Use and limitations of imatinib mesylate (Glivec), a selective inhibitor of the tyrosine kinase Abl transcript in the treatment of chronic myeloid leukaemia

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Accepted: 26 February 2004

ABSTRACT

Chronic myeloid leukaemia is associated with a specific translocation between chromosomes 9 and 22 that results in the formation of a chimaeric gene. This gene, when transcribed, produces the BCR-Abl oncoprotein which has tyrosine kinase activity and the ability to prevent apoptosis, but has no effect on cellular proliferation. Imatinib mesylate, an inhibitor of the BCR-Abl transcript modelled on the ATP binding pocket of the Abl oncoprotein, prevents phosphorylation of effector molecules and induces apoptosis. Imatinib has limited effectiveness when BCR-Abl cells are in the quiescent cell-cycle state of G_0. A life-long regimen of imatinib should reduce the risk of relapse from cells leaving G_0. Up-regulation of BCR-Abl expression, ATP binding pocket mutations, up-regulation of MDR1 and over-expression of Pgp are all thought to limit the effectiveness of imatinib. Advanced BCR-Abl positivity is associated with complex mutations, which are thought to have a cumulative effect on the BCR-Abl oncoprotein in disrupting normal signal transduction, making these cells refractory to monotherapy alone. Combination therapy is thought to overcome this. Research studies have identified imatinib as a potential treatment option for a diverse range of malignancies associated with BCR-Abl, platelet-derived growth factor receptor (PDGFr) and c-Kit pathways. This may extend the application of this special therapy in the future.

KEY WORDS: Glivec.
Imatinib.
Leukemia, myeloid, chronic.
Philadelphia chromosome.

Introduction

Chronic myeloid leukaemia (CML) is a myeloproliferative disorder associated with a quantitative increase in the number of granular leucocytes at all stages of maturation within the peripheral blood and bone marrow. The mean age of presentation is 40–50 years, although any age group can be affected. CML affects both sexes in a male:female ratio of 1.4:1.[1]

At the time of presentation, approximately 11% of all patients are asymptomatic,[2] and CML is diagnosed from a full blood count for the investigation of an unrelated disorder. If the patient is symptomatic, some common ones include sweating (especially at night), abdominal tenderness, lethargy, weight loss and shortness of breath. Spontaneous bruising may also be a feature.[3]

Initially, CML evolves from a single pluripotent stem cell that undergoes one or more genetic mutations, resulting in a clonal proliferation of transformed myeloid precursors. All the cells derived from this precursor will be affected. These daughter cells do not undergo maturational arrest but show an accelerated rate of differentiation and a failure to undergo apoptosis. These changes lead to a bone marrow microenvironment deficient in the majority of normal architectural features.

Splenic and hepatic extramedullary haemopoiesis are common features, with splenomegaly identifiable in approximately 90% of patients at presentation, 50% of which also demonstrate hepatosplenomegaly,[2] and a peripheral white blood cell count frequently between 50–500 x 10^9/L.[1] Additional changes associated with the leucocytosis recognised as a feature of CML are a normocytic, normochromic anaemia, thrombocytosis (but occasionally thrombocytopenia) and a basophilia, the degree of which appears to be linked to disease progression.[2,3]

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The pathology of CML is divided into three stages: chronic phase, accelerated phase and blast transformation.[3] Most patients present in the chronic phase, with diagnosis made initially from a full blood count and peripheral blood film. A definitive diagnosis is only made after a bone marrow biopsy and cytogenetic analysis. The chronic phase is usually a slowly progressive disease, which is readily treated and can be maintained for a period of between two and seven years.[3]

Following the chronic phase, CML usually progresses to the accelerated phase. This tends to become rapidly progressive, with an increase in bone marrow fibrosis and a reduced platelet count. The basophilia becomes more

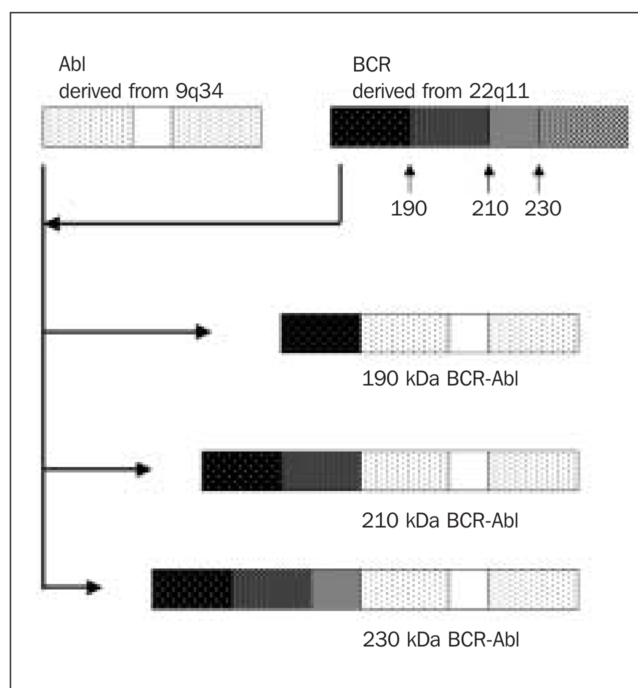
prominent and there is an increasing population of blasts, both in the circulation and in the marrow. The patients express more severe clinical symptoms including worsening night sweats, severe weight loss and anaemia, lymphadenopathy and bone pain.[4] Approximately 30% of patients die before this phase progresses to blast transformation,[4] although the accelerated phase may last for months or occasionally years.

In patients who progress to blast transformation, which tends to be refractory to standard treatment, survival is usually from two to six months.[3] Morphologically, this phase is indistinguishable from acute leukaemia, and immunophenotyping and cytogenetic studies are required to confirm the evolution and lineage of the disease. Approximately 30% of transformed leukaemias become acute lymphoblastic leukaemias. Transformation is associated with a higher incidence of more complex genetic insults that further complicate therapy and have a detrimental effect on prognosis.

Molecular biology of CML

Chronic myeloid leukaemia is a malignancy associated with a translocation of genetic material between the long arms of chromosomes 9 and 22 – t(9;22)(q34;q11) – resulting in the karyotype 9q+ and 22q–. The Abelson (*Abl*) proto-oncogene, located on the long arm of chromosome 9, is translocated to the breakpoint cluster region (BCR) on the long arm of chromosome 22, with a reciprocal translocation between chromosome 22 and chromosome 9. The shortened chromosome 22 (22q–) is called the Philadelphia (Ph) chromosome.

Fig. 1. The (t9;22)(q34;11) translocation responsible for forming one of three common chimaeric oncoproteins (190, 210 or 230 BCR-Abl). Each has the intact *Abl* gene, thus maintaining the intrinsic tyrosine kinase ability.



The *BCR* gene has three main areas associated with an increased risk of breakage, and these breakpoints are called the major BCR (M-*bcr*), minor BCR (m-*bcr*) and the micro-BCR (μ-bcr).[5]

The breakpoints found within the *Abl* gene are localised within a 300-kb segment limited to exons in the 5` region of the gene. Depending upon the position of the junction of the two genes when hybridised, a fusion protein of one of three sizes (p190, p210 or p230) is commonly produced (Fig. 1).[5]

The total incidence of patients with CML and the BCR-Abl transcript is in excess of 95%, with the most common phenotype associated with CML being the expression of a p210 fusion product. This is produced by either a b2a2 or a b3a2 junction between the chimaeric genes.

There is thought to be little functional distinction between the two fusion products. Each has tyrosine kinase activity and can activate and inactivate genes through a series of phosphorylation events via several transduction pathways including RAS, phosphatidylinositol 3′ kinase, Janus-activated kinase signal transducers and activators of transcription[6] (many others have yet to be elucidated). These fusion products may also be associated with approximately 5% of cases of juvenile acute lymphoblastic leukaemia and up to 20% of adult cases.[5] The homogeneity of this chimaeric transcript provides the potential for a targeted therapy against these malignancies.

Identification of the Ph chromosome in patients tentatively diagnosed morphologically with CML confirms the diagnosis. However, in approximately 10% of patients with CML, karyotypic studies fail to identify the presence of the Ph chromosome. Further investigation using gene probes and fluorescence *in situ* hybridisation techniques (FISH) identify the insertion of the *Abl* gene into the *BCR* gene without the reciprocal 22q translocation.

This group is characterised as Ph-negative and accounts for approximately 5% of the CML population. In these patients there is a BCR-Abl fusion product with altered tyrosine kinase activity, resulting in a malignant phenotype, progressive leucocytosis and the development of chronic-phase CML. The remaining 5% of patients fail to have the Ph chromosome and the *BCR-Abl* chimaeric gene identified and are categorised as Ph-negative CML.[5]

Signal transduction and cellular control

BCR-Abl transcript and signal transduction

The *Abl* gene is affiliated with a non-receptor protein tyrosine kinase family, and is responsible for phosphorylation events that promote and regulate the cell cycle. Consequently, experiments conducted by Sawyers *et al*.[6] provided evidence that over-expression of the *Abl* gene results in a failure of the normal development of cells. Conversely, the function of the *BCR* gene has not been shown to be directly involved in signal transduction or regulation of the cell cycle.

The Abl transcript contains three src homology (SH) domains essential for its function as a signal transducer. SH1 has tyrosine kinase activity and is closely associated with the two distal amino terminal SH2 and SH3 domains that are essential for protein binding.

In a recent study, Barila *et al*.[7] showed that SH3 is

responsible for the inhibition of Abl, and if SH3 is inhibited or deleted then Abl becomes oncogenic. Conversely, BCR does not contain SH domains but does contain an SH2 binding domain with which BCR may bind to the Abl SH2 domain.[8]

A GTPase-activating protein (GAP) and a serine threonine kinase domain are essential in the transformation to CML, and a dimerisation domain allows binding with other *BCR* gene products. The BCR-Abl fusion product binds to actin via the specialised actin-binding domain located at the distal carboxyterminal region of the Abl oncoprotein maintained after the BCR insertion. This enables binding to the cytoskeleton. The SH1 domain phosphorylates numerous proteins associated with BCR-Abl, leading to cellular dysfunction within the transformed cells.[9]

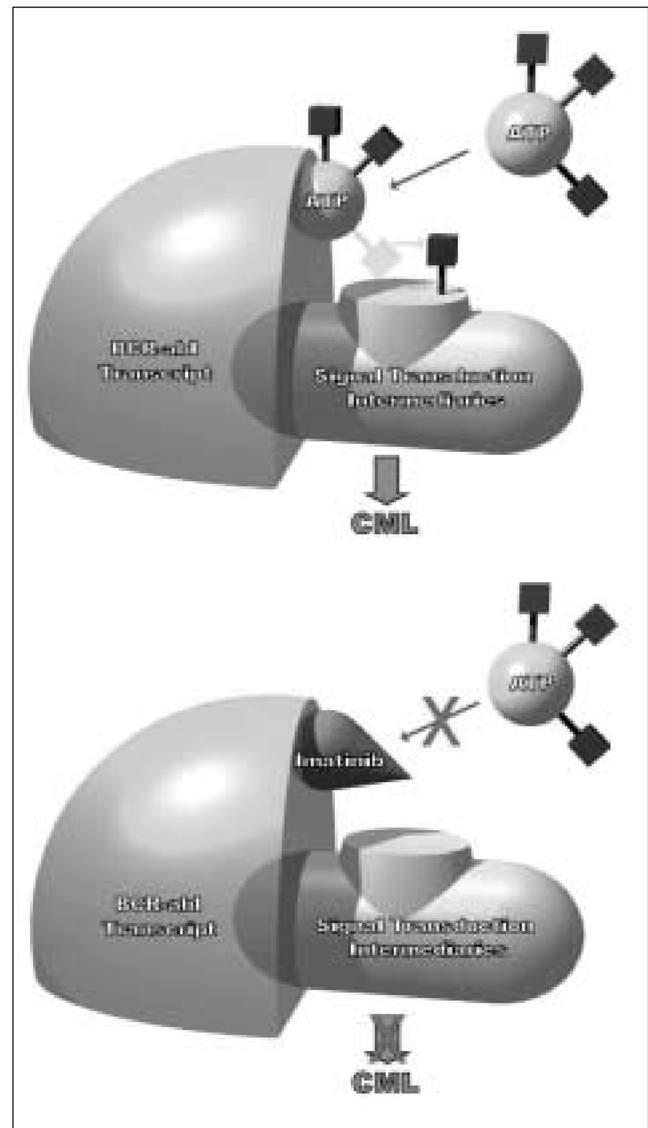
The role of BCR-Abl in the signal transduction events of CML-transformed cells is not fully understood; however, it is recognised that the mutant protein has increased tyrosine kinase activity. This is believed to be due to the failure to conserve an inhibitory loop (SH3) present within the Abl segment of the fusion product. The result is unregulated kinase activity producing increased differentiation and reduced apoptotic regulation.[7]

Apoptotic dysregulation in CML

The mechanism by which apoptosis is lost has yet to be fully elucidated. Horita *et al.*[10] demonstrated that stimulation of the anti-apoptotic protein BCL-x_L by BCR-Abl in an interleukin (IL)-3-independent manner may explain the refractory nature by which cells containing the t(9;22) mutation respond to normal apoptotic stimuli. This would include conventional chemotherapy.

It is believed that BCR-Abl activates the signal transduction and activation of transcription (STAT) pathways stat1, stat3 and stat5, which bind to the BCL-x_L gene promoter.[10] By inhibiting the tyrosine kinase activity of BCR-Abl, stat5 is no longer activated, reducing the transcriptional rate of BCL-x_L. As a negative regulator of apoptosis, decreased BCL-x_L will cause the cell to become more sensitive to normal apoptotic mechanisms.

Fig. 2. The competitive binding of imatinib mesylate to the ATP binding pocket prevents phosphorylation of signal transduction intermediaries by ATP and subsequent activation of a pathway leading to a CML phenotype.



Therapeutic options for the treatment of CML

Imatinib

Use of signal transduction inhibitors specific to the Abl transcript in routine clinical practice has enabled clinicians to gain better control of BCR-Abl-positive pathologies. Imatinib (also known by several names including CGP57148B, STI571, Glivec and imatinib mesylate), a derivative of 2-phenylaminopyrimidine, is effective in reducing tyrosine kinase activity within cells by inhibiting both Abl and BCR-Abl transcripts.

Imatinib competitively binds to the ATP binding cleft within the SH1 domain, inhibiting normal ATP binding. This prevents terminal (γ) phosphate groups being removed from ATP molecules and subsequently used in the phosphorylation of target proteins[11] which would result in the activation of these proteins and the generation of ADP (Fig. 2).

Initially, it was found that imatinib (then known as CGP57148B) was able to bind to many of the tyrosine kinase receptors at the ATP binding cleft. Subsequently, modifications of the signal transduction inhibitor were made according to results obtained from X-ray diffraction studies. These modifications altered the specificity with which the inhibitor binds; thus, selectivity for the Abelson tyrosine kinase, the platelet derived growth factor receptor (PDGFr) and c-Kit was established.

The first clinical studies using imatinib began in 1998, with a group of patients diagnosed with CML and testing positive for the Ph chromosome. The criteria for the clinical studies were set using results obtained from animal studies primarily on mice. From these experiments, the circulating half-life of imatinib was found to be between 10 and 23 hours,[11] suggesting that a single daily dose would be sufficient to provide a clinically beneficial effect.

Dosing was found to be tolerated between 25 and 1000 mg/day, although the optimal dose for producing beneficial results, measurable by haematological and cytogenetic

response, was found to be 300 mg/day.[11] Imatinib had no dose-limiting factors, and side effects, if reported, were mild anaemia, nausea and some abdominal discomfort.

As with all chemotherapeutic agents used in the modulation of malignancy, cells with a high proliferation index, including hair follicles, endothelial cells of the gastrointestinal tract and haemopoietic stem cells, are affected to a greater extent than those with a lower proliferation index. However, imatinib affects cells with a high proliferation index provided they are positive for the BCR-Abl fusion product.

Holyoake *et al*.[12] documented the presence of a small sub-population of leukaemic cells in CML that do not actively undergo rapid proliferation and are retained in the quiescent cell-cycle state of G_0. While this tyrosine kinase inhibitor is taken routinely, active cells are forced to undergo apoptosis, possibly through the inhibition of stat5 and reduction in BCL-x_L or via another mechanism.

Cells in G_0 are not affected. While the treatment is maintained, cells that actively leave G_0 will have apoptosis induced, controlling the disease. Once imatinib therapy is stopped, cells leaving G_0 will begin to proliferate and the patient will relapse. This suggests that imatinib needs to be administered for life to prevent this mechanism of relapse from occurring.

Resistance to imatinib

Recent studies suggest that mechanisms exist by which resistance to imatinib can develop. Several mechanisms have been identified, resulting in both haematological and cytogenetic relapse when imatinib has been in continuous administration.

The first mechanism is the up-regulation of BCR-Abl transcription, leading to a greater intracellular concentration of fusion products with tyrosine kinase activity. It is not clear why increasing the daily dose of imatinib would not succeed in reinitiating remission in such cases. Conceivably, large daily doses would produce adverse side effects.

Second, additional complex mutations within these transformed cells would mean that additional transcripts could bypass BCR-Abl function, stimulating cell proliferation or further inhibiting apoptosis. Third, up-regulation of the multidrug resistance (*MDR*) gene in transformed cells may act as an effective method of reducing the intracellular concentrations of imatinib to sub-therapeutic levels.[13]

*MDR*1 and *MDR*2 are two genes belonging to the *mdr* family, one of which, *MDR*1, is associated with resistance to chemotherapeutic agents.[14] The transcript from *MDR*1, P-glycoprotein (Pgp), is associated with an energy-dependent efflux mechanism[15] that reduces intracellular concentrations of cytotoxic compounds and leads to drug resistance. This may prove to be a particular problem in haemopoietic progenitors,[16] as a strong association has been made between the expression of Pgp and CD34.[17] Increased Pgp expression may influence treatment progression in some malignancies where the genetic insult responsible for the malignancy is at such an early stage.

Weisberg *et al*.[18] studied two cell lines, K562 and Ba/F3.p210, obtained from CML patients and cultured them in increasing concentrations of imatinib. Over a period of several months the group was unable to culture cell lines resistant to concentrations of imatinib greater than 0.5 µmol/L and 2.5 µmol/L for each respective cell line.

Of the cells cultured, several mechanisms appeared to be responsible for the observed resistance. The authors believe an increase in the transcribed BCR-Abl protein cannot account for the level of drug resistance observed.[18] Intracellular MDR transcription rates were measured, and Pgp was found not to be over-expressed. This suggests that there are alternative mechanisms to resistance of imatinib as yet unidentified, or the over-expression of BCR-Abl is sufficient to result in imatinib resistance.

It is conceivable that exposure to high doses of imatinib *in vivo* may induce increased expression of enzymes, perhaps within the liver, that may favour the metabolism or binding of free imatinib, thus reducing the plasma concentrations of active imatinib or resulting in its modification. It is clear from the evidence available that much research needs to be performed to identify this mechanism.

A similar study, culturing cells at increasing concentrations of imatinib using FISH, showed that the caspase-3 response is no longer detectable in cells resistant to imatinib.[19] This failure in caspase-3 induction was associated with an increased intracellular BCR-Abl transcript concentration, and a four- to five-fold increase in BCR-Abl messenger RNA (mRNA).[19] This suggests that over expression of BCR-Abl mRNA in response to imatinib is the result of a mechanism induced to promote cell survival. In contrast to the findings of Weisberg *et al*.,[18] le Coutre[19] believes that this mechanism alone can account for the resistance found within these mutated cell lines.

In vivo studies performed by Mahon *et al*.[20] demonstrate the relative difficulty associated with producing clonal cells that exhibit resistance to imatinib. Concentrations of the signal transduction inhibitor in excess of 0.2 µmol/L produced no cell survival in 50% of the cell lines used for the study. The most common modification found within the cell lines was an over-expression of BCR-Abl, suggesting that this mechanism is the first step in becoming resistant to imatinib.

Furthermore, one of the cell lines studied showed elevated Pgp expression, which contradicts the findings of Weisberg.[18] However, of eight cell lines studied, only one was positive for Pgp expression, suggesting that products of this multidrug resistance gene play only a limited role in the resistance to imatinib.

An indirect measure of imatinib was demonstrated by Gorre *et al*.[21] Instead of measuring the effects of imatinib through proliferation assays, they decided to dissect the signal transduction pathway and measure the direct effect imatinib had on the phosphorylation of specific target proteins. They chose the adapter protein Crkl, which is specifically phosphorylated by the Abl tyrosine kinase and allows an association between tyrosine kinases and downstream effector molecules.[22]

Gorre's group showed that Crkl and BCR-Abl are both dephosphorylated in a dose-dependent manner to imatinib.[21] When compared to cells from patients refractory to imatinib, a much greater dose of the inhibitor was required to inhibit phosphorylation of Crkl than before. The group believes that this resistance is due to mutations in the ATP binding pocket of the tyrosine kinase, reducing the binding capabilities of the inhibitor.

In six of nine patients, a cytosine→thymine point mutation was identified at position 944, resulting in an amino acid substitution from threonine to isoleucine at amino acid 315. This prevents critical hydrogen bonding

with imatinib, reducing its efficacy.

A further study by Corbin *et al*.[23] examined the effect of common BCR-Abl mutations on imatinib binding and subsequent inhibition of autophosphorylation in the presence of escalating imatinib concentrations. Densitometric quantitation was used to calculate the 50% inhibitory concentration (IC_{50}) of imatinib when added to BCR-Abl-mutated Ba/F3 cells. Common mutations at the amino acid sites 315, 253, 255 and 351 were investigated, together with the less frequently seen mutations Met244Val, Gly250Glu, Gln252His, Phe311Leu, Phe317Leu, Glu355Gly, Phe359Val, Val379Ile, Leu387Met and His396Pro/Arg.

The results of this investigation show that mutations within the ATP binding pocket, which are directly responsible for imatinib binding, play a significant role in reducing imatinib sensitivity. In addition, mutations within the activation loop lead to different levels of inhibition, where the IC_{50} is increased and imatinib resistance is demonstrated.

By increasing the dose of imatinib, Corbin's group maintains that those mutations responsible for a lower level of resistance will still be sensitive to inhibition. They demonstrated that mutations outside critical functional regions previously associated with imatinib resistance do not reduce imatinib sensitivity, according to cellular proliferation assays and the IC_{50}. Here it is possible that mechanisms other than Bcr-Abl mutation are responsible for a failure to respond to therapy.

One criticism of this study is that the IC_{50} is not truly representative of the intracellular concentration of imatinib and thus it cannot be used to effectively demonstrate a representative increased requirement for imatinib in these cell lines. Gambacori-Passerini *et al*.[24] maintain that binding of imatinib to plasma proteins such as α1 acid glycoprotein will lead to an underestimation of intracellular concentrations of imatinib, although this may not be the case *in vitro*.

Combination therapy

The latent risk of becoming refractory to imatinib has resulted in the use of combination therapy to avoid resistance mechanisms. By combining imatinib with the current therapeutic agents interferon (IFN), daunorubicin (DNR) and hydroxyurea (HU), Thiesing *et al*.[25] demonstrated that combination therapy using a conventional chemotherapeutic agent and a signal transduction inhibitor increased the success rate and reduced the risk of resistance.

Proliferation assays showed that all the conventional agents except HU are effective in the control of transformed cells. Colony forming assays showed that all traditional chemotherapeutic agents, including HU, were successful when combined with imatinib in the control of transformed cells. Thiesing *et al*.[25] argue that the encouraging results obtained from combination therapy could be due to the activation of different apoptotic pathways within the malignant cell lines, thus reducing the total risk of resistance.

Current treatment methodologies favour the use of IFNα, either as a monotherapy or in combination with a chemotherapeutic agent (either HU or cytosine arabinoside [ara-C]). There is a slight survival advantage in combining IFNα and ara-C, compared with IFNα alone, although this is more successful as a treatment regimen than the chemotherapeutic agents alone.

Success is measured as the duration of disease-free survival. IFNα conferred approximate 20 months' advantage compared with other therapeutic options. The mechanism by which IFNα controls the leucocyte count remains unclear. In a recent study, however, Mayer *et al*.[26] provide evidence for the activation of the p38 MAPK pathway, resulting in the down-regulation of BCR-Abl transcripts and an anti-leukaemic effect.

Bone marrow transplantation

Bone marrow transplantation (BMT) is an option that usually follows either mono- or combination therapy. A more radical form of therapy, it is the only option reportedly capable of being curative. However, significant morbidity and mortality (20% or greater) is associated with allogeneic BMT, with post-transplantation complications including graft-versus-host disease (GvHD), acute respiratory distress syndrome, multi-organ failure and infection being most likely to prove fatal.

The upper age limit for those patients offered allogeneic BMT is now 30 years of age, as studies have shown that patients below this age have the highest rate of disease-free survival.[27] Rates of survival are best in those patients receiving a viable, matched allogeneic BMT who have not been given previous chemotherapeutic treatment containing busulphan, which is associated with a greater degree of post- transplant relapse, presumably due to the nature of its toxicity.[28]

Interferon-*α *and hydroxyurea

The greater survival advantage associated with IFNα compared with conventional chemotherapies suggests that this is the best choice for the first-line treatment of CML. However, the control of the leucocyte count using this treatment alone is much more difficult, and takes longer than using a combination therapy or HU alone as a first-line therapy.

Hydroxyurea inhibits the enzyme ribonucleotide reductase and, having antimetabolite activity, enables rapid control of the peripheral leucocyte count.[29] Hydroxyurea has no effect in the reduction of Ph+ cells within the bone marrow, and cannot limit the progression of the chronic phase into accelerated phase or blast crisis. Conversely, the anti-leukaemia effect conferred by INFα does have a limiting effect on the Ph+ population, and is associated with prolonged chronic phase and associated delay in the accelerated phase, due to this Ph+ reduction.

Until recently, INFα has been the gold standard in the successful treatment of CML, and, as previously highlighted, its additive effect with ara-C means that maximum long-term survival is achieved when used as a combination therapy. The successful introduction of signal transduction inhibitors warrants the use of comparability studies to highlight the *in vivo* effects of Ph+ elimination using INFα and imatinib.

Recently, Marin *et al*.[30] suggested that evidence of a cytogenetic response can be used as a surrogate marker for survival. This group investigated 143 patients diagnosed with CML, classified as unresponsive to INFα, and compared them to historical records of 246 patients, all of whom had been maintained on conventional treatment. They found that the adjusted eight-year survival after diagnosis was significantly higher in imatinib responders

than in controls (those on INFα alone) and non-responders. As imatinib has been documented as more successful in achieving a cytogenetic response than other treatments, this group concludes that all patients failing to achieve an early cytogenetic response on standard therapies should be offered alternative treatments at an early stage.

Kantarjian *et al.*[31] evaluated the consequences of adding imatinib to standard INFα therapy for treating early chronic-phase CML by comparing a current group of 201 patients with early chronic-phase Ph+ CML with an historical group of 293 patients without access to imatinib during their treatment. Imatinib was shown to have a survival advantage, with 101 patients achieving a complete cytogenetic response. In addition, of 80 patients tested by quantitative polymerase chain reaction studies, 20 had no detectable disease. These researchers calculate the five-year survival to be 86%, with a significant advantage (P=0.03) over those treated with IFNα alone. Apparently, this compares with a 5–10 % annual mortality rate for the first two years and a 15–20% annual mortality in subsequent years.

Clinical application of imatinib

Previous guidelines produced by the British Committee for Standards in Haematology (BCSH) on the treatment of CML were not complete, and therefore left protocols to the discretion of the prescribing clinician. Tried and tested treatment regimens were used locally, with guidance usually taken from trials rather than printed standards.

The results of the IRIS (International Randomised Trial of Interferon and Ara-C *vs* STI571) trial were published prematurely, due to the unexpected nature of the results found. The data show that of 1106 patients recruited for the IRIS trial, 83% showed major cytogenetic responses compared with 20% prescribed the IFN + Ara-C regimen. Furthermore, 68% of imatinib patients showed a complete cytogenetic response, compared with only 7% on the alternative treatment.[32]

The Imatinib position paper[33] states that imatinib is not licensed as a first-line therapy for the treatment of CML, and, as such, is used for those patients who fail to have a major cytogenetic response to IFN. Imatinib may also be prescribed if patients fail to tolerate the side effects of IFN, or have an increasing Ph+ population (>35%) previously treated with IFN. All patients entering accelerated phase or blast crisis may also be prescribed imatinib.[33]

The National Institute for Clinical Excellence (NICE),[34] in August 2003, published its final appraisal determination for the use of imatinib in CML. This determination instructs explicitly on the use of imatinib while comparing its efficacy and cost with standard therapeutic regimens. The licence for imatinib has now been extended to newly diagnosed Ph+ CML for whom stem cell transplantation is not considered first-line treatment,[34] and supersedes guidance given by the imatinib position paper.

The determination also stipulates that patients presenting with Ph+ CML in accelerated phase or blast crisis who have not received imatinib previously should be considered for the inhibitor. The committee has also directed research regarding the continued application of imatinib in individuals who have progressed to accelerated phase or blast crisis while receiving imatinib treatment. The group plan to evaluate this evidence in 2006.

Imatinib in advanced and complicated disease

The effects of imatinib in the management of patients with CML in blast crisis or Ph+ acute lymphoblastic leukaemia (ALL) was evaluated by Druker and colleagues.[35] Of the patient population in blast crisis, 32% had a marrow myeloid blast count of less than 5%, over half (55%) of the patients responded to treatment. Of the patients studied, 11% entered complete remission and seven were still in remission up to 349 days later. In the ALL group, 70% of patients responded to therapy, 20% of whom had complete remissions. Bone marrow blast counts of less than 5% were observed in 55% of patients.

Druker *et al.*[35] argue that, even in blast crisis, these results indicate that the disease is still governed by the effects of the BCR-Abl transcript, explaining the responses attributed to imatinib in the late stages of the disease. This group postulates that the application of imatinib in advanced disease may contribute to the successful use of stem cell transplantation. Such transplants completed during blast crisis are less successful than those performed on patients who have returned to the chronic phase;[36] therefore, imatinib may contribute to increased long-term survival when used as an induction therapy.

In addition, Sawyers *et al.*[37] suggest a combination of imatinib and fludarabine or cytarabine to treat patients who have progressed to blast crisis, as these therapies are associated with a measurable response by the bone marrow. This group believes that imatinib acts synergistically with these chemotherapies, permitting better control of Ph+ advanced disease.

Imatinib is associated with less severe cytopenias than are conventional therapies, although cytopenia is frequent.[37] This is thought to be due to a reduction of the tumour burden, with a reduced complement of unaffected precursors in the marrow compartment.

Mutations in the derivative chromosome 9 adjacent to the breakpoint region are associated with poor prognosis in CML.[38] These mutations are found in approximately 10–15% of all patients and have a significant deleterious effect on survival.[38] Huntley *et al.*[39] studied the effect of imatinib on this group of patients compared with patients without deletions, and found that the response rates of the chromosome 9 deletion group were lower than those in uncomplicated disease. However, in the short term, there was no significant difference in survival of both groups. Although the prognosis of the group with chromosome 9 deletions is improved, in the longer term the lower haematologic and cytogenetic response rates will reduce the survival period, reflecting the findings of both Marin *et al.*[30] and Kantarjian *et al.*[31]

Schittenhelm *et al.*[40] used imatimib to achieve complete remission for a patient diagnosed with acute monoblastic leukaemia (FAB:M5) following two relapses using conventional chemotherapy. The leukaemia had a standard immunophenotype, including blasts strongly positive for CD34 and CD117 (c-Kit). After two successive attempts to treat this patient with standard chemotherapeutic protocols, a third relapse proved refractory to hydroxyurea and was

characterised by the presence of 74% blasts in the bone marrow.

A dose of 600 mg/day imatinib was commenced and after 80 days' treatment, with breaks for cytopenia and pneumonia, malignant blasts could no longer be detected in the bone marrow. The patient was maintained on 100 mg/day and, after 20 weeks, remains in complete remission. Schittenhelm *et al*. highlight the risk of prolonged cytopenias at higher imatinib doses and suggest that an imatinib-sensitive pathway may be responsible for non-pathological haemopoiesis.

Future applications of imatinib

The specificity with which imatinib binds to its receptor means that targeted therapy for specific diseases that utilise these receptors is possible. Provided the pathway containing the imatinib receptor is intrinsic to the pathology of the disease, this inhibitor can be used to control disease progression.

Although this review article has specifically examined the effects of imatinib in relation to the treatment of CML, many other applications are being investigated. The ability of imatinib to bind PDGFr and c-Kit means that other malignancies can be treated using this targeted therapy.

Many of the other applications, however, remain laboratory-based, although its use against tumour models is well documented. For example, cells derived from patients with colorectal cancer have shown a response to imatinib via the inhibition of c-Kit. Such tests have resulted in a reduction in tumour growth associated with increased apoptosis.[41]

Gastrointestinal stromal tumours (GISTs) are derived from mesenchymal tissue and the only successful treatment option is surgical resection as both chemotherapy and radiotherapy have proved unsuccessful. Research by Demetri *et al*.[42] into the treatment of gastrointestinal tumours examined the effect of imatinib in the treatment of metastatic or unresectable stromal tumours. Of 147 patients, 53.7% showed a partial response to therapy, with stabilisation of the disease in a further 27.9%.

The European Organisation for Research and Treatment of Cancer (EORTC) Soft Tissue and Bone Sarcoma Group,[43] using a phase II clinical trial, investigated the use of imatinib in GISTs and soft tissue sarcomas (STS), expressing c-Kit and PDGFr, respectively. This group documented differential sensitivities to imatinib that correlate with the mutational status within the c-Kit protein. Imatinib was found to inhibit c-Kit more effectively in GISTs with activating mutations within exon 11 compared to tumours containing wild-type c-Kit or those with exon 9 mutations.

In-depth investigation of c-Kit mutations has yet to be completed so evidence about why exon-11 mutants are preferentially inhibited is limited. Within the GIST group, 4% achieved complete remission, 67% achieved partial remission and 19% had stable disease. Of the 27 patients studied, 11% showed disease progression.

These results accord with previously published data by Demetri *et al*.[42] Of the patients diagnosed with STS, 29% had stable disease following imatinib therapy, but all failed to respond or regress. It is believed that PDGFr is less important to the pathology, and the malignant clone is maintained by alternative pathways.

Similarly, in a study of 17 patients diagnosed with GISTs, Bumming *et al*.[44] acquired a partial response in eight out of nine patients with exon-11 mutations. Furthermore, three of the patients with progressive or stable disease prescribed imatinib did not have exon-11 mutations. However, this group identified two patients who responded to imatinib but failed to have an exon-11 mutation identified. It is unclear whether a separate mutation within the c-Kit protein has similar effects, causing malignant transformation while maintaining its sensitivity to imatinib.

Imatinib has applications also in the treatment of Kaposi's sarcoma. Infection of cells with Kaposi's sarcoma-associated herpes virus (KSHV) causes the induction of c-Kit that can be inhibited by imatinib.[45] Research in this area still continues.

Although just a sample of the applications of imatinib, these illustrate that selective inhibitors of signal transduction pathways intrinsic to disease pathogenesis show considerable potential. As more is discovered about the molecular pathogenesis of disease, new ways of inhibiting disease progression will become available. Currently, research is underway on another haematological condition, hypereosinophilic syndrome.

In summary

Imatinib is a special treatment for CML. Developed specifically to interact with the ATP binding pocket of the BCR-Abl oncoprotein, imatinib prevents phosphorylation of effector molecules that bind to the BCR-Abl molecule by competitive inhibition. Owing to its tyrosine kinase properties, BCR-Abl, phosphorylates effector molecules and this results in an inhibition of the apoptotic mechanism, thereby increasing the total number of mature granulocytes within the bone marrow and peripheral blood. Prevention of ATP binding to the BCR-Abl transcript inhibits this signal transduction pathway and reinitiates the apoptotic mechanism, reducing the tumour burden.

The specificity with which imatinib binds and the nature of its action ensure that only cells containing the BCR-Abl oncoprotein are destroyed, although evidence suggests that cells within the G_0 phase of the cell cycle are protected from its effects. The half life of this signal transduction inhibitor is measured as 10 to 23 hours; thus, it can be administered once a day for convenience, but must be treated as a life-long therapy to prevent CML relapse by the activation of Ph+ quiescent cells in G_0.

Extensive work has been completed on the effects of imatinib, the conclusions of which are similar. Results demonstrate that imatinib is an effective treatment for CML, and is associated with a greater degree of success in producing and maintaining both haematological and cytogenetic responses than other therapeutic regimens, and is the least cytotoxic. The IRIS study highlights the efficacy of imatinib compared to conventional INFα in combination with ara-C, which, with the exception of bone marrow transplantation, had been the most successful treatment option for CML.

Further research has shown that the effectiveness of imatinib may be short-lived. Evidence of resistance is now documented and proposals of various mechanisms of resistance are convincing. The most controversial

mechanism is also the simplest. Up-regulation of BCR-Abl transcription, producing higher intracellular concentrations of the BCR-Abl oncoprotein, reduces the efficacy with which the signal transduction inhibitor can function. Secondly, up-regulation of MDR1 transcription and over-expression of the Pgp protein have been shown to reduce intracellular concentrations of imatinib. This mechanism is known for its effects in reducing the intracellular concentration of conventional chemotherapeutic regimens by the same mechanism.

Point mutations within the ATP binding pocket that prevent signal transduction inhibition have also been documented. The nature of these mutations and their position within the three-dimensional conformational structure of the protein may have a direct effect on imatinib binding. Few studies have focused on this issue in any depth, although Corbin *et al*.[23] successfully demonstrated variations in IC_{50} with different mutants. It is not known if this mechanism is due to a spontaneous mutation or occurs in response to imatinib. Carefully controlled studies must be performed to ensure that these mutations are not part of the normal pathophysiology of CML before further conclusions can be drawn.

As more patients are given the option of treatment with a signal transduction inhibitor, it will prove easier to measure the effects and determine survival rates. At present, true five-year survival statistics have yet to be compiled but the results so far are encouraging. The results may be improved further by introducing combination therapy to allow the activation of apoptosis by different mechanisms, reducing the risk of drug resistance. Furthermore, the application of imatinib in advanced disease has already shown positive results both in prolonging survival and in inducing chronic phase where BMT can be performed.

Ultimately, it is the signal transduction pathway that controls the function of the cell. Stimulation of different pathways by different ligands produces different effects, but understanding how these effects are brought about will help scientists and clinicians understand human cancers in a way that will encourage the development of new treatments and new inhibitors. Recent research has studied the effects of imatinib on other cancers, including GISTs, and, although there is some doubt about the importance of exon-11 mutations in *c-Kit*, the use of this inhibitor in controlling the cancer prior to surgical resection appears promising. □

The basis of this review was used in partial fulfilment of an MSc in biomedical science at the University of Portsmouth. The authors would like to thank Andrew Earle for production of Figure 2.

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