to inhibit Src-mediated phosphorylation of STAT3 (A431 cervical carcinoma cells) and paxillin (HT- 29 colon carcinoma cells. 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] 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. [58-59]

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. In naı¨ve mice, although oral administration of 50 mg/kg (suspension in 10 % aqueous DMSO) only gave mean plasma levels of 98T15 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 hrs 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.

AZD1152

AZD1152 is another selective Aurora-B inhibitor developed by AstraZeneca. It is a highly soluble acetanilide-substituted pyrazole-aminoquinazolone pro-drug that is cleaved completely in human plasma to yield the active drug substance AZD1152 hydroxy-QPA. AZD1152 hydroxy-QPA inhibits Aurora-A, Aurora B-INCENP, and Aurora C-INCENP with respective inhibitory coefficients of 687, 3.7, and 17.0 nmol/L, indicating a 100-fold selectivity for Aurora-B over Aurora-A. Cell line studies reveal inhibition of histone H3 phosphorylation at Ser10 and progression with normal kinetics through anaberrant mitosis, resulting in polyploidy and cell death. [60] Xenograft studies of AZD1152 show reduced phosphorylation of histone H3 on Ser10, increased polyploidy and enhanced apoptosis in athymic nude rodents bearing various human tumors, including colorectal cancer (SW620, HCT116, and Colo205) and lung cancer (A549 and Calu-6). When AZD1152 was dosed as a 48-hour continuous infusion, statistically significant, durable inhibition of tumor growth was observed in all xenograft models. Transient, reversible myelosuppression was the most significant adverse event observed. [61]

KW-2449

KW-2449 is an oral multikinase inhibitor with potent activity against Aurora A (IC50, 48 nmol/L), FLT3 (IC50, 7 nmol/L), FGFR1 (IC50, 36 nmol/L), BCR-ABL1 (IC50, 14 nmol/L), and BCR-ABL1 T315I (IC50, 4 nmol/L) kinases. In a phase I study, KW-2449 is administered at daily doses ranging from 25 to 500 mg divided into 12-hour dosing either on a 14-day or a 28-day schedule. Twenty-nine patients have been enrolled to date, including four with CML, of who three carried T315I. The mean half-life of KW-2449 was 2.8 to 3.9 hours. No treatment-related deaths have been reported. After one cycle of therapy, seven patients had stable disease. [62] Accrual is ongoing and different dosing schedules will be explored given the short half-life of KW-2449.

XL228

XL228 is an Aurora A inhibitor that has shown potent biochemical activity against ABL1 (Ki, 5 nmol/L), as well as the BCR-ABL1 T315I (Ki, 1.4 nmol/L) kinases. [63] In vitro, XL228 inhibits phosphorylation of BCR-ABL1 and STAT5 in K562 cells with IC_{50} values of 33 and 43 nmol/L, respectively, resulting in marked inhibition ofcell proliferation (IC50 < 100 nmol/L). [64] When tested against Ba/F3 cells expressing BCR-ABL1 T315I, XL228 was more effective than MK-0457, imatinib, or dasatinib in downregulating BCR-ABL1 phosphorylation, with IC50 values of 406, 6,912, >10,000, and >10,000 nmol/L, respectively, and in xenografts in vivo. In an ongoing multicenter phase I study, XL228 is administered as a weekly 1-hour infusion in patients with CML or BCR-ABL positive B-ALL who failed therapy with imatinib and dasatinib.

DISCUSSION

It has become apparent that Bcr-Abl is a protein-tyrosine kinase is a very important drug target for treatment of chronic myeloid leukemia. BCR-ABL kinases regulate many cellular processes, including growth and survival, and deregulated activity of these enzymes has been implicated in malignant transformation in various neoplasms. However, BCR–ABL signaling is highly complex and has numerous outputs that promote leukemogenesis. Therefore, specific inhibitors of BCR-ABL kinases are attractive therapeutic agents. BCR–ABL inhibition of apoptosis plays in myeloid cell expansion, tumor progression, and resistance to cytotoxic therapy. Imatinib is proof of principle that rationally designed, molecularly targeted therapy works. Equally important, though not the subject of this review is the efficacy of imatinib in GISTs, chronic myelomonocytic leukemia with rearrangements of PDGFR, and the hypereosinophilic syndrome. Imatinib represents a paradigm shift in cancer drug development. Apart from highly active and durable primary therapy (imatinib) and there are other convincingly proven molecules, dasatinib and nilotinib, dual SRC and ABL kinase inhibitor, which are approved drug for CML therapy as a second generation of BCR-ABL kinase inhibitor.

Nilotinib is in the range of 20- to 30-fold more potent than imatinib. Dasatinib is a novel with more than 100-fold greater potency than imatinib that has been shown to inhibit the kinase activity of 14 out of 15 imatinib-resistant forms of BCR–ABL. In future we will be able to see the developments expanding for novel therapies for leukemias like multikinase (Abl, Src, Lyn, etc) inhibitors such as SKI-606 and INNO-406 and the aurora kinase inhibitor MK0457.

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