GLIVEC (STI571, IMATINIB), A RATIONALLY DEVELOPED, TARGETED ANTICANCER DRUG

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In the early 1980s, it became apparent that the work of pioneers such as Robert Weinberg, Mariano Barbacid and many others in identifying cancer-causing genes in humans was opening the door to a new era in anticancer research. Motivated by this, and by dissatisfaction with the limited efficacy and tolerability of available anticancer modalities, a drug discovery programme was initiated with the aim of rationally developing targeted anticancer therapies. Here, we describe how this programme led to the discovery and continuing development of Glivec (Gleevec in the United States), the first selective tyrosine-kinase inhibitor to be approved for the treatment of a cancer.

LEUKAEMIA
Leukaemia is an uncontrolled proliferation of one type of white blood cell (leukocyte).

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Until the early 1980s, drug discovery programmes for cancer were focused almost exclusively on DNA synthesis and cell division, and resulted in agents such as antimetabolites, alkylating agents and microtubule destabilizers. These drugs showed efficacy, but at the price of high toxicity due to lack of selectivity. Also, resistance was frequently observed after initial stabilization or regression of the disease. The discovery of cancer-causing genes, later called oncogenes, represented a radical departure — all of a sudden, genes were identified that were uniquely associated with cancerous cells. The molecular epidemiology of these genes was established over many years of studying clinical tumour samples, but as described below, it was clear at the outset that chronic myelogenous LEUKAEMIA (CML) — a haematological stem-cell disorder that is characterized by excessive proliferation of cells of the myeloid lineage — represented a particularly interesting case.

Target selection: BCR–ABL

CML is characterized by a reciprocal translocation between chromosomes 9 and 22 (REF. 1). The shortened version of chromosome 22, which is known as the Philadelphia chromosome, was discovered by Nowell and Hungerford[2], and provided the first evidence of a specific genetic change associated with human cancer. The molecular consequence of this inter-chromosomal exchange is the creation of the *BCR–ABL* gene, which encodes a protein with elevated tyrosine-kinase activity. The demonstration that *Bcr–Abl* as the sole oncogenic event could induce leukaemias in mice[3–5] has established *BCR–ABL* as the molecular pathogenic event in CML. As the tyrosine-kinase activity of BCR–ABL is crucial for its transforming activity[6], the enzymatic activity of this deregulated gene could plausibly be defined as an attractive drug target for addressing BCR–ABL-positive leukaemias.

For the first time, a drug target was identified that clearly differed in its activity between normal and leukaemic cells. It was conceivable that this enzyme could be approached with classical tools of pharmacology, as its activity — the transfer of phosphate from ATP to tyrosine residues of protein substrates — could clearly be described and measured in biochemical as well as cellular assays. Furthermore, cell lines that were derived from human leukaemic cells with the same chromosomal abnormality were available. Such cell lines were instrumental for *in vitro* and animal studies, which laid the groundwork for the clinical trials. So, the essential tools were assembled to go forward with the aim of identifying potent and selective inhibitors of the ABL tyrosine kinase.

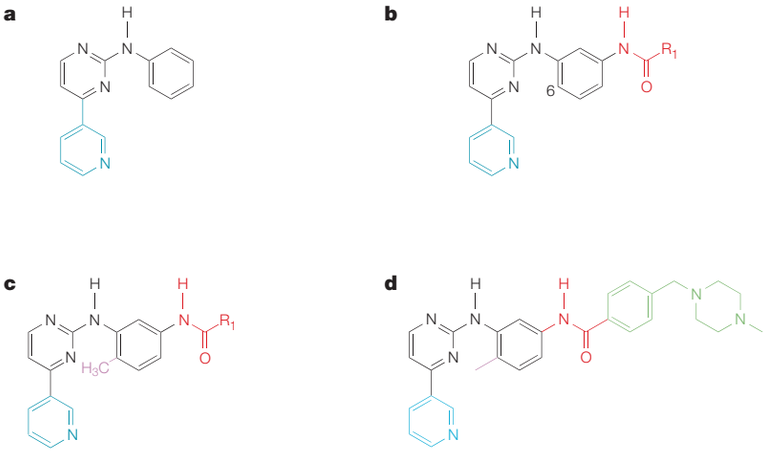

Figure 1 | **Summary of the chemical optimization.** The core structure of the lead compound, a phenylamino derivative, is indicated in black. **a** | The addition of a 3′-pyridyl group (blue) at the 3′-position of the pyrimidine enhanced the cellular activity. **b** | An amide group (red) attached to the phenyl ring provided activity against tyrosine kinases. **c** | A 'flag methyl' (purple) attached to the diaminophenyl ring abolished the undesirable protein-kinase-C inhibitory activity. **d** | The final attachment of an *N*-methyl piperazine moiety (green) markedly increased the solubility and oral bioavailability.

Medicinal chemistry

The starting point for every medicinal-chemistry project is a lead compound with a given pharmacological activity. However, the biological activity of a molecule must be complemented by other properties that make the molecule a good drug — it is estimated that a large proportion of molecules fails in late stages of drug development due to drug–drug interactions or poor ADME (absorption, distribution, metabolism and excretion) features. Not detecting 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[7].

In the case of Glivec, a lead compound was identified in a screen for inhibitors of protein kinase C (PKC). This compound — a phenylaminopyrimidine derivative — had promising 'lead-like' properties[8] and a high potential for diversity, allowing simple chemistry to be applied to produce compounds with more potent activity or selectivity. Strong PKC inhibition in cells 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 relationships was that a substitution at position 6 of the diaminophenyl ring abolished PKC inhibitory activity completely. Indeed, although the introduction of a simple 'flag-methyl' led to loss of activity against PKC, the activity against protein tyrosine kinases was retained or even enhanced (FIG. 1c). However, the first series of selective inhibitors that was prepared originally showed poor oral bioavailability and low solubility in water. The attachment of a highly polar side chain (an *N*-methylpiperazine) was found to improve markedly 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 a methylpiperazine derivative that was originally named STI571 (imatinib, now known as Glivec or Gleevec), which was selected as the most promising candidate for clinical development[9,10] (FIG. 1d).

Docking studies[11] and X-ray crystallography[12] showed that binding of Glivec occurs at the ATP-binding site. Analysis of the crystal structure[12] 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 conformation, which favours binding, might contribute to the high selectivity of the compound. Unexpectedly, these analyses indicated that the *N*-methylpiperazine group (added to increase drug solubility) also interacted strongly with ABL by means of hydrogen bonds to the backbone carbonyl group of isoleucine (Ile)360 and histidine (His)361.

In an *in vitro* screen against a panel of protein kinases, the compound was found to inhibit the autophosphorylation of essentially three kinases: BCR–ABL, c-KIT and the platelet-derived growth factor (PDGF) receptor (TABLE 1). More recently, activity against ARG kinase has also been reported[13].

Pharmacological profile

In collaboration with Brian Druker, the selective inhibitory activity of Glivec was shown at the cellular level on the constitutively active $p210^{BCR–ABL}$ tyrosine kinase[14]. Subsequently, a similar inhibitory activity was also shown on other ABL fusion proteins, such as $p185^{BCR–ABL}$ (REFS 15,16) and TEL (ETV6)–ABL[15]. The inhibition of autophosphorylation of 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 induced cell killing in primary leukaemia cells from patients with Philadelphia-chromosome-positive (Ph^+) CML and acute lymphoblastic leukaemia[14,16–20].

In *in vivo* experiments, once daily intraperitoneal treatment with 2.5–50 mg kg^{-1} of Glivec, started one week after injecting BCR–ABL-transformed 32D cells into SYNGENEIC mice, caused dose-dependent inhibition of tumour growth[14]. In nude mice implanted with KU812 cells, oral treatment with 160 mg kg^{-1} daily in three divided doses for 11 consecutive days was associated with continuous blockage of $p210^{BCR–ABL}$ tyrosine phosphorylation, and resulted in tumour-free survival of the animals[20]. The antitumour effect of Glivec was specific for *BCR–ABL*-expressing cells, as no growth inhibition occurred in mice that were given injections of U937, a *BCR–ABL*-negative myeloid cell line. Recently, Glivec was shown to be orally active in a mouse model of CML, based on retroviral $p210^{BCR–ABL}$

APOPTOSIS
Programmed cell death.

SYNGENEIC MODEL
An animal model in which the injected tumour cells are derived from the same animal species as the host animal.

transduction of transplanted bone marrow. Survival of animals was significantly prolonged, together with a marked improvement in peripheral-white-blood-cell counts and splenomegaly[21].

Table 1 | **Cellular profile of Glivec**

Assay	IC_{50} (μM)
Inhibition of autophosphorylation	
v-ABL	0.1–0.3
$p210^{BCR-ABL}$	0.25
$p185^{BCR-ABL}$	0.25
TEL–ABL	0.35
TEL–ARG	0.5
PDGF receptor	0.1
TEL–PDGF receptor	0.15
c-KIT	0.1
FLT3	> 10
c-FMS and v-fms	> 10
EGF receptor	> 100
c-ERBB2	> 100
Insulin receptor	> 100
IGF-1 receptor	> 100
v-SRC	> 10
JAK2	> 100
Inhibition of MAPK activation	
PDGF dependent	0.1–1
SCF dependent	0.1–1
Inhibition of AKT activation	
SCF dependent	0.1–1
Inhibition of IP release	
PDGF induced	0.25
Inhibition of c-FOS mRNA expression	
PDGF induced	0.3 –1
EGF, FGF or PMA induced	> 100
Antiproliferative activity*	
32D, MO-7e, BaF3 cells	> 10
BCR–ABL-transfected 32D, MO-7e, BaF3 cells	< 1
BCR–ABL-positive human leukaemia lines‖	0.1–1
BaF3 TEL-ARG	0.5
BALB/c 3T3 v-SIS (PDGF autocrine)	0.3
BaF3 TEL–PDGF receptor	< 1
U-87 human glioma‡	~1.5
U-343 human glioma‡	~1.5
MO-7e, SCF stimulated	~0.1
H526 human SCLC, SCF stimulated§	0.8
Human GIST882 line¶	< 1
Human mast-cell leukaemia line HMC-1#	0.01–0.1

Glivec concentrations that cause 50% inhibition (IC_{50}) are given[13–20,47,48,53,54,61,63,66]. EGF, epidermal growth factor; FGF, fibroblast growth factor; FLT3, fms-related tyrosine kinase 3; IGF-1, insulin-like growth factor-1; IP, inositol phosphate; MAPK, mitogen-activated protein kinase; PDGF, platelet-derived growth factor; PMA, phorbol 12-myristate 13-acetate; SCF, stem-cell factor; SCLC, small-cell lung cancer. *Antiproliferative experiments were carried out in 10% fetal calf serum, except for those that were carried out in ‡5% human-platelet poor plasma or under §serum-free conditions. ‖K562, KU812, MC-3, MBA-1, KBM-5, Z-33, Z-119, Z-181. ¶Expresses the activating KIT mutation K642E (lysine 642 to glutamic acid). #Expresses the activating KIT mutation V560G (valine 560 to glycine).

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 signalling molecules of BCR–ABL has been examined. A link between constitutive activation of STAT5 (signal transducer and activator of transcription 5) and enhanced viability of *BCR–ABL*-transformed cells has been shown[22,23]. Glivec had a profound inhibitory effect on STAT5 activation *in vitro* and *in vivo*[21–23]. Furthermore, inhibition of the BCR–ABL kinase activity by Glivec in *BCR–ABL*-expressing cell lines and fresh leukaemic cells from CML patients induced apoptosis by suppressing the capacity of STAT5 to activate the expression of the anti-apoptotic protein $BCL\text{-}X_L$[23]. The adaptor molecule CRKL is a prominent target of BCR–ABL, and its tyrosine phosphorylation has been a useful marker of BCR–ABL kinase activity[24]. 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 below).

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 (REF. 25). Glivec prevented downregulation of p27 levels in *BCR–ABL*-expressing cells[25,26].

The effects of Glivec on cytoskeletal changes and adhesion have been investigated using *BCR–ABL*-transfected fibroblasts[27]. Glivec was shown to restore normal architecture and to increase adhesion in this model of *BCR–ABL* expression.

Clinical development in CML

Because of the three known targets of Glivec, many potential cancers can be speculated to be good candidates for clinical testing of this new drug. However, in most cancers, tumorigenesis is complex and involves the disruption of multiple genes and signalling pathways. By contrast, CML can be considered as one of the few examples of a malignancy in which a single signalling-pathway defect is thought to cause the disease. In addition, in contrast to most of the solid tumours, for which the measurement of tumour response is complex, pharmacodynamic response in CML can be measured easily using blood leukocyte count as the end point. For these reasons, CML was selected as the first indication for Phase I clinical testing.

Clinically, CML is a chronic disease that evolves through three successive stages, from the chronic phase to the end stage of blast crisis that resembles acute leukaemia (FIG. 2). Overall, the median survival time of patients with newly diagnosed CML is approximately 5–6 years with an interferon-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 with doses

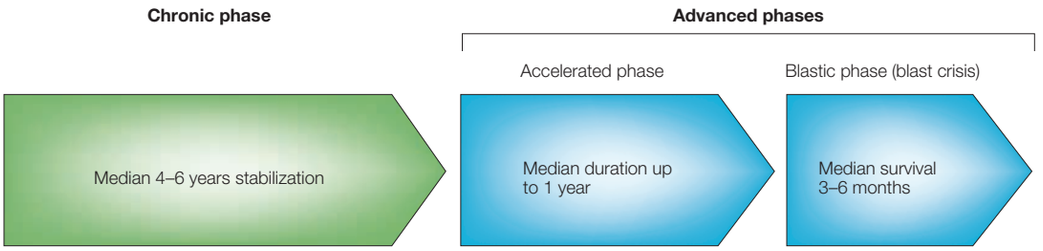

Figure 2 | **Clinical course of chronic myelogenous leukaemia.**

ranging from 25 to 1,000 mg per day, and no maximal tolerated dose was identified, despite a trend for a higher frequency of GRADE III–IV 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 haematological response, and trough serum levels were above the concentrations required for *in vitro* activity[28,29]. Subsequently, a mathematical modelling of the relationship between dose and response, as measured by leukocyte counts after four weeks of therapy, confirmed that doses of 400 mg and higher were optimal in inducing a haematological response[30] (FIG. 3). In addition, effective inhibition of the BCR–ABL kinase was documented in patient samples by inhibition of the phosphorylation status of the downstream target CRKL[27]. From this study, doses ranging from 400 mg (for chronic-phase patients) to 600 mg (for advanced-phase CML) were recommended for subsequent studies.

Subsequently, three large multinational studies have been carried out in 532 patients with late chronic-phase CML in whom previous interferon therapy had failed[31], in 235 patients with accelerated-phase CML[32], and in 260 patients with myeloid blast crisis[33]. Treatment was given at a dose of 400 mg in the chronic-phase trial and 600 mg in the two other studies. The results of these three studies indicated that the rate of both haematological and cytogenetic response increased as the treatment was started earlier in the course of the disease (FIG. 4). Importantly, the achievement of a haematological and/or cytogenetic response was associated with improved survival and progression-free survival[31–33]. In the chronic-phase study, in which patients started treatment within a median of 32 months after their initial diagnosis, the estimated probability of being free of progression at 18 months was 89.2%[31]. The most frequently reported adverse events were mild nausea, vomiting, oedema and muscle cramps. However, rare but serious adverse events, such as liver

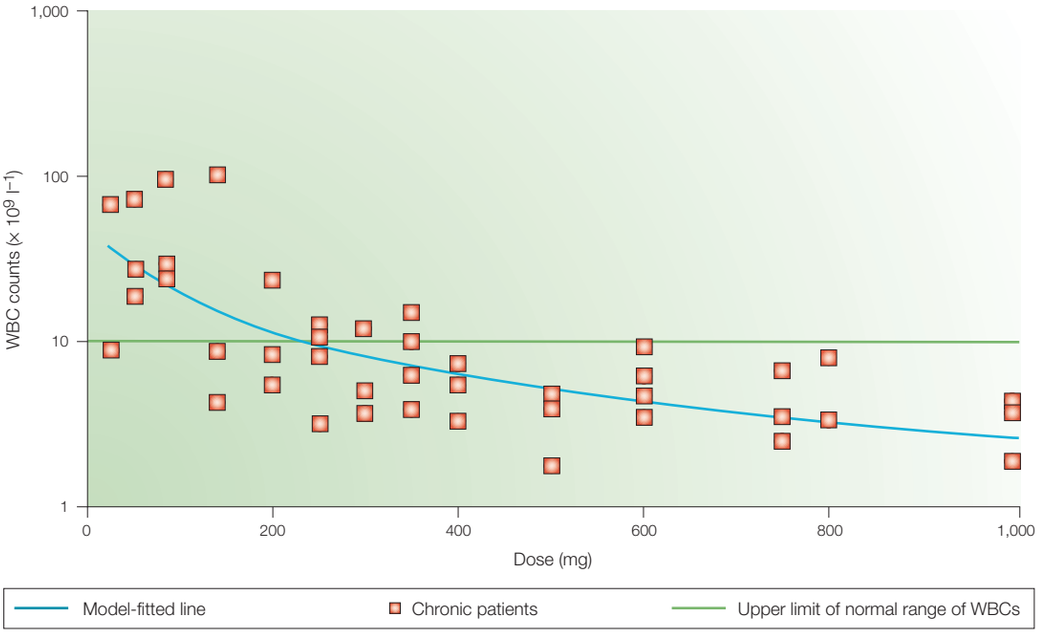

Figure 3 | **Dose–response relationship of Glivec in CML (Phase I study).** Using the leukocyte (white blood cell; WBC) count after 28 days of treatment as a pharmacodynamic marker, the relationship between dose and response was modelled using an E_{max} model , which makes the assumption that once the maximal effect is achieved (E_{max}), increasing the dose further does not translate into additional benefit. The data indicate that at doses of 400 mg per day or higher, all the patients are predicted to achieve a reduction of their leukocyte counts within normal range below 10×10^9 l^{-1}. Adapted with permission from REF. 30 © (2001) American Society of Clinical Oncology. CML, chronic myelogenous leukaemia.

GRADE III–IV ADVERSE EVENTS
For each adverse event that is associated with a specific treatment, grades are assigned and defined using a scale from 0 to V. Grade III, severe and undesirable adverse event; grade IV, life-threatening or disabling adverse event.

toxicity or fluid-retention syndromes, were also reported. Neuropaenias and thrombopaenias were more common in patients with advanced disease, which indicates that haematological toxicity might be related more to an underlying compromised bone-marrow reserve than to toxicity of the drug itself through inhibition of c-KIT-driven haematopoiesis. Taken together, these findings have established Glivec as a safe and effective therapy in all stages of CML, and were the basis for marketing approval by the FDA on 10 May 2001 — less than three years after the start of the first Phase I study (FIG. 5).

Resistance. In CML blast crisis, even though the rate of haematological responses with Glivec is high, these responses are usually short lived, and most patients will ultimately develop resistance and undergo disease progression. A prerequisite to optimally develop strategies to prevent or overcome this resistance is to get a good understanding of the potential mechanisms of resistance in these patients.

On the basis of preclinical and clinical data that are available at present, several potential mechanisms of resistance have been described, which are summarized in BOX 1. They can be categorized into two main groups: mechanisms whereby BCR–ABL is reactivated and cell proliferation remains dependent on BCR–ABL signalling, and mechanisms whereby the BCR–ABL protein remains inhibited by Glivec, but alternative signalling pathways become activated.

BCR–ABL overexpression and *BCR–ABL* gene amplification has been shown in $p210^{BCR-ABL}$-transformed mouse haematopoietic Ba/F3 cells that are resistant to Glivec[34,35], as well as in human *BCR–ABL*-positive leukaemia lines LAMA84 and AR230 (REFS 35,36).

In treated patients, there is now increasing evidence that amplification of the *BCR–ABL* gene and mutations in the BCR–ABL kinase domain are two common mechanisms of resistance to Glivec. The occurrence of these mechanisms was first reported by Sawyers' group[37]. In a study of 11 patients with blast crisis and overt clinical resistance when treated with Glivec, 3 had amplification of the *BCR–ABL* gene and 6 had a point mutation in the ABL kinase domain, which resulted in a T315I (threonine 315 to isoleucine) amino-acid substitution. Following this initial report, the T315I mutation as well as further mutations in the ABL kinase domain have been reported by various investigators[38–41]. Even though these mutations vary in their type and frequency, it is speculated that they might all lead to a reactivation of BCR–ABL-driven signal transduction. To understand the molecular mechanism by which such mutations might cause resistance to Glivec, current studies are using X-ray crystallography to analyse the three-dimensional structure of a complex between the drug and the human c-ABL kinase domain. Glivec binds to an unusual, inactive conformation of ABL with the amino terminus of the activation loop, which contains the highly conserved DFG (asparagine-phenylalanine-glycine) motif, folded into the ATP-binding site[42]. This conformation has been observed by Kuriyan and co-workers[12] in a complex between mouse c-Abl and a Glivec analogue, and cannot bind ATP. The knowledge of the crystal structure allows a better understanding of the decreased sensitivity of mutated BCR–ABL to Glivec, and can be a powerful tool in the design of new BCR–ABL inhibitors that maintain inhibitory activity against these mutated kinases.

Resistance to Glivec might also be related to pharmacokinetic factors. Glivec is a substrate of the multidrug-resistance-associated P-glycoprotein (PgP).

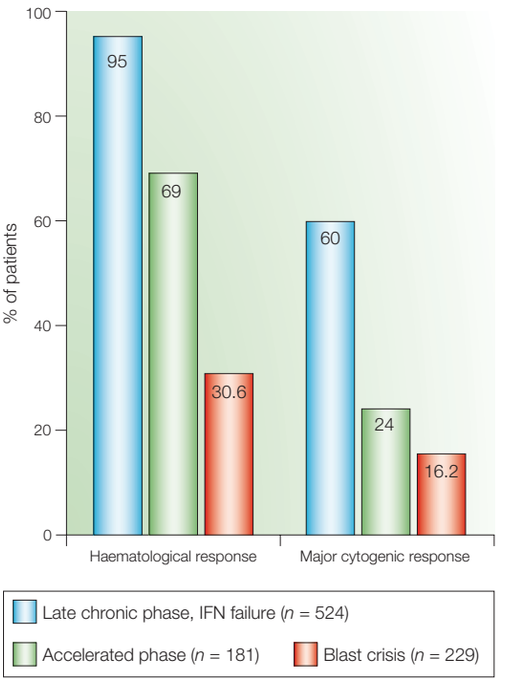

Figure 4 | **Haematological and cytogenetic response in CML: Phase II data.** In all studies, results are expressed as the percentage of responding patients among the patients for whom the diagnosis of the correct phase of chronic myelogenous leukaemia (CML) was confirmed after a central review of the data. A major cytogenetic response combines both complete (0% Ph^+ metaphases) and partial (1–35% Ph^+) responses. Haematological response was defined as complete haematological response (CHR) in the chronic-phase study, and as either a CHR, a marrow response or a return to chronic phase (RTC) in the advanced-phase studies, all to be confirmed after at least four weeks. In the chronic-phase study, CHR was defined as white blood cells $<10 \times 10^9\ l^{-1}$, platelets $<450 \times 10^9\ l^{-1}$, myelocytes and metamyelocytes <5% in blood, no blasts and promyelocytes in blood, basophils <20% and no extramedullary involvement. In advanced-phase studies, CHR was defined as neutrophils $= 1.5 \times 10^9\ l^{-1}$, platelets $= 100 \times 10^9\ l^{-1}$, no blood blasts, marrow blasts <5% and no extramedullary disease. A marrow response was defined by the same criteria as for CHR, but with neutrophils $= 1 \times 10^9\ l^{-1}$ and platelets $= 20 \times 10^9\ l^{-1}$. An RTC was defined as <15% blasts in marrow and blood, <30% blasts and promyelocytes in marrow and blood, <20% basophils in blood and no extramedullary disease. IFN, interferon; Ph^+, Philadelphia chromosome positive.

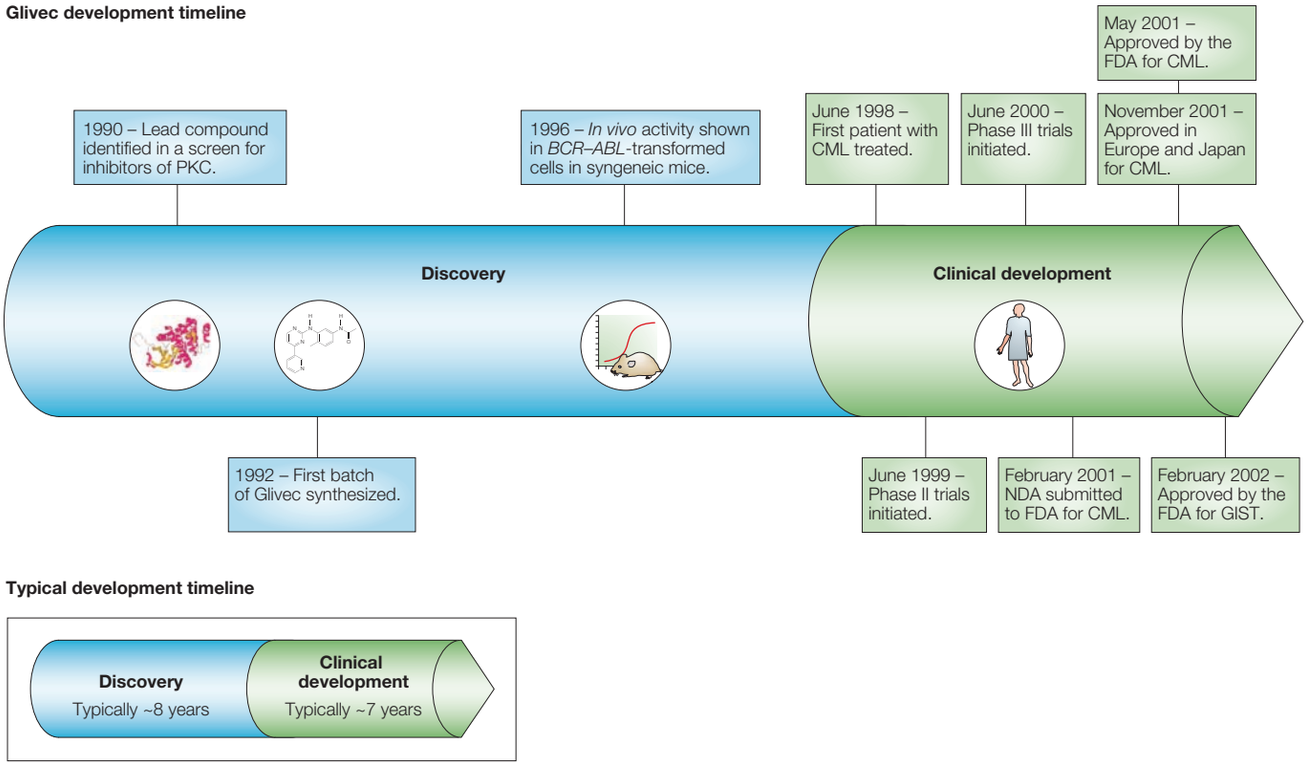

Figure 5 | **Key points in the discovery and development of Glivec.** The clinical development was particularly rapid, as can be seen by comparison with the typical drug discovery and development times shown in the inset. An NDA for Glivec was submitted just two years and nine months after treatment of the first patient with CML, and FDA approval was given less than three months after application. CML, chronic myelogenous leukaemia; GIST, gastrointestinal stromal tumour; NDA, new drug application; PKC, protein kinase C.

Accordingly, the uptake of Glivec was reduced in Glivec-resistant LAMA84 cells in association with an overexpression of the PgP protein. Sensitivity to Glivec was recovered when cells were treated with the PgP inhibitor verapamil[35]. At clinically relevant concentrations of Glivec, binding to plasma proteins is approximately 95%, mostly to albumin and α1-acid glycoprotein (AGP). It has been suggested that a potential mechanism of resistance might relate to this high binding to increased levels of AGP, which would lead to insufficient availability of free drug for antileukaemic activity[43]. However, the clinical significance of this hypothesis is uncertain, in particular in view of the finding that AGP purified from CML patients failed to block the effect of Glivec on the proliferation of leukaemic cells[44].

Recently, Hofmann *et al.*[45] studied a small group of patients with Ph^+ acute lymphoblastic leukaemia who were resistant to Glivec by using DNA-microarray expression profiling. They described an association between the occurrence of resistance to Glivec and upregulation of genes encoding proteins such as Bruton tyrosine kinase and two ATP synthetases (ATP5A1 and ATP5C1), and downregulation of other genes, such as the pro-apoptotic gene *BAK1* and the cell-cycle-control gene *INK4B*[45](also known as *p15*). This is the first report to identify dysregulation of genes that are unrelated to BCR–ABL signalling, and further studies will be necessary to fully assess the significance of these findings and their relevance to CML patients.

Current and future development in CML

The activity of Glivec in patients with newly diagnosed CML is being further investigated by a large randomized Phase III study to compare 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 versus STI571), has enrolled 1,106 patients. The results of an interim analysis with a median follow-up of 14 months indicate a better tolerability and a superior efficacy of first-line Glivec compared with interferon and low-dose cytarabine in terms of cytogenetic response, haematological response and, more importantly, time to progression to accelerated phase or blast crisis[46].

Preclinical studies have shown that the combination of Glivec with various anticancer agents might have synergistic effects. Consequently, several Phase I/II studies are evaluating the feasibility of combining Glivec with interferon, polyethylene glycol (PEG)ylated interferon, cytarabine and other single-agent or combination chemotherapy regimens, in patients with either chronic-phase or advanced CML.

Box 1 | **Mechanisms of resistance to Glivec in CML**

BCR–ABL-dependent mechanisms (cells remain dependent on BCR–ABL signalling)

- Amplification of *BCR–ABL* gene
- Mutations in BCR–ABL kinase domain prevent correct binding of Glivec
- Efflux of Glivec (for example, by PgP-associated MDR protein)
- Protein binding of Glivec (for example, to circulating AGP)

BCR–ABL-independent mechanisms (BCR–ABL is inactivated)

- Activation of signalling pathways downstream of BCR–ABL
- Activation of leukaemogenic pathways unrelated to BCR–ABL

AGP, α1-acid glycoprotein; CML, chronic myelogenous leukaemia; MDR, multidrug resistant; PgP, P-glycoprotein.

c-KIT is another target

In addition to various oncogenic forms of the BCR–ABL tyrosine kinase, Glivec also inhibits the receptor for stem-cell factor (SCF) — c-KIT, a member of the type III group of receptor kinases. Preclinical studies have established that the drug blocks c-KIT autophosphorylation, as well as SCF-stimulated downstream signalling events, such as activation of the mitogen-activated protein kinases (MAPKs) ERK1 and ERK2, and AKT (also known as protein kinase B)[47,48].

Development in c-KIT-positive GISTs. Gastrointestinal stromal tumours (GISTs) represent a rare subset of soft-tissue sarcomas that involve the gastrointestinal tract and are thought to be derived from the interstitial cells of Cajal. Scientific rationale for the use of Glivec in the treatment of these tumours comes from the landmark work of Hirota *et al.*[49], who first identified somatic gain-of-function mutations in the *c-KIT* gene in patients with GIST. Oncogenic *c-KIT* mutations in GISTs have been localized to the extracellular domain, kinase domains 1 and 2 and predominantly in the juxtamembrane domain of the c-KIT protein[50–52]. As c-KIT serves as a phenotypic marker of GISTs and has a key role in their pathogenesis, it provides an ideal target for molecular-based therapy. The first evidence that Glivec might inhibit GIST cells that express mutated *c-KIT* was obtained from studies in a mast-cell leukaemia line expressing a mutated *c-KIT* similar to that found in GISTs[48,53]. Furthermore, Glivec rapidly and completely abolished constitutive phosphorylation of c-KIT in the human cell line GIST882, which expresses an activating *c-KIT* mutation in the first part of the cytoplasmic split-tyrosine-kinase domain, and inhibited proliferation in this GIST line[54]. Similarly, a primary GIST cell culture that expressed a *c-KIT* exon 11 juxtamembrane mutation was also inhibited by Glivec[54].

As reported recently, a pronounced tumour response was first observed in a single patient with progressing GIST[55]. Following this case report, the high level of efficacy of Glivec in GIST has been shown in two subsequent Phase I (REF. 56) and Phase II studies (REF. 57). Two large Phase III studies are being carried out at present to compare the effectiveness of two doses of Glivec (400 mg or 800 mg daily). On the basis of the Phase II data, the FDA approved the use of Glivec for GISTs on 1 February 2002.

Other c-KIT-expressing tumours. In human systemic mastocytosis, most cases show a point mutation in codon 17 of *c-KIT*, which results in a D816V (aspartic acid 816 to valine) amino-acid substitution in the kinase-2 domain of c-KIT. Interestingly, this mutated c-KIT is resistant to inhibition by Glivec[53,58].

Expression of c-KIT and SCF has been reported in a retrospective small-cell lung cancer (SCLC) series, indicating that SCLC growth might involve an AUTOCRINE loop. Inhibition of c-KIT activation by transfection of a dominant-negative *c-KIT* gene results in loss of growth-factor independence[59,60]. Furthermore, the c-KIT/PDGF-receptor inhibitor AG1296 inhibits growth of SCLC cells in serum-containing medium[60]. In H526 SCLC cells, pretreatment with Glivec inhibited SCF-mediated c-KIT activation with an IC_{50} (half-maximal inhibitory concentration) of 0.1 μM (REF. 61). The compound also blocked downstream signal transduction, as evidenced by inhibition of SCF-mediated activation of MAPK and AKT, and potently inhibited SCF-mediated growth in serum-free medium, with a marked increase in apoptosis. Glivec also inhibited the growth of SCLC cell lines in a dose-dependent fashion when grown in serum-containing medium; however, the average IC_{50} was in the range of 5 μM (REFS 61,62).

Although *c-KIT* expression has been documented in various other human tumours, including acute myelogenous leukaemia, ovarian and testicular cancer, it will be important to determine the activation status of the receptor and its importance in the pathogenesis (for a review, see REF. 58). Furthermore, it needs to be explored whether pharmacological inhibition of PARACRINE or autocrine activation of this kinase will be successful therapeutically. Exploratory clinical studies are continuing at present in patients with *c-KIT*-expressing SCLC and acute myelogenous leukaemia.

PDGF receptor as a target

The third target of Glivec is the PDGF-receptor tyrosine kinase. Cellular studies have shown potent inhibition of the two structurally similar PDGF-α and PDGF-β receptors (PDGFR-α and PDGFR-β), as well as blockade of PDGF-mediated cellular events[47,63]. PDGF is a connective-tissue-cell mitogen with *in vivo* functions that include embryonal development, wound healing and control of interstitial-fluid pressure in soft connective tissue. There is increasing evidence that the PDGF ligand–receptor system also has an important role in tumorigenesis[64]. Paracrine and/or autocrine activation of the PDGFR kinase has been postulated in numerous malignancies, and the presence of PDGF autocrine loops is most well documented in gliomas[65]. Glivec inhibited the *in vitro* and *in vivo* growth of cells with autocrine PDGF signalling, including the formation of tumours by the human glioblastoma lines U343 and U87, which had been injected into the brains of nude mice[66]. The inhibitory effects were mediated predominantly through promotion of growth arrest rather than apoptosis.

AUTOCRINE
Describes an agent secreted from a cell that acts on the cell in which it is produced.

PARACRINE
Describes an agent secreted from a cell that acts on other cells in the local environment.

Autocrine PDGFR activation is also well documented in tumour cells of dermatofibrosarcoma protuberans (DFSP), a highly recurrent, infiltrative skin tumour that is characterized by a chromosomal rearrangement involving chromosomes 17 and 22. The resulting fusion-gene product collagen I, α1 polypeptide (COL1A1)–PDGF-β triggers the autocrine stimulation of the PDGFR[67]. *COL1A1–PDGFβ*-transformed fibroblasts, as well as primary DFSP and giant-cell fibrosarcoma cell cultures, were inhibited by Glivec *in vitro* and *in vivo*[67–69]. The main mechanism by which Glivec affected DFSP tumour growth was through induction of apoptosis[69]. Preliminary data indicate that Glivec might also be active in patients with DFSP[70].

Relatively little is known about the ligand-independent activation of PDGFR. However, rearrangement of *PDGFRβ* has been described in chronic myeloproliferative diseases. The best known of these is the t(5;12) chromosomal translocation in chronic myelomonocytic leukaemia (CMML), in which *PDGFRβ*, which is located on chromosome 5, is fused to the *TEL* gene on chromosome 12. Transformation of haematopoietic cells occurs through oligomerization of the TEL–PDGFR-β fusion protein, which causes ligand-independent constitutive activation of the PDGFR kinase[71]. Glivec inhibited the growth of cells expressing *TEL–PDGFRβ*[15], and in transgenic mice that expressed the *TEL–PDGFRβ*, treatment with Glivec inhibited tumour formation and prolonged survival of the animals[72]. A remarkable haematological and complete cytogenetic response has been observed in two patients with chronic myeloproliferative disorders associated with a t(5;12) translocation — one of them with a well-characterized TEL–PDGFR fusion gene and the second with a rearranged PDGFR gene with an as yet unidentified partner gene[73]. Other exploratory clinical trials are being carried out in gliomas and in prostate cancer.

Targeting the tumour microenvironment

An alternative strategy to influence tumour growth is to interfere with the tumour stroma and microvasculature. Paracrine PDGF signalling in the connective-tissue tumour stroma has been described in various types of solid tumour[64]. Several lines of evidence indicate a role for PDGF in the regulation of interstitial fluid pressure (IFP)[74–76]. As most solid tumours have an increased IFP, pharmacological reduction might be a way to increase the uptake of anticancer drugs into tumours[77]. Recent experiments have shown that Glivec significantly reduced tumour IFP in subcutaneously growing PROb rat-colon carcinomas, and a concomitant increase in *trans*-capillary transport of a radiolabelled tracer compound into the tumour interstitium was observed[78]. These effects were mediated by inhibition of the expression of PDGFR on blood vessels and stromal cells, as tumour epithelial cells in this tumour model do not express PDGFRs.

The angiogenic activity that has been described for PDGF might not only be explained by its direct effects on capillary endothelial cells, pericytes and smooth-muscle cells[79], but might also be influenced indirectly through paracrine action on PDGF-responsive stromal and perivascular cells, which are a principal source of vascular endothelial growth factor (VEGF)[80]. PDGF has also been shown to induce the expression of VEGF in endothelial cells, which in turn causes an autocrine VEGF loop[81]. Anti-angiogenic activity of Glivec has been shown *in vitro* through inhibition of serum-stimulated capillary sprouting from rat aorta, and *in vivo* in a subcutaneous implant model in which the drug inhibited PDGF- and also VEGF- and basic fibroblast growth factor (bFGF)-stimulated vascularization[82]. Blockade of PDGFR signalling by Glivec has also been shown to inhibit angiogenesis and tumour growth in an experimental model of bone metastasis[83]. Glivec treatment of nude mice injected with PC-3MM human prostate-cancer cells into the tibia inhibited tumour-cell growth and induced apoptosis, both in tumour cells and tumour-associated endothelial cells. The effects were pronounced when mice were treated with the combination of Glivec and taxol. Interestingly, immunohistochemical studies showed that tumour cells growing in the bone (but not those in surrounding musculature) expressed high levels of PDGF-α, PDGF-β, PDGFR-α and PDGFR-β. Tumour-associated endothelial cells within the bone also expressed PDGFR-α and PDGFR-β. These data indicate that inhibition of the PDGFR in combination with chemotherapy might provide a new approach for the treatment of bone metastasis.

Conclusion

The discovery and development of Glivec has shown that is possible to produce rationally designed, molecular-targeted drugs for the treatment of a specific cancer. The research programme has also clearly shown that it is possible to define *in vitro* and animal models with high predictive quality, as 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 identical genetic abnormalities as those found in man. The case of Glivec also shows that compounds that do not only affect one, but two or more targets (which is frequently the case), can be beneficial in allowing several diseases with differing molecular abnormalities to be addressed, without paying too high a price in terms of toxicity.

The clinical data available so far in CML, GIST and chronic myeloproliferative disorders that involve rearrangement of the PDGFR gene indicate that the inhibition of BCR–ABL, c-KIT and PDGFRs can be achieved with Glivec in humans, and translated into clinically meaningful patient benefit. Providing clinical 'proof of concept', these data validate the initial hypothesis of this programme, 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.

Beyond these reasonably well-understood malignancies, Glivec could have potential in the treatment of other malignancies that involve any of these signalling

pathways, or through targeting of the tumour microenvironment. However, most human cancers are likely to be heterogeneous with regard to molecular abnormalities, such as oncogene activation, and involve multiple signalling pathways in addition to either c-KIT and/or the PDGFR. Consequently, careful attention will have to be paid in designing clinical trials in these more complex indications as to how patients should be selected on the basis of the expression or activation of the molecular target in their tumour, as far as is technically feasible. This point has been crucial in the successful outcome of the CML, GIST and CMML trials. The activity of Glivec in more common cancers with multiple and more complex molecular abnormalities remains to be determined, and is the objective of continuing research in diseases such as SCLC, prostate cancer and gliomas. The potential activity of the combination of Glivec with other signal-transduction inhibitors or anticancer agents is also being investigated.

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