

Kinase Inhibitors in Chronic Myelogenous Leukemia

Alfonso Quintás-Cardama, MD, and Jorge Cortes, MD

Dr. Quintás-Cardama is a Fellow in the Department of Leukemia at The University of Texas M. D. Anderson Cancer Center in Houston, where Dr. Cortes is Professor of Medicine, also in the Department of Leukemia.

Address correspondence to:
Jorge Cortes, MD, Department of Leukemia, The University of Texas, M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Unit 428, Houston, TX 77030;
Tel: 713-794-5783; Fax: 713-794-4297;
E-mail: jrcortes@mdanderson.org.

Abstract: The Bcr-Abl tyrosine kinase inhibitor imatinib mesylate launched the era of molecular targeted therapy and constitutes a milestone in oncology history. However, despite impressive cytogenetic response rates achieved with this agent 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 have been described among patients that develop clinical resistance to imatinib. Point mutations in the Bcr-Abl kinase domain that impair the ability of imatinib to inhibit the kinase activity represent the leading cause of resistance. Several approaches are being pursued to overcome these 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. Multiple compounds are being screened to identify inhibitors of these kinases. This article focuses on the current state of development of new kinase inhibitors for the therapy of CML.

The balanced translocation $t(9;22)(q34;q11.2)$ gives rise to the Philadelphia chromosome (Ph) and the BCR-ABL hybrid gene, the hallmark of chronic myeloid leukemia (CML).^{1,2} BCR-ABL encodes an 8.5-kb chimeric mRNA that translates into the constitutively activated tyrosine kinase p210BCR-ABL oncoprotein, which drives the pathogenesis of CML.^{3,4} Importantly, the expression of this protein is distinctly restricted to leukemic cells, thus making it an optimal therapeutic target. Imatinib inhibits the Abl kinase activity *in vitro* and *in vivo* at concentrations of 0.1–1.0 μM .⁵ Not surprisingly, imatinib (Gleevec, Novartis) is associated with an 80–90% complete cytogenetic response rate in early chronic phase CML patients^{6–8} and 7–40% in advanced-phase CML.^{9,10} Despite these remarkable results, two phenomena have proved to be of particular concern during imatinib therapy thus far: the elimination of primitive quiescent Bcr-Abl-positive progenitor cells and the emergence of resistance to this drug in a small but important proportion of patients.^{11,12} The most frequent mechanism of clinical resistance to imatinib is the development of point mutations in the kinase domain of Bcr-Abl, occurring in 30–90%

Keywords

CML, tyrosine kinase inhibitors, dasatinib, nilotinib

of patients.¹³⁻¹⁶ Crystallographic studies have revealed that imatinib binds the canonical ATP-binding site lining the space between the C and the N lobes of Bcr-Abl when the kinase is not phosphorylated, thus stabilizing the oncoprotein in this inactive conformation.¹⁷ Mutations within the ATP pocket impair imatinib-binding and render patients insensitive to the drug, which leads to loss of cytogenetic responses and eventually hematologic progression. In recent years, new insights into the pathogenesis of CML have revealed that several protein kinases modulating signaling pathways downstream of Bcr-Abl are critical in CML transformation. The development of agents that inhibit these Bcr-Abl downstream kinases and more potent direct Bcr-Abl inhibitors, with improved binding properties that make them less susceptible to the known mutations in the kinase domain, is the subject of the present review.

Bcr-Abl Mutations and Downstream Signaling Pathways

More than 30 different Bcr-Abl point mutations have been reported thus far.¹⁸ The actual frequency of Bcr-Abl mutations is difficult to determine because of the lack of standardization and differences in sensitivities among the techniques employed for this purpose, as well as the variability in the selection of patients screened for mutations. However, most current studies suggest that mutations account for approximately 40% of patients who fail imatinib therapy.^{16,23} Mutations tend to occur in relapsed patients, suggesting clonal selection following imatinib exposure. These anomalies affect different regions of the kinase domain and confer varying degrees of insensitivity to imatinib with IC₅₀ levels ranging from 220 to over 5,000 nM/L.¹⁹ Mutations occur most frequently in the P-loop region of the kinase domain.²⁰⁻²² The P-loop region is a glycine-rich structure that serves as a nucleotide-binding loop for phosphate groups of ATP. This is the region where G250E, Q252H, and the highly imatinib-insensitive Y253F/H and E255V/K mutations occur. Despite the fact that these residues are not directly involved in imatinib binding, they have been suggested to confer a poor prognosis.^{16,23,24} The second region involved is the region proximal to T315, where the F317L and the extremely imatinib-insensitive T315I mutations occur in a highly conserved “gatekeeper” threonine residue near the Bcr-Abl catalytic domain leading to steric interference with imatinib binding. These residues are directly responsible for the adequate binding of imatinib to Bcr-Abl.²³ The catalytic domain of Abl spans residues 351 through 359. Common mutations encountered in this region are F359V, M351T, and E355G. Finally, mutations can impact the activation (A)-loop, impairing the ability of

the kinase to achieve the inactive conformation necessary to bind imatinib.²⁶ Thus, one possible strategy to counteract imatinib resistance is to develop more potent Bcr-Abl tyrosine kinase inhibitors with improved binding that are less affected by the different known Bcr-Abl mutations that prevent imatinib inhibition. However, it is possible that direct inhibition of Bcr-Abl alone is insufficient to eradicate all CML cells, particularly the quiescent leukemic stem cell that is insensitive to imatinib even in its wild-type Bcr-Abl state. Thus, other approaches are also being investigated. One of them is to interfere with other protein kinases located downstream from Bcr-Abl that have been implicated in the pathogenesis of CML. In this regard, phosphorylation at the Y177 site of Bcr is essential for Bcr-Abl leukemogenesis.^{27,28} This site provides a high-affinity docking site for the SH2 domain of growth factor receptor-bound protein 2 (Grb2). In turn, Grb2 recruits the scaffold adapter Grb2-associated binding protein 2 (Gab2) through its SH3 domain and SOS. Upon phosphorylation, Gab2 recruits phosphatidylinositol 3-kinase (PI3K) and SHP2, whereas SOS activates Ras.²⁹ Mutation of Y177 to phenylalanine (Y177F) prevents Grb2 binding and abrogates BCR-ABL-induced Ras activation, thus rendering the Y177F mutant unable to transform primary bone marrow cultures.²⁷ Of note, SHP2 can also activate Ras and the SH2 domain of ABL can bind SHC, which, following phosphorylation, can also recruit Grb2.²⁹ In addition, Bcr-Abl also phosphorylates, the Src kinases Lyn, Hck, and Fgr. Once phosphorylated, Hck activates signal transducer and activator of transcription 5 (STAT5).^{30,31} Therefore, Bcr-Abl promotes hematopoietic cell transformation through Ras, PI3K, and STAT5 activation. All these elements have been shown to upregulate the expression of cyclin D1, thus inducing cell cycle progression from G1 to S phase.³²⁻³⁴ When Bcr-Abl-positive K562 cells were induced to express dominant negative forms of Ras, PI3-K, or STAT5, apoptosis was induced in cells coexpressing two of three dominant negative mutants in any combinations.³⁵ Some of these kinases involved in Bcr-Abl signal transduction may be useful therapeutic targets.

New Bcr-Abl Tyrosine Kinase Inhibitors

The emergence of mutations conferring imatinib resistance has led to the development of more potent inhibitors of the ATP-binding site. Some have improved binding and increased selectivity for Bcr-Abl. The lead compound of this family is nilotinib (AMN107), which is currently being evaluated in phase II trials. Other compounds with different mechanisms of action such as dual Abl/Src kinase inhibitors and drugs targeting the Bcr-Abl substrate-binding site of Abl have shown significant *in vitro* activity

and some, such as dasatinib (Bristol-Myers Squibb), have reached advanced stages of clinical development.

Selective Bcr-Abl Inhibitors

Nilotinib (AMN107) The development of the phenylamino-pyrimidine derivative nilotinib has followed a rational drug design, based on the crystal structure of imatinib-Abl complexes. Imatinib fits tightly into the canonical ATP-binding site of the Bcr-Abl kinase domain and captures a specific inactive conformation of the activation loop of Abl, preventing further changes in the core of imatinib.³⁶ The replacement of the N-methylpiperazine ring in the imatinib molecule, which participates in H-bond interactions with both Ile360 and His361, has led to a compound 20- to 30-fold more potent as an Abl inhibitor while preserving similar activity against c-Kit ($IC_{50} = 60$ nM) and PDGFR β ($IC_{50} = 39$ nM).³⁷ Crystallographic models suggest that this increased potency is a result of nilotinib representing a better topographical fit for the Abl kinase pocket. However, nilotinib, like imatinib, binds the Abl protein only in its inactive conformation. The main advantage of nilotinib over imatinib is its ability to inhibit the tyrosine kinase activity of the majority of clinically significant Bcr-Abl mutants, except for T315I. This mutant remained insensitive to nilotinib even at concentrations of approximately 10 μ M, probably because this residue makes direct contact with both imatinib and nilotinib. There was significant inhibition of proliferation of Ba/F3 cells transfected with many other point mutants, although four of them had IC_{50} values over 500 nM (E255K, E255V, L248R, and Y253H), indicating intermediate sensitivity.³⁷ Nilotinib was equally effective at inhibiting autophosphorylation of Bcr-Abl on Tyr177, the binding site for the Grb2 adapter protein. Sublethally irradiated NOD-SCID mice inoculated with 32D.p210 cells engineered to express Bcr-Abl mutants confirmed the predicted biologic activity of this compound.³⁷ Besides the nilotinib-insensitive T315I mutant, other mechanisms of resistance may potentially impair the inhibitory effect of nilotinib. In fact, the imatinib-resistant cell line K562-R may become both imatinib- and nilotinib-insensitive by modification of chaperone proteins (such as heat shock proteins). The IC_{90} for nilotinib at day 4 was 1 μ M versus 4 μ M for imatinib.³⁸ It is also possible that mutations may occur after exposure to nilotinib. In an induced mutagenesis study, nine different mutations were recovered at drug concentrations corresponding to two-fold the IC_{90} , compared to 18 mutations induced with imatinib at equivalent concentrations. Importantly, no additional mutations were recovered with nilotinib compared with imatinib, suggesting that the difference in structure did not generate new vulnerable sites.³⁹ It is possible that by

eliminating more residual Bcr-Abl-bearing cells at a faster rate, the combination of imatinib and nilotinib could prevent the emergence of resistance. Recent studies have indeed suggested that the combination of imatinib and nilotinib may have additive or synergistic activity against cell lines, including some resistant to imatinib but not those with the T315I mutation.⁴⁰

Results of a phase I study of nilotinib in 106 patients with imatinib-resistant CML in chronic, accelerated, and blast phase and 13 patients with Ph-positive acute lymphoblastic leukemia (ALL) treated at doses ranging from 50 mg to 1,200 mg daily have been reported⁴¹ (Table 1). Significant clinical responses were obtained in all CML phases, with hematologic response rates of 30–40% in blast phase, 72% in accelerated phase, and 92% in chronic phase. The rate of cytogenetic response was 22% in lymphoid blast phase and 29% in myeloid blast phase, 48% in accelerated phase and 53% in chronic phase. Among patients with Bcr-Abl kinase domain mutations prior to treatment with nilotinib, 60% achieved a hematologic response and 41% a cytogenetic response.⁴¹ Dose escalation was carried out up to 1,200 mg daily with good tolerance. The pharmacokinetics were linear up to a dose of 400 mg daily with no significant increase in C_{max} or area under the curve with higher doses. However, when the schedule of administration was changed from a single dose to a twice-daily administration, further increases in plasma levels were observed at equivalent total doses. More important, patients who were not responding adequately to a dose of 800 mg daily responded after their total dose was administered in two divided doses (ie, 400 mg twice daily). Thus the twice-daily dose appeared to be more appropriate. The dosing estimated to be the maximum tolerated dose was 600 mg twice daily but this dose level was associated with more neutropenia and hyperbilirubinemia. Overall, the most common drug-related adverse events were gastrointestinal toxicities, rash, and hematologic toxicities; most of these were grade 1 and 2. The steady state of the drug was reached by day 8 of administration, with a T_{max} of 3 hours postadministration and an apparent half-life of 15 hours. Importantly, greater exposure to the drug and inhibition of Bcr-Abl phosphorylation was observed with twice-daily dosing schedules.

Dual Abl/Src Inhibitors

Dasatinib (BMS354825) Src kinases are nonreceptor intracellular tyrosine kinases that modulate signal transduction involved in cell growth, differentiation and survival through multiple oncogenic pathways including platelet-derived growth factor receptor and vascular endothelial growth factor receptor.⁴² At least eight members of this family have been identified: Src, Fyn, and Yes,

Table 1. Response to Nilotinib and Dasatinib in Patients with CML Who Have Developed Resistance or Intolerance to Imatinib

Study Phase	Agent	CML Phase	Patients, n	Response, %			
				Hematologic		Cytogenetic	
				Overall	Complete	Overall	Complete
Phase I	Nilotinib	CP	17	92	92	53	35
		CE	10	100	100	90	20
		AP	46	72	46	48	13
		MyBP	24	42	8	29	4
	Dasatinib	LyBP	9	33	-	22	11
		CP	40	93	93	63	35
		AP	11	81	45	36	18
		MyBP	23	61	35	52	26
	LyBP	10	80	70	90	30	
Phase II	Dasatinib	CP	186	90	90	45	33
		AP	107	59	33	36	21
		MyBP	74	51	24	42	27
		LyBP - Ph+ALL	78	40	26	54	46

AP = accelerated phase; CE = clonal evolution; CML = chronic myeloid leukemia; CP = chronic phase; LyBP = lymphoid blast phase; MyBP = myeloid blast phase; Ph+ ALL = Philadelphia chromosome-positive acute lymphoblastic leukemia.

which are ubiquitously expressed, and Hck, Lyn, Fgr, Lck, and Blk, which are mainly restricted to hematopoietic cells.⁴² It has been shown that Bcr-Abl activates Src kinases both through phosphorylation and direct binding. Furthermore, the Src-related kinase Lyn was highly overexpressed and activated in the imatinib-resistant K562-R cell line and its inhibition reduced proliferation and survival of K562-R cells while having limited effects on the imatinib-sensitive K562 cell line.⁴³ Cell lysates from imatinib-resistant patients with advanced CML who progressed on imatinib therapy have also been found to overexpress Lyn kinase. Analysis of samples taken prior to and after imatinib failure revealed that activation of Src kinases such as Lyn and Hck occurs during disease progression, suggesting that overexpression of these tyrosine kinases may mediate Bcr-Abl-independent imatinib resistance in some patients.⁴³ Therefore, targeting simultaneously Bcr-Abl and Src kinases could overcome Src-mediated resistance.

Dasatinib is an ATP-competitive, dual-specific Src- and Abl-kinase inhibitor with broad-spectrum anti-proliferative activity against hematologic and solid tumor cell lines.⁴⁴ Dasatinib is structurally unrelated to imatinib and is 100- to 300-fold more potent as Bcr-Abl-kinase activity inhibitor, showing also significant activity against c-kit (IC₅₀ = 5 nM) and PDGFRβ (IC₅₀ = 28 nM).⁴⁴ In addition, dasatinib is a potent inhibitor of Hck, Fyn, Src,

and Lck. Inhibition of proliferation of cells overexpressing these kinases can be achieved at IC₅₀ values of 0.5 nM.⁴⁴ Most of the ABL kinase point mutations associated with imatinib resistance impair the ability of the kinase domain to adopt an inactive conformation, thus precluding imatinib binding. Dasatinib binds the ATP-binding site in a position similar to that bound by imatinib, but makes fewer contact points than nilotinib or imatinib, hence offering less stringent conformation requirements for Abl-binding.⁴⁴ As a consequence dasatinib binds both the inactive and active configurations of Bcr-Abl and has a 100-fold higher inhibitory activity against the ABL kinase than imatinib, exhibiting a remarkable activity against cells transfected with wild-type Bcr-Abl as well as 14 of 15 Bcr-Abl mutants.⁴⁵ Burgess and colleagues⁴⁶ determined that BCR-ABL mutations that confer resistance to dasatinib map almost exclusively to structural contact points between the kinase domain and the drug. For imatinib, mutations at noncontact residues, particularly in the P-loop and activation domains, account for most resistant clones. Studies using saturation mutagenesis screen reported by Azam and colleagues⁴⁷ have given insight into the significance of different mutants for imatinib and dasatinib. This technique employs a DNA-repair-deficient *Escherichia coli* strain to elicit mutagenesis of a BCR-ABL retroviral plasmid that is then used for infection of Ba/F3 cells and creation of Ba/F3 clones expressing drug-resis-

tant Bcr-Abl isoforms. Using this approach, 10 Bcr-Abl mutant isoforms resistant to dasatinib were generated with the most frequent being T315A, T315I, F317V, and F317L. All these mutants represent points of contact between dasatinib and the Abl kinase in crystallographic models and will probably account for clinical resistance to this drug.⁴⁶ In a different mutagenesis model, six different mutations were induced with dasatinib, all of them also in drug contact residues.³⁹ Some of these novel mutations (ie, T315A, F317I) have been reported in patients developing resistance to dasatinib.^{48,49} Because T315A and F317V retain sensitivity to imatinib, it is possible that combination therapy with dasatinib and imatinib may overcome or prevent the development of these mutants. In one recent study, dasatinib combined with imatinib showed additive or synergistic effects on the growth of Ba/F3 cells expressing wild-type BCR-ABL.⁴⁶ Of note, dasatinib does not inhibit the T315I mutant even in the presence of μM concentrations, which has been borne out in SCID mice injected with Ba/F3 cells expressing this mutant.⁴⁵ Von Bubnoff and coworkers⁵⁰ established a cell-based screening strategy for detection of clinically relevant point mutations using Bcr-Abl-transformed Ba/F3 cells. A total of 32 different Abl kinase domain point mutations were identified, with a pattern and frequency resembling that encountered in imatinib-resistant patients. Interestingly, the use of the tyrosine kinase PD166326 yielded mutants with a frequency significantly lower than with imatinib. PD166326 produced a distinct pattern of Bcr-Abl mutations. PD166326 at high doses overcame the majority of imatinib- and PD166326-induced mutants.⁵⁰ Whether this assay can be used clinically to identify mutations that affect different tyrosine kinase inhibitors, and modify therapy accordingly, remains to be defined.

Importantly, dasatinib suppressed the proliferation of bone marrow progenitors from CML patients, but had no significant effect on the proliferation of bone marrow progenitors from healthy volunteers.⁴⁵ In addition, dasatinib has been shown to be significantly more effective than imatinib against the leukemic stem cells, although the most primitive quiescent stem cell may still be relatively insensitive to dasatinib.⁵¹

In a phase I dose-escalating study, dasatinib was administered to CML patients in all phases of the disease after failure or intolerance to imatinib therapy (Table 1). In chronic phase, the drug was given at 15–180 mg once or twice daily for 5–7 days per week. Plasma concentrations in the range of 100–200 nM were obtained within two hours after the administration of the higher doses. Twenty-six (84%) of 31 imatinib-resistant and 8 (100%) of 8 imatinib-intolerant patients had a complete hematologic response whereas cytogenetic responses were obtained by 16 (9 complete) and 7 (4 complete) patients

in each group, respectively.⁵² Patients in accelerated or blast phases received dasatinib at 35–90 mg twice daily. A hematologic response was obtained in 81% of 11 patients in accelerated phase, 61% of 23 in myeloid blast phase, and 80% of 10 with lymphoid blast phase or Ph-positive acute lymphoblastic leukemia. Cytogenetic responses were reported in 36% of patients in accelerated phase, 52% in myeloid blast phase, and 90% with lymphoid blast diseases.⁵³ Therapy was generally well tolerated, with gastrointestinal, hepatic, and hematologic toxicities as the major adverse events. Responses were observed through a wide range of Bcr-Abl mutants in 63 evaluable patients (36 in chronic phase, 8 in accelerated phase, and 19 who had blastic phase CML or Ph-positive ALL), but not in patients with T315I (2 of them detected during dasatinib therapy).⁵⁴

Recently, results from a series of phase II studies of dasatinib, administered at 70 mg twice daily to patients with CML in all phases after imatinib failure or intolerance, have been reported (Table 1). In chronic phase, 24 of 186 patients enrolled were available for analysis at the time of this report.⁵⁵ A complete hematologic response was achieved in 21 (90%) of 24 patients, and 7 (45%) of 16 assessable patients for cytogenetic analysis had a complete cytogenetic response (33% complete). Imatinib-resistant mutations were detected in 6 of 12 patients for whom data were available at the time of the analysis. The most common nonhematologic toxicities were diarrhea (6 patients), rash (5 patients), edema (3 patients) and pleural effusion (1 patient), whereas grade 3–4 neutropenia or thrombocytopenia were reported in 6 patients each.⁵⁵ Data from 35 of 107 patients with accelerated-phase CML treated with dasatinib in another phase II study are available.⁵⁶ After 2 months of follow-up, 23 (66%) patients achieved a major hematologic response, while cytogenetic responses were observed in 13 (54%) of 24 patients, including 4 complete and 2 partial responses.⁵⁶ Preliminary data on 34 of the first 74 patients with myeloid⁵⁷ and 28 of 77 patients with lymphoid blast-phase CML or Ph-positive acute lymphocytic leukemia⁵⁸ showed that dasatinib rendered major hematologic responses in 16 of 29 (55%) patients (7 complete) with myeloid blast phase and in 13 patients (7 complete) with CML in lymphoid blast phase/Ph-positive acute lymphocytic leukemia. Major cytogenetic responses were attained by 13 (45%) of patients with myeloid blast phase, including 6 (21%) and 5 (17%) with complete and partial cytogenetic responses, respectively. In patients with lymphoid blast phase/Ph-positive ALL, cytogenetic responses were attained by 12 patients within 1 to 3 months, including 11 complete and 1 minor.^{57,58}

An analysis of molecular responses to dasatinib among imatinib-resistant/intolerant patients (19 in

chronic phase and 14 in accelerated or blast phase) treated in the phase I dose escalation trial of dasatinib has been recently reported.⁴⁸ In this analysis, a ≥ 2 -log reduction of Bcr-Abl below the standardized baseline, which approximates to a CCGR, was considered significant. A ≥ 2 -log reduction was observed in 43% of patients in advanced phase and 37% of patients in chronic phase, including 4 patients in each group who had a major molecular response.⁴⁸ More importantly, responses were maintained in 2 of 6 patients with advanced disease and 6 of 7 patients in chronic phase achieving ≥ 2 -log reductions.⁴⁸

SKI-606 SKI-606 is a 4-anilino-3-quinolinecarbonitrile compound that inhibits phosphorylation of c-Abl and c-Src with an IC_{50} less than 10 nM. This translates into potent inhibition of the Bcr-Abl-positive cell lines MEG-01, KU812, and K562 with IC_{50} levels of 20, 5, and 20 nM, respectively.⁵⁹ Tyrosine phosphorylation of Bcr-Abl is eliminated at concentrations of SKI-606 greater than 100 nM, thus it is at least one order of magnitude more potent than imatinib. In addition, it also ablates STAT5 phosphorylation in K562 cell lines at a concentration of 25 nM and inhibits Lyn and Hck phosphorylation, resulting in apoptosis of imatinib-sensitive and -resistant CML cells.⁶⁰ More importantly, SKI-606 at a dose of 75 mg/kg twice daily orally for 10 days resulted in complete regression of K562 xenografts in nude mice.⁵⁹ G1 arrest and apoptosis was also induced in CD34+ cells isolated from CML patients in blast crisis harboring Y253H, E255V, E255K, or F359V Bcr-Abl mutants.⁶¹ A phase I trial of SKI-606 in CML patients who failed imatinib therapy has started.

Other Dual Src/Abl Inhibitors AZD0530 is a different structural type of orally bioavailable, dual Abl (IC_{50} = 30 nM) and Src (IC_{50} = 1–5 nM) inhibitor, based upon the quinazoline scaffold.⁶² This drug has demonstrated good tolerance in phase I trials in healthy individuals at a dosage of 250 mg, with a terminal half-life of approximately 40 hours.⁶³ However, its profile against Bcr-Abl kinase resistance mutants has not been yet reported. NS-187 is a dual inhibitor of Abl and Lyn that is 20–55-fold more potent than imatinib in vitro and inhibits 12 of 13 mutant Bcr-Abl proteins against which it has been tested. The one exception is T315I.

Some of the first dual Abl/Src inhibitors derived from the pyrido[2,3-d]pyrimidine scaffold. Among them, the best characterized are PD166326, PD173955, and PD180970, which inhibit phosphorylation of Abl, Src, and Lck in cell-free assays with IC_{50} levels below 50 nM. PD180970 added to K562 cells inhibits Bcr-Abl (IC_{50} = 170 nM), Gab-2 (IC_{50} = 80 nM), and Crkl (IC_{50} =

80 nM) phosphorylation.⁶⁴ Pyrido[2,3]pyrimidines are active against a variety of Bcr-Abl mutants in vitro and in murine CML models, but fail to inhibit T315I.^{65,66}

AP23464 is a purvalanol-purine derivative with important activity against Ba/F3 cells transfected with several Bcr-Abl mutants but not T315I.⁶⁷ Despite having remarkable preclinical activity, all these compounds have unfavorable pharmacokinetic and tolerability profiles. Other dual Abl and Src kinase inhibitors have been described, including PPI, CGP76030, AG957, and NSC680410, and INNO-206 (NS-187), and new ones with improved toxicity profiles are being developed.

Non-ATP-competitive Bcr-Abl Inhibitors

A different approach to Bcr-Abl inhibition consists of blocking the substrate-binding site instead of the ATP-binding site. The tyrphostin AG957 (NSC 654705) that inhibits Bcr-Abl and other kinases, induces dose- and time-dependent p210Bcr/Abl downregulation in K562 cells, followed by mitochondrial release of cytochrome c, caspase activation, and apoptotic morphological changes.⁶⁸ The adamantyl ester of AG957 had the same effect at lower concentrations, inhibiting granulocyte colony formation in CML specimens but not in normal progenitors.⁶⁸ Adaphostin induced apoptosis in imatinib-resistant cell lines and acted synergistically with imatinib.^{69,70}

ON012380 is another inhibitor of the substrate-binding site, that has demonstrated a 10-fold stronger inhibition of wild-type Bcr-Abl (IC_{50} = 9 nM), compared to imatinib (IC_{50} = 98 nM).⁷¹ The combination of both drugs demonstrated synergism in Bcr-Abl kinase inhibition assays (IC_{50} = 0.83 nM), in agreement with their different mechanism of action on Bcr-Abl. More important, ON012380 exhibited very strong in vitro inhibitory activity against all 17 tested imatinib-resistant Bcr-Abl mutants, including T315I (IC_{50} = 1.5 nM). In addition, ON012380 at 100 mg/kg administered to athymic nude mice after tail vein injection of 1×10^6 32Dcl3 cells expressing BCR-ABL T315I, showed dramatic reductions in the number of T315I-expressing leukemic cells at 7 and 14 days, compared to imatinib- or saline-treated mice.⁷¹ Moreover, ON012380 given at doses of 300 mg/kg produced no signs of toxicity in mice, suggesting a very good safety profile. Together, these data suggest that ON012380 may have an important impact in CML therapy, particularly for patients with imatinib-resistant mutations.

BIRB796 is a picomolar inhibitor of the stress-activated protein kinases p38alpha and p38beta, which belong to the MAPK family and are crucial in the signal transduction cascade leading to production of proinflammatory cytokines.⁷² For this reason, BIRB796 is currently being tested in phase II trials of rheumatoid arthritis.

Interestingly, BIRB796 binds with excellent affinity to the Bcr-Abl mutant T315I (K_d = 40 nM) and inhibits autophosphorylation of this mutant in Ba/F3 cells with an IC₅₀ value of 1–2 μM.⁷³ Paradoxically, this compound has significantly less affinity for wild-type and other Bcr-Abl mutants (K_d >1 μM), thus complementing the Bcr-Abl binding profile of imatinib. Although this promising in vitro activity will require substantiation in patients harboring the imatinib-insensitive T315I mutation, BIRB796 has demonstrated the feasibility of inhibiting imatinib-insensitive mutations with ATP-competitive molecules.

Targeting Bcr-Abl Downstream Signaling Pathways

The transforming activity of Bcr-Abl relies upon activation of downstream signaling pathways modulated by different protein kinases. Therefore, interference with one or several these regulatory elements could be a means to abrogate leukemic transformation in CML, either alone or in combination with Bcr-Abl tyrosine kinase inhibitors. Among the downstream pathways being pursued as therapeutic targets are the following.

PI3K/Akt Pathway

Phosphorylation of site Tyr177 in the Bcr-Abl protein leads to successive activation of Grb2 and the scaffolding adaptor protein Gab2,^{74,75} which in turn engages phosphatidylinositol 3-kinase (PI3K) and activates the PI3K/Akt pathway.⁷⁶ Akt is regulated by PI3K and promotes apoptotic signals required for transformation and leukemia development. In addition, it has been shown that PI3K signaling is essential for the growth of Ph chromosome-positive CML cells but not for normal hematopoietic precursors.^{76–78} LY294002 is a PI3K inhibitor that enhances apoptosis of BCR-ABL-transformed Gab2 (+/+) B lymphoblasts.⁷⁶ The combination of imatinib with LY294002 or with wortmannin, another PI3K inhibitor, is synergistic, inhibiting the clonogenic growth of cells from patients with both chronic and blastic phase CML.⁷⁷ The celecoxib-derived compound OSU-03012 has inhibitory properties over PDK1, a protein kinase downstream from PI3K.⁷⁵ OSU-03012 successfully inhibited the proliferation of imatinib-resistant mutants T315I and E255K in BaF3 cells and showed synergism with imatinib.⁸⁰ The clinical applications of these interesting properties remain to be determined.

mTOR Pathway

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase downstream of PI3K/Akt that regulates cell growth and proliferation through mRNA translation

(via phosphorylation of p70S6 kinase and eukaryotic initiation factor 4E-binding protein-1) and inhibition of the production of vascular endothelial growth factor.^{81–83} Of note, imatinib-induced compensatory mTOR activation has been demonstrated both in vitro and in patients with CML during the early phase of imatinib resistance, and it has been proposed as a novel mechanism for the persistence of Bcr-Abl-positive cells in imatinib-treated patients.⁸⁴ In this regard, the use of mTOR inhibitors could be a means to overcome this mechanism of resistance. The complex formed by rapamycin and the intracellular immunophilin FKBP12 binds with high affinity and inhibits mTOR. Importantly, rapamycin enhances the inhibitory effect of imatinib on Bcr-Abl-transformed cells, including some imatinib-resistant mutant isoforms,⁸² including T315I.⁸³ In vivo, rapamycin at a dose of 2 mg daily and imatinib given to a patient with CML in blast crisis resistant to imatinib for 17 consecutive days rendered a partial response for 4 weeks.⁸² Other compounds in this class, such as RAD001 and CCI779 are being evaluated in clinical trials.

Ras Pathway

The Ras oncogene is frequently mutated in human cancer. Its signaling pathway is located downstream of Bcr-Abl and is constitutively activated in CML.⁸⁵ Raf is a tyrosine kinase protein involved in Ras signaling and modulates the MEK/ERK pathway. The Raf-1 inhibitor sorefenib (Bayer) induces proliferation arrest of cell lines harboring both wild-type Bcr-Abl and the kinase domain mutants E255K and T315I.⁸⁶ The MEK/MAPK plays an important role in apoptosis regulation. Apoptosis of Bcr-Abl-positive leukemia cells can be induced by disrupting MEK/MAPK activation with inhibitors of the enzymes that activate the MAPK kinases MEK1/2.⁸⁷ Several of these compounds have been developed, including PD184352, PD98059, and U0126. In addition, a synergistic apoptotic effect can be achieved in imatinib-resistant Bcr-Abl-positive cell lines combining these drugs with imatinib.⁸⁸ These findings suggest a therapeutic potential of inhibitors of the MEK/MAPK pathway for CML, alone or in combination with imatinib.

Other Potential Targets in CML

Inhibition of Cyclin-dependent Kinases

Cell proliferation control is ensured by a group of proteins named cyclin-dependent kinases (CDKs). Flavopiridol (L86-8275, HMR 1275) induces cell cycle arrest in G1-S and G2-M due to potent inhibition of multiple CDKs.⁸⁹ Flavopiridol is synergistic with imatinib against imatinib-resistant K562 cell lines and has already entered clinical trials.⁹⁰

Aurora Kinase Inhibitors

Aurora kinases are key elements for chromosome segregation and cytokinesis during mitosis.⁹¹ However, overexpression of Aurora-A and -B leads to aneuploidy and carcinogenesis. VX-680 (MK0457) is an Aurora kinase inhibitor that has caused leukemia regression in an in vivo xenograft model.⁹² In addition, it inhibits several other kinases, including Abl. Recently, it has been shown that VX-680 binds the imatinib-insensitive Bcr-Abl T315I mutant (Kd = 5 nM) causing its inhibition,⁷³ suggesting a role for this kinase inhibitor in patients carrying this currently untreatable Bcr-Abl mutant. Studies with MK0457 in CML with T315I are ongoing, and several clinical trials with other aurora kinase inhibitors in CML are planned.

Conclusion

A better understanding of the molecular pathogenesis of CML and the mechanisms of resistance to tyrosine kinase inhibitors has led to the development of a wide array of new kinase inhibitors in hopes of improving the already impressive results obtained with imatinib mesylate. However, major aspects of the treatment remain unclear, such as the impact of these new kinase inhibitors on quiescent leukemic stem cells or the actual role of Src kinases in the pathogenesis of CML. In addition, new point mutations may arise against these novel agents. The use of high-dose imatinib may help in preventing the spontaneous development of resistant clones, but once they have evolved, a multistep approach similar to that currently used in HIV therapy, combining synergistic kinase inhibitors that act upon different molecular targets, could be a reasonable strategy to overcome this foreseeable occurrence.

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