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# Glivec: A New Treatment Modality for CML: A Case History

Jürg Zimmermann*
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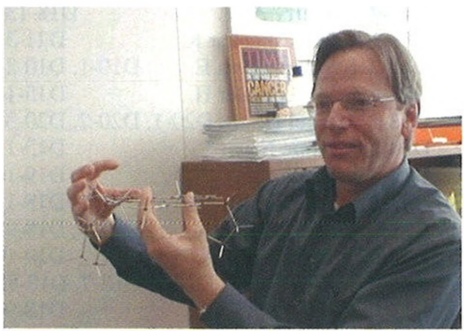
*Abstract:* Glivec (the brand name in the US is Gleevec™) is a protein-tyrosine kinase inhibitor which potently inhibits the Abl tyrosine kinase *in vitro* and *in vivo*. The compound specifically inhibits proliferation of *v-abl* and *bcr-abl* expressing cells, suggesting that it is not a general antimitotic agent. In addition, Glivec is a potent inhibitor of the platelet-derived growth factor receptor kinase (PDGF-R) and of the receptor kinase for stem cell factor (SCF), c-Kit, and inhibits PDGF- and SCF-mediated biochemical events. In contrast, it does not affect signal transduction mediated by other stimuli including epidermal growth factor (EGF), insulin and phorbol esters. Pharmacokinetic studies in various animal species demonstrate that pharmacologically relevant concentrations are achieved in the plasma following oral administration of the drug. STI571 shows antitumor activity as a single agent in animal models at well-tolerated doses. On May 10, 2001, the U.S. Food and Drug administration announced the fast track approval of Gleevec™ (imatinib mesylate), our treatment for patients with chronic myeloid leukemia (CML) in blast crisis, accelerated phase or chronic phase after failure of interferon-alpha therapy. The FDA approval came in just over 10 weeks after Novartis filed its New Drug Application, and just two months after the FDA notified us that it had granted Glivec a priority review.

**Keywords**: Bcr-abl Protein kinase · Chronic myeloid leukemia (CML) · Glivec (Gleevec™) · Lead optimisation · Phenylamino pyrimidines · Philadelphia chromosome



*Correspondence: Dr. J. Zimmermann
Novartis Pharma AG
Präklinische Forschung
WSJ-507.7.51
CH–4002 Basel
Tel.: +41 61 324 47 17
Fax: +41 61 324 67 26
E-Mail: juerg.zimmermann@pharma.novartis.com

## Jürg Zimmermann

*Jürg Zimmermann* was born on 5th May 1957 in Adelboden, Switzerland. After an apprenticeship as a laboratory technician he studied chemical engineering at the Engineering School in Burgdorf, Switzerland. He then studied organic chemistry at the ETH in Zürich, where he attained his PhD under the supervision of Professor Dieter Seebach. Afterward he joined as a postdoctoral fellow Professor Beckwith's group at the Australian National University for the study of the cyclization of radicals and afterward Professor Lown's group at the University of Alberta in Edmonton for the design and synthesis of DNA-binding ligands. In 1990, he joined the Oncology Research Department of Ciba-Geigy in Basel. He headed various protein kinase projects and during this period he invented the active ingredient of Glivec (the US brand name is Gleevec™). He moved then to Core Technology of Novartis to become head of combinatorial chemistry in 1998. He was awarded the Max-Lüthi medal (1982), the Dolphin Prize (1993), the Sandmeyer Prize (2002), the Bruce-Cain Memorial Award (2002) and the Inventor of the Year Award (2002).

## 1. Introduction, Chronic Myeloid Leukemia (CML)

Chronic myeloid leukemia (CML) is one of the four most common types of leukemia. In the United States, there are approximately 20,000–23,000 patients with CML at any given time. Worldwide, CML has an incidence of one to two cases per 100,000 population per year, and is responsible for 15 to 20% of all adult cases of leukemia. CML is a hematological stem cell disorder caused by an acquired or induced abnormality in the DNA of the stem cells in bone marrow. This abnormality results in a gene that produces an abnormal protein. This protein disrupts the bone marrow's normally well-controlled production of white blood cells. The resulting proliferation of white blood cells leads to a massive increase in their concentration in the blood.

CML progresses through three distinct phases: the chronic phase (typically lasting from four to six years), the accelerated phase (typically lasting from six to twelve months), and blast crisis (typically lasting from three to six months), which are marked by a progressive increase in the

number of white blood cells. As a patient moves through these stages, the disease becomes biologically more complex, making it increasingly refractory to therapy and, therefore, more difficult to treat.

The current treatment options include an allogeneic bone marrow transplant – using healthy stem cells from the bone marrow of a closely matched donor – which currently offers patients with CML the best hope for a cure. However, only about 20% of patients are eligible for such a transplant, either because a suitable donor cannot be located, because of advanced age, or because of other complicating medical factors. Another option for some patients is an autologous bone marrow transplant using the patient's own blood stem cells after they have undergone intensive chemotherapy and radiation treatment. Although this therapy prolongs survival, almost all patients eventually relapse, in part because their bodies still harbor malignant CML cells.

A range of drugs is used in the treatment of CML, including interferon-alpha, hydroxyurea, cytarabine, bisulfan. Although it is not a cure, interferon (given by injection) may prolong the survival of some CML patients. It has been shown in some patients to elicit both a hematological response and, to a lesser extent, a cytogenetic response. However, as CML becomes more aggressive, many patients become refractory to interferon therapy.

In CML, the genetically altered stem cell is distinguished by the presence of the Philadelphia (Ph) chromosome [1], which is detected in most patients. The Philadelphia chromosome (so-called because University of Pennsylvania researchers identified it) is created by an exchange of parts between two chromosomes. A detached portion of chromosome 9 shifts to chromosome 22, and a section of chromosome 22 shifts to chromosome 9 in a phenomenon known as 'translocation' [2]. This cytogenetic alteration is detected in 95% of patients with CML and is the hallmark of the disease (Fig. 1).

The Philadelphia chromosome produces an enzyme that plays a central role in aberrant cell growth and division. This enzyme, a tyrosine kinase known as Bcr-Abl [3][4], changes the cell's normal genetic instructions, jamming the signal that tells the body to stop producing white blood cells. The result is that while a cubic millimeter of blood from a healthy person contains 4,000 to 10,000 white blood cells, the same volume of blood from a CML patient contains 10 to 25 times this number. The massive increase in the number of white blood cells characterizes CML. The fact that the expression of Bcr-Abl in mice could induce a disease resembling CML, provided strong evidence that the Bcr-Abl protein was indeed one of the mayor driving forces in the pathophysiology of CML [5]. Thus, an inhibitor of the Abl protein tyrosin kinase would be predicted to be an effective and selective therapeutic agent for this leukemia (Fig. 2).

## 2. Medicinal Chemistry

The starting point for every medicinal chemistry project is a lead compound with a given pharmacological activity. The biological activity of a molecule however must be complemented by other properties that make the molecule a good drug substance. It is estimated that a large proportion of molecules fail in late stages of drug development due to reasons of drug–drug interaction or poor ADME (absorption, distribution, metabolism, and excretion) features. Failure to detect these liabilities early in the drug discovery process can be extremely costly and time consuming. On the basis of physical and calculated properties for known drugs, criteria for 'drug-likeness' have been established [6].

In the case of Glivec (the US brand name is Gleevec™), a lead compound was identified in a screen for inhibitors of protein kinase C (PKC), (see Fig. 3). A high cellular activity was obtained in derivatives bearing a 3'-pyridyl group at the 3-position of the pyrimidine, the phenylamino pyrimidine core was absolutely essential for PKC inhibitory activity (Fig. 4). During the optimization of this structural class on the inhibition of PKC, a serine/threonine kinase, it was observed that the presence of an amide group on the phenyl ring gives rise to inhibition also of tyrosine kinases, such as the bcr-abl kinase (**A**, Fig. 5). The amide bond is required to be stable towards hydrolysis because the release of an unprotected diamino phenyl moiety has to be avoided in order to avoid mutagenicity. In fact a high stability against hydrolysis could be achieved with derivatives bearing a phenyl group at the amide bond (**B**, Fig. 5). The low selectivity was the next hurdle to overcome since this type of compound is a dual inhibitor of PKC and Bcr-Abl.

At this point a key observation was made from analysis of the structure-activity relationships: substituents at position

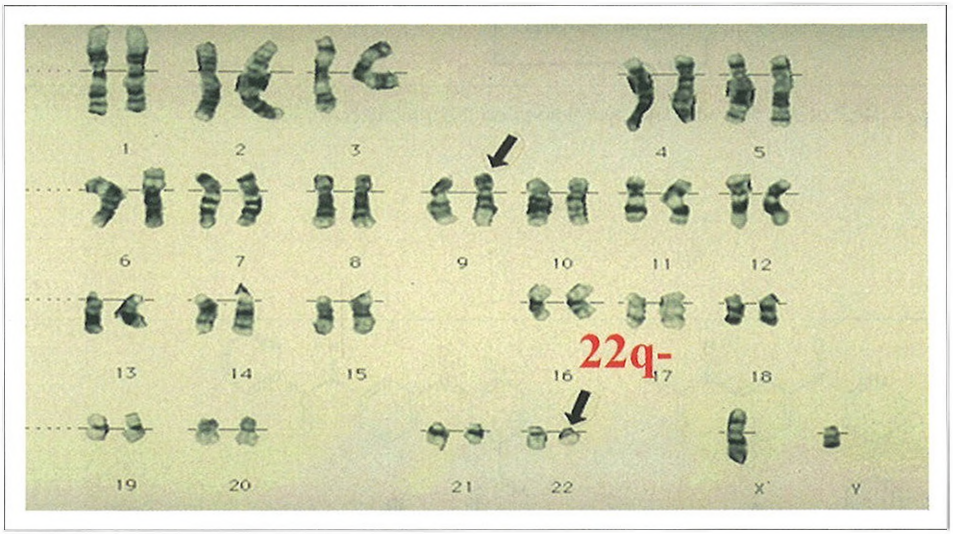



Fig. 1. Philadelphia chromosome 22

- **Bcr-Abl is detected in 95 % of patients with CML**
- **Bcr-Abl is the causative abnormality of CML**
- **Bcr-Abl tyrosine kinase is constitutively activated intracellularly**
- **Tyrosine kinase activity is required for CML cell function**
- **Abl null mice are viable**

Fig. 2. Bcr-abl as a theraputic target for CML

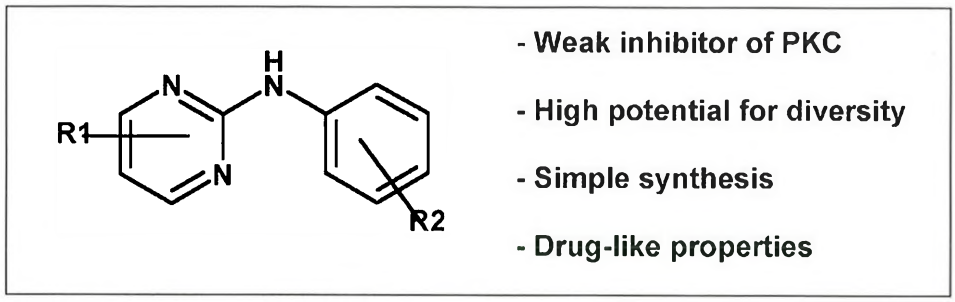



Fig. 3. Lead compound: Phenylamino pyrimidine

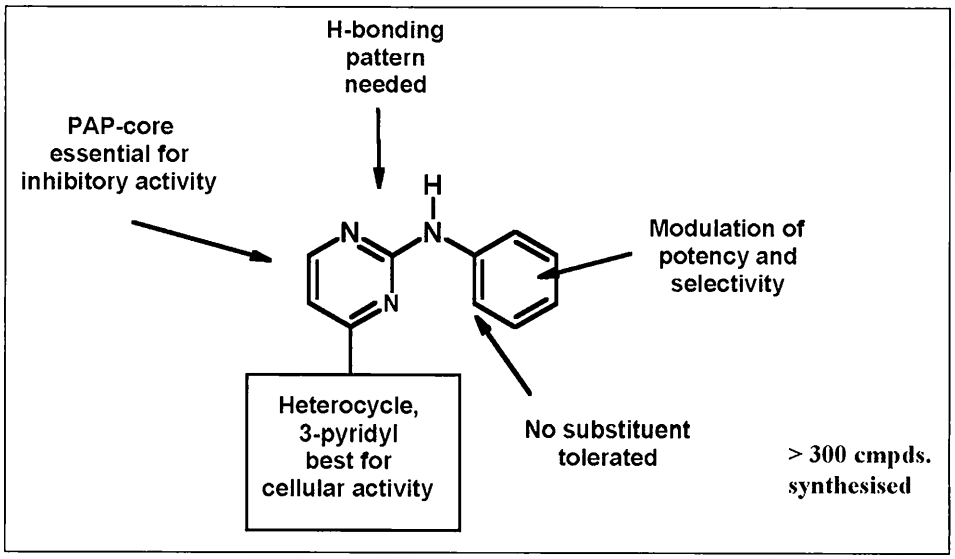



Fig. 4. SAR of the phenylamino pyrimidines on the inhibition of PKC

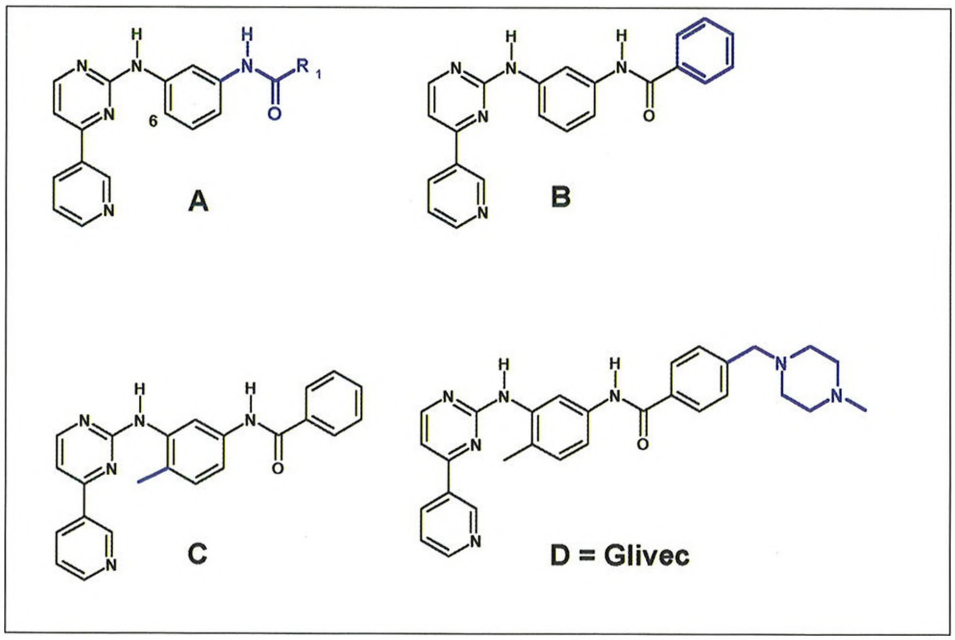


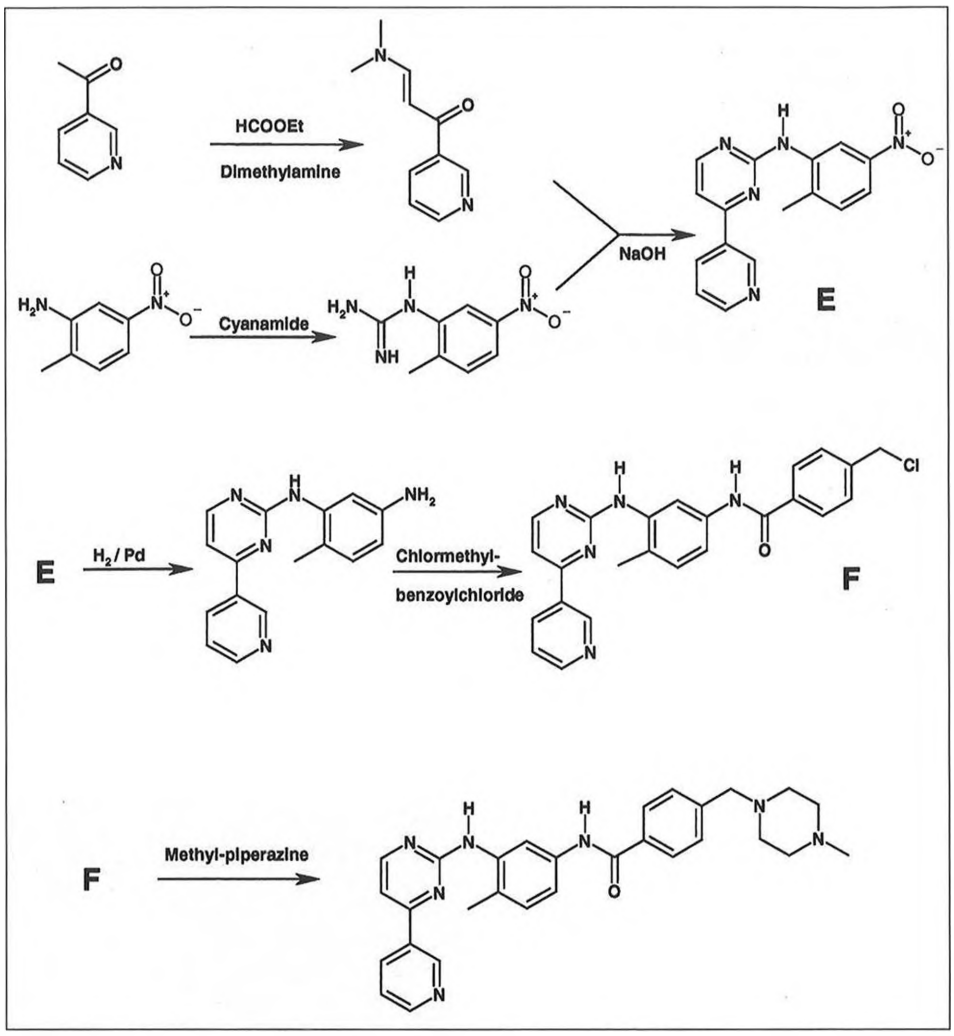

Fig. 5. Chemical formulaes of the compounds mentioned in the text

6 of the diamino phenyl ring were not tolerated for PKC inhibition (Fig. 4). Indeed, the introduction of a simple 'flag-methyl' led to loss of activity against PKC, while the activity against Bcr-Abl could be retained or even enhanced (**C**, Fig. 5). Unfortunately, the first series of selective inhibitors originally prepared showed poor oral bioavailability and low solubility in water. This drawback was eventually circumvented by the introduction of a solubilizing side chain in a region of the molecule that does not interfere with the binding capability to the targeted enzyme. The attachment of basic groups at the 4-position did not significantly alter the potency but dramatically improved aqueous solubility. Again the 'aniline-alert' (mutagenic potential) had to be avoided; this was done in this case by the introduction of a spacer between the phenyl ring and the nitrogen atom. The best compound from this series was the methyl-piperazino derivative **D** [7] which was selected as the most promising candidate for clinical development. The synthesis starts from the acetyl-pyridine, which is converted to an enaminone. The condensation with a guanidine, prepared from the corresponding aniline with cyanamide, yields the phenylamino pyrimidine in high yield. Catalytic reduction of the nitro group, acylation, and benzylic substitution with N-methyl piperazine eventually gives the active ingredient of Glivec (Scheme).

X-ray crystallography [8] showed that binding of a derivative of Glivec occurs at the ATP binding site by binding with high specificity to an inactive form of the kinase. The need for the kinase to adopt this unusual conformation favoring binding may contribute to the high selectivity of the compound.

## 3. Pharmacological Profile and Clinical Development

The compound inhibits the v-Abl kinase with an IC50 of 38 nM but is inactive against serine/threonine kinases. The compound does not inhibit the kinase activity of the receptor for the epidermal growth factor (EGF)-receptor, the vascular endothel growth factor (VEGF-R1 and VEGF-R2) and the fibroblast growth factor (FGF-R1). Tie-2 (Tek), c-Met and the kinase activity of the Src-family (c-Scr, c-Fgr., c-Lyn, Lck) are all also not inhibited. At the cellular level an inhibition of the PDGF-R-K (platelet derived growth factor receptor coupled kinase) and c-kit, the receptor coupled kinase for the stem cell factor (SCF) are inhibited. Glivec showed a strong antiproliverative




Scheme. Synthesis of the active ingredient of Glivec

effect on cell lines which express Bcr-Abl. A selective inhibition on the CML-colony formation was shown in *ex vivo* samples derived from patients. A validation of the antiproliverative effect was also done *in vivo*, a once-daily i.p. dose of 2.5 and 50 mg/kg showed a dose-dependent inhibition of the growth of the bcr-abl transformed cells on syngenic mice. Pharmacokinetic studies showed a rapid uptake upon p.o. administration to give a pharmacologically relevant blood level in the plasma [9]. These data prompted us to start an evaluation of the drug in CML patients. The first trial with Glivec was a phase I study in patients with chronic phase and subsequently also with blast phase CML. The dose given was 25 to 1000 mg, no maximal tolerated dose was identified. At a dose of 300 mg and higher, 98% of patients showed a hematological response with moderate side effects. In later trials in newly diagnosed patients in early chronic phase, data show that use of Glivec can result in high cytogenetic response rates. Complete cytogenetic response, the elimination of the cells that characterise CML, is regarded as the ultimate goal of CML treatment. The rates reported are significally higher than those historically documented with other CML therapies in the same disease setting. Taken together, all these findings have established Glivec as a safe and effective therapy in all stages of CML and have been the basis for the marketing approval by the FDA on May 10, 2001, *i.e.* less than three years after the start of the first phase I. On March 2002 the FDA approved Glivec also for the treatment of patients with Kit (CD 117) positive unresectable and/or metastatic malignant gastrointestinal stromal tumors (GISTs).

In summary, Glivec has shown that a rationally designed, molecularly targeted therapy based on the specific abnormality present in human malignancy can be a very efficient therapy. It represents a new paradigm in cancer drug development and will hopefully be followed by a new generation of specific, targeted therapies in oncology.

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