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BCR/ABL: from molecular mechanisms of leukemia induction to treatment of chronic myelogenous leukemia

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Introduction

Chronic myelogenous leukemia (CML) is a myeloproliferative disorder resulting from the clonal expansion of a transformed multipotent hematopoietic stem cell. CML is a biphasic disease with an initial chronic phase characterized by a massive expansion of myeloid precursors and mature cells that leave the bone marrow (BM) prematurely but retain their capacity to differentiate normally. This is invariably followed by progression to a fatal acute phase termed 'blast crisis', which resembles acute leukemia (Spiers, 1977). Allogeneic stem cell transplantation (SCT) is the only curative treatment for CML patients, but it is available to a limited number of patients due to age of the patient and donor availability. Interferon-α constitutes a second therapeutic option for CML patients, and complete hematological and cytogenetic remission could be achieved in 10–20% of CML patients. At the molecular level, CML is characterized by the Philadelphia chromosome (Ph) resulting from a balanced translocation between chromosome 9 and 22 which leads to the formation of the BCR/ABL fusion gene (Nowell and Hungerford, 1960). Over the last two decades, a large number of studies has evaluated the molecular and cellular mechanisms contributing to CML, and a number of signaling pathways activated by BCR/ABL were elucidated. This progress in the understanding of the molecular pathophysiology of CML has led to the development of several novel therapeutic approaches targeting various steps of the malignant transformation. Some of these show promising results. This review will first describe the molecular pathophysiology of CML, then discuss novel therapeutic strategies developed for the treatment of CML targeting specific molecular events and preclinical and clinical studies.

Molecular pathophysiology of CML

The crucial genetic events in CML is the generation in a hematopoietic stem cell of a t(9;22)(q34;q11) reciprocal chromosomal translocation. This translocation between the long arms of chromosome 9 and 22 results in a shortened chromosome 22, commonly known as the Philadelphia chromosome (Ph) and found in over 90% of CML patients (Nowell and Hungerford, 1960). The molecular consequences of this translocation event is the formation of the chimeric gene BCR/ABL on chromosome 22 (Rowley, 1973) and a reciprocal ABL/BCR on chromosome 9. The later gene, although transcriptionally active, does not appear to have any functional role in CML and no ABL/BCR protein has, as yet, been identified (Diamond et al., 1995; Melo et al., 1993).

Depending on the breakpoint in the BCR gene, three main types of BCR/ABL genes can be formed (Melo, 1996) (Figure 1). The majority of patients with CML have breakpoints in introns 1 or 2 of the ABL gene and in the major breakpoint cluster region (M-bcr) of the BCR gene, either between exons 13 and 14 (b2), or 14 and 15 (b3) (Figure 1). These breakpoints produce BCR/ABL fusion genes that transcribe either a b2a2 or b3a2 mRNA. The final product of this genetic rearrangement is a 210 kDa cytoplasmic fusion protein, $p210^{BCR/ABL}$, which is essential and sufficient for the malignant transformation of CML, and responsible for the phenotypic abnormalities of chronic phase CML (Daley and Baltimore, 1988; Daley et al., 1990; Gishizky et al., 1993). Less frequent, CML is caused by atypical BCR/ABL transcripts, for example involving ABL exon a3 instead of a2 (van der Plas et al., 1991), or transcripts with an e1a2, e19a2 (Hermans et al., 1987) or even e6a2 junction (Hochhaus et al., 1996). In contrast to ABL, BCR/ABL exhibits deregulated, constitutively active tyrosine kinase activity (Ben-Neriah et al., 1986) and is found exclusively in the cytoplasm of the cell (Van Etten et al., 1989), complexed with a number of cytoskeletal proteins. These two features appear to underlie the ability of BCR/ABL to induce a leukemic phenotype. Several functional domains have been identified in the Bcr-Abl

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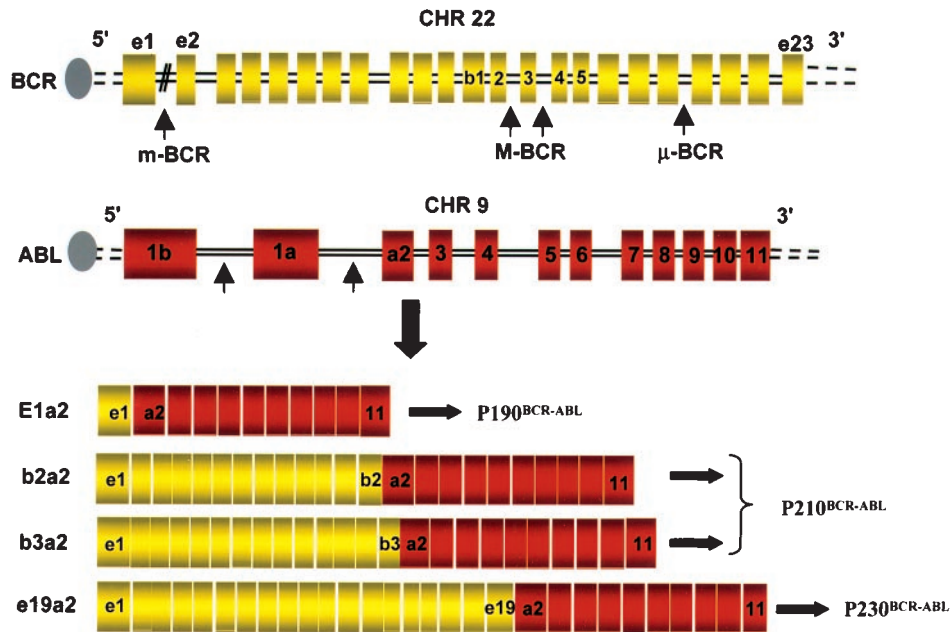
Figure 1 Locations of the breakpoints in the ABL and BCR genes and structure of the chimeric BCR/ABL mRNA transcripts derived from the various breaks

protein that may contribute to cellular transformation (Figure 2). In the Abl portion, these domains are the SH1 (tyrosine kinase), SH2 and actin-binding domains; in the BCR portion, they include the coiled-coil oligomerization domain comprised between amino acids (aa) 1–63, the tyrosine at position 177 (Grb-2 binding site) and the phosphoserine/threonine rich SH2 binding domain.

The increased tyrosine kinase activity of p210$^{BCR/ABL}$ results in phosphorylation of several cellular substrates and in autophosphorylation of p210$^{BCR/ABL}$, which in turn induces recruitment and binding of a number of adaptor molecules and proteins. Activation of a number of signal pathways by p210$^{BCR/ABL}$ leads to malignant transformation by interfering with basic cellular processes, such as control of cell proliferation and differentiation (Afar et al., 1994; Jiang et al., 2000; Puil et al., 1994; Sawyers, 1993), adhesion (Bhatia et al., 1999; Gordon et al., 1987) and cell survival (Bedi et al., 1994; Cortez et al., 1995; Cotter, 1995; McGahon et al., 1994) (Figure 3).

In brief, p210$^{BCR/ABL}$ activates signal transduction pathways such as RAS/MAPK, PI-3 kinase, c-CBL and CRKL pathways, JAK-STAT and the Src pathway. Of these, the ras, Jun-kinase, and PI-3 kinase pathways have been demonstrated to play a major role in transformation and proliferation (Raitano et al., 1995; Sawyers et al., 1995; Skorski et al., 1995, 1997a). Inhibition of apoptosis is thought to result from activation of the PI-3 kinase and RAS pathways, with induction through AKT of c-myc and BCL-2 (Raitano et al., 1995; Sawyers et al., 1995; Skorski et al., 1995, 1997a; Warmuth et al., 1999). p210$^{BCR/ABL}$ effects on CRKL, c-CBL, and on proteins associated with the

organization of the cytoskeleton and cell membrane, such as paxillin, actin, talin, vinculin and FAK/PYK2, result in adhesion defects and cytoskeletal abnormalities, characteristic of CML cells (Salgia et al., 1997; Sattler et al., 2002; Sattler and Salgia, 1998).

Therapies for CML

Standard treatment options

Standard treatment option for patients in the chronic phase of CML are hydroxyurea, interferon-α, or allogeneic stem cell transplantation.

Hydroxyurea Hydroxyurea is a ribonucleotide reductase inhibitor often used for initial cytoreductive therapy. Hydroxyurea is generally well tolerated, effective at controlling blood counts in a majority of patients. Unfortunately cytogenetic responses are rare and the onset to blast crisis is not delayed, with transformation occurring within a median of 4–6 years.

Interferon-α IFN-α has become the treatment of choice in patients with Ph-positive CML who are not candidates for allogeneic stem cell transplantation. It induces durable and complete cytogenetic responses in 10–20% of IFN-α-treated patients, and increases duration of chronic phase and survival compared with conventional chemotherapy. Unfortunately, many patients (up to 20%) tolerate IFN-α poorly, necessitating discontinuation of treatment. Its combination with other treatments, such as cytosine arabinoside (ara-C), showed significantly improved response rates compared
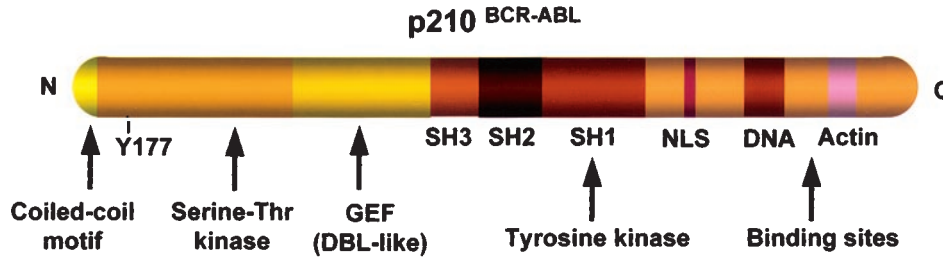
Figure 2 Functional domains of p210[BCR/ABL]. Some of the important domains of p210[BCR/ABL] are illustrated, such as the oligomerization domain (coiled-coil motif), the tyrosine 177 (Grb-2 binding site), the phosphoserine/threonine-rich SH2-binding domain and the rho-GEF (dbl-like) domain on the BCR portion, and the regulatory src-homology regions SH3 and SH2, the SH1 (tyrosine kinase domain), the nuclear localization signal (NLS), and the DNA- and actin-binding domains in the ABL portion
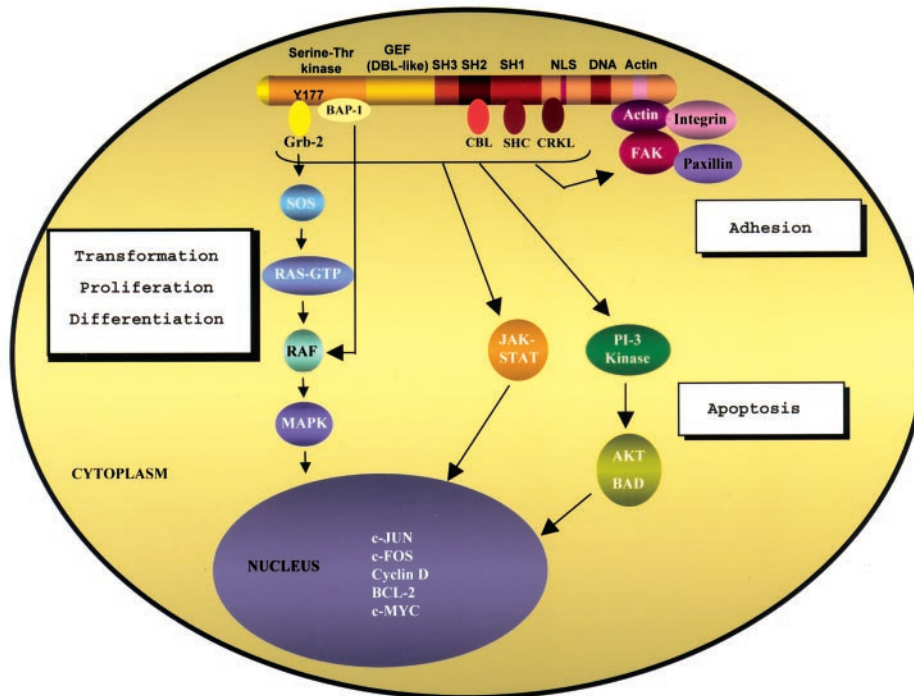

Figure 3 Signaling pathways of p210[BCR/ABL]. Activation of RAS, Jak/Stat, PI-3 kinase pathways and focal adhesion complexes results in increased proliferation, differentiation, and decreased apoptosis and adhesion to the bone marrow stroma of the CML progenitors. Activation of these different pathways is mediated through a series of adapter proteins, such as GRB2, CBL, SHC, and CRKL. BAP-1 denotes BCR-associated protein 1, GRB2: growth factor receptor-bound protein 2, CBL: casitas B-lineage lymphoma protein, SHC: SRC homology 2-containing protein, CRKL: CRK-oncogene-like protein, JAK-STAT: Janus kinase-signal transducers and activators of transcription, FAK: focal adhesion kinase, SOS: son-of-sevenless, GEF: GDP-GTP exchange factor. Note that this is a simplified diagram and that many more associations between BCR/ABL and signaling proteins have been reported

to IFN-α alone, but is associated with increased toxicity. The mechanisms of action of IFN-α are poorly understood. IFN-α may act by a direct antiproliferative effect, or restoration of the adhesive properties of CML cells, or via an indirect effect through the immune system by enhancing antileukemic cell-mediated immune responses.

Allogeneic stem cell transplantation Allogeneic stem cell transplantation, remains the only proven curative treatment for CML. When young (<40 years) chronic-

phase patients are treated with an HLA-matched transplant within 1 year of diagnosis, long-term survival reaches to 70–80%. However, donor availability is limited and only 20% of the patients match criteria listed above. Therefore, for the majority of patients with CML, allogeneic stem cell transplantation is not an option. In addition, graft-versus-host disease remains a major limiting factor of this approach.

Autotransplantation Autotransplantation, was proposed as an alternative for patients refractory to

IFN-α and who are not a candidate for allogeneic stem cell transplantation. This approach is based on the presence in the graft of Ph-negative progenitors capable of reconstituting normal hematopoiesis. Auto-transplantation failed to fulfil its promises mainly because of absence of graft-versus-leukemia effect, and persistence in the graft of Ph-positive cells giving rise to relapse. However, this approach remains of interest when considered in association with purging protocols.

Novel and experimental therapies

Insights in the molecular and cellular pathophysiology of CML has led to the development of several experimental therapies that target various steps in the pathogenesis of CML (Figure 4). The BCR/ABL gene, its mRNA and fusion protein are unique to CML progenitors and therefore constitute a good target for therapy. In addition, molecules in signal transduction pathways constitutively activated by BCR/ABL also constitute new molecular targets, provided that their inhibition does not affect normal hematopoietic cells. Attempts at designing new therapeutic tools have concentrated on three main areas: The inhibition of gene expression at the translational level by 'antisense'

strategies, the modification of protein function by specific signal transduction inhibitors and stimulation of the immune system's capacity to recognize and destroy leukemic cells.

Antisense strategies

CML could be considered an 'ideal' disease for antisense-based therapeutic approaches. Indeed, the presence of the unique nucleotide sequences at the fusion site between BCR and ABL and the requirement of the encoded tyrosine kinase for malignant transformation make CML an attractive target for antisense strategies (Figure 4).

Principle Antisense strategies rely on the formation of DNA–RNA or RNA–RNA complexes between the reverse complement (antisense oligonucleotide) and the mRNA to be disrupted. Antisense oligonucleotides may be short DNA or RNA nucleotides. If hybridization between the target mRNA and the exogenous antisense oligonucleotide occurs, a duplex is created which prevents the ribosomal complex from reading the message (Galderisi *et al.*, 1999; Gewirtz *et al.*, 1998).

However, RNA–RNA and DNA–RNA duplexes can be unwound by a variety of repair/editing enzymes
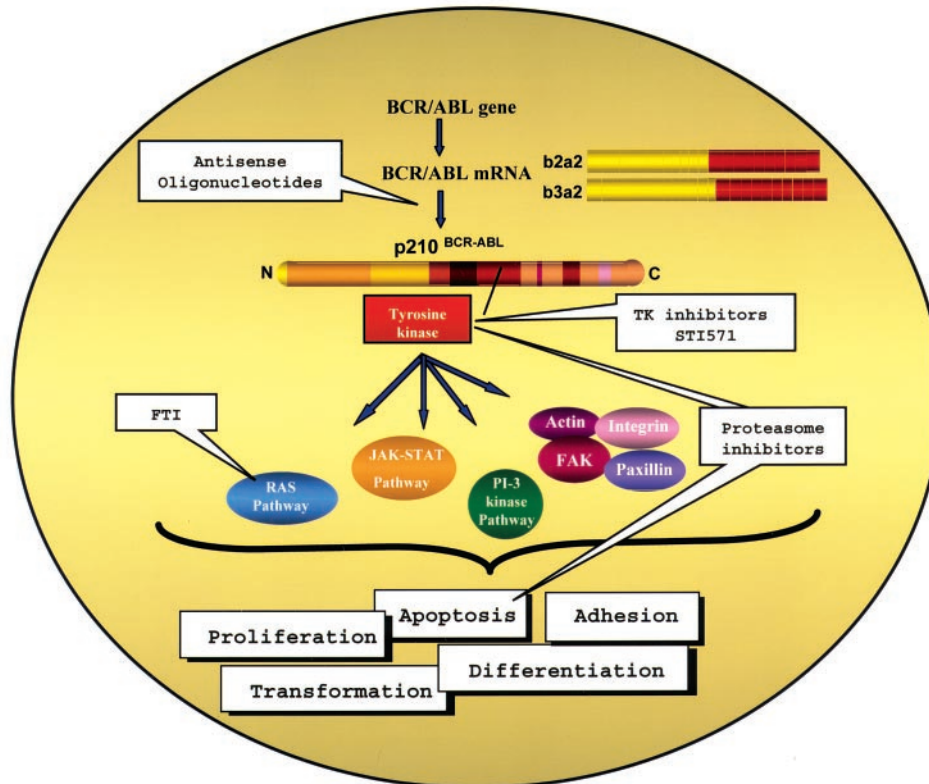
Figure 4 Novel and experimental therapies for CML. Novel and experimental therapies for CML are illustrated, such as antisense oligonucleotides that inhibit translation, Tyrosine kinase inhibitors and STI571 that inhibit the tyrosine kinase activity of p210$^{BCR/ABL}$, Farnesyl Transferase Inhibitors (FTI) that inhibit activation of the Ras protein, and Proteasome inhibitors that decrease P210$^{BCR/ABL}$ tyrosine kinase activity and activate cell apoptosis

such as helicase and RNA unwindase (Nellen and Lichtenstein, 1993). Oligodeoxynucleotides (ODN) support the binding of RNase H at sites of RNA–DNA duplex formation. Once bound, RNase H, functions as an endonuclease that recognizes and cleaves the RNA in the duplex (Crooke, 1999; Wu *et al.*, 1999a). Alternatively, the RNA–RNA duplex may serve as a substrate for editing enzymes such as double-stranded RNA adenosine deaminase (DRADA) (Kim *et al.*, 1994a,b). When DRADA deaminates adenosine, inosine is formed and may tag the mRNA molecule for destruction.

In an attempt to enhance destruction of the mRNA target, ribozymes (James and Gibson, 1998) and DNAzymes (Wu *et al.*, 1999b) have been investigated. Ribozymes and DNAzymes are catalytic molecules that have site-specific self-cleaving enzymatique activity (Gibson and Shillitoe, 1997; Pyle, 1993). When the site-specific cleaving motif of the ribozyme is flanked with 5′ and 3′ ends designed to hybridize with specific sequences within an mRNA target, a specific mRNA cleavage results. A number of ribozymes have been described, hammerhead ribozymes or artificially engineered types (Sigurdsson and Eckstein, 1995; Warashina *et al.*, 1997).

Antisense oligonucleotides can be introduced directly in the target cell, by electroporation, streptolysin permeabilization or lipophilic conjugation (Spiller *et al.*, 1998b). Alternatively, delivery of antisense oligonucleotides into target cells can be achieved by transfecting or transducing target cells with viral or plasmid vectors. This results in expression of antisense RNA or ribozymes that may be more powerful to eliminate proteins with long half-life such as p210$^{BCR/ABL}$ (Garcia-Hernandez and Sanchez-Garcia, 1996).

Antisense oligonucleotides against BCR/ABL Antisense oligodeoxynucleotides (AS-ODNs) targeting the breakpoint junction of BCR/ABL or the translation start site of BCR mRNA have been shown to selectively inhibit proliferation (de Fabritiis *et al.*, 1997; Szczylik *et al.*, 1991), survival (Rowley *et al.*, 1996; Smetsers *et al.*, 1994, 1995) and restore β1-integrin-mediated adhesion and proliferation inhibition (Bhatia and Verfaillie, 1998) of BCR/ABL expressing cell lines and primary cells. Some studies suggest that the inhibition was sequence dependent but not sequence specific (Mahon *et al.*, 1995; O'Brien *et al.*, 1994). Other investigators reported that inhibition of CML cell proliferation by AS-ODNs is sequence specific but nonantisense mediated (Clark, 2000; Vaerman *et al.*, 1995, 1997).

Ribozymes have been used to target BCR/ABL. They decrease BCR/ABL mRNA and p210$^{BCR/ABL}$ protein levels, inhibit growth and survival of BCR/ABL expressing cell lines, and decrease tumorigenicity of BCR/ABL expressing cells in SCID mice (Lange, 1995; Lange *et al.*, 1993, 1994; Shore *et al.*, 1993). However, ribozymes result in only imperfect cleavage of target mRNAs (James and Gibson, 1998). New modifications to the antisense system, such as DNAzymes (Hamada *et al.*, 1999; Kuwabara *et al.*, 1998, 2001a,b; Tanabe *et al.*, 2000; Warashina *et al.*, 1999), BCR/ABL junction-specific catalytic subunits of RNase P (Cobaleda and Sanchez-Garcia, 2000) or maxizymes; novel allosterically controllable ribozymes (Hamada *et al.*, 1999; Kuwabara *et al.*, 1998, 2001a,b; Tanabe *et al.*, 2000); may increase specificity and increase cleavage of BCR/ABL mRNA (Maran *et al.*, 1998; Mendoza-Maldonado *et al.*, 2002; Rowley *et al.*, 1999).

The therapeutic potential of AS-ODNs has been assessed in murine models of CML. BCR/ABL expressing cell lines or primary leukemic bone marrow cells were pretreated with AS-ODNs prior to transplantation in SCID mice as a model of *ex vivo* bone marrow purging. Alternatively, CML-bearing SCID mice were treated *in vivo* with AS-ODNs sequences. Most studies demonstrated a sequence-specific AS-ODN effect on leukemic cell growth and animal survival (Skorski *et al.*, 1993, 1994b). To improve these results, strategies combining AS-ODNs targeting BCR/ABL and c-MYC, or AS-ODNs with traditional chemotherapy such as cyclophosphamide, have been reported (Skorski *et al.*, 1996, 1997b). Both strategies demonstrated a specific synergistic antiproliferative effect of the combined treatment and a markedly increased survival of leukemic mice treated with the combined treatment.

These encouraging results have led to clinical trials with AS-ODNs directed to the BCR/ABL mRNA for *ex vivo* purging of autografts. De Fabritiis *et al.* transplanted eight CML patients with bone marrow cells purged *in vitro* with junction-specific BCR/ABL AS-ODNs. Most patients were in accelerated phase or in second chronic phase (de Fabritiis *et al.*, 1998). The low toxicity of the protocol and the hematopoietic reconstitution observed in all patients made this approach promising. However, despite the marked karyotypic response observed in some patients and the prolonged duration of the second chronic phase in one patient, no obvious long-term therapeutic benefit of purging of the graft was seen, and the overall antileukemia effect of the protocol needs to be improved.

Zhao *et al.* (1997) reported a clever strategy for inhibiting growth of CML cells with an anti-BCR/ABL antisense delivered by retroviral vector that also delivered a methotrexate (MTX) resistance gene. The hypothesis underlying these experiments was that expression of the resistance gene would make the normal stem cells MTX resistant and expression of the anti-BCR/ABL antisense sequence would render CML progenitors functionally normal. Transduction of CD34$^+$ cells from CML patients rendered 20–30% of the cells MTX resistant and reduced BCR/ABL mRNA by 10-fold. *In vivo* tumorigenicity of P210-transduced 32D cells was decreased by three to four logs in a sequence specific manner. These encouraging preclinical results will now be tested in a clinical study in our institution.

Limitations of BCR/ABL antisense strategies Antisense strategies received a lot of attention during the last decade but, due to a number of technical problems, have in general failed to fulfil their theoretical promise. Although BCR/ABL may in theory be the most attractive target for antisense therapy, the long half life of P210$^{BCR/ABL}$ (more than 24 h) poses a significant obstacle (Clark, 2000; Spiller *et al.*, 1998a,b). Prolonged *ex vivo* culture would therefore be needed to induce cell death in most leukemic cells, which may interfere with engraftment ability of hematopoietic progenitors. Furthermore, there is evidence that BCR/ABL mRNA and protein may not be expressed in CML stem cells. *Ex vivo* treatment with anti-BCR/ABL AS-ODNs may therefore not eliminate the leukemic stem cell.

Antisense oligonucleotides against other genes Because of these shortcomings in approaches targeting BCR/ABL using AS-ODNs, Gewirtz and colleagues have examined antisense strategies against downstream targets of BCR/ABL, such as MYC (Calabretta and Skorski, 1997; Skorski *et al.*, 1997c), CRKL, GRB2 (Tari *et al.*, 1997), KIT (Luger *et al.*, 1996; Ratajczak *et al.*, 1992c), VAV (Luger *et al.*, 1996) and MYB (Gewirtz *et al.*, 1989). Of these, only anti-c-Myb-ODNs have been tested in clinical trials.

c-myb Myb family members play a major role in regulating the G1/S transition in cycling hematopoietic cells (Gewirtz *et al.*, 1989), and c-*myb* in particular, functions as a transactivator of a number of important cellular genes, such as CD34 (Melotti *et al.*, 1994), and the kit receptor (Ratajczak *et al.*, 1998). Myb's ability to control in normal hematopoietic cells critical functions such as cell proliferation and growth (Anfossi *et al.*, 1989; Caracciolo *et al.*, 1990; Gewirtz *et al.*, 1989; Gewirtz and Calabretta, 1988; Luger *et al.*, 2002), suggests a potential role for Myb in leukemic transformation (Anfossi *et al.*, 1989). In addition, c-*myb* plays a role in regulating c-*myb* which plays an important role in BCR/ABL-mediated transformation. The very short half-life of c-Myb mRNA and protein, make it an ideal target for antisense strategies. Furthermore, malignant cells may be more sensitive to the growth inhibitory effect of anti- c-*myb* AS-ODNs than normal cells (Calabretta *et al.*, 1991). c-*myb* AS-ODNs selectively inhibit colony formation of chronic and blast crisis CML cells (Ratajczak *et al.*, 1992a,b), and improve survival of CML-bearing SCID mice. A pilot bone marrow purging study in CML patients with c-*myb* AS-ODNs was initiated (Anfossi *et al.*, 1989; Caracciolo *et al.*, 1990; Gewirtz *et al.*, 1989; Gewirtz and Calabretta, 1988; Luger *et al.*, 2002). After purging, c-*myb* mRNA levels and BCR/ABL expression in LTC-IC declined in approximately 50% of the patients and was AS-ODNs dependent. Six of 14 patients transplanted with purged grafts obtained a major cytogenetic response. However, a significant proportion of patients exhibited graft-failure when transplanted with 72-h-purged marrow compared with 24-h-purged marrow consistent with the known role c-*myb* in normal hematopoietic cells. Although, this study was primarily designed to assess the safety of this approach, the results showed that delivery of AS–ODNs, targeted to critical proteins downstream of BCR/ABL that have a short half-life, might lead to the development of more effective purging approaches or even *in vivo* therapy of CML.

Signal transduction inhibitors

Inhibitors of the BCR/ABL fusion protein

As the deregulated tyrosine kinase activity of p210$^{BCR/ABL}$ is known to be the essential transforming event in CML, studies aimed at inhibiting this TK activity were initiated (Lugo *et al.*, 1990; Oda *et al.*, 1995).

Several tyrosine kinase inhibitors have been evaluated in CML cells (Boutin, 1994; Levitzki and Gazit, 1995). The first to be tested were isolated from natural sources, such as the antibiotics herbimycin-A, genistein and erbstatin which inhibit p210$^{BCR/ABL}$ TK activity *in vitro*, inhibit growth of BCR/ABL$^+$ cell lines *in vitro*, and induce erythroid differentiation of K562 cell line (Carlo-Stella *et al.*, 1996; Honma *et al.*, 1989, 1990; Kawada *et al.*, 1993; Okabe *et al.*, 1992). Synthetic compounds, tyrphostins, were then developed and AG957 and AG568 were identified that inhibit p210$^{BCR/ABL}$ TK activity *in vitro*, and induce erythroid differentiation and apoptosis of the K562 cell line (Anafi *et al.*, 1993). Furthermore, AG957 restores β1-integrin-mediated adhesion of CML primary cells (Bhatia *et al.*, 1998). AG957 also has a synergistic antiproliferative effect with the anti-fas receptor on CML progenitors (Carlo-Stella *et al.*, 1999). However, low specificity for the BCR/ABL TK activity is a major limitation of these TK inhibitors.

STI571 In the late 1980s, a 2-phenylaminopyrimidine with specific tyrosine kinase inhibitory activity against platelet-derived growth factor receptor (PDGF-R), C-kit and ABL tyrosine kinase, STI571 (formerly CGP57148, now Gleevec or imatinib mesylate) was identified (Buchdunger *et al.*, 1996; Druker and Lydon, 2000). Like tyrphostins, STI571 functions by binding to the highly conserved nucleotide-binding pocket of the catalytic domain of the ABL-TK and competitively blocking the binding of ATP (Schindler *et al.*, 2000).

Preclinical studies showed that STI571 specifically inhibits proliferation of leukemic cells, and restores interleukin-3 (IL-3) dependent growth and differentiation of BCR/ABL$^+$ cell lines. Growth of CML myeloid colony-forming cells is strongly inhibited by STI571 with minimal effect on growth of normal colonies (Carroll *et al.*, 1997; Deininger *et al.*, 1997; Druker *et al.*, 1996; Gambacorti-Passerini *et al.*, 1997). This is due to inhibition of proliferation and to a lesser extent cell death (Holtz *et al.*, 2002). Long-term culture of BM cells with prolonged exposure with STI571 showed inhibitory effect on CML progenitors with

little toxicity to normal cells (Kasper *et al.*, 1999). However, up to 30–40% of Ph$^+$ LTC-IC survive STI571 treatment (Holtz *et al.*, 2002). Moreover, inhibition of BCR/ABL kinase activity by STI571 results in transcriptional modification of various genes involved in control of cell cycle, cell adhesion and cytoskeletal organization (Deininger *et al.*, 2000), leading to apoptotic death of at least some Ph$^+$ cells. Studies in mice demonstrated an *in vivo* effect of STI571 against BCR/ABL$^+$ cells. However, continuous exposure to STI571 was necessary to eradicate 32D$^{BCR/ABL}$-generated tumors (le Coutre *et al.*, 1999). Before clinical testing, STI571 was shown to have an acceptable animal toxicity profile (Druker and Lydon, 2000).

A phase I clinical trial with STI571 was started in June 1998 (Druker *et al.*, 2001b). This trial was a dose escalation study, designed to establish the maximum tolerated dose in 54 patients in chronic phase CML who had failed IFN-α therapy. The results are summarized in Table 1. Side effects have been minimal, with no dose-limiting toxicity.

The phase I studies were expanded to CML patients in myeloid and lymphoid blast crisis and patients with relapsed or refractory Ph-positive ALL. Patients have been treated with daily doses of 300–1000 mg of STI571. The results are summarized in Table 2. STI571 has remarkable single-agent activity in CML blast crisis and Ph-positive ALL, but responses tend not to be durable (Druker *et al.*, 2001a).

The phase I was followed by a large international phase II study between December 1999 and May 2000, to assess the safety and efficacy of STI571 in interferon-refractory and interferon-intolerant Ph-positive CML patients, as well as accelerated-phase CML patients, CML in myeloid blast crisis, and Ph-positive ALL patients (Kantarjian *et al.*, 2002; Sawyers *et al.*, 2000; Talpaz *et al.*, 2002). This study enrolled over 1000 patients in 27 centers in six countries over a period of 6–9 months. The results are summarized in Table 3. The study confirmed the results seen in phase I and served as the basis for accelerated Food and Drug Administration (FDA) approval of STI572.

Since then, a phase III randomized study comparing STI571 with interferon and cytarabine in newly diagnosed patients accrued over 1000 patients in a six month period, and data collection is ongoing.

Clinically, the majority of patients who relapse after an initial response to STI571 have reactivation of the BCR/ABL kinase (Gorre *et al.*, 2001). *In vitro* studies in murine and human BCR/ABL-positive cell lines resistant to STI571 have demonstrated that a frequent mechanism of resistance to STI571 is amplification and overexpression of the BCR/ABL gene (le Coutre *et al.*, 2000; Mahon *et al.*, 2000). Overexpression of the Pgp glycoprotein, the product of the multidrug resistance (MDR) gene, may also contribute to the resistant phenotype. Approximately, one-third of the patients who relapse after an initial response have BCR/ABL amplification (Gorre *et al.*, 2001). Interestingly, half of these patients have developed point mutations in the ABL kinase domain that result in decreased sensitivity to STI571 (Barthe *et al.*, 2001; Gorre *et al.*, 2001; Hochhaus *et al.*, 2001). At least one of the point mutations is at a site predicted to be a contact site between the ABL kinase and STI571 (Gorre *et al.*, 2001). Several point mutations are at residues adjacent to contact points, whereas others are in the kinase activation loop (Barthe *et al.*, 2001; Gorre *et al.*, 2001; Hochhaus *et al.*, 2001). However, BCR/ABL mutation or amplification have not been commonly seen in patients with *de novo* STI571 resistance and studies are ongoing to identify the mechanism of primary resistance in these patients.

Table 1 Phase I clinical trials of response to STI571 in CML patients

Number of patients	Dose	Hematologic response (%)	Cytogenetic response (%)	Myelosuppression (%) Grade 2	Grade 3
54	⩾300 mg	98	53	21	8

Table 2 Phase I clinical trials of response to STI571 in CML patients

Disease phase	Number of patients	Response to therapy (%)	BM clearance of blasts ⩽5% (%)	Relapse between 42–123 days	Remission on STI571 101–349 days
Myeloid blast crisis Lymphoid blast crisis	38	55	21		7
Relapsed or refractory Ph-positive ALL	20	70	55	19	

Table 3 Phase II clinical trials of response to STI571 in CML patients

Disease phase	Number of patients	Hematologic response (%)	Cytogenetic response (<35% Ph+) (%)	Relapse rate at 18 months (%)
Chronic	532	95	60	9
Accelerated	235	53	26	40
Myeloid blast crisis	260	29	15	78

The inhibition of ABL, PDGF receptor and c-*kit* receptor kinase activity by STI571 may potentially interfere with normal cellular function. However, the negligible degree of side effects observed in STI571 clinical trials suggests that alternative pathways may compensate for suppression of the normal ABL, PDGF and c-*kit* kinases.

Inhibitors of other signal transduction proteins

Farnesyl transferase inhibitors (FTI) This strategy is based on the notion that RAS activation plays a central role in leukemogenic transformation by BCR/ABL (Cortez *et al.*, 1996; Goga *et al.*, 1995; Mandanas *et al.*, 1993; Pendergast *et al.*, 1993; Puil *et al.*, 1994; Sanchez-Garcia and Martin-Zanca, 1997; Sawyers *et al.*, 1995; Senechal *et al.*, 1996). Inhibition of RAS signaling by expression of dominant-negative RAS, blockage of Grb2 adaptor protein function or incubation with antisense oligonucleotides to p21Ras, prevents BCR/ABL transformation in several cell line models (Gishizky *et al.*, 1995; Sawyers *et al.*, 1995; Sakai *et al.*, 1994; Skorski *et al.*, 1994a). Ras function depends on proper subcellular localization at the plasma membrane through addition of a 15-carbon farnesyl group to Ras, a reaction that is catalysed by the farnesyl protein transferase (FPT) enzyme (Gutierrez *et al.*, 1989; Hancock *et al.*, 1989; Long *et al.*, 2001; Reiss *et al.*, 1990; Stokoe *et al.*, 1994). Farnesyl protein transferase inhibitors (FTI) are a class of drugs designed to specifically block oncogenic Ras signaling and Ras-dependent cellular transformation (Gibbs *et al.*, 1994). FTI disrupt Ras prenylation and without proper subcellular localization, Ras is not longer oncogenic (Kato *et al.*, 1992). Several studies have demonstrated the potent antitumor activity of FTI *in vitro* against Ras-transformed murine and human cancer cells and *in vivo* against Ras-specific tumor formation in transgenic and xenograft murine models (End, 1999; Gibbs *et al.*, 1997; Kohl *et al.*, 1993, 1994; Nagasu *et al.*, 1995; Rowinsky *et al.*, 1999). However, it was reported that FTI also inhibit the growth of transformed cells that lack mutant Ras, suggesting that other mechanism are also involved (Liu *et al.*, 1998; Sepp-Lorenzino *et al.*, 1995). For example, in the presence of inhibitory doses of FTI, some proteins substrates become alternatively prenylated by the geranyl-geranyl protein transferase. As an alternatively prenylated form of RhoB exerts anti-proliferative effects on transformed cells (Lebowitz *et al.*, 1997; Lebowitz and Prendergast, 1998). The latter may be responsible for the effect seen by FTI on cell proliferation. Encouraging preliminary studies documented that FTI inhibit *in vitro* proliferation of ALL and juvenile chronic myeloid leukemia cells (JCML) (Emanuel *et al.*, 2000). A phase I dose-escalation trial was conducted with the FTI R115777 in 35 adults with refractory and relapsed acute leukemias (Karp *et al.*, 2001). Clinical responses occurred in 29% of the 34 evaluable patients, including two complete remissions. Results of this trial provide the first evidence for successful inhibition of FT in neoplastic cells *in vivo* and suggest that FTI may be a promising antileukemic modality.

Furthermore, SCH66336, an oral FTI, potently inhibits soft agar colony formation, slowed proliferation and sensitized BCR/ABL$^+$ cell lines to apoptotic stimuli (Peters *et al.*, 2001). When administered to mice with BCR/ABL-induced leukemia, SCH66336 increased survival from 4 weeks (without therapy) to more than a year. However, when SCH66336 was withdrawn animals developed leukemia. The ability of SCH66336 to inhibit colony formation of primary CML cells was also demonstrated (Peters *et al.*, 2001). These results show that FTI compounds are highly effective as single agents against BCR/ABL-transformed hematopoietic cells, identifying FTIs as a potential clinical treatment for BCR/ABL-induced leukemia. Another study reported the efficacy of SCH66336 in the treatment of BCR/ABL-positive acute lymphoblastic leukemia in P190 transgenic mice (Reichert *et al.*, 2001). Further preclinical animal studies will determine the merits of using FTI in combination with other treatments, such as tyrosine kinase inhibitors, to treat BCR/ABL-induced leukemia.

Proteasome inhibitors The proteasome is a multi-catalytic protease present in all eukaryotic cells and constitute the primary component of the protein degradation pathway of the cell. By degrading regulatory proteins (An *et al.*, 2000; Dietrich *et al.*, 1996; Pagano *et al.*, 1995; Wu *et al.*, 2000), the proteasome is key to the activation or repression of many cellular processes, including cell-cycle progression and apoptosis (Adams *et al.*, 1999; Imajoh-Ohmi *et al.*, 1995). *In vitro* and mouse xenograft studies have shown antitumor activity of proteasome inhibitors in a variety of tumor types including pancreatic, prostate, and colon cancers, myeloma and chronic lymphocytic leukemia (Adams, 2002; Hideshima *et al.*, 2001; Shah *et al.*, 2001). Several studies have investigated the hypothesis that the proteasome may play a role in the regulation of BCR/ABL function. Effects of Proteasome inhibitors such as tripeptide aldehydes, lactacystin, and PSI were investigated in different human leukemic cell lines. Proteasome inhibition results in increased apoptotic death and enhancement of the effect of cytotoxic drugs in a number of myeloid cell lines (Dou *et al.*, 1999; Drexler, 1997; Shinohara *et al.*, 1996; Soligo *et al.*, 2001). This process involves activation of caspases, perturbation in the expression of Bcl-2 family proteins and decreased expression of p210$^{BCR/ABL}$. Interestingly, proteasome inhibitors first decrease levels of p210$^{BCR/ABL}$ tyrosine kinase activity and, subsequently, activate the apoptotic death program in K562 cells (Soligo *et al.*, 2001). These results suggest that inactivation of BCR/ABL function by proteasome inhibitors is essential for induction of apoptosis in leukemic cell lines. In primary cells, the sensitivity to PSI is threefold higher in CML CD34$^+$ progenitors than normal progenitors. The observation that transformed cells are more sensitive to blockade of

the proteasome than normal cells, was reported in leukemic cells compared to normal cells (Adams, 2002). While the exact mechanism for this differential susceptibility is not fully understood, proteasome inhibition may reverse some of the changes that permit proliferation and suppress apoptosis in malignant cells. The proteasome inhibitor PS-341 was the first proteasome inhibitor to enter human trials. Six phase I clinical trials for PS-341 in hematologic malignancies or solid tumors have been completed or are in progress (Papandreou et al., 2001; Stinchcombe et al., 2000), and the safety and efficacity of PS-341 treatment for refractory multiple myeloma and CLL are being tested in two ongoing phase II trials.

Immunomodulation

This will only be briefly discussed and we refer the readers to excellent reviews that were recently published (Apperley et al., 1998; Campbell et al., 2001; Clark and Christmas, 2001; Claxton et al., 2001; Dazzi et al., 1999; Goodman et al., 1998; Pinilla-Ibarz et al., 2000b).

Infusion of donor lymphocytes in CML patients that relapsed after allogeneic stem cell transplantation (SCT) significantly increases long term remission rate (Kolb et al., 1995). Although the mechanism is not completely understood, this proves that immuno-regulatory cells can specifically eliminate leukemic progenitors and stem cells. GVHD associated with this therapy constitutes the major limitation for this therapy. However, selective depletion of donor CD8 + T-lymphocytes or transduction of donor T-lympho-cytes with herpes simplex thymidine kinase gene may allow clinicians to control GVHD (Ackerman et al., 1978; Barrett et al., 1998; Giralt et al., 1995; Nimer et al., 1994; Tiberghien et al., 1994). Coculture of donor lymphocytes with host leukemic cells or antigen presenting leukemic dendritic cells from CML patients can be done to generate and expand CTLs specifically reactive against CML progenitors (Choudhury et al., 1997; Faber et al., 1995; Falkenburg et al., 1993; Jiang and Barrett, 1995; Molldrem et al., 1997; Warren et al.,

1998) ex vivo. Development of CML vaccines is another valuable approach. In this therapy, BCR/ABL-specific peptides are expressed on MHC mole-cules to generate a leukemia-specific CTL response. Several studies have shown the development of specific immune response. Whether such vaccines will suffice to effectively treat CML remains to be seen (Bocchia et al., 1995, 1996; Bosch et al., 1996; Pinilla-Ibarz et al., 2000a; ten Bosch et al., 1999).

Administration of low or intermediate doses of IL2 following allogeneic SCT or expansion ex vivo of autologous NK cells with IL2 before reinfusion increases the number and activate the NK cells in CML patients and may be helpful to eliminate minimal residual the disease (Robinson et al., 1996; Soiffer et al., 1994; Vey et al., 1999).

Conclusion

Much progress has been made in the understanding of the molecular pathophysiology underlying CML and has led to the development of targeted and effective therapies. Despite effective ex vivo and animal studies, antisense oligonucleotides have in general failed to fulfil their theoretical promise and have shown limited success in clinical studies. However, new modifications of the antisense system, and new delivery methods are being developed and may improve their efficacy. STI571 is one of the most promising of the new therapies developed recently against BCR/ABL. Initial clinical trials of STI571 were very encouraging and a phase III clinical trial is ongoing. As resistance to this single apart therapy appears to develop, studies aimed at evaluating the mechanism(s) underlying resistance development will be very valuable. The development of inhibitors of other signal transduction proteins like the Farnesyl Transferase Inhibitors (FTI) or protea-some inhibitors may allow additional therapeutic alternatives. Preclinical studies and clinical trails suggest that these new approaches present promising antileukemic modalities.

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