Imatinib (STI571) Resistance in Chronic Myelogenous Leukemia: Molecular Basis of the Underlying Mechanisms and Potential Strategies for Treatment

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Abstract: Following the paradigm set by STI571, protein tyrosine kinase inhibitors are emerging as a promising class of drugs, capable of modulating intracellular signaling and demonstrating therapeutic potential for the treatment of proliferative diseases. Although the majority of chronic phase CML patients treated with STI571 respond, some patients, especially those with more advanced disease, relapse. This article reviews the reasons for relapse and, in particular, analyses resistance resulting from Bcr-Abl tyrosine kinase domain mutations at the molecular level. Arguments are based upon the structure of the STI571-Abl complex, which is compared to the crystal structures of PD173955-Abl and PD180970-Abl, which bind to the kinase differently. Strategies to potentially circumvent or overcome resistance are discussed.

1. CHRONIC MYELOGENOUS LEUKEMIA AND THE PHILADELPHIA CHROMOSOME

In the bone marrow, hematopoietic stem cells differentiate into both lymphoid and myeloid progenitor cells. Whereas lymphoid stem cells are the precursors of B and T lymphocytes and natural killer cells, myeloid cells give rise to the granulocytes (neutrophils, eosinophils, basophils and mast cells), erythrocytes, macrophages and thrombocytes. Chronic myelogenous leukemia (CML) is a clonal abnormality involving the myeloid lineage of stem cells. The disease, which constitutes about 15% of adult leukemias and affects 1-2 people per hundred thousand general population, progresses in three phases [1]. In the initial chronic phase, which has a median duration of 4 - 6 years, the patient may be asymptomatic. Chronic phase CML can be characterized as a clonal myeloproliferative disorder of functional granulocytes at all stages of differentiation [2-4]. The disease can then progress via an intermediate, accelerated phase, characterized by the appearance of undifferentiated blast cells in the blood and bone marrow. Ultimately, all untreated patients will progress to a terminal blast-crisis phase of the disease, which is clinically analogous to the acute myelogenous leukemias (AML). In the blastic phase, for which median survival is 18 weeks, more than 30% of the blood and bone marrow cells are blasts and myeloid precursors may also form tumors in the lymph nodes, skin and bone [5].

The underlying cause and prime diagnostic feature of CML is the 22q-, Philadelphia chromosome (Ph), which results from a reciprocal t (9;22) chromosome translocation in a hematopoietic stem cell [6, 7]. Whereas the Abelson proto-oncogene (*ABL*) on chromosome 9 only fragments between exons 1a and 2 such that the fusion transcript always contains exons 2 – 11 of *ABL*, there are three breakpoint regions on the breakpoint cluster region (*BCR*) gene (Fig. **1**). The resulting *BCR-ABL* hybrid genes e1/a2, b2/a2 (or b3/a2, not illustrated), and c3/a2 thus incorporate either exon 1 alone, exons 1 - 13 (1 – 14), or alternatively exons 1 – 19 of *BCR*. These fusion genes encode either a 190-, 210- or 230-kDa chimeric Bcr-Abl protein. The p210 Bcr-Abl is expressed in 95% of CML patients and in approximately 33% of patients with the less common, acute lymphoblastic leukemia (ALL). Although the p210 Bcr-Abl predominates, expression of p190 Bcr-Abl mRNA is detected in many patients with chronic phase CML and in most of those in blast crisis. Conversely, in the more aggressive ALL, the p190 Bcr-Abl predominates and is present in 80% of pediatric and 50% of adult cases of the disease [8]. The larger 230 kDa fusion protein is associated with the rare chronic neutrophilic leukemia, whose progression to blast crisis is slow [9].

While formation of the *BCR-ABL* fusion gene in a pluripotent stem cell is clearly recognized as the initial event causing CML that gives rise to the clonal expansion of Ph+ cells, the reasons for disease progression are unclear. However, since the aggression and lineage of the leukemia is clearly influenced by the Bcr domain of the Bcr-Abl fusion proteins, this might well be associated with disease progression [10]. By the terminal phase of the disease, Ph+ as well as Ph- blast cells have acquired additional cytogenic aberrations, such as chromosomal translocations, deletions, or karyotypic abnormalities [4, 11-13]. These contribute to a massive enhancement in the proliferation of cells, blockage of differentiation, and the release of undifferentiated, immature cells into the blood, leading to a rapid clinical deterioration.

Expression levels of the Wilms tumor suppressor gene, *WT1,* significantly increase in CML patients as they progress from chronic to accelerated phase and on to blast crisis [14]. Although not directly implicated in disease progression, WT1 has a potential use as a prognostic marker

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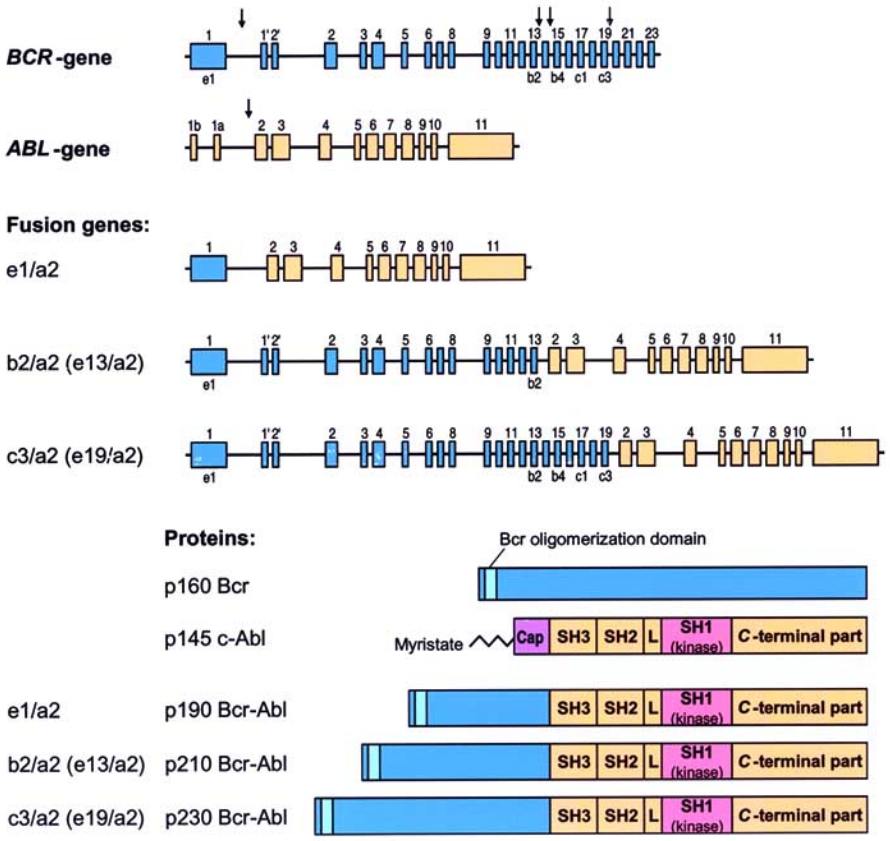


Fig. (1). The structures of the *BCR, ABL* and *BCR*-ABL fusion genes, together with the corresponding encoded proteins (not to scale). For the genes exons are illustrated as colour-coded numbered boxes. Protein regions derived from *BCR* are shown in blue and those derived from ABL labelled and coloured according to domain structure.

for relapse, and may be monitored through reverse-transcriptase polymerase chain reaction (RT-PCR) assays of either bone marrow or peripheral blood.

In blast crisis, the cells become increasingly more genetically unstable and additional mutations in the *BCR-ABL* gene often emerge, potentially leading to drug resistance as discussed in detail in Section 3.

2. ROLE OF Bcr-Abl IN INTRACELLULAR SIGNALING

Human c-Abl is a structurally complex 145 kDa protein (reviewed in Pendergast [15]), possessing three SRC homology domains (SH1-SH3), located towards the amino terminus (Fig. **1**). The SH-1 domain possesses tyrosine kinase function, whereas the SH2 and SH3 domains enable interaction with various proteins. Nuclear localization sequences and DNA-binding motifs are located towards the carboxy-terminus of the protein, which are important for the role of c-Abl in regulation of the cell cycle. The tyrosine kinase activity of c-Abl is normally tightly regulated by the phosphorylation / de-phosphorylation of residues Tyr245, Tyr412 and Ser465, together with a number of inter- and intra-molecular interactions [15]. PDGFR-β and the Src family kinases are believed to be important for the activation of c-Abl [16, 17], with PEST-type phosphotyrosine phosphatases being important for negative regulation [18]. The intra-molecular regulatory interactions involve the SH3 domain [19], the *N*-terminal cap [20], the SH2 domain [21, 22] and the catalytic domain. Thus Pluk *et al.* [20] have described the role of the *N*-terminal 80 residues in suppressing the kinase activity of full length c-Abl, which is analogous to the role of the *C*-terminal phosphotyrosine in down-regulating c-Src (reviewed by Sicheri and Kuriyan, [23]). A recent structure of c-Abl in the assembled inactive state shows that critical interactions, between the SH2 domain and the kinase domain, depend upon a conformational change induced by the binding of myristate within the *C*-terminal lobe of the kinase domain [22]. The c-Abl protein is expressed in two splice forms, 1a and 1b, which differ in the lengths of their *N*-terminal regions. The 1b form is 19 residues longer and contains a myristoylation site on the second residue, which helps regulate enzymatic activity. Mutation of this glycine to alanine prevents myristoylation and results in an activated kinase [21]. It is possible that the shorter 1a form of c-Abl, which has hydrophobic residues at the *N*-terminus but no myristoyl group, is regulated by a similar mechanism with another structural element mimicking the role of the myristoyl group. Loss of the *N*-terminal "cap" is observed in all of the Bcr-Abl fusion proteins (Fig. **1**) and contributes to the

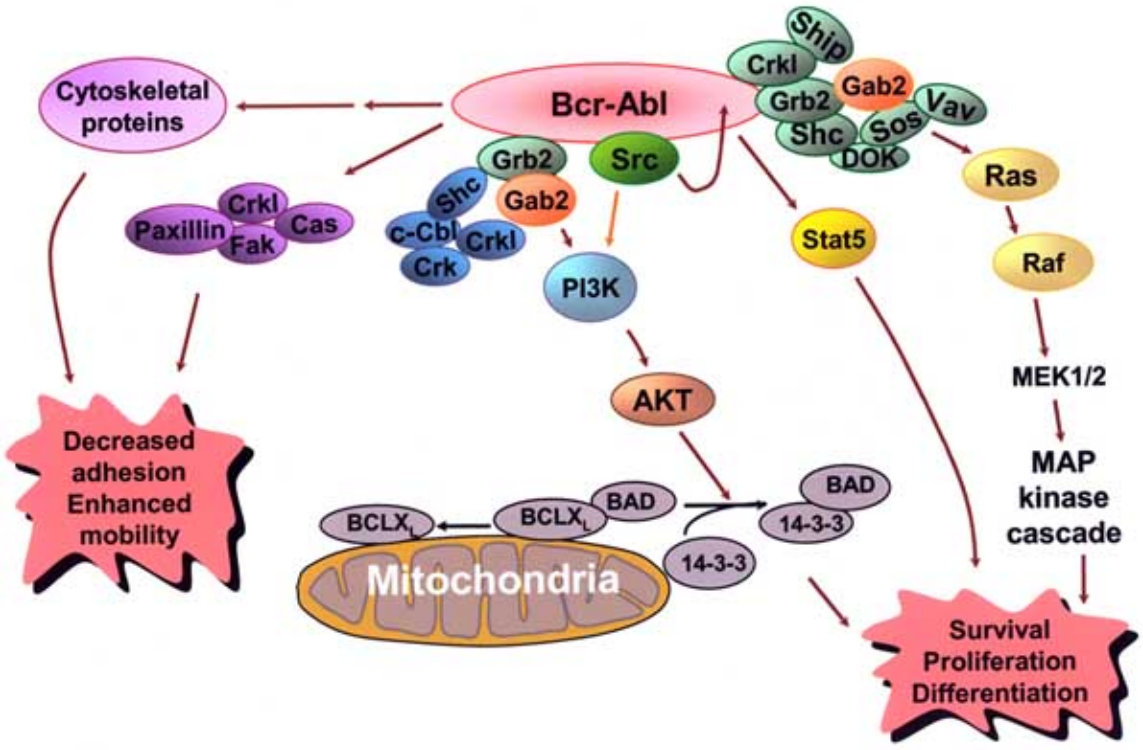
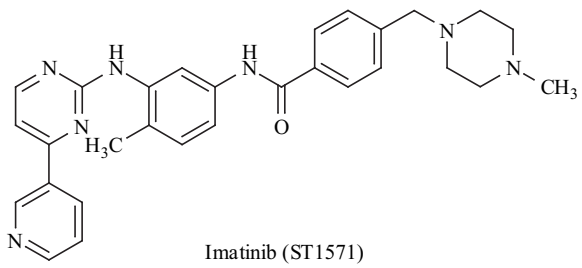


Fig. (2). Cartoon illustrating the interactions of Bcr-Abl with cellular proteins and its influence on downstream signal transduction pathways. Complex protein-protein interactions involving adaptor proteins such as Grb2 (growth factor receptor-bound protein 2) and Gab2 (Grb2-associated binding protein 2), Crk (avian sarcoma virus CT10 (v-crk) oncogene homolog), Crkl (Crk-like protein), Shc (Src homology 2 domain-containing transforming protein), Sos (Son of sevenless) Dok (Downstream of tyrosine kinases), Vav (a guanine nucleotide exchange factor) and Ship (SH2-containing inositol-5-phosphatase) enable Bcr-Abl to associate with and phosphorylate proteins that connect the Bcr-Abl kinase activity with downstream signaling pathways. Grb2 and Gab2 were described to be essential for the activation of the PI3K (Phosphatidylinositol-3 Kinase)/AKT (protein kinase B) pathway by Bcr-Abl, which enhances survival of cells in the presence of apoptotic stimuli by a mechanism that involves phosphorylation of BAD (BCL2-antagonist of cell death) and sequestration of phosphorylated BAD by 14-3-3 (phosphoserine / phosphothreonine binding proteins) causing dissociation of the proapoptotic complex of $BCLX_L$ (BCL2-related gene) and BAD. This results in the inhibition of mitochondrial cytochrome C release and prevention of the caspase cascade activation. The activation of the Ras-Raf/MAP kinase pathway via MEK is essential for the transformation by Bcr-Abl and involves the association of Bcr-Abl with the adapter proteins Grb2, Gab2 and Crkl, allowing interaction with the Ras activator Sos. Furthermore, interaction of Bcr-Abl with structural proteins of the cytoskeleton (e.g. actin) and with proteins that are involved in the formation of the focal adhesion points – e.g. Crkl, Cas (Crk-associated substrate), Paxillin and Fak (Focal adhesion kinase) may probably influence cell-adhesion properties and cell-mobility, thereby affecting the retention of the cells in the bone marrow and their premature release into circulation.

acquisition of the oncogenic, constitutive tyrosine kinase activity. The tyrosine kinase activity of Bcr-Abl is further up-regulated via the Bcr oligomerisation domain, which brings multiple Abl kinase domains into proximity and facilitates their intermolecular transphosphorylation [24-26]. This intermolecular, trans-autophosphorylation results in the formation of phosphotyrosine residues located in the catalytic domain, as well as in other regions of Bcr-Abl. These phosphorylated domains then serve as docking sites for SH2 containing proteins, which are either phosphorylated themselves, or which recruit other substrates for phosphorylation, leading to the activation of a number of signaling pathways.

The Bcr protein also includes a Serine/Threonine kinase domain, which is encoded within the first *N*-terminal exon of the *BCR* gene, and therefore, by all transcripts of *BCR-ABL* (reviewed in Arlinghaus [27]). However, few substrates have been identified for this kinase and it is unclear whether or not it plays a role in either CML or ALL [6, 10, 27].

The pathophysiology of CML is directly attributable to the activity of Bcr-Abl and its interaction with intracellular signaling pathways in hematopoietic stem cells. The expression of Bcr-Abl abrogates the growth factor requirements for cell proliferation and survival by three major mechanisms: the constitutive activation and enhancement of mitogenic signaling [28, 29], the reduced responsiveness to apoptotic stimuli [30, 31] and thirdly through altered adhesion to stroma cells and extra-cellular matrix [32-34]. The constitutively activated tyrosine kinase of Bcr-Abl is essential for the transforming activity [35] and induces the phosphorylation of many cellular proteins (Fig. **2**), including Crkl, Shc, paxillin, FAK, Vav, and STAT5 [36-44]. Evidence suggests that activation of the PI-3 kinase and the Ras/Erk MAP kinase pathways are associated with



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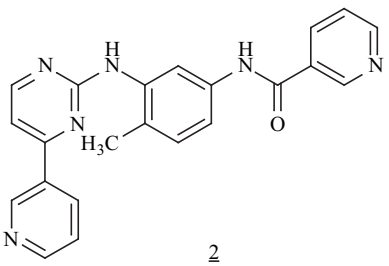

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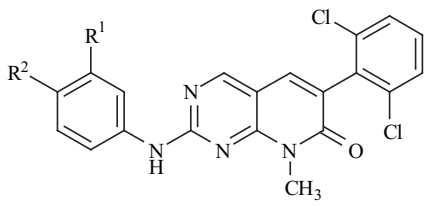

PD173955: $R^1 = SCH_3$, $R^2 = H$
PD180970: $R^1 = CH_3$, $R^2 = F$

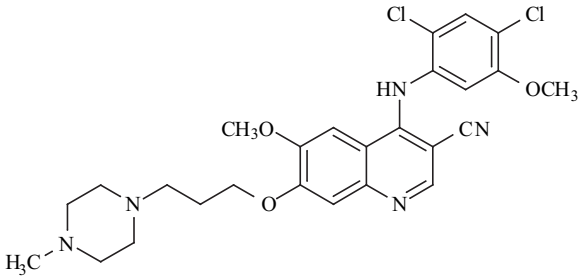

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Fig. (3). Chemical structures of some inhibitors of the Abl / Bcr-Abl kinase.

the increased proliferative, migratory and anti-apoptotic effects of Bcr-Abl [10, 45-48]. Bcr-Abl signaling is coupled to the PI-3 kinase pathway through its interaction with the adaptor protein Gab2, mediated through Grb2 binding to phosphorylated Tyr177 of the Bcr-domain, and possibly through Abl-domain mediated Shc-Grb2 interactions [10, 47, 49, 50]. Both Crkl and Shc have also been reported to link Bcr-Abl to the mitogenic Ras/Erk MAP kinase pathway [28, 36, 51, 52]. Activated Ras can stimulate the Jnk/Sapk pathway and this has been reported as another requirement for malignant transformation [53]. Phosphorylation of Vav by Bcr-Abl probably leads to downstream activation of both the Jnk/Sapk (in cooperation with Ras) and p38 MAP kinase pathways [54]. Stat5 has been shown to be constitutively phosphorylated in Bcr-Abl transformed Ba/F3 cells and probably suppresses apoptosis in CML by increasing the transcriptional expression of cell survival genes, such as *BCL-XL* [55-60]. Interestingly, p190 Bcr-Abl, in contrast to p210, induces strong Stat6 activity, which might contribute to the enhanced aggressiveness of ALL [61]. Bcr-Abl can also associate with the receptors for IL3 (β-subunit), GM-CSF and stem cell factor (SCF), probably influencing downstream signaling to proliferative or survival control mechanisms and mediating growth factor independence via the Jak / Stat5 signaling pathway. Interaction of Bcr-Abl with the cytoskeleton [62] and with proteins of the focal adhesion complexes such as paxillin, vinculin and focal adhesion kinase (FAK), probably contribute to the impaired adhesion properties of transformed cells, promoting the premature release of the progenitor/precursor cells from the bone marrow into circulation [44, 55, 63].

Transfection of primary bone marrow cells with the *BCR-ABL* oncogene increased the expression of 14 genes, all of which are over-expressed in cells from chronic phase CML patients [64]. Of these, the expression of *NUP98* (which has been implicated in CML disease progression [11, 65]), *HSPC150*, *RAN*, *TOPK* and *NME1* (which encodes a protein that inhibits hematopoietic cell differentiation) were dependent upon Bcr-Abl tyrosine kinase activity. Bcr-Abl has also been shown to suppress the expression of the transcription factor *C/EBPα*, which can then lead to impaired hematopoietic stem cell differentiation and may also contribute to disease progression [66, 67].

3. STI571 AS A PROTEIN KINASE INHIBITOR

Imatinib mesylate (STI571; Glivec®, or in the USA Gleevec™; Fig. **3**) is a drug targeted against the Bcr-Abl kinase (reviewed in [68]), which potently inhibits the tyrosine kinase activity of c-Abl, PDGFR-β and c-Kit (Table 1). Kinetic analysis of the inhibition of Abl-catalyzed phosphorylation confirmed that STI571 is an ATP-competitive inhibitor, having a Ki value of 85 ± 19 nM (Fig. **4**; D. Fabbro *et al.*, unpublished results). Although STI571 also inhibits the c-Arg kinase [69], which has high sequence and structural homology with c-Abl [15], it has little effect on other tyrosine or serine/threonine protein kinases at concentrations below 5 μM. In cells STI571 inhibits the tyrosine kinase activity of Bcr-Abl, as well as the proliferation and viability of Bcr-Abl expressing cells, with similar potency to that required to inhibit c-Abl activity in cell-free assays (Table 1). In addition to directly inhibiting Bcr-Abl catalytic activity, by inhibiting PDGFR-β, STI571 might also affect the activation of Bcr-Abl. Furthermore, c-Kit activity is important for the survival and differentiation of hematopoietic cells [70], and therefore, inhibition of this kinase might also contribute to the effects of STI571 on cell viability.

The drug possesses an excellent pharmacokinetic profile in man, with a therapeutic oral dose of 400-600 mg/day resulting in mean plasma trough concentrations in excess of 1 μM, 24 hours after drug administration [71]. As a result of this profile and its mechanism of action, STI571 has proven to be an efficacious therapy for CML. Thus, after a median 19 months of treatment, newly diagnosed patients showed an estimated 96.8% complete hematological response (CHR)

Table 1. Comparitive Effects of STI571 on Kinase Transphosphorylation, Autophosphorylation and Cell Proliferation (n.a.: applicable; n.d.: not determined). Throughout this manuscript, the numbering used for the amino-acid residues of Abl is based upon c-Abl splice-form 1a, as used in most publications; a more appropriate nomenclature based upon splice-form 1b has been suggested [116].

Kinase	Inhibition of kinase transphosphorylation [a]	K_m	Inhibition of kinase autophosphorylation [b]	Inhibition of cell proliferation [c]
***Wild-Type*-Ba/F3**	n.a	n.a	n.a	8604 ± 721; *n = 7*
Bcr-Abl	170 ± 23; *n = 21*	5.0	231 +43; *n = 10*	627 + 60; *n = 11*
M244V Bcr-Abl	640 ± 130; *n = 14*	*6.1*	n. d.	n. d.
G250E Bcr-Abl	1600 ± 190; *n = 15*	3.4	> 10000; *n = 12*	9316 ± 405; *n = 18*
Y253H Bcr-Abl	> 10000; *n = 10*	*4.9*	n. d.	n. d.
E255V Bcr-Ab	3516 ± 295; *n = 7*	4.0	7343 ± 747; *n = 13*	7794 ± 619; *n = 20*
E258G Bcr-Abl	1310 ± 130; *n = 7*	*9.8*	n. d.	n. d.
T315I Bcr-Abl	> 10000; *n = 9*	3.5	> 10000; *n = 14*	9511 ± 264; *n = 16*
F317L Bcr-Abl	380 ± 90; *n = 8*	10	818 ± 99; *n = 10*	1432 ± 256; *n = 11*
M351T Bcr-Abl	260 ± 50; *n = 10*	5.5	595 ± 63; *n = 10*	1338 ± 187; *n = 12*
H396P Bcr-Abl	870 ± 120; *n = 15*	5.7	n. d.	n. d.
F486S Bcr-Abl	670 ± 90; *n = 9*	6.4	1068 ± 153; *n = 6*	1476 ± 304; *n = 3*
Bcr-Abl [d]	n. d.	n. d.	193 ± 7; *n = 8*	282 ± 13; *n = 63*
Bcr-Abl [e]	n. d.	n. d.	470 ± 59; *n = 15*	226 ± 9; *n = 42*
Bcr-Abl [f]	n. d.	n. d.	399 ± 82; *n = 7*	61 ± 4; *n = 21*
PDGFR-β [g]	869 ± 117; *n = 12*	n. d.	69 ± 9; *n = 4*	43 ± 8; *n = 5*
c-Kit [h]	579 ± 92; *n = 11*	n. d.	96 ± 12; *n = 7*	128 ± 9; *n = 10*

[a] In cell free assays Abl (in place of Bcr-Abl), PDGFR-β and Kit kinase activities were evaluated by measuring the phosphorylation of a synthetic substrate (poly[GluTyr]), catalysed by purified GST-fusion kinase domains in the presence of radiolabeled ATP (ATP-concentrations used were optimized within the K_m range for the individual enzymes) and data represent the mean ± SEM (n = number of determinations) drug concentrations required to inhibit kinase activity by 50% (IC_{50} value; nM); [b] In cellular assays, kinase auto-phosphorylation was quantified with a capture ELISA using specific capture antibodies for the kinase, together with an enzyme-labeled anti-phosphotyrosine antibody and a luminescent substrate. Bcr-Abl activity was assessed in transfected Ba/F3 cells, unless indicated otherwise. The effects of compounds on the kinase activity were expressed as percent reduction of the kinase phosphorylation and IC_{50} values were determined from the dose response curves. [c] Cell proliferation was assessed with a luminescent ATP detection assay kit (ATPLite™-M; PerkinElmer Life Sciences) in transfected Ba/F3 cells unless indicated otherwise, and IC_{50} values were determined from the dose response curves. [d] Effects on autophosphorylation and proliferation were assessed in transfected murine 32D cells. [e] Effects on autophosphorylation and proliferation were assessed in human K562 cells. [f] Effect on autophosphorylation and proliferation were assessed in human Ku-812F cells. [g] Effects on auto-phosphorylation and proliferation were assessed in A31 and in Tel-PDGFR-β Ba/F3 cells respectively. [h] Effects on auto-phosphorylation and proliferation were assessed in gastrointestinal stromal tumour cells [133].

and an estimated 76.2% complete cytogenetic response (CCR; no detectable Ph+ cells), significantly improved over that of the standard therapy (69% CHR and 14.5% CCR) of α-interferon combined with cytosine arabinoside (Ara-c) [72]. In addition, imatinib is generally well tolerated with most adverse events being of only mild to moderate severity.

Considerable insight has recently been gained regarding the molecular interaction of STI571 with the Bcr-Abl protein tyrosine kinase. The original hypothesis for the interaction of STI571 was based upon a homology model of the activated kinase domain of c-Abl, [73] derived from the X-ray crystal structure of the activated form of the fibroblast growth factor receptor (FGF-R) kinase in complex with an inhibitor possessing an amino-pyrimidine scaffold [74]. From this it was believed that STI571 was bound within the ATP binding-site of the kinase, primarily via a donor and an acceptor hydrogen bond between the HN-C=N motif of the aminopyrimidine moiety and the backbone carbonyl and NH of Met318, located in the hinge-region of the protein. However, the crystal structure of a pyridine analog (**2**; Fig. **3**) of STI571 in complex with the kinase domain of c-Abl, showed that this inhibitor binds to a catalytically inactive state of the kinase. In this structure the activation loop adopts a conformation, stabilized by a hydrogen-bond between Tyr393 and Asp363, such that Tyr393 and surrounding residues occupy the substrate binding site, effectively blocking the catalytic activity [75]. The inhibitor **2** fills a pocket, which is created in the ATP-binding site by the conserved DFG motif at the *N*-terminal end of the activation loop being displaced from the position which it occupies in the catalytically active conformation of the enzyme. It is not clear from the structure if the conformation of the DFG motif is induced by the binding of inhibitor **2**, or whether this is the natural auto-inhibited state of the enzyme. The inactive state of c-Abl kinase has a similar arrangement of SH3, SH2 and kinase domains as c-Src [22], where the regulatory domains are involved in intramolecular contacts that prevent them from interacting with activating proteins in the cell [76]. In the case of c-Src, the *N*- and *C*-terminal lobes of the kinase domain are forced into a closed state by the SH3 and SH2 domain interactions that, along with a helix in the activation loop, prevent the protein from adopting a catalytically active conformation by holding helix C out of the active site cleft [77]. The inactive state of c-Abl

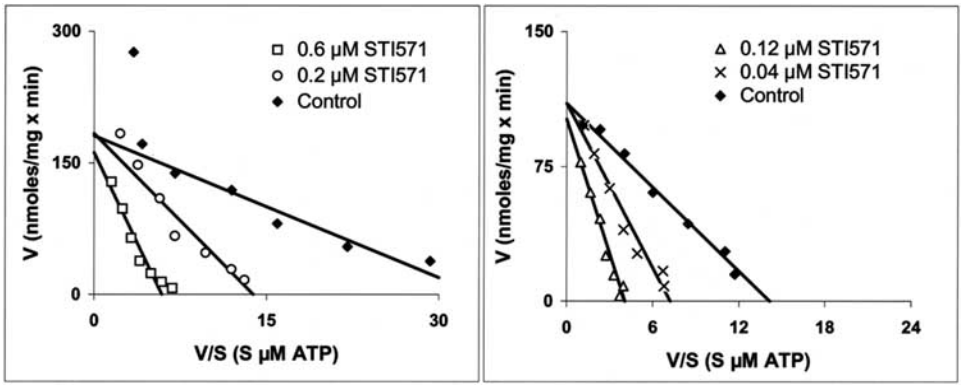

Fig. (4). Eadie-Hofstee representation of the enzyme kinetics for Abl kinase and STI571. The Km for ATP was determined by assaying c-Abl with increasing concentrations of ATP at a constant concentration of poly[AlaGluLysTyr] as exogenous acceptor protein substrate (Abl affinity, Km, 26 ± 5.6 µg/mL, at 15 µM ATP) and *vice versa*. ATP concentrations were varied between 1.25 and 100 µM, while the poly[AlaGluLysTyr] concentration was kept at 50 µg/mL in the absence (◆) or presence of 40 nM (X), 120 nM (△), 200 nM (❍) or 600 nM (□) of STI571. The Ki for STI571 was estimated to be 85 ± 19 nM. The Km for ATP was 6.4 ± 1.0 µM using 50 µg/mL of poly[AlaGluLysTyr]. Results of two independent experiments are shown.

is also stabilized by the orientations of the SH3 and SH2 domains, but the details of the inactive conformation of the kinase are different. The conformations of the DFG motif and the Gly-rich P-loop are unsuitable for the binding of ATP, whereas the position of helix C is similar to that of an active kinase. Recent structures of STI571 itself, in complex with mouse [78] and human c-Abl kinase [79, 80] confirm that the binding of STI571 stabilizes the same inactive conformation of the enzyme as observed for the complex with inhibitor **2** (Fig. **5a**). However, the affinity of STI571 for the assembled inactive state of c-Abl is lower than the affinity for an active conformational state, in which the SH3/SH2 "clamp" is dislodged from the kinase domain by the binding of a phosphopeptide to the SH2 domain (IC_{50} for STI571 of 400 nM versus 150 nM) [21]. This is due to the need for conformational changes in the assembled inactive state in order to allow STI571 binding. Weak binding of STI571 has also been observed with phosphorylated c-Abl kinase [21, 75; G. Fendrich *et al*., unpublished results], raising speculation as to whether this simply requires an alternative conformation of the activation loop or an alternative mode of STI571 binding. Such allosteric inhibition of tyrosine kinase activity involving the binding of an inhibitor to an inactive conformation of the enzyme has subsequently been observed in p38 MAP kinase [81] and VEGFR-2 kinase [82], and taken together these three cases highlight the limitations of homology modeling in predicting the binding modes of kinase inhibitors based upon a single, active conformation of the enzyme.

The pyrido[2,3-d]pyrimidine, PD173955 (Fig. **3**), is structurally unrelated to STI571 but nevertheless, a highly potent inhibitor of c-Abl kinase [78, 83]. The crystal structure of a complex between PD173955 and the kinase domain of c-Abl has been published and reveals that the ligand binds within the adenine-binding site *via* two hydrogen-bonds and a range of van der Waals interactions [78]. Independently, the structure of a close analog, PD180970 (Fig. **3**) [84] has been determined in complex with the human c-Abl kinase domain (1.7 Å resolution; Cowan-Jacob *et al*., unpublished data; Fig. **5b**). Both of these complexes have structures that resemble the catalytically active conformation of other kinases, however, the DFG motif is in a conformation that is unsuitable for optimal binding of ATP. In this case the inactive conformation is due to protonation of Asp381 in the DFG motif, which allows it to flip over and coordinate the backbone carbonyl of Leu299. This may be an artifact of the low pH of the crystallization buffer (about pH 4.8 in the PD180970 complex, but pH 6.5 in the PD173955 complex), or it may be yet another natural inactive state of the protein favored by the particular electrostic environment of Asp381. In comparison to PD173955 and PD180970, STI571 makes close contacts with a larger surface area of the Abl protein and involves six hydrogen-bonds with Met318, Thr315, Asp381, Glu286, His361 and Ile360 (shown from left to right in Fig. **5a**). On the basis of the relative buried surface areas of the two compounds alone, one would expect STI571 to have a higher affinity [85]. However, the potency of STI571 as an inhibitor of c-Abl (IC_{50} 170 ± 23 nM) is significantly less than that of PD173955 [78] or the closely related analog PD180970 (IC_{50} 62 ± 17 nM; J. Mestan *et al.* unpublished results), which has a similar binding mode to that of PD173955 [86]. It has been suggested that the lower potency of STI571 is related to binding only the inactive state of the enzyme, whereas inhibitors such as PD173955, which bind to both the active and inactive states are able to achieve much greater potency [78]. However, the difference may also result from a combination of several other factors: STI571 extends beyond the adenine-binding pocket and probably therefore, requires an induced-fit, largely of the *N*-terminal lobe but maybe also in the DFG motif, resulting in a slightly higher energy conformation of the protein, whereas the smaller PD173955 may bind without inducing any significant changes to the structure in the activated state. This is also supported by the observation that the affinity of STI571 for the assembled inactive state of c-Abl is less than that for the less rigid, unassembled but unphosphorylated form, which can presumably breathe due to the absence of the SH3/SH2/linker clamp [21]. An additional factor may relate to the necessity for STI571 to undergo desolvation /

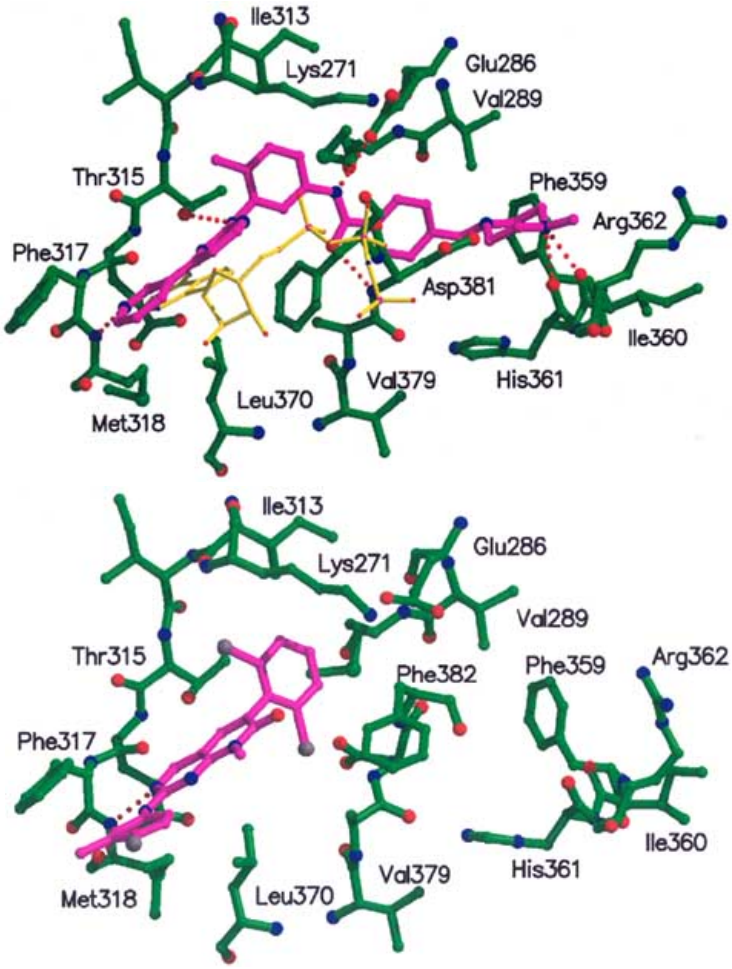

Fig. (5). (**a**) Details of the binding of STI571 (magenta) to the c-Abl kinase domain (green). Residues in the binding site that would be in front of the picture (mainly from the glycine-rich or P-loop), or behind, are not shown for clarity. The structure of AMP-PNP (yellow) as bound to the Insulin receptor kinase (PDB entry 1IRK) is shown to indicate the adenine binding site. Hydrogen bonds are represented by dashed red lines, and involve (from left to right in picture), Met318, Thr315, Glu286, Asp381, His361 and Ile360. The inhibitor has packing interactions with Phe317, Phe382, Val256, Tyr253, Ile313, Lys271, Met290, Val299, Ala380 and Val289. (**b**) Using the same colour scheme, details of the binding of PD180970 (magenta) to c-Abl kinase are shown. PD180970 has two hydrogen bonds with Met318 and packing interactions with Gly321, Phe317, Leu370, Ala269, Tyr253, Thr315, Val256, Lys271, Ala380, Asp381 and Ile313. Both pictures are viewed from the same angle with respect to the kinase domain in order to facilitate comparison of the binding modes.

deprotonation of the *N*-methylpiperazine moiety (pKa 12) prior to binding.

From the crystal structures of various tyrosine kinase domains solved over the past ten years, it is apparent that the conformations of the activated states, which are able to bind ATP and catalyze the transfer of the γ-phosphate group to the substrate, are highly conserved. However, the inactive states of the kinases often adopt distinct conformations, in which there are large variations in the size and shape of the ATP pocket [87]. One of the revelations of the crystal structure of STI571 in complex with Abl kinase is that it is the targeting of the drug to the inactive conformation of the kinase that probably leads to the excellent selectivity profile of the drug against other kinases (Table 1). In addition to occupying the adenosine sub-site of the ATP pocket, and due to the small size of Thr315, the inhibitor is able to reach past this residue, which contributes to the shape of the hydrophobic pocket used to design selectivity into kinase inhibitors [88], and occupy the space left by the conformational change of the DFG motif. This change is required for the tight binding of STI571, which may not be possible in many other kinases due to the fact that they cannot adopt such a conformation, perhaps because they possess different regulatory mechanisms. This method of gaining selectivity is also observed in the binding of BIRB796 to p38 MAP kinase, a serine-threonine kinase that apparently has similar flexibility in the DFG motif [81]. However, a negative aspect of this type of selectivity is that inhibitors, such as STI571 are exposed to residues that are neither involved in binding ATP, nor necessary for the function of the enzyme. These residues can be mutated to give changes in structure that prevent STI571 binding without losing enzyme activity, leading to resistance.

4. IMATINIB RESISTANCE

After 18 months of treatment with imatinib, 96.7% of patients diagnosed in stable, chronic phase CML continue to

Table 2. Summary of Bcr-Abl Mutant Proteins Isolated from Leukemia Patients (Compiled June 2003)

Mutant	References	Mutant	References
Met244Val	104; 109; 111; 114	Phe317Leu	106; 111; 112; 114
Leu248Val	109; 114	Met343Thr	111
Gly250Ala	113	Met351Thr	104; 106; 109-112; 114
Gly250Glu	106; 111; 114	Glu355Gly	104; 109; 111; 114
Gln252His	104; 109; 111; 114	Phe358Ala	112
Gln252Arg	111	Phe359Val	111; 112; 114
Tyr253His	104; 106; 107; 109; 111 ; 112	Val379Ile	111
Tyr253Phe	109; 111; 114	Phe382Leu	111
Glu255Lys	104 - 109; 111; 112; 114, 115	Leu387Met	111
Glu255Val	104; 107; 109; 112; 114	His396Pro	107
Phe311Ile	110	His396Arg	104; 109; 111; 112; 114
Phe311Leu	113	Ser417Tyr	114
Thr315Ile	99; 104; 106 -114	Glu459Lys	114
		Phe486Ser	114

show a major cytogenetic response (< 35% Ph+ cells) [72]. In contrast, patients diagnosed with Ph+ ALL, as well as many patients with CML in accelerated phase and blast crisis, frequently develop resistance to therapy and relapse after initially responding to treatment [89, 90].

Potential extrinsic mechanisms, which could result in STI571-resistance through diminished drug concentrations being presented to Ph+ cells, and hence to the Bcr-Abl kinase, include increased metabolism of the drug and changes in plasma protein binding. Endogenous α1-acid glycoprotein (AGP) binds to STI571 with high affinity, reducing its distribution into tissues and the cellular uptake of drug, thereby inhibiting both its *in vitro* and *in vivo* activity [91, 92]. The consequence of this in patients is that increased levels of AGP could reduce free-plasma levels of STI571. Moreover, displacement of the drug from AGP with an agent that competes for AGP binding, such as in the case of concurrent therapy with erythromycin, could reduce total drug plasma levels through promoting tissue distribution of free STI571 [93]. However, although elevated AGP levels have been reported in some patients with chronic phase CML [94], this mechanism has not been definitively shown to be a major cause of drug-resistance [95].

In advanced leukemias, STI571 resistance primarily arises as a result of the presence of residual Ph+ cells, and the risk of disease progression in chronic phase CML appears to increase with an increasing load of residual BCR-ABL transcripts [96]. Although cytogenetic abnormalities have been observed in recovering Ph- cell populations in CML patients treated with STI571, such genomic damage has not been shown to lead to patient relapse and may be related to prior anti-leukemic therapy [13, 97]. Possible mechanisms of drug resistance, which are intrinsic to Ph+ cells include amplification of the *BCR-ABL* gene, increased Bcr-Abl expression, up-regulation of Bcr-Abl kinase activity, de-regulation of alternative signaling pathways leading to Ph+ cell survival, and the emergence of other mutations affecting cell differentiation, or increased expression of the *multidrug resistance-1* gene-encoded PGP (responsible for drug efflux from cells) [98-102].

Of these mechanisms, only amplified gene expression and increased Bcr-Abl protein expression have been reported for some resistant patients [103, 104]. However, in the majority of relapsed patients, resistance appears to be the result of point mutations in the Abl kinase domain of the Bcr-Abl. These mutant forms of Bcr-Abl are desensitized towards inhibition by STI571, allowing clonal expansion of Ph+ cells resistant to the drug. Following the initial findings of Gorre *et al.* [99], who first isolated the Thr315Ile mutant, studies in which the Bcr-Abl kinase domains from STI571-resistant patients have been sequenced reveal some 30 distinct mutations (Table 2) [99, 103-114]. All of the mutant forms result from single letter changes in the genetic code. Some patients possess multiple cell populations harbouring different *BCR-ABL* mutations and one patient has been observed to have the Thr315Ile mutant, together with a separate *BCR-ABL* clone that contained two mutations, Met343Thr and Phe382Leu, on the same DNA sequence [111]. The most common mutants detected are Glu255Lys/Val, Thr315Ile and Met351Thr, with Gly250Glu, Gln252His/Arg, Tyr253His/Phe, Phe317Leu and Glu355Gly also appearing quite frequently. The resistant clones do not necessarily arise as a consequence of exposure to the drug, since in a number of cases mutant DNA has been detected in STI571 naïve patients [110, 111, 115]. Given the number and diversity of the mutations observed, it seems likely that a vast array of mutant clones are actually produced randomly, and those observed in resistant patients are the ones which expand under the selection pressure of being able to bind ATP, catalyze substrate phosphorylation

and be resistant to inhibition by STI571. This is supported by a recent study, in which BCR-ABL was subjected to random mutagenesis, leading to clones expressing STI571-resistant proteins with amino-acid substitutions affecting 90 separate residues [116]. Twenty-seven of these substitutions affected residues outside of the tyrosine kinase domain, none of which has as yet been detected in STI571 resistant patients, probably due to the focus of such studies having been restricted to the tyrosine kinase domain.

The mutant kinases observed possess varying degrees of imatinib resistance [111], as illustrated in Table 1 for a panel of mutant forms of recombinant enzymes as well as full-length Bcr-Abl expressed in Ba/F3 cells [J. Mestan *et al*., unpublished results]. A comparison of the K_m values for the GST-fusion kinase domains of a number of mutants indicates that they all possess similar intrinsic catalytic activity [V. Guez *et al*., unpublished results], however, their influence on the activation state of the kinase is unknown. In fact point mutations have been found in other kinases, which also result in their constitutive activation, for example, mutation of Thr338Ile (equivalent to Thr315 in Abl) is sufficient to produce a transforming form of c-Src [117]. The most resistant mutants observed in CML patients are Thr315Ile, Gly250Glu, Glu255Lys and Tyr253His (Table 1 and reference [111]). Analysis of the crystal structure of the complex between STI571 and the kinase domain of Abl allows us to afford plausible hypotheses to explain the sensitivities of many of these mutants towards STI571. The point mutations are not just clustered around the inhibitor binding site, but are spread throughout the kinase domain of the protein (Fig. **6**) and, as judged by the proximity of the modified side chain with the inhibitor, very few of these mutations directly affect STI571 binding.

Only the Tyr253Phe (and Tyr253His), Thr315Ile, Phe317Leu, Phe359Val and Phe382Leu mutations are located on the surface of the STI571 binding site. The mutation of Thr315 to isoleucine, which in addition to causing a steric interaction with STI571, also leads to the

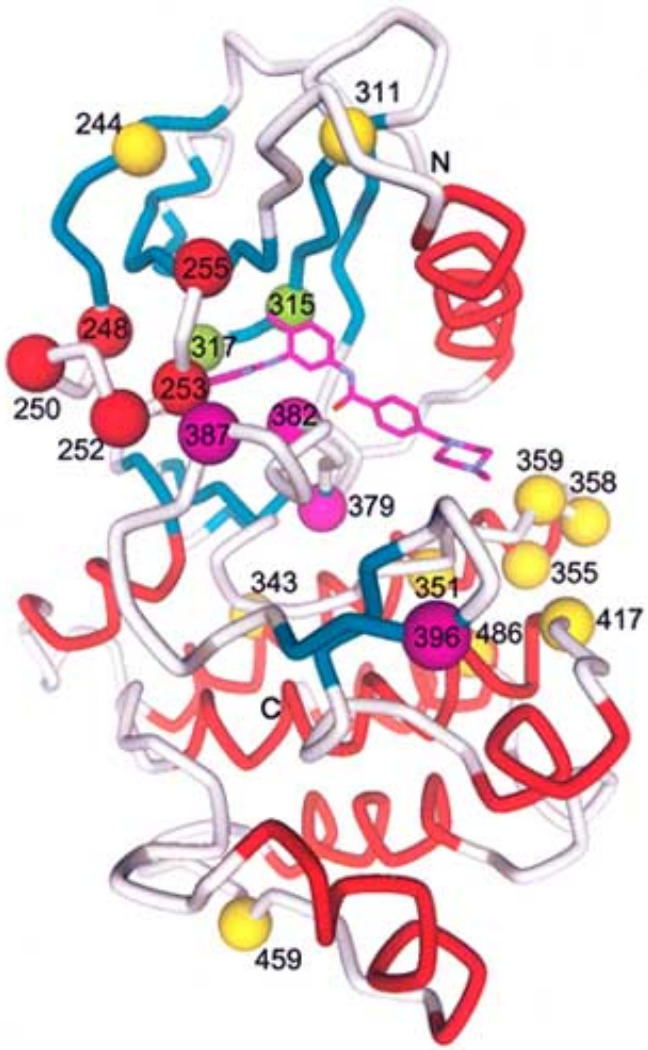

Fig. (6). Locations of mutations (summarised in Table 2), on the structure of Abl kinase in complex with STI571 (magenta). Labels for the spheres indicate the residue number of c-Abl kinase that is found mutated. Highlighted in red are mutations in the Gly-rich loop, in green are those from the hinge region, and in purple are those in the activation loop. The protein is coloured according to the secondary structure elements with alpha helices in red and beta sheets in blue.

loss of an H-bond (Fig. **7a**), resulting in a kinase that is not inhibited by STI571 at concentrations below 10 μM. The mutation of Phe317 to leucine, also located in the hinge region, which links the *C*- and the *N*-terminal lobes of the kinase domain, leads to the loss of the π-π interaction and altered van der Waals contacts with the inhibitor (Fig. **7a**). In contrast to the Thr315Ile mutant, the Phe317Leu mutation causes a relatively minor disturbance in STI571 binding, which is consistent with the IC_{50} for kinase inhibition being only 3-fold that of STI571 against wild type Abl (Table 1).

Mutations in the Gly-rich loop of Abl kinase appear to give a particularly bad prognosis for patients [114]. Three of the most resistant mutants, Gly250Glu, Glu255Lys/Val, and Tyr253His (Table 1), of which Glu255Lys is one of the most common (reported autophosphorylation and proliferation IC_{50} values > 5 μM [104, 107, 111]), are located in this region, along with Leu248Val and Gln252His/Arg (Fig. **6**; Table 1). The Gly-rich loop (or P-loop) forms a cage around the pyridine and pyrimidine ring systems of STI571 that bind in the adenosine sub-site of the ATP pocket, while in the activated state of most kinases this

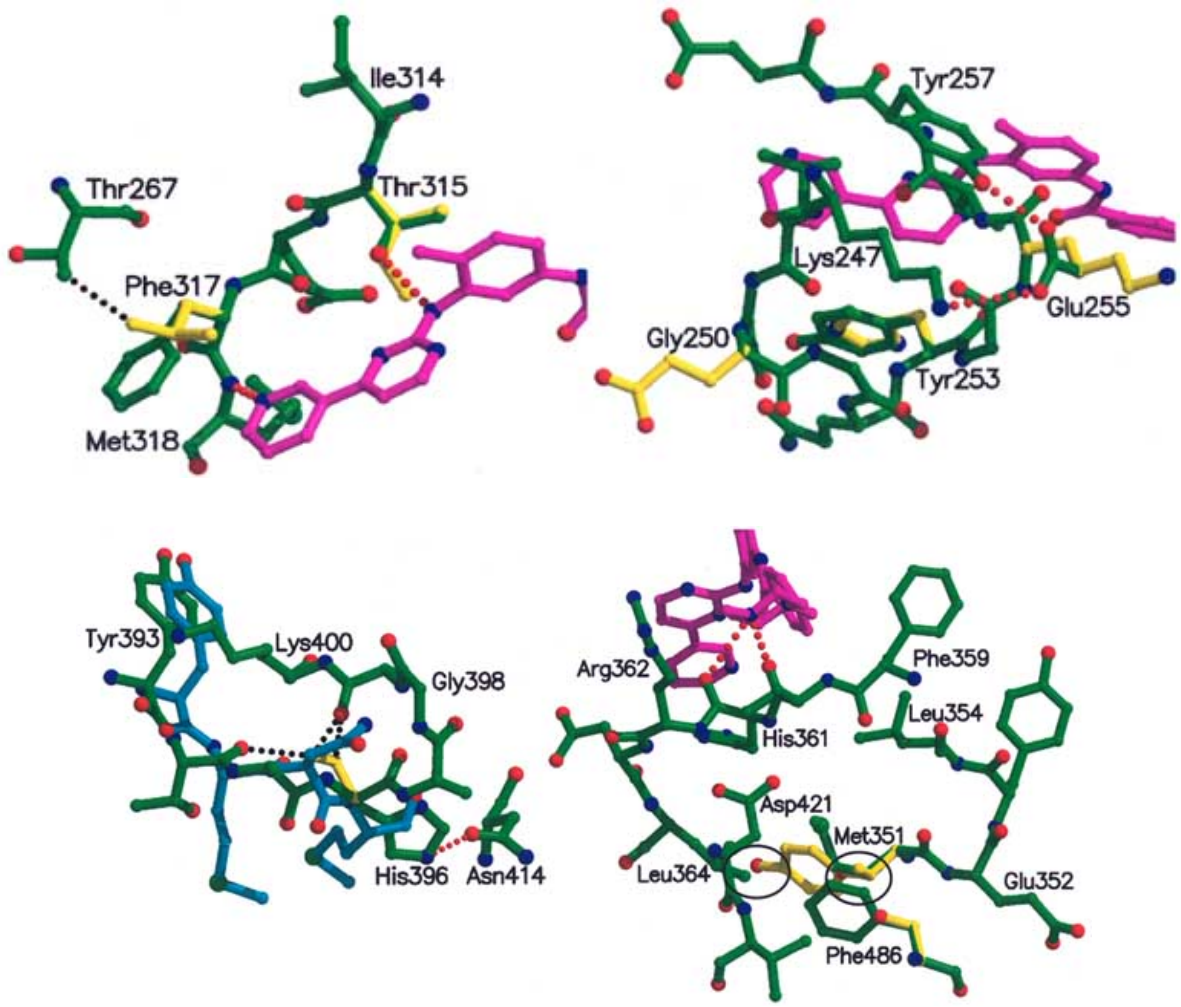

Fig. (7). Modelling of mutations (yellow) into the wild type structure of Abl kinase (green) in complex with STI571 (magenta). In all pictures, black dotted lines indicate steric clashes and red dotted lines show hydrogen bonds. (**a**) The Ile side chain of the Thr315Ile mutation would cause a steric clash with STI571 and loss of a hydrogen bond. To avoid a steric clash with Thr267, the Leu side chain of the F317L mutation would cause a slight adjustment of the postion of this residue and possibly weaken the Met318 hydrogen bond and/or have less favourable packing interactions with STI571 than Phe. (**b**) Mutation of Glu255 in the glycine-rich loop to Lys or Val will result in the loss of hydrogen bonds to Lys247 and Tyr257. These hydrogen bonds appear to be very important for stabilizing the special conformation of the Gly-rich loop. Also important for this conformation is the packing of Tyr253 against the pyrimidine group of STI571. The mutation of this side-chain to His will disrupt this packing, and to Phe will result in the loss of a hydrogen bond with Asn322 that stabilises the position of this loop with respect to the rest of the protein. (**c**) The C-terminal part of the activation loop showing the hydrogen bond between His396 and Asn414, with the binding of the substrate in Irk (PDB code 1IR3) shown in light blue. Mutation of His396 to Pro would cause loss of this hydrogen bond in addition to requiring a change in the backbone conformation due to steric clashes (indicated by dotted black lines to neighbouring carbonyl groups). (**d**) Tyr and Thr mutants of Met351 cause steric clashes (circled) or voids in the hydrophobic core near His 361. Residue 351 is part of helix E and packing in this region is important for the exact positioning of the loop containing residues 360 and 361, which are in contact with STI571. A similar effect would arise with the Phe486Ser mutation.

loop adopts a double stranded beta-sheet conformation. In the Abl kinase structure the special conformation of this loop is stabilized by numerous interactions with other parts of the protein (e.g. Gln252 with Asn322, Tyr253 with Gln252 and Phe382), by packing against the inhibitor (e.g. Tyr253 stacks against STI571) and with internal hydrogen bonds between side chains (Glu255 with Lys247 and Tyr257). Mutations disrupting any of these interactions, or favoring other conformations of the Gly-rich loop, will cause STI571 resistance (Fig. **7b**). The strongly resistant mutation of Gly250 to glutamate is more difficult to explain. In contrast to Gly249, Gly251 and Gly254, this glycine is not highly conserved across kinases, indicating that such a mutation should be well tolerated. However based on the structures of other kinases where this residue is often glutamine, a side chain interaction of Gly250Glu with Lys247 could help to stabilize the active state conformation of the Gly-rich loop. As with all of the observed mutants, it cannot be ruled out that these loop mutations may cause constitutive activation of the enzyme by themselves, and the low affinity of STI571 for the activated form of the enzyme may be the reason that resistance develops.

As already mentioned, the auto-inhibitory conformation of the activation loop is stabilized by the binding of STI571 (Fig. **6**). Mutation of His396 in the activation loop to Pro or Arg causes mild resistance to STI571 [104, 107, 111]. Substitution of this residue will result in a slight shift of the activation loop in the best case and a change in conformation of the backbone associated with a loss of a hydrogen bond to Asn414 in the case of a mutation to proline (Fig. **7c**). The affinity of STI571 for such mutations is reduced, but is only about four-fold less than the affinity for wild-type Abl kinase (Table 1). Mutations in the *N*-terminal part of the activation loop have been detected only very recently in patients [111]. These include Val379Ile, Phe382Leu and Leu387Met. Although close together in space, the reasons for the loss of STI571 affinity for each of these mutations are quite different. In the case of Leu387, the side chain packs against the backbone of Tyr253 and Gly254 in the Gly-rich loop. Any change in this side chain will cause a different packing, which might result in a slight shift or destabilization of the special conformation of the Gly-rich loop. Phe382 is part of the highly conserved DFG motif that is important for the positioning of the magnesium ions that in turn coordinate the phosphate groups of ATP, and help to orientate them correctly for the phosphorylation reaction. In the Phe382Leu mutant, the leucine side-chain is unable to make π-π interactions and cannot form such favourable van der Waals contacts with STI571 as can phenylalanine. The point mutation of Val379 to Ile is more distant from the STI571 binding site, and probably causes slight changes in the packing of the neighboring amino acids, which in turn, have an effect on the potential interactions with STI571. None of these three mutations would be expected to cause a major difference in the affinity of STI571, as is observed for the Phe317Leu mutation (2-fold less affinity) (Table 1).

There is a group of point mutations that lie in the *C*-terminal lobe of Abl kinase in the neighborhood of the STI571 binding site occupied by the piperazinyl group (Fig. **6**). Apart from Phe359Val, none of these mutation sites (Glu355Gly, Met351Thr, Ser417Tyr, Phe486Ser) is in direct contact with STI571. The mutation of Glu355 in helix E to glycine removes the hydrogen bonds that helped to hold this helix against the *C*-terminal domain, namely Ala487 and Lys419 (the latter indirectly via a water molecule). The *C*-terminal end of helix E and the loop that follows form the *N*-methyl-piperazine sub-site of the STI571 binding pocket. Met351 and Phe486 lie just below Glu355 (Fig. **7d**). The remote location of these two residues from the STI571 binding site and the slight changes that they would be predicted to cause to the structure make the interpretation of the reasons for resistance speculative. However, mutation of these residues is likely to affect the packing of the hydrophobic core of Abl kinase in this region, resulting in slight changes that will alter the shape or flexibility of the structure lining the STI571 binding site (Fig. **7d**). Mutation of the neighboring Ser417 to Tyrosine is likely to cause a similar effect because of the bulkiness of the tyrosine side chain and the loss of a hydrogen bond to the main chain of Lys419. Met351Thr is one of the most common point mutations isolated from relapsed patients after treatment with STI571 [104, 106, 109-112, 114]. This mutant retains some sensitivity to STI571 and it may be possible to treat these patients with higher doses or a more potent inhibitor.

There are several point mutations found in the *N*-terminal lobe of Abl kinase that cause resistance in patients (Fig. **6**). These include Met244Val and Phe311Ile [104, 109 – 111, 114]. The *N*-terminal lobe is quite a flexible region that can adapt to the binding of different inhibitors, however, mutations such as Met244Val, which would be predicted to give only a slight change in the packing of the hydrophobic core, are enough to cause resistance. In the case of Met244, it is the importance of this side-chain for the packing against the Gly-rich loop, as mentioned earlier for Leu387 that contributes to the effect. Phe311 is close to helix C, which has direct contacts including hydrogen bonds to STI571. Mutations of these residues causing slight packing rearrangements may, therefore, be enough to weaken these interactions and cause STI571 resistance.

One mutation, Glu459Lys [114], being located at the bottom of the *C*-terminal lobe, is very remote from the STI571 binding site (Fig. **6**). Based upon the crystal structure, the side-chain of this residue is exposed to the solvent and it is difficult to foresee how this point mutation should result in decreased sensitivity of the enzyme to STI571. This example, therefore, serves as a reminder that the mutation of a residue can have effects far more reaching than those predicted directly from the kinase domain structure. The recent structure of assembled, inactive c-Abl shows that Glu459 is close to the myristoyl binding site in the *C*-terminal lobe of the kinase domain [22]. It is possible that this mutation helps to lock the interaction of the *N*-terminal peptide with the kinase, thus reinforcing the stability of the assembled state for which STI571 has a slightly lower affinity (IC_{50} of 400nM) [21]. However, it is also conceivable that the mutation has the opposite effect: destabilizing the assembled inactive state, and thus favoring activation of the enzyme, a state for which STI571 has even lower affinity (estimated Ki of 7 μM) [75]. Resistance to STI571 can be conferred by amino-acid changes both within and outside the kinase domain of Bcr-Abl, as shown by the *in vitro* sensitivities of randomly mutated Bcr-Abl [116]. Many of these resistant mutants result from allosteric destabilization of the auto-inhibited conformation to which

STI571 preferentially binds. These include residues involved in the interactions between the kinase domain and the regulatory *N*-terminal cap, SH3 and SH2 domains. Therefore, the observed mutations isolated from patients of residues in the kinase domain can affect self regulatory mechanisms within Bcr-Abl, and could also affect interactions with substrates or other regulatory proteins in the cell. In the future, when Bcr-Abl samples from relapsed patients are sequenced beyond the kinase domain, it will be interesting to see if mutations are observed as predicted by this *in vitro* study [116].

5. CIRCUMVENTING OR OVERCOMING RESISTANCE

As has been discussed, resistance to STI571 therapy frequently emerges during genetically unstable blast crisis of CML, while additional mutations can occur in the Ph+ cells, promoting their proliferation and viability, as well as blocking their differentiation [4, 89, 90]. This complexity, coupled with the short median survival time for patients in blast crisis and the lack of an established prognostic marker for relapse, emphasizes the need to aggressively treat CML patients in early chronic phase. Such a strategy aims to minimize the presence of residual Ph+ cells, and thereby, hinder the progression of the disease. In this respect, current evidence suggests that chronic therapy with STI571 is effective in the majority of newly diagnosed patients in managing CML and preventing disease progression [72, 96]. However, for those patients at risk for relapse, as for example those diagnosed with ALL, combination therapy with STI571 to prevent the emergence of mutants could be proposed. Unfortunately, in view of the variety of resistance mechanisms, together with the number and diversity of kinase-domain point mutations, it is unlikely that a single, well-tolerated therapeutic agent could be designed that would be able to overcome all cases of STI571 resistance.

Concerning kinase domain mutations, these can result in enzymes with sensitivities ranging from weak to relatively strong inhibition by STI571 (Table 1). It is probable that resistance mutants, which act by destabilizing the STI571-binding conformation of Bcr-Abl could be overcome with a more potent kinase inhibitor that interacts with the STI571-binding mode. However, to overcome the resistance caused by mutants in the Gly-rich loop, or the Thr315Ile mutant, it may be necessary to use a completely different type of inhibitor. Such a compound could either target a conformation of Abl different to that targeted by STI571, or target the same conformation, but not rely upon the topology and the hydrogen bonding capabilities of the Thr315 side-chain. Compounds, which target the active, ATP-binding conformation of Abl, should be able to inhibit these strongly resistant mutants. Drugs of this latter class would have an additional advantage in that the emergence of resistant mutants would be impeded by the need for the active conformation to maintain kinase function and abrogate inhibitor binding. A prototype compound in this respect is PD180970 (Figs. **3** and **5b**), which has been shown to inhibit the Gln252His, Tyr253Phe and Glu255Lys mutants of the Gly-rich loop, together with Met351Thr and His396Pro, but not the Thr315Ile mutant [84, 86]. However, a drawback of such compounds is their lack of selectivity against other kinases, which might limit their tolerability when employed for chronic therapy [83].

A similar philosophy applies, if mutations in domains other than the kinase domain are responsible for resistance, due to destabilization of the self-regulating inactive conformation of the protein. Weak and medium resistance mutants can probably be targeted with more potent inhibitors that target the inactive mode, taking advantage of the dynamic nature of the shift between active and inactive states. A more potent inhibitor could also overcome resistance arising from *BCR-ABL* gene amplification or increased Bcr-Abl protein expression. Conversely, it has also been suggested that resistance arising from the emergence of resistant *BCR-ABL* clones could be combated by stopping treatment with STI571, and continuing with cytotoxic therapy, to enable the unmutated clone to become reestablished [109]. Over-expression of other kinases, as in the case of alternative cell survival mechanisms, such as BTK [118] will require different kinase inhibitors.

A means to circumvent resistance arising from the up-regulation of Bcr-Abl expression, as well as from the emergence of Bcr-Abl point mutations and to some extent the de-regulation of alternative signaling pathways, could be to target a down-stream component of the Bcr-Abl intracellular signaling pathways. This in itself is unlikely to provide a single solution for all cases of STI571-resistance, due to the number of signaling pathways that are modulated by Bcr-Abl (Fig. **1**). However, key targets such as the c-Src [87, 119], Mek/Erk [120], PI-3 [121], mTOR, c-Raf or AKT kinases, where some inhibitors have already been shown to exhibit additive and/or synergistic effects with STI571 in cellular systems, could provide benefit in at least some patient populations. Alternatively, combinations with agents which affect post-translational protein processing have also been proposed. For example, the farnesylation and membrane translocation of Ras proteins is critical for their full activation, and combinations of STI571 with farnesyl transferase inhibitors, such as SCH66336 and L-744832, have been shown to have enhanced antiproliferative effects in Bcr-Abl expressing cells and induce apoptosis in STI571 resistant cells [122-124]. In addition agents modulating protein degradation, such as the proteasome inhibitor PS341 [125] and inhibitors of the molecular chaperone Hsp90 [126], have also shown encouraging results upon evaluation in combination studies with STI571 in cellular assays. Another potential strategy involves combinations with agents which modulate the transcription of genes associated with regulating the cell cycle, such as the histone deacetylase inhibitors, trichostatin A or LAQ284, which have been reported to induce apoptosis in STI571-resistant cells [129].

Although the above discussions have been limited to the treatment of CML with STI571, that the fact that the drug also targets the c-Kit and PDGFR kinases is also of therapeutic relevance. This has already been established in the case of gastrointestinal stromal tumors (GIST), where as an inhibitor of c-Kit, STI571 is an effective therapy with a response rate of 62% after 15 months of treatment [130, 131]. However, drug resistance and relapse has been observed in up to 20% of patients and it seems likely that one of the underlying mechanisms for this is the emergence of clones carrying point-mutations in the kinase domain of c-Kit.

In conclusion, it is probable that the optimum treatment of (STI571-resistant) CML would come from a cocktail of drugs to be taken in combination, with each component being designed to combat either a particular class of mutations or mechanism of resistance, similar to the therapeutic approach that has been shown to be effective for the treatment of HIV [132]. It is possible that this multifaceted attack will be more robust in avoiding the development of resistance in the treatment of patients with late stage CML or Ph+ ALL. However, a balance would need to be drawn between the degree of aggressiveness of the combination and the tolerability of the treatment regime, and therefore, the identification of factors predisposing patients to relapse and the early diagnosis of emerging STI571 resistance will be important.

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