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Novel targeted therapies for Bcr–Abl positive acute leukemias: beyond STI571

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In the pathophysiology of CML, the constitutive activity of the Bcr–Abl tyrosine kinase (TK) is, most likely, the sole molecular abnormality of the chronic phase. It also remains a critical molecular determinant of malignant behavior of the leukemic progenitors in the accelerated and blastic phase of CML. Therefore, downregulation of the levels and activity of Bcr–Abl is clearly the lynchpin of a rational therapeutic strategy against all phases of CML. Support for this has only been strengthened by the observations that resistance to imatinib mesylate (imatinib) commonly involves a breakthrough and the persistent activity of Bcr–Abl TK. This is due to either mutations that inhibit imatinib action on Bcr–Abl TK or amplification of the *bcr–abl* gene. Recent studies have demonstrated that other small molecule tyrosine kinase inhibitors that also inhibit Bcr–Abl TK may be highly active in inducing differentiation and apoptosis of CML progenitors, regardless of their sensitivity to imatinib. Small molecule inhibitors that downregulate the levels of Bcr–Abl by inhibiting its translation, e.g., arsenic trioxide, or promoting its proteasomal degradation, e.g., geldanamycin analogues, have also been identified. Finally the identification of other potent survival and antiapoptotic signaling pathways in imatinib-resistant CML progenitors indicates that inhibitors of these pathways will eventually be treatment strategies for advanced phases of CML.
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Introduction

CML is a pluripotent hematopoietic stem cell disorder, characterized by the Philadelphia chromosome (Ph) (Nowell and Hungerford, 1960; Rowley, 1973). The Ph chromosome is the result of a t(9:22) reciprocal chromosomal translocation involving the c-*abl* oncogene on chromosome 9 and the *bcr* oncogene on chromosome 22. Studies in several experimental models have established that the deregulated tyrosine kinase (TK) activity of the Bcr–Abl fusion oncoprotein, encoded by the *bcr–abl* fusion gene alone, is sufficient to produce CML-like disease both *in vitro* and in mice (Daley *et al*., 1990). *Abl* gene normally encodes a tightly regulated non-receptor TK. Abl TK has an important role in signal transduction, and in the regulation of cell growth and cellular response to genotoxic stress (Wang, 1993; Yuan *et al*., 1999; Raitano *et al*., 1997). The N-terminal segment of Abl includes two SRC homology domains (SH2 and SH3), which regulate the tyrosine kinase function of Abl. The N-terminal fusion of Bcr to Abl, which is typical of Ph chromosome, adds a large amino acid sequence to the SH2 segment of Abl. This fusion of Bcr to Abl interferes with the adjacent SH3, the tyrosine kinase regulatory domain of Abl. This causes Bcr–Abl to become constitutively active and results in autophosphorylation of Bcr–Abl (Raitano *et al*., 1997). The structure of Bcr–Abl also allows multiple protein interactions, which trigger the involvement of diverse intracellular signaling pathways responsible for cell proliferation and survival (Raitano *et al*., 1997).

Several mechanisms have been implicated in the malignant transformation due to Bcr–Abl (Figure 1). These include altered adhesion to stromal cells and extracellular matrix, activation of mitogenic signaling and reduced apoptosis. Bcr–Abl induces mitogenesis in fibroblast and hematopoietic cell transformation models (reviewed in Deininger *et al*., 2000a) and protects cells from apoptosis induced by numerous stimuli including cytokine withdrawal, DNA damage and death receptor activation (McGahon *et al*., 1994, 1995; Cortez *et al*., 1995; Nishii *et al*., 1996). The deregulated Bcr–Abl TK phosphorylates several substrates including Ras, which collectively results in the activation of multiple signaling pathways (Faderl *et al*., 1999; Sawyers, 1999; Sawyers and Druker, 1999). Autophosphorylation of Bcr–Abl induces recruitment of adapter molecules such as Grb-2 and Crkl, which are known to be responsible for the activation of Ras/MAPK pathway (Sawyers *et al*., 1995). Activation of JAK/SAP kinase pathway by Bcr–Abl has also been demonstrated and is involved in the malignant transformation due to Bcr–Abl (Dickens *et al*., 1997). Bcr–Abl TK activity leads to the phosphorylation of and increased transactivation by STAT5 (signal transducer and activator of transcription), resulting in increased expression of the anti-apoptotic Bcl-x_L in Bcr–Abl positive cells (Carlesso *et al*., 1996; Ilaria and

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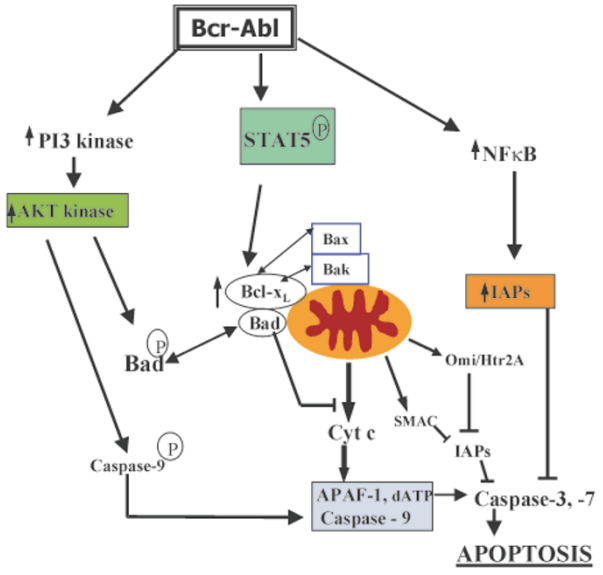

Figure 1 Mechanisms underlying the anti-apoptotic effects of the constitutive cytoplasmic activity of Bcr–Abl tyrosine kinase mediated by increased activities of AKT kinase STAT5 and NF-κB. IAP: Inhibitory apoptotic proteins; Apaf-1: Apoptosis activating factor -1 and STAT5: Signal transducers and activators of transcription

van Etten, 1996; Shuai *et al*., 1996). Bcr–Abl forms complexes with and activates phosphatidylinositol 3-kinase (PI3-kinase), which in turn, phosphorylates and activates Akt kinase (Skorski *et al*., 1997). The activity of Akt kinase is known to inhibit apoptosis by several mechanisms (Skorski *et al*., 1997; Scheid *et al*., 1998). Ectopic expression of Bcr–Abl in interleukin (IL)-3-dependent murine myeloid cells has also been shown to activate p65 NF-κB (Rel A), which is known to suppress apoptosis due to a variety of apoptotic stimuli (Wang *et al*., 1996; Hamdane *et al*., 1997; Fang *et al*., 2000). Ectopic or endogenous expression of Bcr–Abl has been shown to block, during the mitochondrial pathway of apoptosis, the mitochondrial permeability transition and release of cytochrome *c* (cyt *c*). This inhibits the activation of caspase-3 through the Apaf-1-cyt *c*- dATP-caspase-9 assembled 'apoptosome' and apoptosis (Evans *et al*., 1993; Bedi *et al*., 1994; Fang *et al*., 2000). Collectively, Bcr–Abl mediated activation of multiple survival signaling pathways as well as direct inhibition of the pathways of apoptotic signaling results in the expansion of the myeloid precursors (Afar *et al*., 1994; Sawyers *et al*., 1992; Deininger *et al*., 2000b). This review summarizes the recent pre-clinical and clinical experience with novel Bcr–Abl targeted or other active agents being developed for the treatment of chronic or advanced stages of CML.

Inhibition of Bcr–Abl TK activity

Bcr–Abl TK is ideal for targeted therapy for several reasons. The Bcr–Abl mutation is present in almost all patients with CML and is expressed at high levels only in the leukemic cells. In addition, since the mitogenic and anti-apoptotic effects are dependent on the TK activity of Bcr–Abl, its inhibition triggers growth arrest and apoptosis of the leukemic cells. This was first confirmed by Druker *et al*. (1996) who reported that STI571 (formerly GCP57148, now known as Imatinib mesylate/Gleevec) while sparing normal cells, selectively inhibited the proliferation and exerted lethal effects on Bcr–Abl positive leukemic cells. Imatinib was shown to suppress the *in vitro* colony growth of Bcr–Abl positive leukemic progenitor cells (Beran *et al*., 1998; Carroll *et al*., 1997; Deininger *et al*., 1997). Later, it was also demonstrated that exposure to Imatinib induced differentiation and apoptosis of Bcr–Abl positive human leukemic cells (Fang *et al*., 2000; Deininger *et al*., 1997). Imatinib/Gleevec treatment was shown to downregulate anti-apoptotic XIAP, cIAP1, and Bcl-x_L, without affecting Bcl-2, Bax, Apaf-1, Fas (CD95), Fas ligand and Bcr–Abl levels (Fang *et al*., 2000). Imatinib also inhibited STAT5, Akt kinase and NF-κB activities in the Bcr–Abl-positive cells (Deininger *et al*., 2000b). The novel pyrido (2,3-d)pyrimidine derivative, PD180970, originally identified as a Src tyrosine kinase inhibitor (Kraker *et al*., 2000), has also been shown to potently inhibit Bcr–Abl TK activity and induce apoptosis of Bcr–Abl positive leukemic cells (Dorsey *et al*., 2000). Treatment with PD180970 inhibited the phosphorylation of Gab2 and Crkl. It is noteworthy that PD180970 did not affect the growth or viability of Bcr–Abl negative human leukemic cells (Dorsey *et al*., 2000). These results highlight PD180970 as a promising therapeutic agent against Bcr–Abl positive leukemias.

Emerging evidence that imatinib as a Bcr–Abl TK inhibitor has limitations in eradicating the Bcr–Abl positive clone

Based on the promising data from *in vitro* and *in vivo* pre-clinical models, a Phase I trial of imatinib was initiated in patients with CML (Druker *et al*., 2001). Patients with interferon refractory CML or those who were intolerant to the drug were enrolled in the study. Imatinib was well tolerated and dose-limiting toxicity was not encountered. Remarkably, of the 54 patients who received oral doses of at least 300 mg per day, 53 (98%) had normalization of leukocyte and platelet counts, usually within 4 weeks of initiation of the treatment. Cytogenetic responses occurred in 29 patients (54%), including 17 (31%) who experienced a major response and 7 (13%) who had complete responses. Due to the high level of efficacy of imatinib, this study was expanded to include patients with myeloid or lymphoid blast crisis of CML or with relapsed or refractory Ph-positive ALL (Savage and Antman, 2002). Fifty-five per cent with myeloid blast crisis responded to therapy, 21% had clearance of blasts from their bone marrows. Seventy per cent with the lymphoid phenotype disease, i.e., CML in

lymphoid blast crisis or Ph-positive ALL, responded, with 55% clearing their marrow blasts down to 5%. Unfortunately, all but one of the lymphoid phenotype patients suffered an early relapse between days 42 and 123 (Druker, 2002).

The success of the phase I studies in CML prompted phase II studies in which single-agent STI571 was further tested in IFN-refractory, accelerated phase or myeloid blast crisis patients. Of the 532 patients with the chronic phase of CML treated with imatinib, 95% showed a hematologic response. At least 60% experienced a cytogenetic response, and the relapse rate at 18 months was 9%. In accelerated phase of the disease, out of 235 patients treated 53% showed a hematologic response and 26% experienced a cytogenic response. In myeloid blast crisis, out of 260 patients, 29% showed hematological response and 15% experienced a cytogenitic response. The relapse rate was 40% in accelerated, and 78% in patients with myeloid blast crisis (Savage *et al*., 2002).

Mechanisms of resistance of CML to imatinib

Although complete remissions are observed on treatment with imatinib in patients with blast crisis of CML or Ph-positive ALL (Druker *et al*., 2001), most patients enjoy only a short duration of response, with eventual emergence of imatinib-resistant leukemic cells and a clinical relapse (Sawyers, 2001). To understand the possible mechanism of resistance to STI-571, several groups have recently reported the isolation of imatinib-resistant, Bcr–Abl positive, human acute leukemia cells that were selected for resistance after prolonged culture in progressively higher concentrations of imatinib (Le Coutre *et al*., 2000; Weisberg and Griffin, 2000; Mahon *et al*., 2000). Analyses of these cells have shown several mechanisms of resistance to imatinib (Table 1). These include amplification of the *bcr–abl* gene, increased expression of the Bcr–Abl protein without amplification of the gene or increased expression of the MDR-1 gene-encoded P-glycoprotein (PGP) (Le Coutre *et al*., 2000; Weisberg and Griffin, 2000; Mahon *et al*., 2000). Human CML-BC cells growing in mice have also been shown to acquire resistance to STI-571, based on increased serum levels of the α-1-acid glycoprotein (AGP) which binds and blocks the uptake of STI-571 in Bcr–Abl positive CML-BC cells (Gambacorti-Passerini *et al*., 2000). Biochemical and molecular analyses of leukemic cells from patients with CML-BC who had developed resistance to imatinib showed that acquired resistance to STI-571 was associated with reactivation of the Bcr–Abl TK (Gorre *et al*., 2001). This resistance was either due to a point mutation in the kinase domain of Bcr–Abl that renders the kinase variably less sensitive to imatinib or the amplification of *bcr–abl* gene (Barthe *et al*., 2001; Hochhaus *et al*., 2001). Other potential and novel mechanisms of resistance include, markedly decreased mRNA and protein expression of Bcr–Abl (Nimmanapalli *et al*., 2001a). Whatever the mechanism, the high incidence of resistance to imatinib suggests that additional treatment strategies may have to be employed alone or in combination with imatinib to achieve durable responses in Bcr–Abl positive acute leukemias (Table 2).

Table 1 Mechanisms of Resistance to Gleevex (imatibib mesylate)

Leukemic Cell-Based
1. Bcr-Abl Kinase Dependent
   - Mutations in Bcr-Abl (T315I, E255K, M351T, G250E, F3171)
   - Bcr-Abl Amplification
2. Bcr-Abl Independent
   - Other Mutations
   - Survival Signaling: LYN kinase, MAP kinases
3. Drug Efflux

Host-Based
1. Alpha-Acid Glycoprotein Binding
2. Drug metabolism

Downregulation of Bcr–Abl levels as a strategy against Bcr–Abl positive acute leukemias

Historical perspective: antisense and ribozymes

In vitro studies have shown that Bcr–Abl antisense oligodeoxyneucleotides (AS-ODNs) can suppress expression of Bcr–Abl m-RNA and protein and specifically inhibit growth of CML but not normal cells (Wu *et al*., 1999; Fabritis *et al*., 1998; Skorski *et al*., 1991; Szczylik *et al*., 1991). These AS-ODNs have also been used systemically to treat animals with previously established leukemia (Skorski *et al*., 1994). Although animal (Delong *et al*., 1997) and human (Bayever *et al*., 1993; Zhang *et al*., 1995) studies have demonstrated the safety of the systemic infusions of phosphorothioate ODNs, such therapy has not been easily applicable in humans since it requires large quantities of AS-ODNs, which makes the treatment costly. Bcr–Abl protein has a long half-life (Dhut *et al*., 1990), which requires that the AS-ODN be physically present in the cell longer than 24–48 h to significantly decrease the level of p210 Bcr–Abl and cause cell death. Ribozymes of various lengths and compositions have been used to target the Bcr–Abl junctional sequence and have shown varying degrees of target specificity and efficiency (Lange *et al*., 1993, 1994). Transfection with ribozymes or transient expression of ribozymes suppresses expression of Bcr–Abl m-RNA and protein levels, thereby inhibiting growth and inducing apoptosis of Bcr–Abl positive

Table 2 Novel Treatment Strategies for Bcr-Abl Positive Leukemias

A. *Inhibition of Bcr–Abl TK Activity*
   1. Imatinib Mesylate (Gleevec/STI-571/CGP57148B)
   2. PD180970

B. *Downregulation of Intracellular Bcr-Abl Protein Levels*
   1. Antisense ODNs
   2. Ribozymes
   3. Geldanamycin analogues
   4. Arsenic trioxide (Trisenox)

cells (Wright *et al*., 1993; Shore *et al*., 1993). Tumorigenicity of Bcr–Abl containing cells in SCID mice has also been shown to be reduced by treatment with ribozymes (Mills *et al*., 1996). These results suggest that ribozymes directed against Bcr–Abl m-RNA can effectively suppress Bcr–Abl gene expression and alter the leukemic nature of CML cell lines. However, these observations need to be substantiated in clinical studies. Like AS-ODNs, ribozymes are limited by their low resistance to nucleases and less-than-ideal specificity of cleavage of the Bcr–Abl junctional sequence (James and Gibson, 1998).

Activity of Arsenic trioxide against Bcr–Abl positive leukemic cells

Arsenicals have a long history of use in the treatment of leukemia. Arsenic trioxide (Trisenox, AT) induces a high rate of complete remissions in APL (Soignet *et al*., 1998). Recently, AT was approved by the Food and Drug Administration for use in the treatment of relapsed/refractory acute promyelocytic leukemia (APL). Treatment of APL with AT is associated with the downregulation of the PML–RARα fusion protein (the gene product of the chromosomal translocation t(15;17), characteristic of APL), resulting in documented molecular remissions (Soignet *et al*., 1998, 2001). In other studies, using a variety of leukemia cell lines, cultures of murine embryonic fibroblasts and bone marrow progenitor cells, AT and melarsoprol (an organic arsenical compound) have been demonstrated to inhibit cell growth, downregulate Bcl-2, and induce apoptosis (Wang *et al*., 1998). It has been shown that AT has direct effects on mitochondria. AT-induced apoptosis is associated with a loss of inner mitochondrial transmembrane potential and the release of cyt *c* into the cytosol, resulting in caspase activation (Chen *et al*., 1998; Cai *et al*., 2000; Perkins *et al*., 2000; Zhu *et al*., 1999). In several experimental models, AT-induced apoptosis is associated with the generation of reactive oxygen species (ROS) with subsequent intracellular accumulation of H_2O_2 (Jing *et al*., 1999; Dai *et al*., 1999). Following treatment with AT, apoptosis of Bcr–Abl positive acute leukemia HL-60/Bcr–Abl and K562 cells was associated with a decline in the levels of Bcr–Abl protein (Perkins *et al*., 2000). Recently, AT mediated decline in Bcl–Abl levels has been shown to be due to inhibition of translation of Bcr–Abl. This may be mediated by inhibition of ribosomal p70S6 kinase activity (Nimmanapalli *et al*., 2001b). In these cells AT was also demonstrated to induce the acetylation of the histones H3 and H4 (Perkins *et al*., 2000). In addition to its mitochondrial toxicity, other mechanisms by which AT promotes apoptosis of Bcr–Abl positive leukemia cells are only beginning to be understood. These mechanisms appear to be distinct from those activated by conventional cytotoxic agents. Since AT induces cell differentiation and apoptosis by multiple mechanisms in a number of human malignant cell-types, this highlights its potential role in combination therapies against Bcr–Abl positive acute leukemias.

Activity of Gledanamycin analogues (17-Allylamino-17-demethoxygeldanamycin (17-AAG) against Bcr–Abl positive leukemic cells

Heat shock proteins (HSP), otherwise referred to as 'Molecular chaperones', help proteins to avoid misfolded, inactive or aggregated states. HSP90 is an abundant cytosolic protein that acts in concert with other chaperones and partners (Hsp70, p23, Hop etc) to help in maturation and folding, as well as the trafficking of a number of client proteins (Blagosklonny, 2002). Inactivation of Hsp90 using Benzoquinone ansamycins such as Geldanamycin (GA), or its less toxic analogues such as 17-allylamino-17-demethoxygeldanamycin (17-AAG), results in a rapid degradation of client proteins (Creagh *et al*., 2000; Young *et al*., 2001; Richter *et al*., 2001). Recently, Bcr–Abl was shown to be a client protein for Hsp90. Consistent with this, 17-AAG was shown to down-regulate intracellular levels of Bcr–Abl, in addition to c-Raf and Akt kinase. This was associated with the cytosolic accumulation of cyt *c* and the processing and activities of caspase-9 and -3, triggering apoptosis of Bcr–Abl positive leukemia cells (Nimmanapalli *et al*., 2001c). 17-AAG was shown to shift the binding of Bcr–Abl from Hsp90 to Hsp70 and induce proteasomal degradation of Bcr–Abl. Co-treatment with proteasome inhibitor PS-341 reduced 17-AAG mediated downregulation of Bcr–Abl and inhibited apoptosis of Bcr–Abl positive acute leukemia cells (Nimmanapalli *et al*., 2001c; An *et al*., 2000) Blagosklonny *et al*. (2001) demonstrated that GA sensitized Bcr–Abl positive leukemia cells to doxorubicin (Dox) and paclitaxel. GA selectively increased apoptosis of Bcr–Abl expressing cells (Blagosklonny *et al*., 2001). These reports have established the *in vitro* activity of GA and 17-AAG against Bcr–Abl-positive leukemic cells. These findings also support the *in vivo* investigation of Hsp 90 either alone or in combination with other therapeutic inhibitor agents and inhibitors in the treatment of Bcr–Abl positive leukemias (for review see Blagosklonny, 2002).

Other agents that synergize with imatinib against Bcr–Abl positive leukemic cells

Because of a high incidence of resistance to imatinib and the need to improve its efficacy, other conventional antileukemic drugs have been tested in combination with imatinib against Bcl–Abl positive leukemic cells. Fang *et al*. (2000) demonstrated that co-treatment with imatinib significantly increased Ara-C- or doxorubicin-induced apoptosis of Bcr–Abl positive leukemic cells. This was associated with greater cytosolic accumulation of cyt *c* as well as processing and activity of caspase-3 (Fang *et al*., 2000). Other reports have also confirmed the superior activity of imatinib in combination with

other antileukemic agents currently used in the treatment of CML. These include interferon-alpha (IFN), hydroxyurea (HU), daunorubicin (DNR), and cytosine arabinoside (Ara-C) (Thiesing *et al*., 2000). In colony growth assays, the combination of imatinib with IFN, DNR or Ara-C showed additive or synergistic effects against patient-derived CML progenitor cells (Thiesing *et al*., 2000). These findings have also been reported by Kano *et al*. (2001) who studied the cytotoxic effects of imatinib in combination with conventional antileukemic agents against Ph(+) leukemia cell lines. Apo-2L/TRAIL is a member of the TNFα family of death ligands, which has been shown to exert selective cytotoxic effects on cancer versus normal cells (Ashkenazi and Dixit, 1998). Recently, co-treatment with imatinib was shown to significantly enhance Apo-2L/TRAIL induced apoptosis without affecting the levels of its DR4 and DR5 death receptors, decoy receptors, or c-FLIP (Nimmanapalli *et al*., 2001d). These studies suggest that a co-treatment with imatinib may be an effective strategy to selectively sensitize Bcr–Abl-positive leukemic blasts to Apo-2L/TRAIL. As described above, treatment with AT lowers Bcr–Abl protein levels and induces apoptosis of the Bcr–Abl-positive leukemic blasts (Perkins *et al*., 2000). Combined treatment with AT and imatinib has also been shown to induce significantly more apoptosis of Bcr–Abl positive cells, as compared to treatment with either drug alone. AT and imatinib caused more decline in the levels of Bcl-x_L, XIAP and Akt, as well as a greater inhibition of Akt kinase activity (Porosnicu *et al*., 2001). These data demonstrate that a treatment strategy based on combining an agent that lowers Bcr–Abl levels, e.g., AT, with an agent that inhibits Bcr–Abl TK activity, e.g., imatinib, has the potential to deliver potent cytotoxic effects against Bcr–Abl-positive human leukemic cells. Support for this has also been provided by la Rosée *et al*. (2001), who showed that the co-treatment of imatinib with AT has superior inhibitory effects on the colony growth of Bcr–Abl positive leukemic cells, as compared to treatment with either agent alone. Exposure to imatinib in combination with the Mek kinase inhibitor PD184352 has also been shown to cause greater mitochondrial dysfunction, e.g., loss of mitochondrial membrane potential, cytosolic accumulation of cyt *c* and PARP (poly ADP-ribose polymerase) cleavage activity of caspase-3 (Yu *et al*., 2002). Similar results were obtained using other pharmacological MEK1/2 inhibitors (e.g., PD98059 and U0126) in combination with imatinib.

Inactivation – nuclear trapping – activation: lethal effect of Bcr–Abl

One of the most intriguing differences between Abl and Bcr–Abl lies in their respective sub-cellular localizations. Abl is found both in the nucleus and in the cytoplasm, and shuttles between these two compartments because it contains three nuclear localization signals (NLS) and a nuclear export signal (NES) on its carboxy-terminus (Daley *et al*., 1992; Renshaw *et al*., 1995). In spite of retaining both the NLS and NES domains of Abl, Bcr–Abl is exclusively cytoplasmic (McWhirter and Wang, 1993; Wen *et al*., 1996). Interestingly, nuclear Abl is a positive inducer of apoptosis in response to DNA damage (Gong *et al*., 1999; Wang, 2000), whereas cytosolic Bcr–Abl inhibits apoptosis through activation of PI-3 kinase and other pathways as explained above (McWhirter and Wang, 1993). Inhibition of the Bcr–Abl TK activity stimulates the nuclear entry of the Bcr–Abl protein. Hence by combining imatinib with leptomycin B (LMB), which blocks nuclear export, Bcr–Abl is trapped in the nucleus and the nuclear Bcr–Abl TK activates apoptosis (Vigneri and Wang, 2001). As a result, the combined treatment with imatinib and LMB causes irreversible and complete killing of Bcr–Abl transformed cells. In contrast, the effect of either drug alone is fully reversible. The combined treatment with imatinib and LMB has also been shown to preferentially eliminate mouse bone marrow cells that express Bcr–Abl (Vigneri and Wang, 2001). These results indicate that nuclear entrapment of Bcr–Abl can be used as a therapeutic strategy to selectively kill chronic myelogenous leukemia cells.

Strategies or agents that exert cytotoxic effects against imatinib resistant leukemic cells

Targeting Bcr–Abl TK or protein

Recently, studies have highlighted the activity of novel agents against imatinib resistant cultured leukemic cells with disparate levels and the activity of Bcr–Abl TK. Imatinib-resistant cell-types have been shown to retain sensitivity to 17-AAG or PD180970-induced apoptosis. Treatment with 17-AAG or PD180970 also induced apoptosis of CD34+ leukemic cells from three patients with CML-BC who had progressive leukemia while receiving imatinib therapy. These observations support the rationale to test the *in vivo* efficacy of 17-AAG and PD180970 against STI-571 resistant Bcr–Abl positive acute leukemias (Nimmanapalli *et al*., 2000). In a recent report by Donato *et al*. (2001) In imatinib resistant K562 cells, where Bcr–Abl downstream signaling was significantly impaired, imatinib failed to reduce Bcr–Abl TK activity, Bcl-X_L expression or induce apoptosis. But PD180970, which targets both Abl and Src kinases, caused not only apoptosis but also reduced the TK activity of Bcr–Abl in imatinib sensitive or resistant cells. These results indicate that multiple tyrosine kinase inhibitors such as PD180970 may have a greater therapeutic activity against advanced or imatinib-resistant CML.

Targeting other survival pathways

Recently, imatinib has been shown to elicit MAPK activation in imatinib-resistant, Bcr–Abl positive leukemic cells. Interruption of this pathway (e.g., by

MEK1/2 inhibitors) in conjunction with imatinib is associated with a highly synergistic induction of mitochondrial damage and apoptosis (Yu *et al*., 2002). Farnesyl transferase inhibitors (FTI) (SCH66336), which are known to block Ras mediated signaling, have been shown to inhibit cell growth of Bcr–Abl positive leukemic cells. A combination of SCH66336 with imatinib or Ara-C was demonstrated to exert synergistic effects against imatinib-resistant, Bcr–Abl positive leukemic cells. The combination of SCH66336 with imatinib also synergistically inhibited colony growth of primary CML cells and caused apoptosis of imatinib resistant Bcr–Abl expressing cells (Nakajima *et al*., 2002).

Summary

In summary, this review has highlighted the recent successes with imatinib-based targeted therapy of Bcr–Abl positive leukemias. Accumulating experience with treatment of advanced stages of CML with imatinib has indicated that resistance to this agent is common. Novel, Bcr–Abl-targeted agents that either inhibit its TK activity, e.g., PD180970, or reduce its intracellular levels, e.g., AT or 17-AAG, hold great promise in either preventing the emergence or effectively treating imatinib-resistant disease. Combination therapy involving imatinib and conventional antileukemic drugs, or these novel agents, clearly need to be tested in the clinic. Evidence suggests that in imatinib-resistant leukemic cells, survival signaling mediated by pathways other than Bcr–Abl may also emerge. Targeted therapies for these pathways may also potentially be combined with imatinib or other Bcr–Abl targeted agents to achieve more complete eradication of Bcr–Abl positive leukemic cells.

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