7 Glivec® (Gleevec®, Imatinib, STI571)

A Targeted Therapy for Chronic Myelogenous Leukemia

Elisabeth Buchdunger, PhD
and Renaud Capdeville, MD

CONTENTS

1. CONCEPT AND TARGET SELECTION: BCR-ABL

Chronic myelogenous leukemia (CML) is a clonal hematological disorder characterized by a reciprocal translocation between chromosomes 9 and 22 *(1,2)* known as the Philadelphia (Ph) chromosome. The molecular consequence of this interchromosomal exchange is the creation of the *bcr-abl* gene coding for a protein with elevated tyrosine kinase activity. The demonstration that the expression of Bcr-Abl is both necessary and sufficient to cause a CML-like syndrome in murine bone marrow transplantation models *(3–5)* and the finding that the tyrosine kinase activity of Bcr-Abl is crucial for its transforming activity *(6)*, has established the enzymatic activity of this deregulated protein as an attractive drug target addressing Bcr-Abl-positive leukemias. For the first time,

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a drug target was identified that very clearly differed in its activity between normal and leukemic cells. It was conceivable that this enzyme could be approached with classical tools of pharmacology since its activity, the transfer of phosphate from adenosine triphosphate (ATP) to tyrosine residues of protein substrates, could clearly be described and measured in biochemical as well as cellular assays. Furthermore, cell lines were available that were derived from human leukemic cells that had the same chromosomal abnormality. Such cell lines were instrumental for in vitro and animal studies that laid the groundwork for the clinical trials. So, the essential tools were assembled to go forward aiming at identifying potent and selective inhibitors of the Abl tyrosine kinase.

2. MEDICINAL CHEMISTRY: DEVELOPMENT OF AN ABL TYROSINE KINASE INHIBITOR

The starting point for the medicinal chemistry project that led to the synthesis of Glivec was the identification of a lead compound from a screen for inhibitors of protein kinase C (PKC). This compound, a phenyl-amino pyrimidine derivative, had very promising "lead-like" properties *(7,8)* and had a high potential for diversity, allowing simple chemistry to be applied to produce compounds with more potent activity or selectivity. A high cellular PKC inhibitory activity was obtained with derivatives bearing a 3′-pyridyl group at the 3 position of the pyrimidine (Fig. 1A). During the optimization of this structural class, it was observed that the presence of an amide group on the phenyl ring provided inhibitory activity against tyrosine kinases, such as the Bcr-Abl kinase (Fig. 1B). At this point a key observation from analysis of structure–activity relationship (SAR) was that a substitution at position 6 of the diamino phenyl ring abolished PKC inhibitory activity completely. Indeed, the introduction of a simple "flag-methyl" led to loss of activity against PKC, whereas the activity against protein-tyrosin kinases was retained or even enhanced (Fig. 1C). However, the first series of selective inhibitors originally prepared showed poor oral bioavailability and low solubility in water. The attachment of a highly polar side chain (an *N*-methylpiperazine) was found to dramatically improve both solubility and oral bioavailability. To avoid the mutagenic potential of aniline moieties, a spacer was introduced between the phenyl ring and the nitrogen atom. The best compound from this series was the methyl piperazine derivative originally named STI571 (imatinib, now known as Glivec® or Gleevec®), which was selected as the most promising candidate for clinical development (Fig. 1D) *(9,10)*.

Docking studies *(11)* and X-ray crystallography *(12,13)* showed that binding of Glivec occurs at the ATP binding site. Analysis of the crystal structure showed that Glivec inhibits the Abl kinase by binding with high specificity to an inactive form of the kinase. The need for the kinase to adopt this unusual

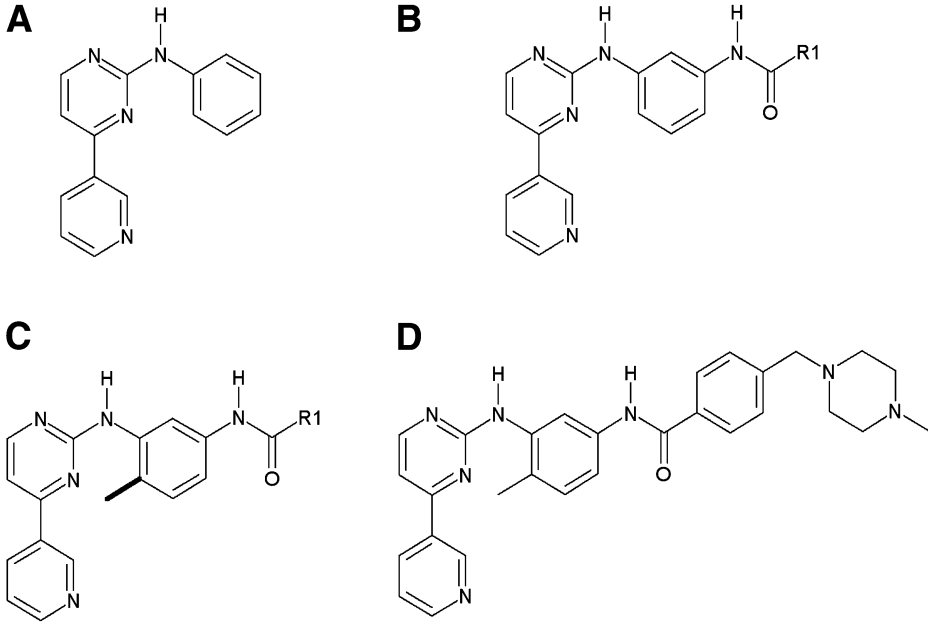

Fig. 1. Summary of the chemical optimization.

conformation favoring binding may contribute to the high selectivity of the compound. Unexpectedly, these analyses indicated that the *N*-methylpiperazine group (added to increase drug solubility) was also interacting strongly with Abl via hydrogen bonds to the backbone carbonyl of Ile360 and His 361.

3. PHARMACOLOGICAL PROFILE OF GLIVEC

3.1. In Vitro/Cellular Activity

In vitro studies using purified enzymes expressed as bacterial fusion proteins or immunoprecipitations of intact proteins showed that Glivec potently inhibited all of the *Abl* tyrosine kinases, including cellular Abl (c-Abl), v-Abl, the oncogenic form contained in the Abelson murine leukemia virus, and Bcr-Abl (Table 1) *(14–16)*. STI571 is an ATP-competitive inhibitor of Abl with a K_i value of 85 nm *(17)*. Extended profiling against various serine/threonine and tyrosine kinases revealed that the compound was also an inhibitor of the platelet-derived growth factor (PDGF) receptor and c-KIT tyrosine kinases, devoid of activity against most other kinases. The selective inhibitory activity of Glivec was also demonstrated at the cellular level *(18–20*; Table 1). The compound inhibited the constitutively activated fusion forms of Abl, such as the p210Bcr-Abl *(14)*, p185Bcr-Abl *(15,16)*, and Tel-Abl *(15)* tyrosine kinases with IC_{50} values between 0.1 and 0.35 μ*M*. The inhibition of autophosphorylation of

Table 1
Inhibition of Protein Kinases by Glivec

Enzyme	*In vitro substrate phosphorylation* IC_{50} [μM]	*Cellular tyrosine phosphorylation* IC_{50} [μM]
c-Abl	**0.17 ± 0.023; 0.025**[a]	
p210Bcr-Abl	**0.025**[a]	**0.25**
p185Bcr-Abl	**0.025**[a]	**0.25**
TEL-Abl		**0.35**
PDGFRβ	**0.87 ± 0.012**	**0.1**
TEL-PDGF-Rβ		**0.15**
c-Kit	**0.56 ± 0.092**	**0.1**
FGF-R1	>10	
c-Fms and v-Fms		>10
VEGFR1 (Flt-1)	>10	
VEGFR2 (Kdr)	>10	>10
Flt-3	>10	>10
Flt-4	5.7 ± 1.1	
EGFR (HER1)	>100	>100
ErbB2 (HER2)	>10	>10
ErbB4 (HER4)	>10	
IGF-IR	>10	>100
Insulin receptor	>10	>100
c-Met	>10	
Tie-2 (Tek)	>10	
Jak-2	>100[a]	>100
c-Fgr	>100	
Lck	9.0	
c-Lyn	>100	
Syk (TPK-IIB)	>100	
c-Src	>10	
Akt (PKB)	>10	
Cdk1/cyclin B	>10	
Jnk2	>10	
p38 MAPK	>10	
PDK1	>10	
PKA	>10	
PKCα, β1, β2, γ, δ, ε, ζ, or η	>10	
PPK	>10	
Protein kinase CK-1, CK-2	>10	
c-Raf-1	0.97 ± 0.16	>10

[a]IC_{50} was determined in immunocomplex assays. Data represent the mean ± SEM drug concentrations causing a 50% reduction in kinase activity (IC_{50} value; μ*M*). PDGFR, platelet-derived growth factor receptor; FGFR1, fibroblast growth factor receptor 1; VEGFR, vascular endothelial

Bcr-Abl was closely related to the antiproliferative activity of Glivec. Incubation with submicromolar concentrations of Glivec selectively induced apoptosis in Bcr-Abl-positive cell lines and also induced cell killing in primary leukemia cells from Ph chromosome-positive CML and acute lymphoblastic leukemia patients, whereas Ph chromosome-negative cells were not affected *(14,16,21–24)*. The IC_{50} values for inhibition of the KU812 and MC3 Bcr-Abl-positive CML blast crisis cell lines were 0.1–0.3 μ*M (24)*. Selective inhibition of CML colony formation by Glivec has been demonstrated. At concentrations of 1 μ*M*, the compound selectively inhibited colony formation from peripheral blood and bone marrow from Ph-positive CML patients, with a 92–98% decrease in Bcr-Abl-positive colonies but little effect on normal hematopoiesis *(14,22)*. The findings were confirmed by assessing the effects of Glivec on proliferation of peripheral blood progenitors under stroma-dependent long-term culture (LTC) conditions *(25)*.

Fundamental phenotypic features in Bcr-Abl-positive cells involve resistance to apoptosis, enhanced proliferation, and altered adhesion properties. The impact of Glivec on some known downstream signaling molecules of Bcr-Abl has been examined. A link between constitutive activation of signal transducer and activator of transcription (STAT) 5 and enhanced viability of Bcr-Abl-transformed cells has been demonstrated *(26,27)*. Glivec had a profound inhibitory effect on STAT5 activation in vitro and in vivo *(26–28)*. Furthermore, inhibition of the Bcr-Abl kinase activity by Glivec in Bcr-Abl-expressing cell lines and fresh leukemic cells from CML patients induced apoptosis by suppressing the capacity of STAT5 to activate the expression of the antiapoptotic protein Bcl-x_L *(27)*. The adapter molecule CrkL is a prominent target of Bcr-Abl, and its tyrosine phosphorylation has been a useful marker of Bcr-Abl kinase activity *(29)*. As expected, a decrease in tyrosine phosphorylation of CrkL has been observed in Glivec-treated cell lines and has also served as an indicator of Bcr-Abl kinase activity in patients (*see* Section 4).

There is increasing evidence that cell cycle regulation is disturbed in Bcr-Abl-positive cells; however, the underlying molecular mechanisms are poorly understood. Recently, Bcr-Abl has been shown to promote cell cycle progression and activate cyclin-dependent kinases by interfering with the regulation of the cell cycle inhibitory protein p27 *(30)*. Glivec prevented downregulation of p27 levels in Bcr-Abl-expressing cells *(30,31)*.

The effects of Glivec on cytoskeletal changes and adhesion have been investigated using Bcr-Abl-transfected fibroblasts *(32)*. Glivec was shown to restore normal architecture and to increase adhesion in this model of Bcr-Abl expression.

Table 1 (*Continued*) growth factor receptor; EGFR, epidermal growth factor receptor; ErbB, oncogene B of Avian Erythroblastosis virus; HER, human EGF receptor family; IGF-IR, insulin-like growth factor receptor I; TPK, tyrosine-protein kinase; PKB, protein kinase B; Jnk2, c-Jun N-terminal kinase 2; MAPK, mitogen-activated protein kinase; PDK1, 3-phosphoinositide-dependent protein kinase-1; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PPK, phosphorylase kinase; CK, casein kinase.

3.2. Activity in Animal Models

The antiproliferative activity of Glivec has been confirmed in animal models. Once-daily intraperitoneal treatment with 2.5 to 50 mg/kg of Glivec starting 1 wk after injection of Bcr-Abl-transformed 32D cells in syngeneic mice caused dose-dependent inhibition of tumor growth *(14)*. In contrast, Glivec showed no anti-tumor activity against tumors derived from v-src-transformed 32D cells, in line with the lack of inhibition of Src kinase by the compound. The in vivo activity of Glivec against Bcr-Abl-driven tumors was confirmed using the KU812 cell line derived from a CML patient in blast crisis injected into nude mice. Oral treatment with 160 mg/kg daily in three divided doses for 11 consecutive days was associated with continuous blockage of $p210^{bcr\text{-}abl}$ tyrosine phosphorylation and resulted in tumor-free survival of the animals *(24)*. These data suggested that continuous exposure to Glivec would be important for optimal anti-leukemic effects. The anti-tumor effect of Glivec was specific for Bcr-Abl-expressing cells as no growth inhibition occurred in mice given injections of U937, a Bcr-Abl-negative myeloid cell line. Glivec has also been shown to have oral activity in a murine model of CML based on retroviral $p210^{bcr\text{-}abl}$ transduction of transplanted bone marrow, where survival of animals was significantly prolonged, together with a marked improvement in peripheral white blood counts and splenomegaly *(28)*.

4. CLINICAL DEVELOPMENT IN CML

Clinically, CML is a chronic disease evolving through three successive stages from the chronic phase to the end stage of blast crisis that resembles acute leukemia. Overall, the median survival of patients with newly diagnosed CML is approx 5–6 yr with interferon (IFN)-based treatment regimen. The first trial with Glivec was a phase I study in patients with chronic phase and subsequently also with blast phase CML. In this trial, patients were treated at doses ranging from 25 to 1000 mg daily, and no maximal tolerated dose was identified despite a trend for a higher frequency of grade 3–4 adverse events at doses of 750 mg or higher. On the other hand, a clear dose–response relationship with respect to efficacy was described in patients with chronic-phase CML. At doses of 300 mg or higher, 98% of the patients achieved a complete hematological response, and trough serum levels were above the concentrations required for in vitro activity *(33,34)* (Fig. 2). In addition, effective inhibition of the Bcr-Abl kinase was documented in patient samples by the inhibition of the phosphorylation status of the downstream target CrkL *(34)*. From this study, taking into account a large interpatient variability in pharmacokinetic parameters, doses ranging from 400 mg (for chronic-phase patients) to 600 mg (for advanced-phase CML) were recommended for subsequent studies.

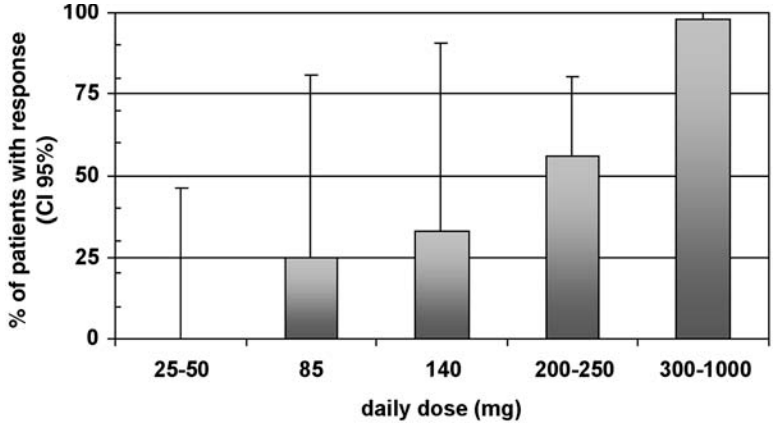

Fig. 2. Dose–response relationship of Glivec in chronic-phase chronic myeloid leukemia (CML) (phase I study): percentage of patients with a hematological response as a function of dose cohort. Hematological response was defined as complete hematological response (CHR) to be confirmed after at least 4 wk. CHR was defined as WBC $<10 \times 10^9$/L, platelet $<450 \times 10^9$/L, myelocytes + metamyelocytes <5% in blood, no blasts and promyelocytes in blood, basophils <20%, no extramedullary involvement.

The clinical development program then focused on two main objectives: to better define the role of Glivec in the management of various stages of CML by characterizing efficacy and safety in large phase II and III trials; and to explore the role and signaling activity of the KIT and PDGF receptors in other malignancies as well as the potential therapeutic use of Glivec in these indications. Three large multinational studies have been performed in 532 patients with late-chronic-phase CML failing prior IFN therapy *(35)*, in 235 patients with accelerated-phase CML *(36)*, and in 260 patients with myeloid blast crisis *(37)*. Treatment was given at a dose of 400 mg in the chronic-phase trial and 400–600 mg in the two other studies. The results of these three studies indicated that the rate of both hematological and cytogenetic response increased as the treatment was started earlier in the course of the disease (Fig. 3). Importantly, the achievement of a hematological and/or cytogenic response was associated with an improved survival and progression-free survival *(35–37)*. In the chronic-phase study where patients started treatment within a median of 32 mo after their initial diagnosis, the estimated probability of being free of progression at 18 mo was 89.2% *(35)*. The most frequently reported adverse events were mild nausea, vomiting, edema, and muscle cramps. However, rare but serious adverse events such as liver toxicity or fluid retention syndromes were also reported. Neutropenias and thrombopenias were more common in patients with advanced disease, suggesting that hematological toxicity may be related more to an underlying compromised bone marrow reserve rather than a toxicity of the drug itself through inhibition of c-KIT-driven hematopoiesis.

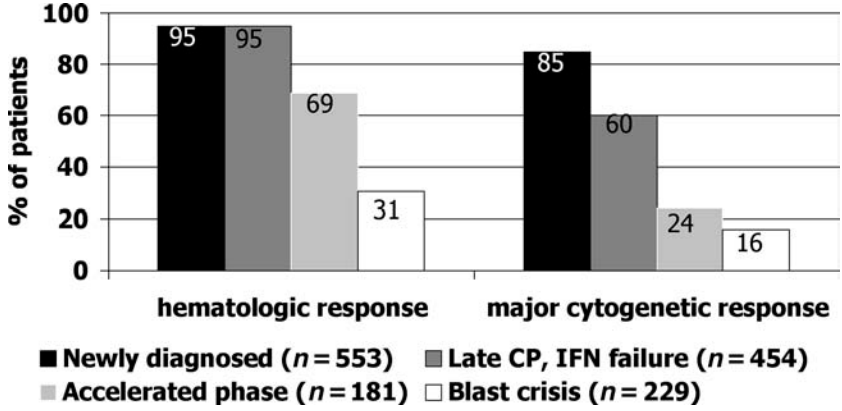

Fig. 3. Hematological and cytogenetic response in CML. In all studies, results are expressed as the percentage of responding patients among the patients for whom the diagnosis of the correct phase of chronic myeloid leukemia (CML) was confirmed on a central review of data. A major cytogenetic response combines both complete (0% Ph + metaphases) and partial responses (1–35%). Hematologic response was defined as complete hematological response (CHR) in the chronic phase study, and as either a CHR, a marrow response, or a return to chronic (RTC) phase in the advanced-phase studies, all to be confirmed after at least 4 wk. In the chronic phase study, CHR was defined as WBC $<10 \times 10^9$/L, platelet $<450 \times 10^9$/L, myelocytes + metamyelocytes <5% in blood, no blasts and promyelocytes in blood, basophils <20%, no extramedullary involvement. In advanced-phase studies, CHR was defined as neutrophils $\geq 1.5 \times 10^9$/L, platelets $\geq 100 \times 10^9$/L, no blood blasts, marrow blasts <5% and no extramedullary disease. A marrow response was defined with the same criteria as for CHR but with neutrophils $\geq 1 \times 10^9$/L and platelets $\geq 20 \times 10^9$/L. An RTC phase was defined as <15% blasts in marrow and blood, <30% blasts + promyelocytes in marrow and blood, <20% basophils in blood, and no extramedullary disease.

The activity of Glivec for patients with newly diagnosed CML has been investigated in a large randomized phase III study comparing first-line therapy with Glivec against standard interferon in combination with low-dose cytarabine. This study, known as the "IRIS" study (International Randomized study of Interferon vs STI571), has enrolled 1106 patients and is currently ongoing. The results of an interim analysis with a median follow-up of 19 mo indicated a better tolerability and a superior efficacy of first-line Glivec as compared to interferon and low-dose cytarabine, in terms of estimated rates of complete cytogenetic response (76.2% vs 14.5%) and hematologic response (96.8% vs 69%). More importantly, the estimated rate of freedom from progression to accelerated-phase or blast-crisis CML at 18 mo was improved from 91.5% with IFN + cytarabine, to 96.7% with Glivec *(38)*. Importantly, in comparison with IFN + cytarabine, Glivec therapy induced a faster and more profound depletion of residual Bcr-Abl transcripts in patients with a complete cytogenetic response

as measured by quantitative polymerase chain reaction *(39)*. Taken together, keeping in mind the limited follow-up available to date, these findings suggest that Glivec now represents a superior first-line drug therapy for patients with newly diagnosed Ph + CML. In this context, the therapeutic decisions related to the use of bone marrow transplantation are becoming increasingly complex.

5. MANAGING CLINICAL RESISTANCE TO GLIVEC

In CML advanced phase, even though the rate of hematologic responses with Glivec is high, these responses are usually short-lived and the majority of patients will ultimately develop resistance and undergo disease progression. In chronic-phase CML, a small proportion of patients either do not achieve a satisfactory hematological or cytogenetic response or lose a previous response to Glivec therapy *(40–45)*. In these patients experiencing resistance to Glivec, a variety of mechanisms of resistance have been reported, including amplification of the Bcr-Abl gene, the emergence of leukemic clones with mutations in the Abl kinase domain, and the development of new chromosomal abnormalities.

Different strategies may be conceivable to overcome clinical resistance and optimize the therapy with Glivec, by increasing the antileukemic activity and the durability of response: the use of higher doses of Glivec, the combination of Glivec with either established antileukemic agents or new investigational agents affecting signaling pathways downstream of the Bcr-Abl protein, and the development of new potent inhibitors of the Bcr-Abl kinase active against Glivec-resistant mutants. Clinical data have indicated that increasing the daily dose of Glivec from 400 to 600 or 800 mg can improve the hematological or cytogenetic response in selected patients *(46)*. Multiple combination studies with other agents have been performed preclinically in various laboratories. A large number of conventional chemotherapeutic drugs have been tested for synergy, additivity, and antagonism with Glivec using proliferation assays with Bcr-Abl-transfected cell lines and human CML cell lines and colony-forming assays with primary CML patient cells (recently reviewed in refs. *47* and *48*). However, because various experimental designs and different analytical paradigms have been used, results should be interpreted with caution. The data for the various combinations of Glivec with cytotoxic agents and γ-irradiation are summarized in Table 2. A synergistic or additive antiproliferative effect has been documented for a variety of agents including cytarabine (Ara-C), IFN-α, and homoharringtonine. In addition to standard chemotherapeutic agents, several groups have studied in vitro combinations of Glivec with novel antileukemic drugs and signal transduction inhibitors. Targeting downstream signaling pathways, the inhibition of Mek (PD184352) *(49)*, phosphatidylinositol-3 kinase (wortmannin and LY294002) *(50)*, and farnesyltransferase (SCH66336; L-744832) *(51,52)* enhanced the inhibitory effects of Glivec. Moreover, SCH66336

Table 2
In Vitro Combination Studies of Glivec With Chemotherapeutic Agents and Irradiation

Combination partner	*Synergy*	*Additivity*	*Antagonism*	*Reference*
Ara-C	+	+		*61–68*
Busulfan		+		*69*
Carboplatin		+		*65*
Cladribine		+		*65*
Daunorubicin	+	+		*61,62,68,70*
Decitabine	+			*54*
Doxorubicin		+		*63*
Etoposide	+	+		*63–65*
Gemcitabine		+		*65*
Homoharringtonine	+	+		*63,66,68*
Hydroxyurea	+	+	+	*62–65,68*
4-Hydroperoxycyclo-phosphamide		+		*63*
Interferon-α	+	+		*61–63*
Mafosfamide		+		*64*
Methotrexate		+	+	*63,65*
Mitoxantrone	+			*65*
Nimustine (ACNU)		+		*65*
Taxotere		+		*65*
Thiotepa		+		*65*
Topotecan			+	*65*
Treosulfan		+		*69*
Vincristine	+	+		*63*
γ-Irradiation	+			*69*

has been shown to also sensitize Glivec-resistant cells to Glivec-induced apoptosis *(52)*. Alternative strategies included agents that decrease Bcr-Abl levels such as geldanamycin, allylamino-17-demethoxygeldanamycin (17-AAG), or arsenic trioxide *(47,51,53,54)*. Additive to synergistic effects were observed when these agents were combined with Glivec in Bcr-Abl-positive cells. Table 3 summarizes the new experimental approaches that have been tested in combination with Glivec.

Based on the promising preclinical data, clinical trials combining Glivec with standard antileukemic agents or investigational drugs have been initiated. In phase I/II trials, the combination of Glivec appears feasible with either pegylated-interferons *(55,56)* or Ara-C *(57)*, although at the cost of an increased toxicity, particularly hematologic toxicity. Phase III trials are under preparation. Another phase I study is investigating the combination of Glivec

Table 3
In Vitro Combination Studies Of Glivec With Novel Antileukemic Agents

Investigational drug	*Synergy*	*Additivity*	*Antagonism*	*Reference*
17-AAG (Hsp90 chaperone inhibitor)		+		*51*
AG490 (JAK-2 inhibitor)	+	+		*71,72*
Arsenic trioxide	+			*53,54*
Flavopiridol (cyclin-dependent kinase inhibitor)	+			*73*
LY294002, Wortmannin (PI-3K inhibitors)	+			*50,61*
PD184352 (Mek inhibitor)	+			*49*
PS-341 (proteasome inhibitor)	+	+	+	*74*
SCH66336, L-744832 (farnesyl transferase inhibitors)	+			*51,52,75*
Suberoylanilide hydroxamic acid (histone deacetylase inhibitor)		+		*76*
Telomestatin (telomerase inhibitor)		+		*77*
TRAIL (apoptosis inducer)		+		*78–80*

PI-3K, phosphatidylinositiol-3 kinase; TRAIL, tumor necrosis factor–related apoptosis-inducing ligand.

and arsenic trioxide *(58)*. As an alternative approach to the management of resistance, other studies are evaluating the effects of high doses of 800 mg of Glivec *(59,60)*.

6. CONCLUSION

The research program has clearly shown that it is possible to define in vitro and animal models with high predictive quality since the results of the subsequent clinical studies have largely corroborated the preclinical findings. The predictive quality was achieved in this particular case by using models with the identical genetic abnormalities as those found in man. The case of Glivec also shows that compounds that affect not only one but two or more targets (which is frequently the case) can be beneficial in allowing several diseases to be addressed with differing molecular abnormalities, without paying too high a price in terms of toxicity.

The extensive clinical data available in CML in phase I to phase III studies indicate that the inhibition of Bcr-Abl can be achieved with Glivec in humans and translate into clinically meaningful patient benefit, as evidenced by a much

higher rate of cytogenetic response and a lower rate of progression to advanced phases of the disease. These data validate the initial hypothesis of this program, and underscore the importance of rationally selecting the target diseases to be considered in the early phases of development of a molecule such as Glivec.

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