STI-571: an anticancer protein-tyrosine kinase inhibitor

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Abstract

STI-571 (imatinib, Gleevec, Glivec, CGP 57148) is an inhibitor of the Abl group of protein-tyrosine kinases. One of these enzymes, the Bcr-Abl oncoprotein, results from the fusion of the BCR and ABL genes that result from the reciprocal chromosomal translocation that forms the Philadelphia chromosome. The Philadelphia chromosome occurs in 95% of people with chronic myeloid leukemia. ABL is the cellular homologue of the oncogene found in murine Abelson leukemia virus, and BCR refers to breakpoint cluster region. The Bcr-Abl oncoprotein exhibits elevated protein-tyrosine kinase activity, which is strongly implicated in the mechanism of development of chronic myeloid leukemia. STI-571 is effective in the treatment of the stable phase of chronic myeloid leukemia. The c-Abl protein kinase domain exists in an active and inactive conformation. STI-571 binds only to the inactive state of the enzyme as shown by X-ray crystallography. The drug binds to a portion of the ATP-binding site and extends from there into adjacent hydrophobic regions. STI-571 is a competitive inhibitor of Abl kinase with respect to ATP. Resistance to STI-571 is often the result of mutations in residues of the Bcr-Abl kinase that ordinarily bind to the drug. Inhibition of target protein kinases represents an emerging therapeutic strategy for the treatment of cancer.

Keywords: ABL; Actin; ATM; BCR; Bcr-Abl kinase; c-Abl; c-Kit; Chronic myeloid leukemia; c-Src; Cytosine arabinoside; DNA repair; Gleevec; Glivec; Imatinib; Interferon-α; Leukemia; Myristic acid; Nuclear export sequence; Nuclear localization sequence; PD173955; Philadelphia chromosome; Phosphoserine; Phosphothreonine; Phosphotyrosine; Platelet-derived growth factor receptor; Posttranslational modification; Protein kinase A; Protein kinase C; Protein phosphorylation; SH2; SH3; Signal transduction; v-Abl; v-Src; Xeroderma pigmentosum

Protein kinases are enzymes that play a role in nearly every aspect of cell biology [1]. Edwin Krebs and Edmond Fischer showed that protein kinases play a regulatory role in glycogen metabolism; subsequently many other investigators showed that protein kinases participate in transcription, cell cycle progression, cytoskeletal rearrangement, apoptosis, differentiation, development, and the immune response. The brain, moreover, is an especially rich source of protein kinase activity. Recent studies indicate that mutations and dysregulation of protein kinases play causal roles in human disease so that these enzymes represent bona fide drug targets [2].

Protein kinases catalyze the following reaction:

\[ \text{MgATP}^{-1} + \text{protein–OH} \rightarrow \text{Protein–OPO}_{3}^{-2} + \text{MgADP} + \text{H}^{+} \]

Based upon the nature of the phosphorylated –OH group, these enzymes are classified as protein-serine/threonine kinases and protein-tyrosine kinases. Furthermore, there is a small group of dual specificity kinases, which closely resemble serine/threonine kinases, that catalyze the phosphorylation of both threonine and tyrosine on target proteins. The ratio of phosphoserine/phosphothreonine/phosphotyrosine in proteins from normal animal cells in culture is about 3000/300/1 [3]. Despite the scarcity of protein-tyrosine phosphate, it plays a paramount role in cell signaling. Protein kinases form the fourth largest family of genes in humans; it follows C2H2 zinc finger proteins (3% of all genes), G-protein coupled receptors (2.8%), and the major histocompatibility complex protein.

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Abbreviations: GIST, gastrointestinal stromal tumor; JNK, c-Jun amino-terminal kinase; JNK, c-Jun kinase; SH2, Src homology domain 2; SH3, Src homology domain 3.
family (2.8%) [4]. Tony Hunter and co-workers [5] identified 478 typical and 40 atypical protein kinase genes in humans (total 518) that correspond to about 2% of all human genes. These genes encode for 90 protein-tyrosine kinases, 43 tyrosine-kinase like proteins, and 385 serine/threonine kinases. There is thus no shortage of potential drug targets, as protein kinases represent the largest enzyme family in humans. The majority of protein kinases make up a superfamily with a characteristic catalytic domain.

Several protein kinases are implicated in the mechanisms leading to malignancies [6]. In 1978, Ray Erikson found that the Rous sarcoma virus oncogene product was a protein kinase, now called v-Src (where v represents viral and c represents the normal cellular homologue). In 1980, Tony Hunter and Bartholomew Sefton [3] showed that v-Src mediates the phosphorylation of tyrosine residues; this discovery ushered in a new era in signal transduction research. The platelet-derived growth factor receptor protein-tyrosine kinase family, which includes c-Kit, is associated with several human cancers. The Bcr-Abl protein-tyrosine kinase is associated predominantly with chronic myeloid leukemia but also with 5–10% of adults with acute lymphoblastic leukemia [7].

**Chronic myeloid leukemia and the Bcr-Abl protein-tyrosine kinase**

Chronic myeloid leukemia is a malignant hematological disease characterized by an elevated white blood cell count [7]. There is an increased production of granulocytes, especially neutrophils. About 40% of the patients are asymptomatic at the time of presentation, and the diagnosis is based upon an abnormal blood count. Chronic myeloid leukemia accounts for about 20% of all cases of leukemia. The expected incidence of new cases in the United States in 2003 is 4300, compared with 171,000 new cases of lung cancer [8]. The natural history of chronic myeloid leukemia is progression from a stable or chronic phase to an accelerated phase or to a rapidly fatal blast crisis within 3–5 years. Blood cells differentiate normally in the stable phase but not in the blast phase [7,9].

The diagnosis of chronic myeloid leukemia is usually based on detection of the Philadelphia chromosome [7]. Peter Nowell and David Hungerford [10] first described this abnormal chromosome in chronic myeloid leukemia in 1960, and this represents the first specific cytogenetic change associated with a human cancer. Later work showed that the abnormal chromosome was a shortened chromosome 22—the Philadelphia chromosome. Janet Rowley [11] described in 1973 the reciprocal exchange of DNA between the long arms of chromosomes 9 and 22 that results in a shortened chromosome 22 and a lengthened chromosome 9. The Philadelphia chromosome is present in bone marrow cells of 95% of people with chronic myeloid leukemia [7]. It occurs in myeloid cells (granulocytes), erythroid cells (red cell lineage), monocytes, megakaryocytes (platelet precursors), less commonly in B-lymphocytes, rarely in T lymphocytes, but not in marrow fibroblasts.

Owen Witte and co-workers showed in 1980 [12] that the Abelson leukemia virus oncogene product, v-Abl, is a constitutively active protein-tyrosine kinase. Later work showed that the 3’ end of the cellular Abelson leukemia virus homologue gene (c-ABL) is moved from chromosome 9 to chromosome 22 where it is combined with the 5’ portion of the breakpoint cluster region gene (BCR) on chromosome 22 [11,13–16]. The consequence of the BCR-ABL translocation is the creation of a Bcr-Abl fusion protein, a constitutively active cytoplasmic protein-tyrosine kinase [17,18]. Owing to the variability of the sites of disruption in the BCR gene, three chimeric gene products have been described: p210\textsubscript{Bcr-Abl}, p190\textsubscript{Bcr-Abl}, and p230\textsubscript{Bcr-Abl}. These proteins contain the same segment of Abl but variable segments of Bcr [9]. p210\textsubscript{Bcr-Abl} is associated with chronic myeloid leukemia; p190\textsubscript{Bcr-Abl} and p230\textsubscript{Bcr-Abl} are associated with other forms of leukemia. Insertion of a retrovirus encoding p210\textsubscript{Bcr-Abl} into cells of mice leads to the development of a disorder closely resembling chronic myeloid leukemia, giving support to the hypothesis that the BCR-ABL hybrid gene is sufficient to cause this disease [18–20].

Previous treatments of chronic myeloid leukemia include hydroxyurea, interferon-\(\alpha\) with cytosine arabinoside, and bone marrow transplantation [7]. Although bone marrow transplantation can lead to cures, donors are available for only about 20% of affected people.

Chronic myeloid leukemia, at least in the stable phase, is unique among malignancies in that the malady appears to be the result of a single major biochemical defect [7,18]. In contrast, most malignancies are the result of several genetic and biochemical lesions [21]. Additional mutations account for disease progression from the stable phase to the accelerated and blast phases of chronic myeloid leukemia. The Bcr-Abl oncprotein, an activated protein-tyrosine kinase, thus represents a unique drug target that differs between normal and leukemic cells.

**Development of STI-571**

STI-571 (Signal Transduction Inhibitor-571) is an inhibitor of Abl protein-tyrosine kinases. STI-571 is known also as CGP 57148, imatinib, Gleevec (in the United States), and Glivec (in Europe). The chemical precursor of STI-571 was discovered by the time-consuming process of testing a large number of compounds for inhibition of protein kinase C in vitro [22,23].
Protein kinase C is a serine/threonine kinase, which includes numerous isoforms, that participates in many cellular processes and is implicated in tumor formation. The initial chemical was a phenylaminopyrimidine that inhibited both serine/threonine and tyrosine protein kinases.

A number of derivatives of phenylaminopyrimidine were made to increase its efficacy for platelet-derived growth factor receptor protein-tyrosine kinase. Additional modifications were made to enhance cellular activity and to increase solubility and oral bioavailability. This resulted in STI-571 (Fig. 1), originally shown to be an inhibitor of platelet-derived growth factor and v-Abl protein-tyrosine kinases [23].

A significant advantage of STI-571 is that it is effective when administered orally; many anticancer drugs are effective only when injected. In contrast to treatment with antimetabolites and irradiation, side effects from STI-571 are mild [9]. STI-571 suppresses the proliferation of Bcr-Abl-expressing cells in vitro and in vivo. Using specimens obtained from people with chronic myeloid leukemia for colony-forming assays, Brian Druker and co-workers [24] showed that STI-571 produces a 92-98% decrease in the number of colonies formed from Bcr-Abl cells. Of great importance, STI-571 had a minimal effect on colony formation from normal cells. Note that the initial targets of this research were protein kinase C and platelet-derived growth factor receptor kinase and not Bcr-Abl kinase.

Besides v-Abl and platelet-derived growth factor receptor, STI-571 is an inhibitor of c-Abl, Bcr-Abl, and c-Kit (stem-cell factor/mast-cell factor receptor) protein-tyrosine kinases. It is not a potent inhibitor of about 30 other protein kinases that were tested [23]. The concentration that inhibits c-Abl kinase activity 50% in vitro is about 35 nM. Higher concentrations (≈1 μM) are required to inhibit Bcr-Abl in cells, but these concentrations can be achieved in humans by once-daily administration of STI-571 (≥ 300 mg).

c-Abl kinase structure and mechanism

Susan Taylor and co-workers [25] first described in 1991 the three-dimensional structure of a protein kinase: cyclic AMP-dependent protein kinase, or protein kinase A. The enzyme consists of two lobes with a cleft between them. At the time the primary structures of about 65 protein kinases were known or inferred [26]. Based upon the nature of the conserved residues and their location in the protein kinase A structure, Taylor and co-workers [25] postulated that the bilobed structure would occur in all serine/threonine and tyrosine protein kinases, and this prediction has proven correct [27].

Early work showed that v-Abl and p210Bcr-Abl had protein-tyrosine kinase activity [12,17], but the demonstration of this catalytic activity in c-Abl, the normal cellular homologue, required additional work. This was due to the low level of c-Abl expression in cells and the need to work out the appropriate methods for cell lysis and noninhibitory immunoprecipitation of the enzyme [28].

The overall structure of the c-Abl kinase domain consists of the characteristic bilobed protein kinase architecture (Fig. 2) [30,31]. Residues 244–369 (human c-Abl 1b spliceform) make up the amino-terminal lobe of the kinase; residues 373–517 make up the carboxyl-terminal lobe. The amino acid sequence of the catalytic domain of human c-Abl differs from that of mouse.
c-Abl by one residue; an asparagine (human) to serine (mouse) substitution occurs at position 355 on the surface of the carboxyl-terminal lobe. Most biochemical and crystallographic studies are performed with these two enzymes, and the results are interchangeable.

The smaller amino-terminal lobe of protein kinases, including c-Abl, is primarily involved in anchoring and orienting ATP [25,27,30]. This smaller lobe has a predominantly antiparallel β-sheet structure. The larger carboxyl-terminal lobe is responsible for binding the peptide substrate. However, part of the ATP-binding site occurs in the large lobe. The large lobe is predominantly α-helical in nature (Fig. 2). As described for protein kinase A [25], the catalytic site of c-Abl lies in a cleft between the two lobes. The two lobes move relative to each other and can open or close the cleft [32,33]. The open form is necessary to allow access of ATP to the catalytic site and release of ADP; the closed form is necessary to bring residues into the catalytically active state. Any process that can block the interconversion of the open and closed forms of the cleft will be inhibitory.

Tony Hunter and co-workers [26] identified 12 subdomains with conserved amino acid residue signatures that constitute the catalytic core of protein kinases. Of these, the three following residues illustrate the inferred catalytic properties of c-Abl kinase. Lys290 of c-Abl 1b represents an invariant residue of protein kinases that forms ion pairs with the α- and β-phosphates of ATP. Asp382 orients the tyrosyl group of the substrate protein in a catalytically competent state. Asp382 may function as a base that abstracts a proton from tyrosine thereby facilitating its nucleophilic attack of the γ-phosphorus atom of MgATP; Asp382 is called the catalytic base. Asp400 is the first residue of the activation loop found in the large lobe. Asp400 binds Mg2+, which in turn coordinates the β- and γ-phosphate groups of ATP. Mg2+ is required for both high-affinity ATP binding and catalysis by protein kinase A [34]. Mn2+ and Co2+ are equally effective. In contrast, Ca2+ promotes ATP binding, but it fails to support catalysis. Mg2+ and Mn2+ support catalysis by the Abi kinases.

The two lobes can adopt a range of relative orientations, opening or closing the active-site cleft [33]. Within each lobe is a polypeptide segment that has an active and an inactive conformation [27]. In the small lobe, this segment is the major α-helix. It is designated as the C α-helix, or αC-helix (it is preceded by minor A and B helices in protein kinase A) [25]. The αC-helix in some kinases rotates and translates with respect to the rest of the lobe, making or breaking part of the catalytic site. In the large lobe, the activation loop adjusts to make or break part of the catalytic site. In most kinases, including c-Abl, phosphorylation of the activation loop stabilizes the active conformation.

The conformation of the activation loop differs between active and inactive kinases [27,33,35]. The activation loop of nearly all protein kinases begins with Asp–Phe–Gly (DFG) and ends with Ala–Pro–Glu (APE). In protein kinases that are inactive, the activation loop has a compact conformation. In structures of enzymes that are in an active state, the activation loop is in an extended and open conformation (Fig. 3). There are two crucial aspects to this active conformation. First, the aspartate residue (Asp400 in c-Abl 1b) within the conserved DFG motif at the amino-terminal base of the activation loop binds to the magnesium ion as noted above. Second, the rest of the loop is positioned away from the catalytic center in an extended conformation so that the carboxyl-terminal portion of the activation loop provides a platform for protein substrate binding [35].

**Intramolecular regulation of c-Abl kinase**

In a surprising development, the regulation of c-Abl turns out to closely resemble that of c-Src, despite key structural differences. Recent collaborative work by the laboratories of Giulio Superti-Furga and John Kuriyan [31] shows that c-Abl kinase has an elaborate regulatory apparatus made from structural elements within and outside the bilobed kinase domain. c-Abl kinase contains an amino-terminal cap, a Src-homology 3 (SH3) domain, a Src-homology 2 (SH2) domain, a protein kinase domain (SH1), a DNA-binding domain, and an actin-binding domain. There is a connector between the SH3 and SH2 domains, and a linker between the SH2 and kinase domains. SH2 domains are noncatalytic regions of ~100 amino acids that bind to phosphotyrosyl...
residues. SH3 domains are noncatalytic regions of \( \approx60 \) amino acids that bind to proline-containing sequences.

The apparatus controlling c-Abl has three components called the switch, the clamp, and the latch [31,36]. The switch is the kinase-domain activation loop; the activation loop can open between active and inactive conformations as described above. The clamp is an assembly of the SH2 and SH3 domains behind the kinase domain. The SH2 domain contacts the large lobe, and the SH3 domain contacts the small lobe (Fig. 4). The linker between the SH2 and kinase domains contains proline at positions 242 and 249 that function as a motif that binds the SH3 domain and attaches the SH3 domain to the small kinase lobe. The amino-terminal cap of c-Abl 1b contains an N-myristoyl group. The large lobe of the kinase has a deep hydrophobic pocket for the fatty acid chain; the local structure of this pocket depends on whether or not the myristoyl group is bound.

When the myristoyl group binds to the hydrophobic latch in the large lobe, this protein segment keeps the SH3 and SH2 clamp in place. As a result, the SH3 and SH2 domains inhibit the ability of the catalytic cleft to open and admit ATP, thus inhibiting the enzyme (Fig. 4). Note that the phosphotyrosine pocket in the SH2 domain of c-Abl is not occupied with phosphotyrosine as it is in inactive c-Src kinase.

There are several potential mechanisms for converting c-Abl from an inactive state to an active state. One idea is that there is an equilibrium between the enzyme forms with the myristoyl group bound or not bound to the hydrophobic pocket. Since c-Abl activity is fastidiously regulated, the equilibrium favors the inactive bound conformation. In the inactive state, Tyr 412, which occurs in the activation loop, is sequestered and is not a substrate for phosphorylation by another kinase. When the myristoyl group dissociates from the hydrophobic binding pocket, rearrangement near the hydrophobic pocket destroys the docking surface for the SH2 domain; the clamp no longer locks the catalytic domain into an inactive conformation [31]. The activation loop can open, thereby forming an active enzyme. Tyr412 can then be phosphorylated by another c-Abl kinase molecule or another protein kinase. Following phosphorylation, the enzyme is stabilized in its active state. The corresponding interactions of c-Abl 1a, lacking the myristoyl group, may include a group of hydrophobic residues at the amino terminus. c-Abl 1a results from alternative splicing that yields a non-myristoylated protein containing 19 fewer amino acid residues than are found in c-Abl 1b.

This structural design allows for c-Abl regulation at multiple levels including competition between intramolecular and external ligands [31,36]. The intramolecular interactions maintain an inactive state, and external ligands promote an active state. Proteins that bind to the SH2 domain of c-Abl disrupt the clamp, activating the kinase. Similarly, proteins that bind to the SH3 domain of c-Abl domain also disrupt the clamp, activating the enzyme. There is also the possibility that a regulatory protein may bind to the amino-terminal myristoyl group, remove it from the hydrophobic latch, thereby promoting c-Abl activation. Such a protein has not yet been identified.

Yet another mechanism of activation of c-Abl involves the trans phosphorylation of Tyr245 that is in the linker region between the SH2 and kinase domains [37]. Modification of this residue breaks the SH3 linker interaction thereby unclamping and activating the enzyme. Reversal of this activation process requires enzymatic-mediated dephosphorylation, but this process has not been characterized.

**Actions of c-Abl and Bcr**

c-Abl, which is expressed in all types of cells and not just hematopoietic cells, is located in both the cytoplasm and nucleus [9,38]. Localization of a protein-tyrosine kinase in the nucleus is unusual. In contrast, the Bcr-Abl kinase is located exclusively in the cytoplasm. Most cytoplasmic c-Abl is associated with filamentous actin. c-Abl also interacts with cell-cycle regulatory proteins, thereby affecting cell proliferation. c-Abl has DNA-binding activity, which may be involved in initiating transcription, in the DNA damage response, and in meiotic processes. A role for c-Abl in DNA repair has been suggested by its interaction with other molecules involved in this activity, such as the ATM gene product. Mutation of ATM causes ataxia telangiectasia, a disorder characterized by hypersensitivity to radiation damage and predisposition to B-cell lymphoma and T-cell leukemia. c-Abl, which is carefully regulated, is inactive unless it receives stimulatory signals.

The BCR gene product is a complicated molecule with many different functional motifs. It is implicated in protein phosphorylation and GTP function [9]. The first
exon of the BCR gene is pivotal to oncogenesis; it occurs in all known Bcr-Abl fusion proteins. Bcr, which has protein-serine/threonine kinase activity, represents one of the atypical protein kinases [5]. Several SH2-binding domains also occur in BCR. Bcr has both GTPase-activating protein and guanine nucleotide exchange factor functions, suggesting a dual role for this molecule in G-protein–associated signaling pathways. Finally, Bcr and Bcr-Abl interact with the xeroderma pigmentosum gene products. Xeroderma pigmentosum is an inherited disease whose hallmark is increased sensitivity to sunlight coupled with a predisposition to skin cancer and a defect in the DNA damage response. Thus, Bcr may also participate in DNA repair.

**Bcr-Abl kinase activation**

As a result of chromosomal translocation and generation of a chimeric protein, Bcr-Abl is an activated protein-tyrosine kinase [18,28]. The amino-terminal portion of the chimera consists of a fragment of Bcr thereby eliminating the myristoyl group that binds to the latch that shuts down kinase activity. Moreover, the Bcr fragment contains an oligomerization domain [39]. The oligomerization of the Abl kinase appears to be critical in the development of leukemia in animal models [23].

Like the phosphorylation activation following ligand-induced dimerization of receptor protein-tyrosine kinases [40], trans phosphorylation of the residue corresponding to Tyr412 of the c-Abl 1b leads to activation of Bcr-Abl. Trans phosphorylation refers to the phosphorylation of one protein kinase molecule by another one; cis phosphorylation—which is apparently rare—refers to phosphorylation of a protein kinase by itself. The term autophosphorylation, which is common in the protein kinase literature, does not distinguish between cis- and trans-phosphorylation. It is used to describe observations where the mechanism, cis or trans, is unknown. For Bcr-Abl, oligomerization keeps four Abl kinase molecules close together, thereby increasing the probability of trans phosphorylation and activation. c-Abl exists as a monomer; the rate of phosphorylation increases as the concentration of monomer increases providing evidence for trans and not cis phosphorylation [41].

Normal c-Abl, which contains nuclear localization sequences and a nuclear export sequence carboxyl-terminal to the kinase domain, functions in both the cytosol and in the nucleus. Bcr-Abl, which contains these targeting sequences, is found only in the cytoplasm. The reason for the failure of Bcr-Abl to be translocated into the nucleus is unknown. Inhibition of Bcr-Abl by STI-571 promotes nuclear translocation [6].

The constitutively active Bcr-Abl kinase most likely activates a number of signal transduction pathways that influence the growth and survival of hematopoietic cells. Downstream signals may include Ras and Raf, Stat, Jun amino-terminal kinase (JNK), the myc transcription factor, and phosphatidylinositol 3-kinase and protein kinase B (Akt) [7,9,38]. The major tyrosine phosphorylated protein in chronic myeloid leukemia cells is Crk1 (Crk like); monitoring the ratio of unphosphorylated and phosphorylated Crk1 with denaturing gel electrophoresis provides a measure of Bcr-Abl enzyme inhibition in vivo [22].

**Inhibition of Abl by STI-571**

STI-571 is an effective inhibitor of c-Abl and Bcr-Abl protein-tyrosine kinase activity. John Kuriyan and co-workers [30,35], using X-ray crystallography, found that STI-571 binds to an inactive conformation of c-Abl (Fig. 3A). The drug binds in the cleft between the amino and carboxy-terminal lobes of the kinase domain. Only the leftmost portion of STI-571 (the pyridine and pyrimidine rings shown in Fig. 1) 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 [42], which is consistent with the X-ray studies showing that the drug binds to the ATP-binding site.

PD173955, which is another inhibitor of Abl protein kinases, binds in the cleft between the two lobes and occupies a portion of the ATP-binding site (Fig. 3B). In contrast to STI-571, PD173955 can bind to both the active and inactive conformations of the c-Abl kinase domain [35].

In the c-Abl/STI-571 complex, the amino-terminal portion of the activation loop is rotated with respect to the active conformation so that Phe401 rather than Asp400 of the DFG motif points toward the ATP-binding site. Asp400 binds the magnesium ion of the MgATP substrate in the active conformation, but this salt bridge cannot occur in c-Abl/STI-571 owing to the rotation of this segment. Moreover, the conformation with Phe401 pointing toward the ATP-binding site is crucial for binding STI-571. The rest of the activation loop adopts a conformation in which the region surrounding Tyr412 (a site for activating phosphorylation) mimics substrate binding to the enzyme, thereby blocking the protein substrate-binding site. In the non-phosphorylated form, Tyr412, which is folded into the active site of the kinase, forms a hydrogen bond with Asp382 (the catalytic base) [30,35].

In the c-Abl/STI-571 complex, the activation loop mimics the binding of protein substrates like that of the inactive insulin-receptor protein-tyrosine kinase [40].
This result suggests that the loop is in a natural auto-
inhibitory conformation. Although Tyr412 is positioned
like a peptide substrate, the kinase domain is not in a
conformation that is competent for tyrosine phosphor-
ylation since the inward movement of the activation
loop is coupled to the displacement of the DFG motif
away from the active conformation [35]. The interaction
of STI-571 with the amino-terminal lobe involves an
induced-fit mechanism.

A total of 21 amino acid residues of the c-Abl cata-
lytic domain interacts with STI-571 forming six hydro-
gen bonds. The majority of interactions involve van der
Waals forces between protein residues and the aromatic
rings of the inhibitor [35]. The complementary fit limits
any modification of either the inhibitor or the kinase
domain without compromising binding affinity. Once
STI-571 is bound to c-Abl, it occupies the space between
the activation loop and helix αC, preventing the activa-
tion loop from changing conformation. STI-571 is an
effective inhibitor only when c-Abl is unphosphorylated.

In chronic myeloid leukemia, c-Abl is fused with Bcr
causing it to be constitutively active. Thus, the activa-
tion loop in Bcr-Abl would be in the open conformation
and phosphorylated. Given that STI-571 cannot recog-
nize this open conformation, how does STI-571 achieve
its great inhibitory effect? One possibility is that the
phosphorylation state is dynamic. The action of cellular
phosphatases constantly counteracts the kinase activity
of the Bcr-Abl complex. 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.

The residues that contact STI-571 in c-Abl kinase are
either identical in the Src family tyrosine kinases or are
substituted conservatively. Nevertheless, this compound
is inactive against c-Src kinase activity. The residues that
are not identical in c-Abl and the c-Src kinases also vary
in the c-Kit and platelet-derived growth factor receptor
tyrosine kinases, which are the only other kinases re-
ported to be inhibited by STI-571. However, the inactive
conformation of the activation loop in c-Src is much
different from that of c-Abl, thereby accounting for the
inability of STI-571 to inhibit c-Src [35].

Resistance to STI-571

Despite the success with STI-571 in the treatment of
chronic myeloid leukemia, the issue of how to maximize
response and eliminate resistance remains. In the stable
phase of chronic myeloid leukemia, about 2–4% of pa-
tients fail to achieve hematological remission [43].
However, about 60% of those in the accelerated phase
and about 90% in the blast crisis either fail to respond or
relapse soon after an initial response [44]. Mutations
within the Bcr-Abl kinase domain are the most com-
monly identified mechanisms associated with relapse.

The following four amino acid replacements account
for about 60% of the mutations found at the time of
disease relapse: T334I, Y272F, E274K, E274V, and
M370T [45]. Threonine 334 makes a specific hydrogen
bond with STI-571, but this cannot occur in the iso-leu-
cine mutant. Construction of c-Abl containing this al-
teration showed that the enzyme is insensitive to STI-
571. Tyrosine 272 makes van der Waals contact with
STI-571; substitution of phenylalanine at this position
leads to a resistant form of the kinase. Amino acid 273 is
located within the ATP-binding site of the kinase; mu-
tations that convert glutamate to lysine or valine lead to
enzymes that are insensitive to STI-571. Methionine 370
is not part of the ATP-binding loop nor is it within that
activation loop. However, it occurs at the base of the
activation loop, which may explain its ability to influence
binding of STI-571 leading to a resistant enzyme form.

Other mechanisms leading to resistant forms of
chronic myeloid leukemia are being studied. These in-
clude upregulation of multidrug-resistance proteins,
functional inactivation of STI-571, and BCR-ABL gene
amplification [9]. Because of these therapeutic failures,
work is underway to develop additional inhibitors of
Bcr-Abl kinase. Similar to the treatment of AIDS with
several reverse transcriptase inhibitors, successful
treatment of chronic myeloid leukemia may require a
combination of drugs.

Epilogue

The dramatic success in the treatment of the stable
phase of chronic myeloid leukemia by an inhibitor of the
Bcr-Abl kinase is due to the propitious mechanism in-
volved a single major biochemical defect. It is impor-
tant to note that this special characteristic is lacking in
nearly all other forms of malignancy, and development
of an inhibitor to a single target is less likely to be ef-
te in treating other cancers. Studies in animals and
humans are consistent with the notion that the Bcr-Ab1
oncoprotein is the major factor in producing the disor-
der [9,18,23]. However, other factors may play a role in
the development of the malady as indicated by the
presence of BCR-ABL transcripts in the blood of many
healthy persons [9]. Nevertheless, the protein-tyrosine
kinase activity is essential for the ability of Bcr-Abl to
produce the disease [18]. The presence of Bcr-Abl in 95%
of patients with chronic myeloid leukemia and the re-
quirement for kinase activity make Bcr-Abl a theoreti-
cally attractive drug target.

Because of the low incidence of chronic myeloid
leukemia [8], developing treatments for this disease
was given low priority by the pharmaceutical industry.
Ironically, STI-571 (Gleevec, Glivec) has been a
commercial success, and this success has stimulated considerable research [2].

Targeting the ATP-binding site of protein kinases was not thought to be effective because of the large number of protein kinases and other ATP-requiring enzymes and the likelihood that these binding sites would be undistinguishable. The success of STI-571 in the treatment of chronic myeloid leukemia dispelled this notion and serves as a proof of principle for the usefulness of this approach. Other enzymes that are inhibited by STI-571 include c-Kit and platelet-derived growth factor receptor. c-Kit is a receptor protein-tyrosine kinase that is activated by stem cell growth factor. This enzyme is activated in gastrointestinal stromal tumors (GIST); studies on the efficacy of STI-571 in the treatment of these cancers have been reported [46].

As noted above, STI-571 is not specific for the activated Bcr-Abl kinase; the drug also inhibits c-Kit and platelet-derived growth factor receptor protein-tyrosine kinase. Is this pharmacological profile important for successful treatment of chronic myeloid leukemia? Moreover, only about 30 of the more than 500 protein kinases have been tested for STI-571 inhibition and reported in the open literature. How many others are inhibited, and do they play a role in the therapeutic response? The answers to these questions may emerge from proteomic profiles. Assays for about 100 purified protein kinases are currently available so that it may be several years before the entire complement of protein kinases can be tested for inhibition by STI-571 and other drugs.

STI-571 was discovered through the process of empirical high-throughput screening for small molecule inhibitors of protein kinase C [23] rather than through rationale design of drugs based on knowledge of a particular protein kinase domain structure. However, crystallographic data of many target protein kinases in various states of activation promises to lead to structure-based drug design of inhibitors of these enzymes. Structural studies reveal subtle differences in the ATP-binding site and contiguous regions that can be exploited to provide specificity for kinase inhibitors. This approach has great potential.

With STI-571 and c-Abl kinase, could it have been predicted that a portion of the binding site would undergo perturbation to allow the drug to bind as a result of induced fit? Moreover, is it therapeutically advantageous that STI-571 preferentially binds to the inactive form of the Abl tyrosine kinase? As a result of sequestering Tyr412, Abl kinase/STI-571 cannot be activated by trans phosphorylation by upstream pathways, and this property may be important mechanistically. Development of inhibitors of the inactive form of protein kinases may prove to be beneficial.

Chronic myeloid leukemia was first described in Europe in the 1840s. Rudolph Virchow, the noted German pathologist, made important contributions in elucidating the nature of this disease, and he coined the term leukemia. The Philadelphia chromosome was discovered in chronic myeloid leukemia in 1960, the reciprocal translocation was reported in 1973, and the protein-tyrosine kinase activity of the v-Abl oncoprotein product was reported in 1980. Later work showed that Bcr-Abl is a protein-tyrosine kinase that could produce disorders in mice that resembled chronic myeloid leukemia. In the early 1990s researchers at Ciba-Geigy (now Novartis) identified several compounds that were protein kinase inhibitors. Jürg Zimmermann and collaborators synthesized STI-571 in 1992. Brian Druker and colleagues reported the inhibitory effects of STI-571 (also known as CGP 57148 of Ciba-Geigy Pharmaceuticals) on the growth of Bcr-Abl positive cells in 1996 [24]. Clinical trials with STI-571 were begun in June 1998, and the United States Food and Drug Administration approved the drug for the treatment of Philadelphia chromosome positive chronic myeloid leukemia in May 2001. Thus, it required about 20 years from the discovery of the culprit protein kinase to approval of an inhibitory anticancer agent.

Owing to the recent introduction of STI-571 into the clinic, it will be some time before the long-term results of treatment of chronic myeloid leukemia with this drug will be known. Because of the effectiveness and relative safety of STI-571, it is being tested in diseases that may involve c-Kit or platelet-derived growth factor receptor.

References


