

# Chronic Myeloid Leukaemia: Molecular Abnormalities and Treatment Options

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## ABSTRACT

Chronic myeloid leukaemia (CML) is a malignant, myeloproliferative disorder of haemopoietic stem cells. It arises from a stem cell acquiring a specific translocation t(9;22) which results in the formation of a hybrid oncogene, BCR-ABL. Selecting the most appropriate therapy for a patient with CML remains difficult. Currently, stem cell transplantation is generally accepted as offering the best prospect of a cure. However, advances in the study of tyrosine kinase inhibitors and immunological treatments may direct the future of CML treatment.

## INTRODUCTION

Chronic myeloid leukaemia (CML) is a malignant myeloproliferative disorder of haemopoietic stem cells, giving rise to abnormally elevated numbers of myeloid cells in peripheral blood and myeloid hyperplasia in bone marrow. Uncontrolled production of myeloid precursors results in increased levels of maturing granulocytes, mainly neutrophils but also eosinophils and basophils. Elevated numbers of erythroid cells and platelets are also found.

## Clinical Features of CML

Peripheral blood abnormalities account for many of the clinical features of CML (Table 1). Systemically the excess of neutrophils is accompanied by splenomegaly, and occasionally hepatomegaly or adenopathy, and varying degrees of myelofibrosis and extramedullary haemato-poiesis. Symptoms related to leukostasis arising from high white cell count include blurred vision, headaches and rarely in males, priapism. Some 40

percent of patients are asymptomatic, with diagnosis following a routine blood test.

## EPIDEMIOLOGY

CML occurs with an incidence of one to two cases per 100,000 people per year and accounts for 15 percent of adult leukaemias.<sup>1</sup> CML may affect any age group, though it is unusual in children. The peak incidence is between 40 and 60 years with the median age at diagnoses being 53 years.<sup>2</sup>

## CML: A Progressive Triphasic Disease

The clinical course of CML is divided into three distinct phases, characterised by progressive changes in leukocytes and accumulation of genetic abnormalities. The stage of CML is an important consideration in deciding on treatment course.

The chronic phase (present at time of diagnosis in approximately 85 percent of patients) is associated with large increases in the pool of committed myeloid progenitors, leading to peripheral blood

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## PRESENTATION OF CML

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### Clinical Findings

- Fatigue
- Anorexia
- Weight Loss
- Splenomegaly
- Hepatomegaly

40 percent of patients asymptomatic at diagnosis

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### Peripheral Blood:

- White Cell Count > 25,000/mm<sup>3</sup>
- 30-50 percent have elevated platelet count
- Basophilia
- Reduced leukocyte alkaline phosphatase activity
- All stages of granulocyte differentiation visible on peripheral smear

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### Bone Marrow

- Hypercellularity, with reduced fat content
  - Increased ratio of myeloid to erythroid cells
  - Increased numbers of megakaryocytes
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leukocytosis and often thrombocytosis with a prominent left shift in the differential count and basophilia.<sup>1</sup> Left untreated the chronic phase lasts three to five years before developing into the accelerated phase.

In the accelerated phase of CML neutrophil differentiation becomes progressively impaired and leukocyte counts are more difficult to control with myelosuppressive medications.

Finally CML culminates in blast crisis. The blast phase is defined by the presence of 30 percent or more leukaemic cells in peripheral blood or marrow or the presence of extramedullary infiltrates of blast cells. The blast phase of CML resembles acute leukaemia in which myeloid or lymphoid blasts fail to differentiate.

In one third of cases, the blasts have a lymphoid morphology and express lymphoid markers such as terminal deoxynucleotidyl transferase or CD10 (common acute lymphoblastic leukaemia antigen). The remaining two-thirds of cases have a phenotype similar to that of acute myeloblastic leukaemia and form a heterogeneous group.

The distinction is important because CML in the lymphoid blast phase may respond to treatment with regimens that are active against acute lymphoid leukaemia. However, the median survival after progression to blast crises remains approximately six months.

Occasionally, CML does not follow this pattern but transforms into myelofibrosis, in which case death results from bone marrow failure.

### **Molecular Abnormalities**

CML is characterised by the presence of a distinct molecular abnormality. First described by Nowell and Hungerford in the 1960s who dubbed it the “Philadelphia chromosome,” the molecular abnormality has since been identified as a reciprocal translocation between chromosomes 9 and 22.

Now designated t(9;22)(q34;q11) this translocation involves the BCR gene on chromosome 9 and the ABL gene on chromosome 22. The resultant BCR-ABL fusion gene, directs synthesis of a protein with tyrosine kinase activity. This abnormal tyrosine kinase protein product is unique to the leukaemia cells and is the fundamental cause of all the abnormalities observed in

Philadelphia chromosome-positive leukaemias.

How this translocation initially occurs is not fully understood although radiation has been implicated. It has also been suggested that the close proximity of the BCR and ABL genes during interphase may facilitate the mutation.<sup>3</sup>

### **BCR and ABL in Normal Cells**

In a normal cell the BCR gene codes for a 1271-amino acid cytoplasmic phosphoprotein of 160 kDa that has several known domains:

*Amino acids 1-427:* contains a coiled domain that mediates homo-oligomerisation and a novel serine kinase activity.

*Amino acids 490-690:* contains a region of homology to the DBL oncoprotein that functions as a guanine nucleotide exchanger

*C-terminus:* contains a domain with GTPase activating protein homology and GAP activity toward RAC and Cdc42 proteins. Mice lacking BCR show an increased respiratory burst in their neutrophils.

There is less known about the c-ABL gene. It may be involved in cell response to genotoxicity (damage to genetic material) and oxidative stress, and in integrin and platelet-derived growth factor (PDGF) signalling. c-ABL tyrosine kinase activity is tightly controlled in the normal cell, and appears to be regulated at multiple levels by serine and tyrosine phosphorylation, by NH<sub>2</sub>-terminal sequences and by a cellular inhibitor that binds to Src homology 3 domain on c-ABL.

### **BCR-ABL Cell Transformation**

Although BCR and c-ABL have no intrinsic oncogenic properties themselves, BCR-ABL has the ability to transform cell lines and primary cells *in vitro*.

The abnormal BCR-ABL hybrid encodes for an abnormal, constitutively active tyrosine kinase receptor. Simply, BCR-ABL can be thought of as always “switched on.” BCR-ABL has primary mitogenic activity and can stimulate cell cycle entry of haematopoietic cell lines and primary cells in the absence of growth factors. It activates multiple signal transduction cascades including the RAS, MYC and PI3K pathways thereby allowing the growth and survival of haemato-

poetic stem cells to continue independent of their regulatory cytokines.<sup>2, 3</sup> BCR-ABL reduces the expression of cell surface adhesion molecules, facilitating the dissemination of leukaemic cells in the peripheral blood.<sup>1</sup> In addition, the BCR-ABL allows leukaemic cells to evade apoptosis. Philadelphia chromosome positive cells are protected from apoptosis upon cytokine withdrawal, radiation and cytotoxic chemotherapeutic agents.

The malignant clone in CML is genetically unstable and acquires multiple genetic abnormalities during the progression from chronic phase to blast crisis. As the disease progresses, leukaemia cells acquire further mutations including trisomy 8 and alterations in RAS and RB1 genes.<sup>1</sup> Mouse studies have suggested that BCR-ABL may directly induce karyotypic instability.

### CML Histopathology

In contrast to normal marrow, which is approximately 50 percent cellular and 50 percent fat, CML marrow is 100 percent cellular, predominantly composed of maturing granulocytic precursors. Seen on microscopic investigation are increased numbers of megakaryocytes, including small dysplastic forms, erythroid progenitors which are usually in normal or decreased numbers, scattered storage histiocytes with wrinkled, sea-blue cytoplasm, increased deposition of reticulin fibres (but overt marrow fibrosis is rare at presentation) and marked leukocytosis often exceeding 100,000 cells/mm<sup>3</sup>.

### BCR-ABL: Three Forms

There are three principal forms of the BCR-ABL mutation: p190, p210 and p230. They all encode the c-ABL gene sequence (except the first exon) and the entire ABL tyrosine kinase catalytic domain, but differ in the length of the BCR transcription sequence at the N-terminus.

Briefly, all three have increased tyrosine kinase activity *in vivo* and *in vitro* relative to c-ABL with p190 exhibiting the highest intrinsic kinase activity. The mechanism of activation is unknown but may involve oligomerisation of BCR-ABL via the coiled cell domain, and interaction of BCR with the ABL src homology 2 domain, blocking the inhibition of an ABL inhibitor.

### TREATMENT OF CML

CML treatment options can broadly be divided into stem cell transplantation and non-transplant based therapies, which include oral chemotherapeutic agents interferon-alfa (IFN $\alpha$ ) and oral tyrosine kinase inhibitors. Treatment choice depends on the phase of the disease (chronic, accelerated or blast), the age of the patient and the availability of a suitable stem cell donor.

Given the growing range of therapeutic choices, the patient and disease factors which must be taken into account, the question of selecting the correct treatment for the patient who will benefit most is quite complicated.

Unfortunately, less than 20 percent of CML patients are eligible for transplantation either due to age limitations or lack of a suitable donor.<sup>2</sup> For the majority of cases of CML, drug treatment remains the mainstay.

### Imatinib

With the discovery that the product of the Philadelphia chromosome accounted for cell transformation in CML, inhibition of BCR-ABL soon became the focus of research efforts in the search for a cure. BCR-ABL was an ideal molecular therapeutic target because it is unique to leukaemic cells, is expressed at high levels and its tyrosine kinase activity is essential for the induction of leukaemia.

STI-571, later renamed imatinib mesylate (Gleevec, Gleevec, Novartis, Basel, Switzerland), was the most promising tyrosine kinase inhibitor discovered. It binds to the ATP domain of BCR-ABL preventing the phosphorylation of its substrates and thereby blocking the downstream signal cascades. Imatinib stimulates apoptosis of BCR-ABL positive cells without affecting normal stem cells.

*In vitro* studies found that imatinib produced a 92 to 98 percent reduction in CML colony growth without inhibiting growth of normal cells.<sup>4</sup> Phase I trials began using imatinib in patients with chronic phase CML who had failed to respond to IFN $\alpha$  therapy or were intolerant to it. Complete haematological response, defined as reduction in white-cell count to less than 10,000/mm<sup>3</sup> and in the platelet count to less than 450,000/mm<sup>3</sup>, was achieved in 53 of 54 patients treated.<sup>5</sup>

In a multicentre phase II trial using similar patient criteria, complete haematological response was obtained in 95 percent of patients and a major cytogenetic response in 60 percent.<sup>6</sup> Major cytogenetic response is defined by reduction in Philadelphia chromosome-positive cells in metaphase to less than 35 percent of cells in bone marrow. Ninety-five percent of complete responders remained in remission after two years.

In the International Randomised Interferon-alfa versus STI-571 (IRIS) Study, a phase III trial comparing imatinib versus IFN plus cytarabine (Ara-C), imatinib was shown to be a more effective treatment in patients with newly diagnosed chronic phase CML. Imatinib showed greater cytogenetic and haematologic responses and also reduced the incidence of progression to accelerated or blast phase. Imatinib was also tolerated better than the IFN $\alpha$  and Ara-C regimen.<sup>7</sup> A follow up study to the phase III trial measured the amount of BCR-ABL transcripts in the blood of patients who had a complete cytogenetic response. Results from the study showed that the proportion of patients that had at least three log kill at 12 months was greater in the imatinib group compared with controls receiving IFN $\alpha$  plus Ara-C (39 percent vs. 2 percent,  $p < 0.001$ ).<sup>8</sup>

Imatinib is also effective in the accelerated phase of CML. It has produced a major cytogenetic response (less than 35 percent Philadelphia chromosome-positive cells in the bone marrow) in 24 percent of patients in the accelerated phase, short remissions of up to six months and has been used to control the leukaemia before transplantation.

In the blast phase of CML, imatinib induced a major cytogenetic response in 16 percent of patients and therapeutic response was associated with prolonged survival. Imatinib was also shown to be less toxic and it increased median survival when compared with Ara-C.<sup>10</sup>

Imatinib is licensed for first line treatment of CML in patients unsuitable for transplantation, as a second line treatment in patients unresponsive to interferon therapy and has been used to induce remission in the accelerated and blast phases of the disease.<sup>11</sup> It may also be used in the treatment of gastrointestinal stromal tumours, inhibiting the c-kit tyrosine kinase dependent signalling in this cancer.<sup>12</sup>

## **Limitations of Imatinib Therapy**

Although the development of imatinib has significantly improved the outlook for patients diagnosed with CML, there remains two major hurdles to successful therapy.

Firstly, imatinib suppresses CML but does not eradicate all Philadelphia chromosome-positive cells. The persistence of detectable BCR-ABL transcripts in patients treated with imatinib is known as “residual disease.” CML cells which fail to respond to imatinib therapy act as a reservoir for disease.<sup>13</sup> Imatinib therapy suppresses disease and induces remission but does not cure the leukaemia and it remains a life-long treatment.

Secondly, the emergence of resistance to imatinib therapy limits the efficiency of the drug. Resistance to imatinib is common in advanced phases of disease. Patients may relapse during treatment but resistance can occur in previously untreated patients.

Several mechanisms of resistance have been demonstrated, the most common of which is a point mutation in the BCR-ABL gene, impairing binding of the drug to the tyrosine kinase receptor. Mechanisms of resistance include amino acid substitution that changes conformation of the ATP binding site, reactivation of BCR-ABL signal transduction and amplification of BCR-ABL gene.<sup>12</sup>

CML progenitor cells are relatively insensitive to imatinib, using an efflux pump to reduce the intracellular concentration of the drug.<sup>13</sup>

Another possibility is that BCR-ABL may not tell the full story of CML. If this is true, it has significant implications for the development of future tyrosine kinase inhibitors. If the ABL tyrosine kinase is not solely responsible for CML cell survival and proliferation, then no matter how powerful an inhibitor may be, it will still fail to eradicate residual disease. There is some evidence from studies in mouse cell lines to support this idea. Imatinib did not inhibit the growth of mouse myeloid cells in the presence of interleukin-3.<sup>14</sup>

It has been posulated that inhibition of the ABL kinase does not directly stimulate apoptosis, but allows the leukaemic cells to return to their normal cytokine dependent growth and proliferation. Thus, imatinib causes the apoptosis of the excess cells which lack the cytokines to support them.<sup>13</sup>

**Interferon-alfa**

Interferons are glycoproteins produced by cells in response to antigenic stimuli, for example, viral infection or malignancy. IFN $\alpha$  has a range of immunomodulatory effects including activation of cytotoxic T cells. While its precise mechanism of action in CML remains unclear, it has been shown to reduce the survival and inhibit proliferation of CML cells *in vitro*.

IFN $\alpha$  induces haematological remission in the chronic phase of CML. Complete cytogenetic response is achieved in between 20 to 30 percent of patients with the sustained disappearance of CML cells reported in less than 10 percent of those treated.

The combination of IFN $\alpha$  and Ara-C, as compared with IFN $\alpha$  alone, has been shown to increase the rate of major cytogenetic response and prolongs survival in patients with the chronic phase of CML. Guilhot *et al.* demonstrated significant advantages of combination therapy. The survival rate was 85.7 percent with IFN $\alpha$  and Ara-C and 79.1 percent with IFN $\alpha$  alone.<sup>15</sup>

Prior to the introduction of imatinib, IFN $\alpha$  therapy was the treatment of choice in patients unsuitable for transplantation. CML was among the first diseases to be treated with a biological agent. IFN $\alpha$  remains an important tool in the management of the disease.

**Chemotherapeutic Agents**

Hydroxyurea and busulfan are the most commonly used chemotherapeutic agents in the treatment of CML. Both reduce the number of circulating neutrophils and allow patients to avoid the thrombotic complications of a high white cell count. However, neither alters the course of the disease and, as such, are considered palliative treatments. Hydroxyurea may be preferable to busulfan as it has less toxic side effects, particularly in patients who may undergo transplantation.<sup>2</sup>

**Haematopoietic Stem Cell Transplantation**

High-dose chemotherapy with or without total body irradiation followed by allogeneic stem cell transplantation has been shown for many years to be curative in chronic phase of CML.

However, successful transplantation depends on

the availability of suitable donors and the condition of the recipient. Transplantation should ideally occur with a HLA-matched, related donor to a recipient under 40 years of age who has been diagnosed with chronic phase CML within the last year (Table 1). Unfortunately less than 20 percent of patients with CML are suitable for bone marrow transplantation, due to a combination of age and lack of availability of donors.<sup>2</sup>

Transplantation during the chronic phase increases the likelihood of survival. Patients transplanted within one year of diagnosis do best. This suggests

<b>Prognostic Factors for Chronic Myeloid Leukaemia Patients considering Stem Cell Transplantation</b> <small>(European Group for Blood and Marrow Transplantation)</small>	
<b>Disease Phase</b> <ul style="list-style-type: none"> <li>• Chronic</li> <li>• Accelerated</li> <li>• Blast                             <ul style="list-style-type: none"> <li>- Give chemotherapy prior to transplant to achieve second chronic phase of transplant in blast phase or use tyrosine kinase inhibitor</li> </ul> </li> </ul>	
<b>Disease duration</b> <ul style="list-style-type: none"> <li>• Transplant within the first year of diagnosis</li> </ul>	
<b>Age</b> <ul style="list-style-type: none"> <li>• Younger age (&lt;40 years) improves survival</li> </ul>	

Table 1. Prognostic factors for stem cell transplantation.

that CML cells may develop some degree of resistance to treatment even before progression to the accelerated phase. Although different centres have different criteria for eligibility, patients greater than 40 years of age have been shown to suffer a greater incidence of transplantation-related mortality. HLA-matched donors have been shown to reduce some of the risks of stem cell transplantation.<sup>16,17,18</sup>

Stem cell transplantation can cure CML but it carries serious risks of infections and graft-versus-host-disease (GVHD). Therefore, a clinician must balance the prospects of a cure against the morbidity and mortality associated with the treatment.

**Graft-Versus-Leukaemia Effect**

The relationship between the incidence of GVHD, a life-threatening complication of transplantation and the long-term disease free survival of patients are termed the graft-versus-leukaemia (GVL) effect. This correlation illustrates the importance of the immune system in the treatment of CML and may direct future therapies.

The key role of the T cell in GVL is highlighted by a series of transplants performed using T cell depleted marrow, aiming to reduce the incidence of GVHD. Goldman *et al.* demonstrated the probability of relapse was higher for recipients of T cell-depleted bone marrow compared with recipients of non-T cell-depleted bone marrow and for patients who did not develop chronic GVHD compared with patients who did.<sup>19</sup>

T cells are also significant in inducing remission of relapsed CML. Patients who have undergone stem cell transplant and subsequently relapse have been effectively treated by infusion of donor lymphocytes.<sup>20</sup>

### Future Therapies

Finding a way to reduce the danger of the GVHD while retaining the benefits of the GVL is a challenge for the future. One approach has been to remove the T cells for transplantation and reinfuse them at a later date. Another possibility is that two different populations of T cells may mediate the GVHD and GVL effect.

### Vaccination

With the T cell widely acknowledged as playing a vital role in the success of bone marrow transplantation, it is no surprise to find researchers questioning the possibility of a vaccination against the disease. Work is underway to determine if T lymphocytes may be activated specifically against CML cells. Cytotoxic T cells that recognise the PR1 component of proteinase 1 or Wilms Tumour 1 antigen, both over expressed in leukaemia cells, have been shown to kill CML cells *in vitro*.

Trials at the University of Texas of a vaccine for CML based on the PR1 peptide have already met with some success, with half of the subjects remaining disease free three years after receiving the vaccine. The vaccine induces cytotoxic T cells to preferentially kill leukaemic instead of normal progenitor cells.<sup>21</sup>

Researchers at Siena University, Italy have gone further and developed a p210 multi-peptide vaccine against CML. Sixteen patients with stable residual disease who had been treated with imatinib for a minimum of 12 months or interferon alfa therapy for 24 months underwent a course of vaccination. Fifteen of 16 patients demonstrated improved cytogenetic responses, with half of the patients achieving complete cytogenetic remission in three months.<sup>22</sup>

These studies lend support to the argument for the development of a vaccine in addition to existing therapies.

### Further Targets for Molecular Therapy

The second generation of tyrosine kinase inhibitors are also under development. BMS-354825 is a dual inhibitor of SRC and ABL kinase with a 100-fold greater potency than imatinib. It has been shown to inhibit the kinase activity of 14 out of 15 imatinib resistant forms of BCR-ABL.<sup>22</sup>

Other potential therapeutic targets include treatments directed at the downstream targets of BCR-ABL are under development. Inhibitors of heat shock protein 90, a protein that stabilises BCR-ABL and other oncoproteins as well as inhibitors of the RAS and PI3K pathways may offer prospects for future therapies.<sup>12</sup>

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