RESPONSE TO IMATINIB MESYLATE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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CERTIFICATE

This is to certify that Dr M Joseph John has worked under our direct supervision and guidance on his thesis entitled "Response to Imatinib Mesylate in Patients with Chronic Myeloid Leukemia" in the department of Clinical Haematology, Christian Medical College, Vellore, Tamil Nadu. This is his bona fide work during the period from March 2004 to October 2006 as part fulfillment towards the Degree of Doctor of Medicine (Higher specialty) in clinical Haematology towards the examinations to be conducted by the Dr. MGR University in February 2007.

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I thank my Lord for making it all possible.

Dated:

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CONTENTS

INTRODUCTION

It was in the year 1960, Nowell and Hungerford discovered the “minute chromosome”.[1] More than a decade later, Janet rowely’[2] described the presence of t(9;22). This has lead to the evolution in treatment of CML from “carpet bombing” to the modern day targeted therapy, the “magic bullet” – Imatinib Mesylate.

In June 1998, the drug was given to the first human volunteer with CML in the phase I trial [3 4]. Patients were either unresponsive to interferon (IFN), or in advanced phase. In the next three phase II trials, the efficacy of this drug in all the phases of CML were confirmed.[5 6,7]. However, it was the landmark phase III trials that established its present status as the first line drug of choice in the management of newly diagnosed cases of CML. This multicentre prospective trial proved its true virtue with clear superiority in cytogenetic response rates as compared with the “gold standard” of that time; INF administered along with low dose cytosine. [8,9]

Marketed by the Novartis, Switzerland (Gleevec or Glivec) at a prize of approximately Rs 100,000 per month, it was affordable only by the patient of developed countries. In the United States, initially it was approved only for patients with INF unresponsive CML in chronic phase (CP) and in advanced phase. Subsequently, in December 2002, its use in newly diagnosed CML in CP was approved by US-FDA (Food and Drug Administration). Many Indian companies launched Imatinib through reverse engineering (Since January 2003) before the exclusive marketing rights (EMR) were granted to Novartis. Although it was sold at a fraction of cost of Glivec, it was unaffordable to the majority of patients.

CML patients from 60 odd nations benefited when Max foundation (started in memory of Maxmillano from Argentina who died of CML at the age of 17 yrs) supported the developing countries with its generous GIPAP (Glivec International Patient Assistance Program). This

program was initially restricted to patients resistant to INF unresponsive CML-CP or advanced phase disease and subsequently from April 2003, it was opened for first line therapy for CML. With the introduction of this efficient and well run program it is possible for practically every newly diagnosed patient with CML in India to be enrolled and treated with this drug. With the cost of the drug being absorbed by the GIPAP, it has become the choice of front line therapy because of the reduced cost of treatment and proven benefit in this condition. The latter however was based on extrapolation from data generated from the West and it is potentially possible that there could be differences in both the efficacy and toxicity profile of this drug among Indian patients.

This study aims to look into the profile of CML patients in coming to CMC Vellore who are on Imatinib.

REVIEW OF LITERATURE

Chronic myeloid leukemia (myelogenous, myelocytic, granulocytic) leukemia (CML) is a clonal myeloproliferative disorder of a pluripotent hematopoietic stem cell with a specific cytogenetic abnormality, the Philadelphia (Ph+) chromosome. This chromosome results from a balanced translocation between the long arms of chromosomes 9 and 22, resulting in the *bcr/abl* chimeric gene that expresses an abnormal fusion protein with altered tyrosine kinase activity. CML accounts for 7% to 20% of all leukemia and affects an estimated 1 to 2/100,000 persons in the general population.[10] The clinical presentation often includes granulocytosis, marrow hypercellularity and splenomegaly. The natural course of the disease involves three sequential phases (Chronic, accelerated, and blast crisis). Chronic phase can persist for years, but the accelerated phase and blast crises last only for months. Each phase of the disease becomes more resistant to therapy.[11]

History of CML and pathogenesis:

The first description of cases of CML was made by two pathologists, Dr Robert Virchow and Dr John Hughes Bennett in 1845.[12,13] Although a debate ensued as to whose description was first, Virchow publicly acknowledged that Bennett's case report had predated his.[14] These first accounts of CML occurred before staining methods for blood, which were not developed until the late 1800s.

After 115 years of the initial description of the disease, Philadelphia chromosome was described in the seminal paper by Nowell and Hungerford.[1] What ensued was a long history of scientific discoveries that led to an unraveling of the molecular pathogenesis of CML. In 1973, Dr Janet Rowley determined that the shortened chromosome 22, the so-called Philadelphia chromosome, was the product of a reciprocal translocation between the long

arms of chromosomes 9 and 22, t(9:22)(q34;q11).[2] In 1982, by mapping oncogenes to specific chromosomal locations, it was recognized that the c-*ABL* tyrosine kinase, which normally resides on chromosome 9, had been translocated to chromosome 22 in CML patients.[15] Shortly thereafter, Eli Canaani et al showed that a chimeric mRNA called *BCR-ABL* was present in patients with CML that was larger than the normal c-*ABL* mRNA.[16] One year later, Owen Witte and David Baltimore demonstrated that a chimeric, BCR-ABL protein was made and that it possessed tyrosine kinase activity.[17]It was in 1990 that *BCR-ABL* could be put into animal models to demonstrate that *BCR-ABL*, as the sole oncogenic event that induced leukemia, thus establishing *BCR-ABL* as a leukemic oncogene.[18,19]

Cytokinetics

CML develops when a single, pluripotential, haematopoietic stem cell acquires a Ph chromosome carrying the BCR-ABL fusion gene, which confers on its progeny a proliferative advantage over normal haematopoietic elements and thus allows the Ph-positive clone gradually to displace residual haematopoiesis.[20] The proliferative advantage has been postulated to either to constitutive expression by leukemic progenitors of growth factors (G CSF)[21], interleukin-3[22], mitogenic signalling[23] or a defective apoptotic response to stimuli that would have lead to physiologic cell death.[24] The other mechanism implicated in the malignant transformation by *Bcr-Abl* is the altered adhesion to stroma cells and extra cellular matrix.[25]CML cells express adhesion inhibitory variant of β1 integrin that is not found in normal progenitors.[26]

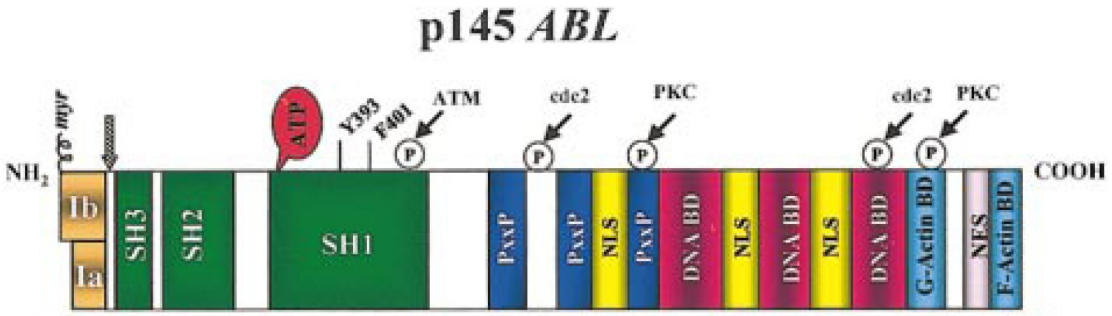
Molecular biology of CML[27]:

ABL

The *ABL* gene is the human homologue of the v-*abl* oncogene carried by the Abelson murine

leukemia virus (A-MuLV), and it encodes a nonreceptor tyrosine kinase. *ABL* gene has 11 exons and spans over 230 kilobases. The breakpoint in the *ABL* gene occurs usually 5' (toward the centromere) of exon 2 of *ABL*. The *ABL* exons 2 to 11 (also called a2 to a11) are transposed into the major breakpoint cluster region (M-bcr) of the *BCR* gene on chromosome 22 between exons 12 and 16 (also referred to as b1 to b5), which extends over 5.8 kb.

Human Abl is a ubiquitously expressed 145-kd protein with 2 isoforms arising from alternative splicing of the first exon. Several structural domains can be defined within the protein (Figure 1). Three SRC homology domains (SH1-SH3) are located toward the NH2 terminus. The SH1 domain carries the tyrosine kinase function, whereas the SH2 and SH3 domains allow for interaction with other proteins. Proline-rich sequences in the center of the molecule can, in turn, interact with SH3 domains of other proteins, such as Crk.13 Toward the 3' end, nuclear localization signals and the DNA-binding and actin-binding motifs are found.



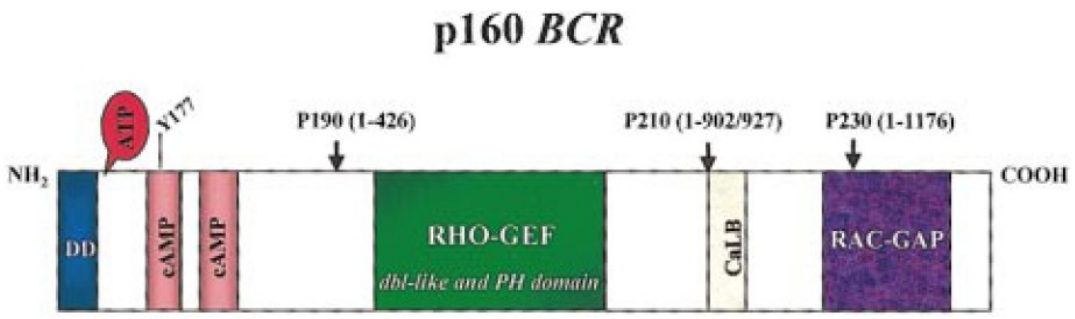
Blood **96**, 3343-56 (2000).

Functionally, Abl protein serves a complex role as a cellular module that integrates signals from various extracellular and intracellular sources and that influences cell cycle and apoptosis. Knock out mice showed neonatal lethality and lymphopenia with a homozygous disruption of the c-abl proto-oncogene in one study[28] and viability with depletion of selected B and T cell populations in another study.[29] The ABL protein is found in both the nucleus and the cytoplasm and can shuttle between these two compartments under the influence of its nuclear-export signal domain, whereas BCR-ABL is exclusively cytoplasmic. Nuclear ABL is

an essentially proapoptotic protein, playing a key part in the cellular response to gentoxic stress. [30]

BCR[27]

The Bcr is a 160-kd protein, like Abl, which is also ubiquitously expressed (23 exons). It has an N terminal exon which encodes a serine–threonine kinase. A coiled–coil domain at the N-terminus of Bcr allows dimer formation in vivo. The center of the molecule contains a region with *dbl*-like and pleckstrin-homology (PH) domains. Bcr can be phosphorylated on several tyrosine residues, especially tyrosine 177, which binds Grb-2, an important adapter molecule involved in the activation of the Ras pathway. However, *BCR* knockout mice are viable and the only recognized defect is increased oxidative burst in neutrophils.[31]



Blood **96**, 3343-56 (2000).

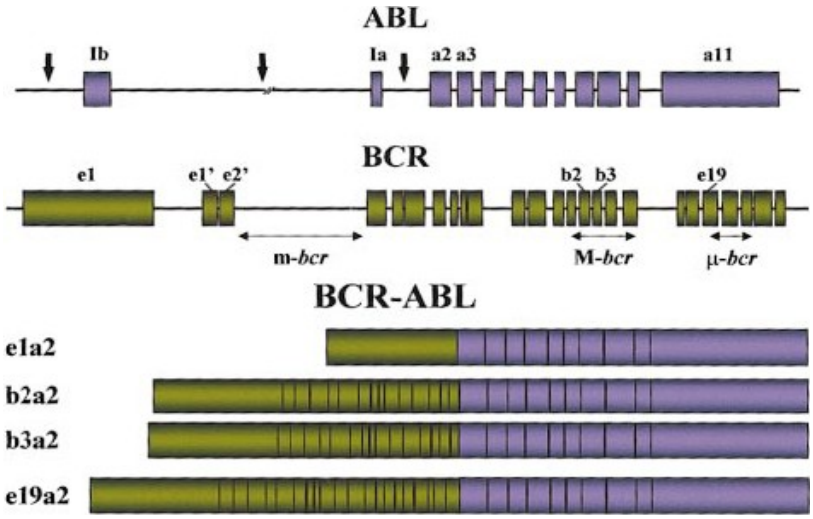
Figure 2: DD – Dimerization Domain or the coiled coil domain at the N terminus of Bcr which allows dimmer formation in vivo. Tyrosine 177 (Y-177) in the BCR portion of the fusion gene is important of the docking of adapter proteins like Grb-2 which has a role in RAS pathway. RHO GEF: GTP-GDP exchange factor which may activate transcription factors such as NF-kB.

BCR-ABL

The breakpoints within the *ABL* gene at 9q34 can occur anywhere over a large (greater than

300 kb) area at its end, upstream of exon 2 (Figure 3) and simultaneously a break occurs in the major breakpoint cluster region of the *BCR* gene. As a result, a 5' portion of BCR and a 3' region of ABL are juxtaposed on a shortened chromosome 22 (the derivative 22q-, or Ph chromosome). Regardless of the exact location of the breakpoint, splicing of the primary hybrid transcript yields an mRNA molecule in which *BCR* sequences are fused to *ABL* exon a2. In contrast to *ABL*, breakpoints within *BCR* localize to 1 of 3 breakpoint cluster regions (*bcr*). In most patients with CML and in approximately one third of patients with Ph-positive acute lymphoblastic leukemia (ALL), the break occurs within a 5.8-kb area spanning *BCR* exons 12-16 (originally referred to as exons b1-b5), defined as the major breakpoint cluster region (M-*bcr*). A *BCR–ABL* fusion gene with a b2a2 (e13a2) or b3a2 (e14a2) junction is created and transcribed into an 8.5-kb mRNA. The fusion mRNA is translated into a chimeric protein of 210 kd called $p210^{BCR–ABL}$.

In the remaining patients with ALL and rarely in patients with CML, the breakpoints are further upstream in the 54.4-kb region between the alternative *BCR* exons e2. (minor breakpoint cluster region (m-*bcr*). The resultant e1a2 mRNA is translated into a 190-kd protein ($P190^{BCR-ABL}$). Recently, a third breakpoint cluster region (m-*bcr*) was identified downstream of exon 19, giving rise to a 230-kd fusion protein ($P230^{BCR-ABL}$) associated with the rare Ph-positive chronic neutrophilic leukemia, though not in all cases.



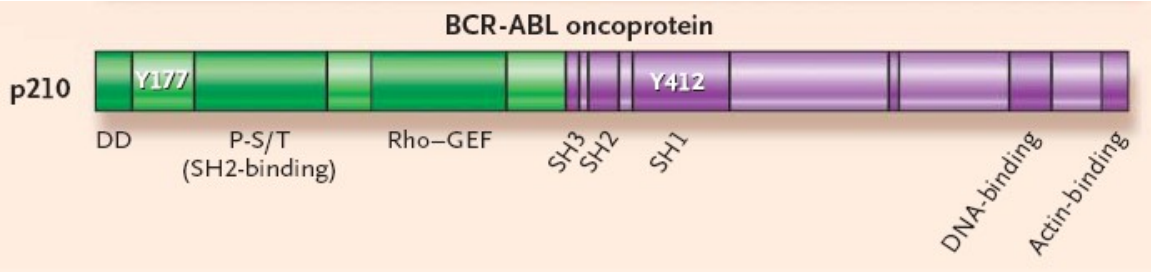
Blood **96**, 3343-56 (2000).

ABL exon 1, even though retained in the genomic fusion, is never part of the chimeric mRNA. Thus, it must be spliced out during processing of the primary mRNA; the mechanism underlying this apparent peculiarity is unknown. Based on the observation that the Abl part in the chimeric protein is almost invariably constant while the Bcr portion varies greatly, one may deduce that Abl is likely to carry the transforming principle whereas the different sizes of the Bcr sequence may dictate the phenotype of the disease.[27]

The leukemogenic potential of $p210^{BCR-ABL}$ resides in the fact that the normally regulated tyrosine kinase activity of the ABL protein is constitutively activated by the juxtaposition of BCR sequences. BCR acts by promoting dimerization of the oncoprotein, such that the two adjacent BCR-ABL molecules phosphorylates each other on tyrosine residues in their kinase-activation loops.[32] The uncontrolled kinase activity of BCR-ABL then up regulates the normal physiological functions of the normal ABL enzyme by interacting with a variety of effector proteins resulting in deregulated cellular proliferation, decreased adherence of leukemia cells to the bone marrow stroma and reduced apoptotic response to mutagenic stimuli.[33]

The tyrosine kinase encoded by the SRC- homology 1 (SH1) domain of the ABL component

of BCR-ABL is most crucial for oncogenic transformation. SH3 domain on the ABL plays a critical role in regulating its tyrosine kinase activity. Fusion of the Bcr sequences to the 5' end of Abl SH3 domain abrogates the physiologic suppression of the kinase. The other important motifs in the ABL portion are the SH2 and C terminal actin binding domains. On autophosphorylation of the protein, SH2 creates binding sites for other adaptor proteins like Crkl and other proteins associated with organization of the cytoskeleton and the cell membrane. Under physiologic conditions, phosphatases regulate the effects of tyrosine kinases; however, its significance in the transformation process is unknown. On the BCR, the coiled-coil motif is responsible for the dimerization of the oncoprotein. A tyrosine at postion 177 (Y177) is crucial for binding of adaptor protein Grb 2 (Growth factor receptor-bound protein 2). The N terminal phosphoserine and phosphothreonine (P-S/T) residues are required for interaction with SH2 containing proteins including ABL. Numerous substrates bind to BCR-ABL and are tyrosine phosphorylated by it resulting in increased mitogenic activity (via Ras, Myc, STAT, PI3K etc) decreased apoptosis (Bcr-Abl may block the release of cytochrome C from the mitochondria and decreased caspases and phosphorylation of pro-apoptotic protein Bad.) and altered adhesion (CML cells express an adhesion inhibitory variant of β integrin).[27,33]



N Engl J Med 2003;349:1451-64.[33]

This phosphorylation of the effector protein requires binding of ATP to transfer the phosphate

group. So any agent which could block the binding of ATP would prevent this transfer of phosphate and prevent conformational change of the substrate protein. This leads to interruption of downstream pathway and interferes with oncogenic signal to the nucleus.

History of treatment of CML:

Fowler's solution (1% solution of Arsenic trioxide) was the mainstay of therapy for all ailments since 1786 and it was being used for the treatment of CML during the late 1800s till the introduction of radiation therapy in 1903.[34] With its introduction, median survival of CML patients increased to approximately 2 ½ years.

An alkylating agent Busulfan was introduced in the 1950s with specific granulocyte series suppression. [35] This drug improved the median survival to 3 ½ - 4 years. In the 1960s, Hydroxyurea (Hydrea) was introduced. Being less toxic than Busulfan and non toxic to stem cells, this became popular. Randomized studies showed prolonged survival over busulfan. In the 60s and in the 80's the median survival prolonged to four and a half years. Both Busulfan and Hydroxyurea remained the mainstay of treatment for the next 35 years. However the onset of blast crisis was not delayed and myeloblastic transformation was typically resistant to chemotherapy. Lymphoblastic crises could be achieved remission with steroids and vincristine.

Interferon was introduced for the treatment of CML in the 1980s. Although side effects were more, the median survival improved to 7 ½ years with interferon and induction of Ph negativity seemed to correlate with a low rate of blastic transformation.[36] Meta-analysis showed that the pooled 5-year survival rate was 57% for recombinant interferon alpha (rIFN-a) and 42% for chemotherapy (Hydroxyurea and Busulfan) (*P* , .0001), which results from a delay in the onset of blast crisis.[37,38]Compared with BUS or HU, the controlled trials suggested that, on average, rIFN-a increased life expectancy by a median of about 20 months. Patients overall have a 50% to 59% probability of being alive 5 years after treatment, which represents an

improvement over the 29% to 44% 5-year survival rate seen with chemotherapy.[39] The French multicentre randomized control trial in 1997 showed further improvement in response with addition of cytosine along with interferon.[40]The combined therapy group had a significantly higher incidence of complete hematologic and cytogenetic remissions (66% *v* 55%, 15% *v* 9%, respectively). The reported 3-year survival rate was 86% in the comibined group and 79% for IFN alone. This improvement of survival was correlated with the achievement of major cytogenetic remission.[40,41]

Allogeneic bone marrow transplantation as a curative therapy for CML was introduced in 1986. [42]Survival rates after sibling donation were at first rather disappointing, but the outlook gradually improved as the importance of the 'antileukemic' effect of T cell activity was appreciated.[43] Allogeneic stem cell transplant is an option in only 40% of CML patients. Long term survival rates Human leucocyte antigen (HLA) matched related bone marrow transplant range from 50% to 75% in patients with chronic phase CML. The major factors influencing survival are patient age, disease phase at the time of stem cell transplant (SCT), disease duration, degree of histocompatibility between donor and recipient and gender of donor.[44] Survival rates after transplantation in accelerated phase approximately 50% lower, and 5 year survival rates are <20% for patients who receive transplants during blast crisis.[45,46] The risk of relapse after allogeneic transplant in CML is about 20%. Many patients who relapse respond to donor leukocyte infusions (adoptive immunotherapy).[47] However, transplant related mortality in CML ranges between 20 – 41% and incidence of grade II-IV acute GVHD between 8% - 63% which is a possible deterrent to survival. The rates for chronic GVHD are 4% to 75%, with 8% to 10% mortality in the next 10 – 20 years.[39] The initial studies showed a survival advantage with BMT over IFN. However, both groups were not comparable with better prognostic markers in the BMT group. Gale et al[48] attempted to control for the differences by comparing the survival of 548 patients from the International Bone Marrow

Transplant Registry with 196 patients who had received rIFN-a or HU in the German RCT.[49] Survival curves were adjusted for different patient characteristics and duration of illness, showing that the percentage of patients surviving was less for BMT patients during the first 18 months of treatment (reflecting early transplant-related mortality), similar between the groups from 18 to 56 months, but significantly better for BMT after 56 months (*P* , .0001). The 7-year probability of survival (and 95% confidence interval) was 58% (50% to 65%) for BMT and 32% (22% to 41%) for rIFN-a/HU, with the survival advantage first becoming statistically significant after 5.5 years. The corresponding rates for patients transplanted within 1 year of diagnosis compared to those treated with rIFN-a/HU were 67% (56% to 75%) and 30% (21% to 40%), respectively, with the survival advantage appearing earlier at 4.8 years. These data supported the view that BMT produced better long-term outcomes, but concerns remained regarding the definitive evidence. Till the year 2003, based on the above findings it was recommended that BMT should preferably be offered to patients within 1 to 2 years of diagnosis to achieve the greatest likelihood of success. Patients with adverse prognostic factors (reflected by a high Sokal score) were offered upfront BMT over rIFN-a. For patients who have had CML for more than 1 year and for those who are considering delaying BMT until more than 1 year from diagnosis, a decision was required of whether the decreased likelihood of benefit justified the risk of transplant. Young patients with CML were advised to undergo BMT if there was a matched related donor.[39]

Era of Imatinib

Imatinib which was earlier called as STI-571 (Signal transduction inhibitor) is a 2-phenlyaminopyridine, is a prototype for signal transduction inhibitors. It is a highly selective inhibitor of the protein kinase family which includes BCR-ABL protein, Platelet derived growth factor receptor (PDGFR) and the c-kit receptor.[50] Imatinib competitively binds to the ATP-binding site of BCR-ABL and inhibits protein tyrosine phosphorylation.[51] Initially this

compound was proven to inhibit BCR-ABL induced tumor formation *in vitro* in immunodeficient mice. In 1996, Druker et al confirmed that STI-571 (present Imatinib) prevented growth of haematopoietic cells that expressed BCR –ABL but did not affect normal cell function.[52]

Pre clinical studies:

From late 1980s, scientists at Ciba Geigy (Now Novartis) had initiated projects on identification of compounds with inhibitory activity against protein kinases. One such lead compound was 2-phenylaminopyrimidine. In 1992 [53], Anafi and colleagues reported a tyrphostin, related to erbstatin, that inhibited the tyrosine kinase activity of BCR-ABL and suggested that it might be possible to design specific compounds for the treatment of ABL-associated human leukemias.

By making a series of modification to the lead compound 2 – phenylaminopyrimidine, it was able to create a drug which specifically bound to BCR-ABL. Later its solubility and oral bioavailability was increased by adding a highly polar side chain. STI571 (formerly CGP57148B, now Imatinib mesylate; Gleevec or Glivec, Novartis, Basel, Switzerland) emerged as the most promising compound for clinical development, since it had the highest selectivity for growth inhibition of BCR-ABL–expressing cells.

Studies using purified enzymes expressed as bacterial fusion proteins or using immuno-precipitations of intact proteins showed that Imatinib potently inhibits all of the ABL tyrosine kinases including cellular ABL(c-ABL), viral ABL (*v*-ABL), and BCR-ABL.[54] The results of the kinase assays were confirmed in cell lines expressing constitutively active forms of ABL such as *v*-ABL, $p210^{BCR-ABL}$, $p185^{BCR-ABL}$ and translocated leukemia (TEL)–ABL, where Imatinib was found to inhibit ABL kinase activity with 50% inhibitory concentration (IC50) values ranging between 0.1 and 0.35μM. [55,56]

In vivo profile of Imatinib: animal models

Once daily intraperitoneal injection of Imatinib (2.5 – 50mg/Kg) in *BCR-ABL* transformed cell lines in syngeneic mice showed a dose-dependent inhibition of tumor growth.[52,57]. Imatinib was also tested in the transduction-transplantation model of CML. In this system, lethally irradiated syngeneic mice receive marrow infected with a BCR-ABL retrovirus and consistently die within 3 weeks from an aggressive CML[58]. Treatment with Imatinib (50 mg/kg in the morning, 100 mg/kg in the evening) led to prolonged survival[59].

Phase I clinical studies

Phase I trials with Imatinib began in June of 1998 and were designed to determine the maximally tolerated dose, with clinical benefit as a secondary endpoint. Patients in the chronic phase of CML who had failed therapy with IFN were eligible[3]. Imatinib, given as daily oral therapy, was well tolerated, without dose limiting toxicity. Hematological responses were seen at doses of 140 mg and greater. Most remarkable was that 53/54 patients treated with at least 300 mg showed complete hematologic responses (CHR). Moreover, at doses of 300 mg and higher, cytogenetic responses were achieved in 31% of patients, which were complete in 13%. Pharmacokinetic studies revealed that above 300 mg, plasma levels equivalent to the effective in vitro level (1μM) were achieved. At 400mg, the current standard dose for CML in chronic phase, peak levels at steady state were approximately 4.6 μM and trough levels approximately 2.13 μM, with a half-life of 19.3 hours, indicating that once-daily dosing was sufficient to provide continuous kinase inhibition[60].

Phase I study was further expanded to patients with myeloid and lymphoid blast crisis of CML and patients with relapsed or refractory Ph-positive ALL[4]. With doses of 300 to 1000 mg/d, 11% of patients with myeloid blast crisis achieved CHR, and another 10% a reduction of bone marrow blasts to <5%, without complete recovery of peripheral blood counts. In patients with

lymphoid disease, the corresponding remission rates were 20% and 15%, respectively. Unfortunately, most patients with myeloid and all but one patient with lymphoid blasts crisis relapsed within weeks to months.

Phase II clinical studies

There were three phase 2 studies which began in the late 1999 using Imatinib as single agent in all phases of CML. Patients in chronic phase who had failed IFN did much better than expected, with rates of CCR of 41% and major cytogenetic remission (MCR) of 60%[5,7]. Importantly, these responses were usually durable, resulting in a progression-free survival of 89.2% at 18 months. The efficacy in patients with accelerated phase was intermediate between chronic phase and blast crisis[6]. And Imatinib in blast crises showed similar responses as in phase 1 study[7]. The results of the phase I and II trials led to the approval by the Food and Drug Administration of Imatinib for the treatment of CML in advanced phase and after failure of IFN

Phase 2 studies [61]

	Overall hematologic response/ CHR (%)	Sustained hematologic responses (> 4 weeks) (%)	MCR (%)	CCR (%)	Median survival (%)
Myeloid blast crisis (n = 229)	52/15	31	16	7	6.8 months
Ph-positive ALL* (%) (n = 56)	52/22	27	NA	NA	4.9 months
Accelerated phase (n = 181)	82/53	69	24	17	Not reached
Chronic phase after failure of IFN	95% sustained CR	95% sustained CR	60	41	Not reached

*Also lymphoid blast crisis of CML. CHR – complete hematologic response; MCR – major cytogenetic response; CCR – complete cytogenetic response

Phase III clinical studies

Imatinib and the combination of IFN plus cytarabine were compared in a randomized trial, (IRIS - The International Randomized Study of Interferon and STI571) which showed Imatinib to be vastly superior with respect to CHR, MCR and CCR as well as progression free survival[8,62].

Responses to Imatinib vs. IFN plus cytarabine in newly diagnosed CML patients in chronic phase[8]

	CHR	MCR	CCR	PFS 14 months
Imatinib (%) (n = 553)	95.3	85.2	73.8	92.1
IFN+ Cytarabine (%) (n = 553)	55.5	22.1	8.5	73.5
p =	.0001	0.001	0.001	.001

A recent update of the same group of patients with a median follow-up of 54-months showed that, 72% of the 553 randomized pts remain on initial IM treatment (5% of pts discontinued due to adverse events, 9.5% due to unsatisfactory therapeutic effect and 11% due to other reasons another 2.5% crossed over to IFN+Ara-C). Overall, the cumulative best response rates of CHR, MCyR and CCyR are 97%, 88% and 82%, respectively. The overall estimated survival was 90% (93% when censored at bone marrow transplant). An estimated 84% of pts have not progressed on treatment and 93% of pts were free from progression to AP/BC. The annual rate of progression to AP/BC of < 1% in the fourth year was lower than each of the first three years (1.5, 2.8, and 1.6%, respectively). Of the pts with MCyR at 12 months (n=436), an

estimated 96% were free of progression to AP/BC at 54 months whereas it was only 81% for the 73 pts who did not achieve a MCyR at 12 months ($p< 0.001$). No patient with a MMR within 12 months progressed to AP/BC within 54 months.[63]

Adverse effects of Imatinib

Haematological toxicity

In the phase III trial with Imatinib therapy in newly diagnosed CML patients, Neutropenia, thrombocytopenia and anemia of all grades occurred in 60.8%, 56.6% and 44.6% patients respectively. However, grades 3 and 4 toxicities in 25%, 16.5% and 4.3% respectively.[62]

It is unclear why some patients develop myelosuppression during therapy. Along with its potent inhibitory effect on Bcr/Abl, Imatinib also inhibits c-kit, which is involved in early hematopoiesis.[52] Thus, myelosuppression may be the result of undesired suppression of normal progenitors.[64] Some reports suggest that Imatinib may impair the colony forming capacity of CD34 positive cells.[65] However, concentrations of Imatinib similar to those achieved in vivo have very minimal effect on colony formation of normal progenitor cells in vitro.[52] Furthermore, in patients without hematologic diseases (e.g., gastrointestinal stromal tumors) who are treated with Imatinib, hematological toxicity is far less common (5% develop neutropenia >/=Grade 3); it is manifested almost

exclusively by neutropenia and is seen mostly at higher doses.[64]

Non haematological toxicity

The most common side effects noted in the phase III trial were superficial edema, nausea, muscle cramps, and rashes. There were only rare occurrences of grade 3 or 4 events.

Table 4 Non Haematological toxicity profile in the IRIS study.[8]

Non Haematological toxicity	All grades %	Grade 3 or 4 %
Superficial oedema	55.5	0.9
Nausea	43.7	0.7
Muscle cramps	38.5	1.3
Musculoskeletal pain	36.5	2.7
Rash	33.9	2.0
Fatigue	34.5	1.1
Diarrhoea	32.8	1.8
Head ache	31.2	0.4
Joint pain	28.3	2.4
Myalgia	21.4	1.5
Dyspepsia	16.2	0
Weight gain	13.4	0.9

Cutaneous reactions to imatinib are common and occur in 9.5% to 69% of patients depending on the series reported.[66] Hypopigmentation was not documented in the initial phases of trial. However, subsequently the same was noted especially in the dark skinned patients. Many case reports are available especially in the African-American[67] and an Indian study has mentioned 65% incidence of generalized hypopigmentation in the patients treated.[68] Many patients noticed pigmentation changes within one month of initiation of treatment and persisted throughout the course of therapy.

Several lines of evidence have previously reported that KIT and its ligand stem cell factor (SCF) play a regulatory role in melanocyte development and survival, suggesting a rational mechanism of action for Imatinib Mesylate in the pathogenesis of hypopigmentation.[69] This is supported by the observation that human mutations in the encoded tyrosine kinase region of KIT have been shown to cause piebaldism, an autosomal dominant disorder characterized by white hair and hypopigmented skin patches on the forehead, torso, and extremities.[70]

Of late, Imatinib has been shown to induce cardiac toxicity in the form of congestive cardiac failure secondary to mitochondrial injury.[71]

Pregnancy and Imatinib

The information on the potential effect of Imatinib on developing infant is limited. However, many young patients frequently face the dilemma of conception and pregnancy while receiving Imatinib. Patriticia et al reported for the first time a series of patients with such experience. Nineteen pregnancies involving 18 patients (10 females and eight males) who conceived while receiving Imatinib for the treatment of CML were followed up. All female patients discontinued therapy immediately on recognition of pregnancy. Three pregnancies (involving two female patients and one male patient) ended in spontaneous abortion, and one patient had an elective abortion. All other pregnancies were uneventful. Two of the 16 babies had minor abnormalities at or shortly after birth (hypospadias in one baby and rotation of small intestine in one baby) that were surgically repaired. All babies have continued normal growth and development. Among female patients who interrupted therapy, five of nine in complete hematologic remission (CHR) at the time of treatment interruption eventually lost CHR, and six experienced an increase in Philadelphia chromosome–positive metaphases. At a median of 18 months after resuming therapy with Imatinib, eight patients had a cytogenetic response (complete in three patients).[72]

Although there is no evidence that a brief exposure to Imatinib during conception and pregnancy adversely affects the developing fetus, most patients lose their response after treatment interruption. Patients receiving Imatinib should be advised to practice adequate contraception.

Monitoring of CML patients in the era of Imatinib

Cytogenetic analysis has been the mainstay of disease monitoring in CML. Response criteria based on the percentage of Ph-positive cells in the bone marrow were established for patients on interferon alpha. They have proved to be good predictors of long term response.[73] This has been used for treatment monitoring in the treatment with Glivec also, which correlated with

the progression free survival and overall survival. Although bone marrow cytogenetics can also detect clonal evolution and other chromosomal abnormalities and fibrosis apart from t (9; 22), it has its limitations in the form of repeated invasive bone marrow aspirations and possible failure to capture adequate metaphases.

Peripheral blood Fluorescent in situ hybridization (FISH) for t (9; 22) in the interphase cells has been evaluated as an alternative to bone marrow cytogenetics. Several studies done earlier proved to be of limited sensitivity with false positivity of upto 10% using single fusion probes.[74] With the availability of including either a second fusion signal on the derivative chromosome 9 or a split of the *ABL* probe, the sensitivity increased to 1%.[75] Steven et al has showed that interface FISH agreed with conventional cytogenetics on bone marrow and interface FISH on bone marrow and peripheral blood specimens showed strong correlation between the two specimens. There are other studies showing correlation between peripheral blood FISH and bone marrow cytogenetics. They have however, cautioned its role at diagnosis and monitoring to entirely replace conventional cytogenetics.[76] Though not widely used, some centers are using peripheral blood FISH to follow up cytogenetic response to Imatinib therapy. Its advantage remains at the early availability (24 hours) of results and avoiding a painful procedure to the patient for frequent monitoring.

Over the past 12 years, several groups have developed quantitative RTPCR assays to measure BCR–ABL transcript levels in the blood and marrow that enabled the dynamics of residual disease to be monitored over time and has provided a viable alternative for disease monitoring.[73,77] The transcript level correlates with the number of leukemic cells present in the blood and marrow and can be used as an accurate barometer of the response to therapy.[78] The clinical usefulness of *BCR-ABL* quantitation by RQ-PCR has been demonstrated by showing a strong correlation between the percentage of Ph positive

metaphases in the bone marrow and simultaneous study of peripheral blood *BCR-ABL* levels measured by RQ-PCR.[79]

Status Imatinib for CML in India

Although Imatinib Mesylate (Glivec) was approved by the FDA for newly diagnosed CML in December 2002, cost was a major deterrent for many patients in India. Four months after the approval, from April 2003, Max foundation through GIPAP (Glivec International Patient Assistance Program) absorbed the cost of the drug and it was made available free of cost to the patients of developing countries.

There has been 2 major studies from AIIMS[80] and Tata Memorial hospital, Mumbai[68]. In the former study 118 patients were analyzed (79 in CP, 23 in AP and 16 in BT) and 96% patients achieved complete haematological remission (CHR) and 30% achieved major cytogenetic remission. In the advanced phase (AP and BT), the results were 35% and 20% respectively. In the second study done from TATA memorial hospital, Mumbai, 174 patients were studied. Of them, 97 were in chronic phase, 47 in accelerated phase and 30 patients in blast crisis. Among the patients in chronic phase, 50.5% achieved a major cytogenetic response, and 21.3%, 23.3% in accelerated phase and blast crisis respectively.

AIM OF THE STUDY

To assess the response to Imatinib Mesylate in patients with chronic myeloid leukemia in chronic and advanced phase

PATIENTS AND METHODS

This was a retrospective and prospective study of 243 patients who came to the department of haematology, CMC Vellore starting from January 2002 to December 2005.

Inclusion criteria:

1. Newly diagnosed Ph positive CML patients.*
2. Interferon unresponsive CML patients.
3. Patients in accelerated or blast phase of chronic myeloid leukemia

*All patients were either peripheral blood or bone marrow FISH (Fluorescent in situ hybridization) positive or bone marrow cytogenetics positive for t (9; 22). In the absence of the above criteria, patient should be RT PCR positive for *BCR/ABL*.

Exclusion criteria: Patients with Philadelphia negative myeloproliferative disorders.

Complete history and physical examination were performed on all patients at diagnosis and spleen size was documented. If clinical findings and complete blood profile are suggestive of CML, then peripheral blood samples were sent for t (9; 22) (FISH) for confirmation of diagnosis. In rare situations if FISH was negative RT PCR was done. Standard criteria for the diagnoses of chronic phase, accelerated phase, and blast crisis were used.[81]

Qualification requirements for GIPAP (Glivec International patient Assistance Programme)

1. Patient should be properly diagnosed by a physician qualified to diagnose, treat and regularly monitor patients with Philadelphia chromosome-positive (Ph+) chronic myeloid leukemia (CML) and/or Kit (CD117) positive unresectable (inoperable) and/or metastatic malignant gastrointestinal stromal tumours (GIST)
2. Patient should meet the inclusion criteria.
3. The patient's physician must follow treatment guidelines outlined in the Glivec® package leaflet and then supply progress information (Patient response every 90 days)

Financial qualifications:

1. Patient should not be insured.
2. Patient is not reimbursed by any other source.
3. Patient has no other financial resources. (Cannot pay for it privately)

Other qualifications.

1. The patient's country is a specified qualifying country for GIPAP.
2. The patient's physician and clinic must be qualified, and be granted approval by The Max Foundation to participate in GIPAP.
3. The country must have drug approval for CML and /or GIST.
4. Generic Glivec® (Imatinib) is not available in that country.

Dose of Imatinib:

Patients in chronic phase were initiated at a dose of 400mg once a day and for advanced phase at 600mg in two divided doses. Dose reductions were done as per the recommendations[82]

CRITERIA FOR DIAGNOSIS[81]

CHRONIC PHASE: (All 5 criteria must be fulfilled)

1. <15% blasts in PB and BM
2. <30% blasts + promyelocytes in PB and BM
3. <20% basophils in PB
4. >/= 100,000 x 10^9/L platelets
5. No extramedullary involvement other than spleen or liver.

   (In general, patient shouldn't have any features of accelerated or blast phase)

ACCELERATED PHASE

	MDACC[5]	IBMTR	WHO[83]
Blasts % in PB and/or of nucleated bone marrow cells	>15%	≥ 10%	10 -19 %
Blasts + Promyelocytes	≥ 30% (but < 30% blasts alone in PB/BM)	≥ 20%	NA
Basophils	≥20 %	≥ 20% (Basophils + Eosinophils)	≥ 20%
Platelets x 10^9	<100 x 10^9/L	Unresponsive increase or persistent decrease	<100 x 10^9 or >1000 x 10^9/L
Cytogenetics	CE	CE	CE
WBC x 10^9	NA	Difficult to control or doubling of counts <5d	Unresponsive to treatment
Anemia	NA	Unresponsive	NA
Splenomegaly	NA	Increasing	Increasing
Others	NA	Chloromas/ Myelofibrosis	Megakaryocyte proliferation, fibrosis

MDACC- MD Anderson Cancer Centre (In the Imatinib era this criterion is followed)
IBMTR – International Bone Marrow Transplant Registry
CE – Clonal Evolution

BLAST CRISIS

MDACC criteria[84]

These two evaluations take preference over chronic and accelerated phase results.

1. ≥ 30% blasts in peripheral blood or bone marrow.
2. Extramedullary involvement other than spleen or liver

IBMTR criteria

30% blasts plus promyelocytes in the blood or bone marrow

WHO criteria[83]

1. Blasts ≥ 20% of peripheral blood white cells or of nucleated bone marrow cells.
2. Extramedullary blast proliferation
3. Large foci or clusters of blasts in the bone marrow biopsy.

PROGNOSIS

Hasford score[85] was calculated in patients with chronic phase using following parameters

1. Age 2. Spleen Size 3. Blasts

4. Basophils 5. Eosinophils 6. Platelets

The score was calculated using the site

http:// www.pharmacoepi.de/cmlscore.html

Low Risk = ≤ 780 (corresponds to median survival 100months)

Intermediate Risk =780-1480 (corresponds to median survival 69 months)

High Risk = ≥1480 (Corresponds to median survival 45 months)

RESPONSE CRITERIA

HEMATOLOGICAL RESPONSE

COMPLETE HEMATOLOGICAL REMISSION[3]:

1. Wbc <10,000, Platelets - <4,50,000
2. No immature cells in perepheral blood, myelocytes, promyelocytes, or blasts

   (Maintained for 4 weeks)

PARTIAL HEMATOLOGICAL RESPONSE

1. Same as complete except, persistence of immature cells
2. Platelets <50% of pre-treatment levels, but >4.5 lakhs
3. Persistance of splenomegaly but <50% of pre-treatment size.

Cytogenetic response[3]

COMPLETE – 0% Ph POS

PARTIAL – 1 – 35%

MAJOR - ≤ 35% (COMPLETE + PARTIAL)

MINOR – 36 – 65 %

MINIMAL – 66 – 95%

NONE - >95%

% of Philadelphia chromosome counted in bone marrow after seeing a minimum of 20 metaphases.

MOLECULAR RESPONSE[9]

Complete molecular remission: >/= 4.5log reduction from base line or undetectable levels.

Major molecular remission: >/= 3 log reduction below the base line or less than 0.045% or <0.05%

(the base line can be calculated by the median value of 30 samples collected from

patients with newly diagnosed chronic phase CML who are untreated or the patient's base line value)

PRIMARY (intrinsic) RESISTANCE[86]

1. Failure to achieve complete hematological remission by 3 months- Primary Hematological Resistance (PHR)
2. Failure to achieve any cytogenetic response by 6 months – Primary cytogenetic resistance (PCR).
3. Failure to achieve MCR by 12 months
4. Failure to achieve CCR by 18 months

Acquired resistance (Relapse)

1. Sustained complete hematological remission followed by transformation to AP or BT.
2. Loss of sustained CHR without transformation*.
3. Loss of MCR**
4. Loss of CCR with a corresponding increase in BCR-ABL levels of at least 1 log.

*Loss of hematologic remission

1. WBC >20,000
2. Platelet >6,00,000
3. Extra medullary disease
4. >5% Myelo + MM
5. Appearance of blasts or promyelocytes in PB

(Done in 2 diff samples 1 month apart)

**Loss of cytogenetic response

Increase in Ph pos cells by 30% in 2 cytogenetic analysis performed 1 month apart.

Or increase to >65%

Toxicity

Toxicities encountered during therapy were graded as per the National Cancer Institute criteria and dose modifications were done as per the toxicity and hematological and cytogenetic responses.[87]

Monitoring

Patients were monitored by weekly counts for the first month and fortnightly if possible thereafter till patient achieved hematological remission and then monthly either in CMC or in their home town as per convenience of the patient. Patients were advised to undergo peripheral blood FISH was done a second time (2^{nd} FISH) between 6 months to 1 year and every 6 months till they achieved CCR. After achieving CCR, they were monitored by RQ PCR.

Peripheral blood fluorescent in situ hybridization (FISH) was performed using LSI BCR/ABL Dual color, Dual fusion *BCR-ABL* translocation probe from Vysis (Vysis Inc. Dowers Grove, IL). It is a mixture of the LSI BCR probe labeled with SpectrumGreen™ and the LSI ABL probe labeled with SpectrumOrange™. The spanning ABL probe has a genomic target of approximately 650 kb extending from an area centromeric of the argininosuccinate synthetase gene (ASS) to well telomeric of the last ABL exon. The BCR probe target spans a genomic distance of about 1.5Mb. The probe begins within the variable segments of the immunoglobulin lambda light chain locus (IGLV), extends along chromosome 22 through the BCR gene, and ends at a point approximately 900 kb telomeric of BCR. A region of about 300 kb containing low-copy number repeats has been eliminated from the probe which introduces a gap in the coverage of the probe target. Both probes span their respective breakpoints.

Interpretation of results: A nucleus lacking the t(9;22) translocation will exhibit the two orange,

two green (2O2G) signal pattern. In a nucleus containing a simple balanced t(9;22), one orange, and one green signal from the normal 9 and 22 chromosomes and two orange/green (yellow) fusion signals, one each from the derivative 9 and 22 chromosomes, will be observed (1O1G2F). In some instances, deletions may occur 3' of the BCR breakpoint and /or 5' of the ABL breakpoint resulting in either an ES (Extra orange or green) signal pattern or a single fusion pattern. These probes allow a detection of residual disease at the 1 – 2 % level.[88] So A value of <2% by peripheral blood FISH was taken as complete cytogenetic response and the rest of the responses were taken as per the cytogenetic criteria.

Statistical analysis

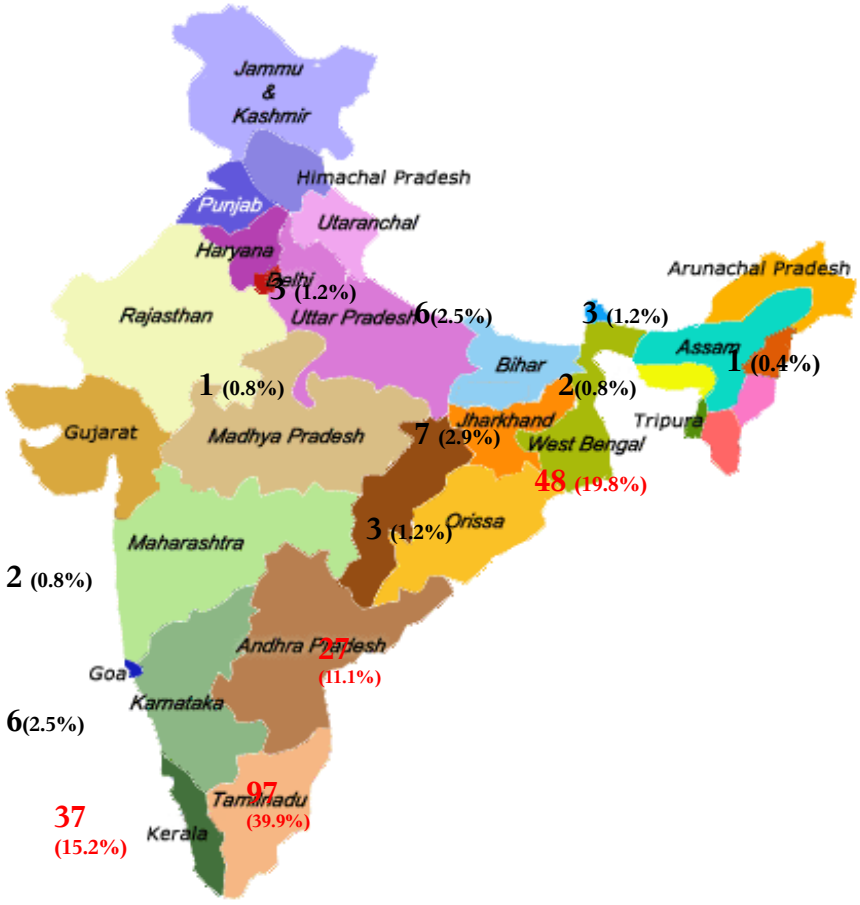
Chi square test was used for discrete variables to find out the statistical difference between the two groups. The probability of survival was estimated with the use of the product limit method of Kaplan Meier with death (any cause defined as the event). The probabilities between groups were compared with the use of the Log rank statistics. For all tests, a 2 sided 'p' value of 0.05 or less was considered statistically significant. SPSS version 11 was used for analysis.

RESULTS

Between January 2002 and December 2005, a total of 243 patients who were diagnosed to have chronic myeloid leukemia (CML) were retrospectively and prospectively analyzed. All these patients were enrolled in the GIPAP (Glivec International Patient Assistance Program) at Christian Medical College, Vellore. Diagnosis of CML was established by peripheral blood FISH for *bcr/abl* translocation or peripheral blood RT PCR for bcr/abl transcripts.

Regional distribution of patients: Figure 1

Patients belonged to 14 different states. The majority came from Tamil Nadu (40%), West Bengal (20%) and the neighboring states of Kerala (15%) and Andhra Pradesh (11%).(Figure – 1)



Base line characteristics (Table -1)

Of the total 243 patients, there were 160 males and 83 females. The M: F ratio was 2:1 and the mean age was 38 years. (Range 7 -74 years). Nine of them were children less than 15 years of age (Range 7 – 15 years).

Table - 1 Base line characteristics

Characteristic	Chronic phase n (%)	Accelerated phase n (%)	Blast crisis n (%)	Total n (%)
Number of patients	226 (93)	2 (0.8)	15 (6.2)	243
Sex				
Male	151 (67)		9 (60)	160 (66)
Female	75 (33)	2(100)	6 (40)	83 (34)
Age (yrs)				
Median (range)	38 (7 – 74)	46 (42 – 50)	37 (11 – 58)	38 (7 – 74)
WBC at diagnosis/cumm				
Median (range)	172500 (50900 – 780000)	3800 (3800 – 31300)	149325 (9100 – 592000)	202541 (3800 – 780000)
Spleen cm				
Mean (range)	9 (0 – 28)	7	15 (2 – 25)	9 (0-28)
Hasford score at diagnosis				
Low risk	72 (32)	NA	NA	
Intermediate risk	66(29)			
High risk	25(11)			
CML phase prior to starting Glivec	**202 (83)** **< 1 year 147 (73)** **>1 year 55 (27)**	**13 (5.3)**	**28 (11.5)**	**243**

At diagnosis of the disease, 226 (93%) were in chronic phase (CP), 2 (0.8%) in accelerated phase (AP) and 15 (6.2%) had progressed to blast transformation (BT). This data is largely based on diagnosis made elsewhere at presentation and subsequent referral to this institution for treatment with conventional drugs. At the initiation of Glivec, 202 (83%), 13 (5.3%) and 28(11.5%) were in CP, AP and BT, respectively. Of the patients in CP, 147 (73%) were initiated on Glivec within 1 year of diagnosis (early CP) and 55 (27%) after 1 year of diagnosis

(late CP). Hasford score was calculated at diagnosis and 72(32%), 66(29%) and 25 (11%) were found to be in low, intermediate and high risk, respectively.

Treatment received prior to starting Glivec in chronic phase

Majority of the patients received Hydroxyurea and Busulfan prior to initiation of Glivec. Twenty three patients had received Imatinib of other manufacturers before starting Glivec. The median duration of treatment prior to starting Glivec was 2 months ranging from 1 – 180 months. (Table – 2)

Table – 2 Treatment prior to Glivec

Drug	Frequency(%)
Hydroxyurea	223 (92)
Busulfan	11(4.5)
Interferon	4 (1.6)
Veenat/Zoleta	23 (9.5)
Others VCR/Pred – 5 Cytosine - 2	7 (2.8)

Median duration of treatment given prior to starting Glivec- 2 months (1 – 180)

Overall response to Imatinib

Although 202 patients in CP, 13 patients in AP and 28 patients in BT were enrolled at the time of diagnosis, only 182 patients in the chronic phase (136 in <1 yr group, 46 in >1 yr group) were evaluable at 3 months to assess haematological response as others were lost to follow up before 3 months. (Table – 3)

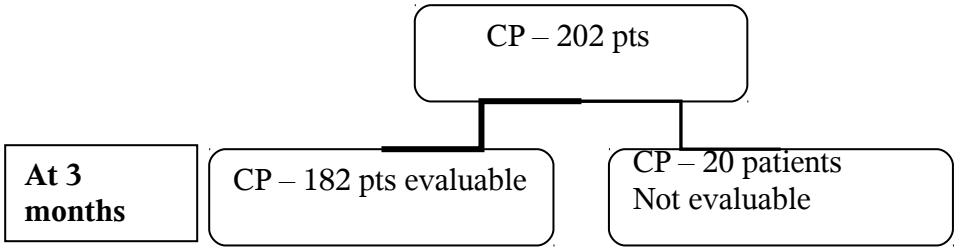


Table - 3

Phase of CML	Chronic phase			Acclerated phase	Blast crisis
	Early and late chronic phase combined	CP <1yr (Early phase) from diagnosis N/total	CP >1 yr (late phase) from diagnosis N/total/		
CHR at 3 months (%)	165/182 (91)	125/136 (92)	40/46 (87)	9/13 (69)	15/28 (54)
MCR (%) Median time range	82/117 (70)	69/89 (Partial CR 19) (77.5) 8 months (3 – 34)	13/28 (Partial CR-3) (46.4) 10 months (3 – 18)	2/6 (Partial CR-0) (33.3) 19 months 19	2/7(Partial CR-1) (28.5) 8.5 months 6-11
CCR (%) Median time range	60/117 (51.2)	50/89 (56) 10 months (4 – 40)	10/28 (35.7) 13 months (6 – 30)	2/6 (33.3) 19 months 19	1/7 (14.2) 6 months 6
MMR (%) Median time range	4/30 (13)	3/22 (13.6 of evaluable CCR pts) 24 months 15 - 40	1/8 (12.5) 20 months	-	-
CMR	1/22	1/22	-	-	-

(%) Median time range	(4.5)	(4.5) 18 18			

At 3 months, complete haematological response (CHR) were noted in 91% of the patients in chronic phase, 69% of patients in accelerated phase and 54% of patients in blast crisis. Of the patients in chronic phase, 92% CHR was observed in early chronic phase and 87% in late chronic phase. Seventy percent patients in chronic phase achieved major cytogenetic response, (77.5% in early CP and 46.4% in late CP) 33.3% in accelerated phase and 28.5% in blast transformation. Complete cytogenetic response was achieved in 51.2% of chronic phase patients (56% in early CP and 35.7% in late CP) but only 33.3% and 14.2% among accelerated phase and blast transformation respectively. The difference between the early and late chronic phase response for MCR alone was statistically significant. (PHR p value = 0.16, MCR- p Value = 0.001 , CCR - p value = 0.12)

Molecular response was evaluated in 30 patients who achieved CCR in the chronic phase and 13% were noted to have major molecular response (MMR). Only one patient achieved complete molecular response (CMR).

Resistance to Imatinib[89]

Primary Haematological resistance (PHR) was noted in 9% of patients in chronic phase (8% in early chronic phase and 13% in late chronic phase: p value – 0.23). It was 23% and 50% in patients with AP and BT respectively. Primary cytogenetic resistance was noted in 12.5% of early chronic phase patients and 46% of late chronic phase patients. This difference was statistically significant. (p value <0.001). In the AP and BT groups, primary cytogenetic resistance was noted in 50% and 57% patients respectively (Cytogenetic response status could be evaluated only in 6 patients in AP and 7 patients in BT) (Table – 4)

Table – 4 Resistance to Imatinib

	Chronic phase			AP	BT
	Overall CP	CP <1 yr	CP >1 yr		
PHR	17/182 (9.3)	11/136 (8)	6/46 (13)	3/13 (23)	14/28 (50)
PCR	21/98 (21.4)	9/72 (12.5)	12/26 (46)	3/6 (50)	4/7 (57)

PHR- Primary haematological resistance, PCR- Primary cytogenetic resistance

Evaluation of cytogenetic responses in patients of chronic phase.

A total of 57% of patients underwent peripheral blood FISH ((t (9; 22)) examination as part of cytogenetic evaluation. 72% of the patients underwent 2nd peripheral blood FISH ((t (9; 22)) examination between 3 and 12 months. (Table – 5)

Table – 5 Cytogenetic evaluation at various time intervals

Months in category	MCR		Min CR		CCR		PCR	
	<1 yr	>1yr	<1 yr	>1 yr	<1 yr	>1yr	<1 yr	>1 yr
3 – 12 (%)	54/75 (72)	9/20 (45)	19/75 (25.3)	7/20 (35)	37/75 (49)	3/20 (15)	2/75 (2.6)	4/20 (20)
13 – 18 (%)	6/8 (75)	8/15 (53)	Nil	Nil	5/8 (52.5)	3/7 (43)	2/8 (25)	4/7 (57)
19 – 24	3/4	4/7	Nil	2/3	3/4	Nil	¼ (25)	1/3

(%)	(75)	(57)		(66.7)	(75)			(33.3)
>24 (%)	2/3 (66.7)	3/6 (50)	Nil	1/3 (33)	Nil	1/3 (33.3)	1/3 (33.3)	Nil

MCR – Major cytogenetic response, Min CR- Minimal cytogenetic response, CCR- complete cytogenetic response, PCR- Primary cytogenetic resistance.

Cytogenetic response status at specific time intervals in patients started on Glivec in Chronic phase

At 6 months Partial cytogenetic response was observed in 24% patients and it was 20.5%, 19.2%, 18% and 19% at 1 year, 1 ½ years, 2 years and >2 years respectively. Complete cytogenetic response was observed in 50%, 50.6%, 53%, 53% and 51.2% at 6 months, 1 year, 1 ½ years, 2 years and >2 years respectively. Major cytogenetic response was observed in 73%, 71%, 72.2%, 71% and 70% at the same intervals. Minor and minimal cytogenetic responses were 26%, 29%, 28%, 29% and 30% also at the same intervals. Differences between any of these responses were not statistically significant. Molecular responses were evaluated in 30 chronic phase patients who achieved CCR and major molecular response was observed in 13% of patients and complete molecular response in 4.5% of patients.

Table – 6 Cytogenetic response status at specific time intervals in CP patients

	6months N / evaluable pts (%)	1 yr N / evaluable pts (%)	1 ½ yr N / evaluable pts (%)	2 yrs N / evaluable pts (%)	>2 yrswe N / evaluable pts	P value
Partial cytogenetic response	10/42 (24)	17/83 (20.5)	20/104 (19.2)	20/111 (18)	22/117 (19)	0.94
Complete cytogenetic response	21/42 (50)	42/83 (50.6)	55/104 (53)	59/111 (53)	60/117 (51.2)	0.99
Major cytogenetic response (Partial CR + CCR)	31/42 (73)	59/83 (71)	75/104 (72.2)	79/111 (71)	82/117 (70)	0.99
Minor and Minimal Cytogenetic Response (Min CR)	11/42 (26)	24/83 (29)	29/104 (28)	32/111 (29)	35/117 (30)	0.99
Major Molecular Response	-	-	-	3/22 (13)		
CompleteMolecular Response	-	-	-	1/22 (4.5)		

Figure – 2 Overall survival from after starting Glivec in chronic phase (CP), accelerated phase (AP) and blast transformation (BT)

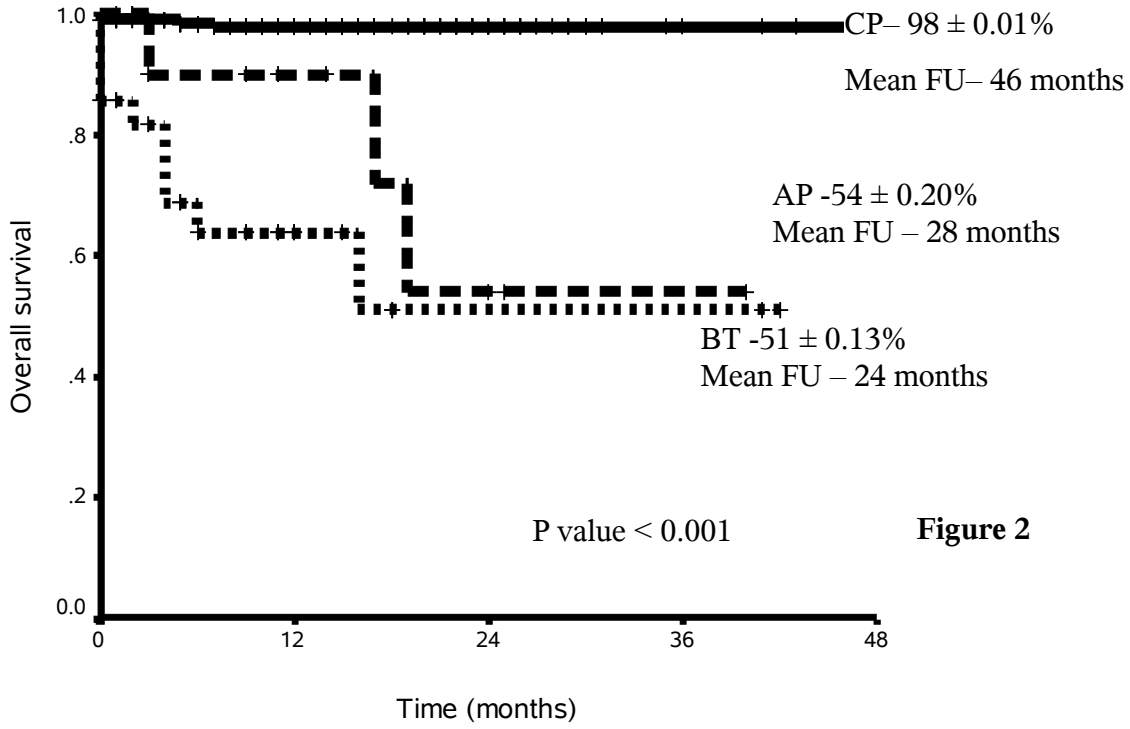


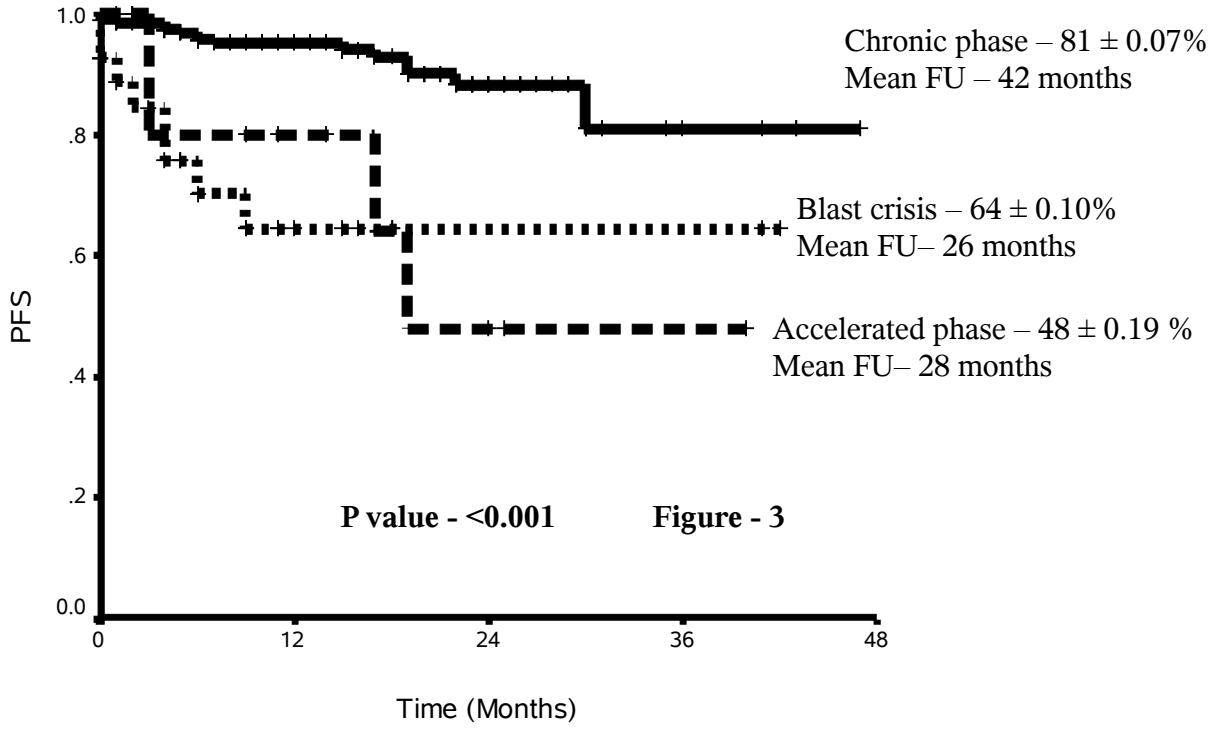
Figure 2

At a mean follow up of 46 months (1 – 47 months), the overall survival in chronic phase was 98 ± 0.01%. For accelerated phase, the OS was 54 ± 0.20% at a mean follow up of 28 months (1 – 40 months) and for blast crisis it was 51 ± 0.13% at a mean follow up of 24 months (1 – 42 months).(Figure 2)

Progression free survival (PFS) after starting Glivec in various phases

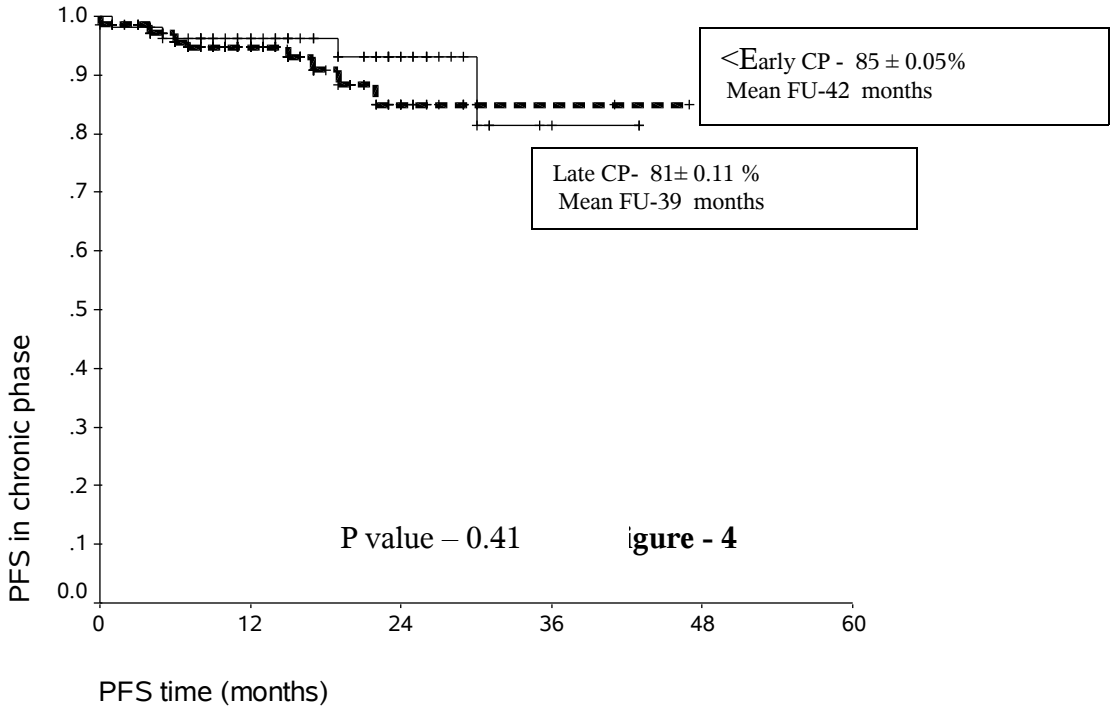
Figure – 3

The PFS in CP, AP and BT were 81 ± 0.07%, 64 ± 0.10%, and 48 ± 0.19% at mean follow up periods of 42 months (1 – 47months) , 28 months(1 – 40 months) and 26 months (0 – 42 months) respectively. (Figure 3)



Progression free survival in chronic phase CML – Figure 4

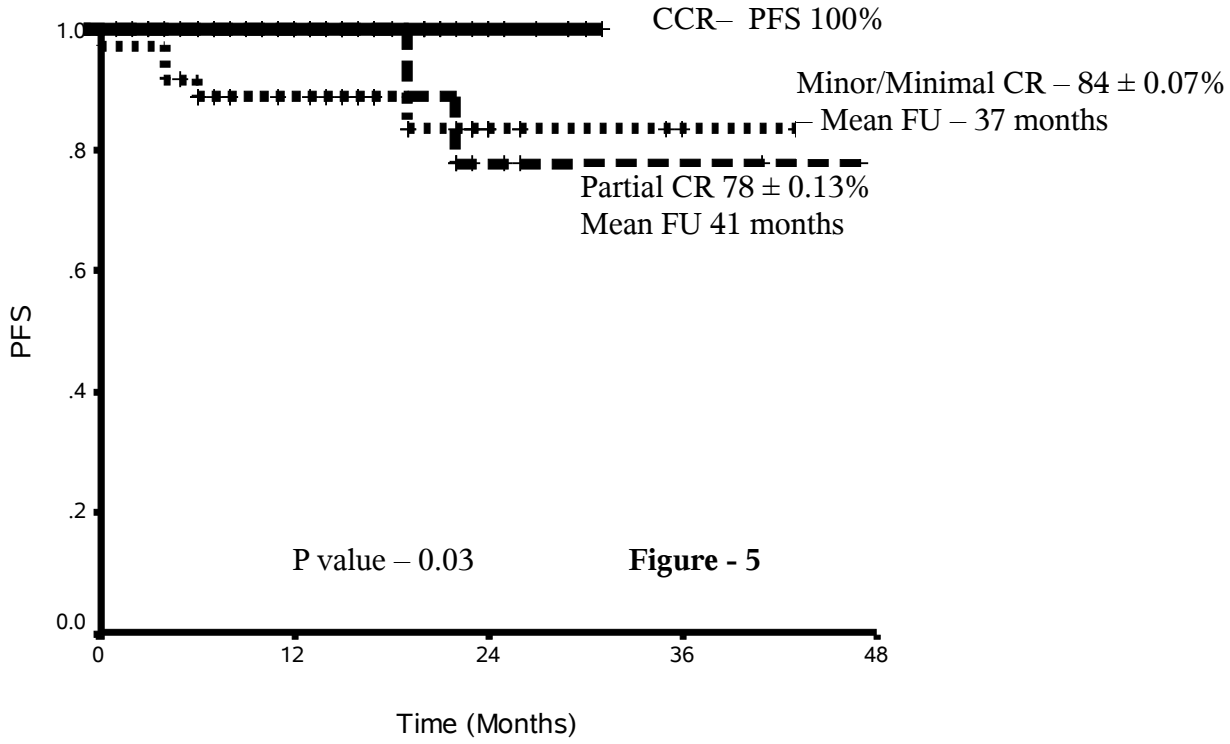
The progression free survival in chronic phase patients in CML was 85 ± 0.05% in early CP at 42 months (1 – 47 months) and 81 ± .011% in late CP at 39 months (1 – 43 months). In log rank analysis, there was no apparent difference between the two groups.



igure - 4

Progression free survival in Chronic phase (early and late CP),according to Cytogenetic response. Figure - 5

PFS in early and late chronic phase patients in CCR were 100% (1 – 31 months). For patients in Minor/minimal CR, at a mean follow of 37 months (4 – 43 months) the PFS was 84 + 0.07% and for patients in partial CR, the PFS was 78 ± 0.13% at a mean follow up of 41 months (3 – 47 months). (Figure – 4)



P value – 0.03 **Figure - 5**

Overall survival on patients with Glivec in relation to Hasford score – Figure - 6

The Hasford score risk stratification did not show any correlation with the survival curves.

(Figure – 6)

High risk – No event 100%
1 – 47months

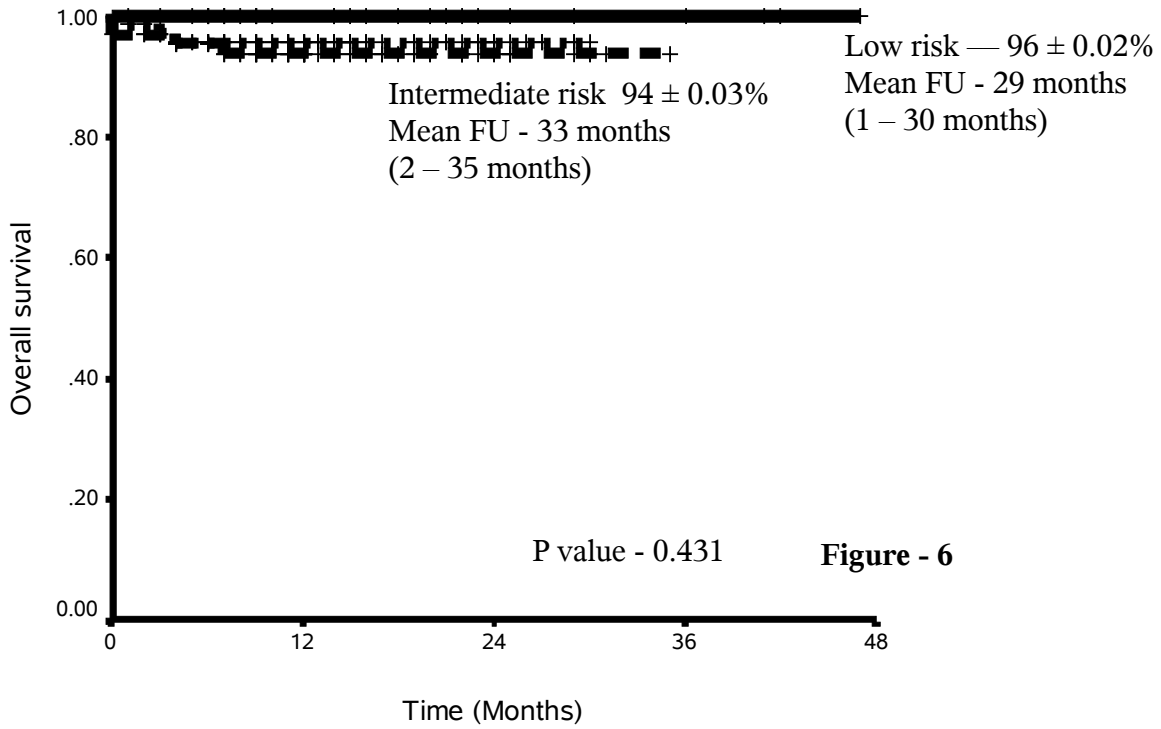


Figure - 6

Haematological toxicity on Glivec

Haematological toxicity was noted in 14% of patient in chronic phase, 23% in Accelerated phase and 50% of patients in blast transformation. (Table – 7)

Table - 7

Toxicity	CP any grade n/total (%)	CP grade 3 or 4 n/total (%)	AP any grade n/total (%)	AP grade 3 or 4 n/total (%)	BT any grade n/total (%)	BT grade 3 or 4 n/total (%)
Haematological toxicity present	28/202 (14)		3/13 (23)		14/28 (50)	
Neutropenia	21/202 (9)	9/202	2/13 (15)	nil	14/28 (50)	11/28
Mean time from start of treatment(weeks)	8 2 -28	-	1.5 1-2	-	2 1 - 8	-
Thrombocytopenia	22/202 (11)	11/202	2/13 (17)	1/13	9/28 (33.3)	9/28

Mean time from start of treatment (weeks)	8.5 1 - 36	-	2 2	-	2 1-4	-
Anaemia	8/202 (4)	6/202	1/13 (8)	1/13	7/28 (25)	5/28
Mean time from start of treatment (weeks)	3 2 - 28	-	2 2	-	2 1-6	-
Bone marrow aplasia	4/202 (2)	-	NA	-	NA	-
Time of onset to BM aplasia (weeks)	12 10 - 28	-	-	-	-	-

Non hematological toxicities observed in various phases of CML

The overall non- haematological toxicity were noted in 17% of patients in chronic phase and 30% of patients in accelerated phase and blast transformation. (Table – 8)

Table - 8

DISCUSSION

Introduction of Imatinib has made a paradigm shift in the treatment of chronic myeloid leukemia in the true sense of “magic bullet” in cancer chemotherapy. As BCR-ABL remains unique to induce leukemogenesis, Imatinib serves as the ideal treatment in CML.

A total of 243 patients from 14 different states in India were analyzed who had enrolled in GIPAP at our centre. Majority (40%) of patients belonged to Tamil Nadu, and West Bengal was second highest (20%) similar to the hospital statistics. This was followed by the 2 neighboring states, Kerala (15.2%) and Andhra Pradesh (11.1%). (Figure – 1)

Chronic myeloid leukemia is noted to be higher among the males and the ratio is usually 1.4:1 in Western countries.[90] However, Indian studies have shown higher male preponderance with ratios of 2.3:1[68] and 3.4:1[80] similar to our study (2:1).

The median age of onset of disease in the Western population ranges from 45 to 55 years. It is noted to be much earlier in the Indian population with 38 (4-79)[68] and 38 (11 – 65)[80] very much similar to the present study (38 (7 – 74)). About 12 to 30 percent of the Western population are more than 60 years of age[91]. In our study, only 5% belonged to that group and 78% of patients were less than 50 years of age, 55% less than 40 years and 44% of population were between 20 to 40 years of age. Only 9 patients belonged to the pediatric population. (<15 years) (Table – 1)

Hasford score was calculated for 163 out of 226 patients in chronic phase and 32%, 29% and 11% were noted to be in low, intermediate and high risk groups. (Figure -6)

Patients in chronic phase on Glivec were subdivided into initiation of treatment before 1 year (early chronic phase) (147 (65.5%)) and more than 1 year from diagnosis (late chronic phase) (55 (34.5%)). (Table – 1)

Majority of the patients were pretreated with Hydroxyurea (92%) followed by Veenat/Zoleta

(23%) and Busulfan (4.5%) prior to the initiation of therapy for a median of 2 months (1 – 180). This time duration was either because patients were initiated on treatment before coming to CMC or during the waiting period of getting the GIPAP approval.

The overall response in chronic phase were categorized into early (<1 yr from diagnosis) and late chronic phase (>1 year from diagnosis). The results were comparable for CHR, MCR and CCR in terms of the other western and Indian studies. (Table-9). The MCR was significantly higher in early versus late chronic phase patients in our study similar to the study by Kantarjian et al in interferon failure group.

However AP and BT responses were marginally lower. The differences between were not statistically significant (p – 0.65).

Table - 9

Study	**Number of patients**	**CHR %**	**MCR %**	**CCR %**
Chronic phase				
S O'Brien et al[8] (IRIS) – Newly diagnose- at 400mg. Median follow up of 19 months	553	95.3	87.1	76.2
Kantarjian et al[92] (Interferon failure)	261	95	62	45
Kantarjian et al[93] Newly diagnosed at 800mg	114	96	96	90
Deshmukh et al[68] Early chronic phase Late chronic phase	 24 73	 100 89	 62.5 46.4	 41.7 27.3
Arora et al[80] Median follow up of 5 months	79	95.8	30	16
Present study Early chronic phase) Late chronic phase)	 147 55	 92 87	 77.5 46.4	 56 35.7
Accelerated phase				
Kantarjian et al[94]	200	80	45	24
Talpaz et al[6]	235	82	24	17

Deshmukh et al[68]	47	55.3	21.3	6.4
Arora et al[80]	23	45	20	
Present study	13	54	33.3	33.3
Blast crisis				
Kantarjian et al[84]	75	21.3	10.6	6.6
Sawyer et al[7]	260	52	16	7
Deshmukh et al[68]	30	36.7	23.3	13.3
Arora et al[80]	16	20	20	
Present study	28	75	28.5	14.2

The molecular responses noted in our study at a median follow up of 24 months were much lower than the earlier studies. The probable reason was that only 30 patients who achieved CCR got RQ PCR analysis. The criteria used here was BCR-ABL/ABL ratio of <0.05[95] to call it Major molecular remission. However, as per the proposed guidelines of keeping the cutoff for MMR at 0.1%[96], the percentage of MMR increases to 31.8% (7/22). (Tables 3 & 11)

Table – 10 Molecular response in chronic phase

Study	**Number of patients**	**MMR** ***(BCR-ABL/ABL)*** **0.05%**	**CMR**
Hughes et al[9] Newly diagnosed at 400mg (Median follow up 12 months)	553	39	3
Kantarjian et al.[93] Newly diagnosed at 800mg (Median follow up 15 months)	114	63	28
Kantarjian et al[95] Post interferon failue (Median follow up 45 months)	261	43	26
Present study Median follow up 24 months	30 evaluable patients of the 53 who achieved CCR	13	4.5

The incidence of haematological resistance in the earlier studies[97] is shown in table 11. The incidence of resistance in early and late chronic phase is similar to the previous studies. There is significant difference in the AP and BT groups, probably due to smaller numbers

analyzed.

Table - 11

Phase of CML	Haematological resistance	Cytogenetic resistance
Newly diagnosed CML-CP. Hughes et al[8]	PHR – 5% at median FU 18 months 4% relapse or progression	14% at 18 months, 12 % after 24 months and 10% relapse or progression
Interferon treated CML – CP	PHR – 5% median FU of 18 months 13% relapse or progression	40% after 18months 36% at 24 months
Accelarated phase	24% PHR and 51% relapse after 2 years.	76 % at 12 months
Blast crisis	66% PHR and 88% relapse at 2 years.	84% at 12 months
Present study at a median follow up of 46 months in CP, 28 months in AP and 24 months in BT	PHR CP <1 year – 8% CP >1 year – 9% AP – 25% BT – 15%	CP <1 year 12.5% CP >1 year 46% AP 50% BT 57%

The cytogenetic responses at various time periods were analyzed separately because there was no uniformity in the timing of 2nd FISH (t (9; 22) due to logistic reasons. Many of our patients are coming from far (West Bengal – 20%) and only 40% of patients belonged to Tamil Nadu. Majority (69%) of the patients got the 2nd Fish done before 1 year (3 – 12 months). Table – 5. Third FISH (t (9; 22) test could only be done in 22 patients.

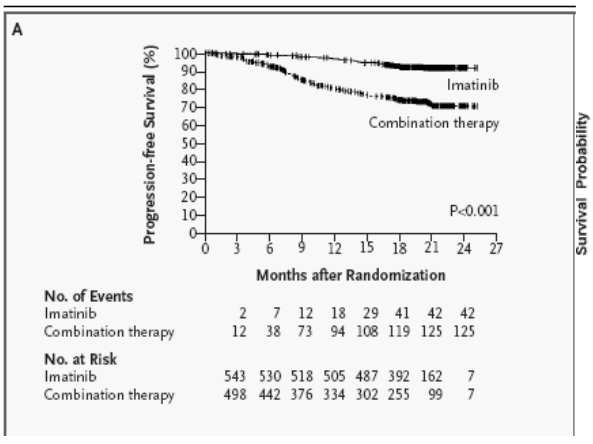
Response status (Table – 7) at various time intervals in chronic phase shows similar trend from 6 months onwards. However, partial cytogenetic responses show a marginal decrement and increase in complete cytogenetic response. The Major cytogenetic response remained almost the same. This could possibly because we have analyzed both the early and late chronic phases together and response evaluation was done at different time points for different patients. The follow up of IRIS[8] study patients shown below.

Table - 12

	CHR		MCR		CCR		PFS	
Months follow up	24	42	24	42	24	42	24	42
Estimated rate (%)	Not reported	98	88	91	79	84	96	94

The overall survival after starting Glivec in CP, AP and BT were 98%, 54% and 51% at mean follow up of 46 months, 28 months and 24 months respectively. Two patients in Blast transformation received Vincristine and prednisolone along with Glivec for lymphoid blast transformation. The OS in patients in chronic phase is similar to the other studies.[8,95] (Figure – 2)

The progression free survival (PFS) in CP, AP and BT were 81%, 64% and 48% at mean follow up of 42 months, 28 months and 26 months respectively. The PFS in the chronic phase is lower as compared to the IRIS study because here we have combined both early and late chronic phase (Figure – 3). When the chronic phase patients were analyzed separately, early phase CP showed PFS of 85% at a mean follow up of 39 months (Figure – 4). The PFS in the late phase CML is comparable to the study by Kantarjian et al. [8,95]



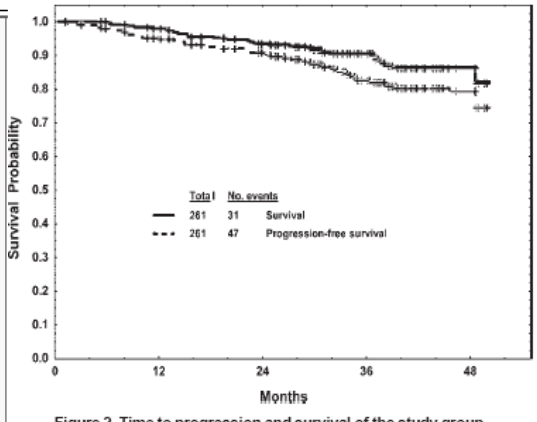


Figure 2. Time to progression and survival of the study group.

IRIS study – PFS – 93% at 24 months[8] **Interferon failure – OS 88% and PFS 83%[95]**

Patients in chronic phase (both early and late chronic phase) were further analyzed for PFS according to the cytogenetic response (Figure 5). The PFS was 100% in the CCR group indicating that no matter whether the patient is in early or late chronic phase, if CCR can be achieved, PFS improves dramatically. It was 78% in the partial CR group (41months) and 84% in minor/minimal CR group (37 months). This is comparable with the IRIS study which showed that achieving CCR by 12 months shows marked reduction in progression (PFS – 100%).

The overall survival was compared with Hasford score which was proved to be an important tool in predicting survival for patients on interferon.[85,98] But it has not shown any correlation treatment with Imatinib. This was consistent in our study also. (Figure – 6)

Haematological toxicity profile was similar to the other studies with 14% in the chronic phase, 23% in the accelerated phase and 50% in blast transformation. (Table–7). Five patients in chronic phase (2%) developed bone marrow aplasia during the course of treatment with a median time onset of 12 weeks. (10 – 28 weeks). There are only case reports of patients developing bone marrow aplasia[99] and pretreatment with interferon and Busulfan has been considered to be a risk factor. Among the 5 patients who developed BM aplasia, 4 of them were initiated on Glivec in late chronic phase (40, 36, 37 and 13 months from diagnosis.) and only 1 patient was started on Glivec in early chronic phase. Assessment of non – haematological toxicity profile was not well co-ordinated and the same cannot be commented upon. (Table- 8).

SUMMARY AND CONCLUSIONS

This study was performed on 243 patients with chronic myeloid leukemia who came to CMC Vellore and was eligible to enroll into GIPAP for Imatinib Mesylate (Glivec). Response to treatment was monitored by regular blood counts and peripheral blood FISH for t (9; 22).

1. Male: Female ratio was 2:1.
2. Mean age of diagnosis was 38 years (7 – 74).
3. A total of 202 (83%) of patients were in chronic phase, 12 (5.3%) in accelerated phase and 28 (11.5%) patients were in blast crisis at the time of starting Imatinib (Glivec). Of the 202 patients in chronic phase, 147 (65.5%) of patients were started on Imatinib < 1 year from (Early chronic phase) from diagnosis and 55 (27%) of patients were >1 year form (late chronic phase) diagnosis.
4. Majority of patients were on Hydroxyurea (92%) and Busulfan (4.5%) prior to initiation of Imatinib (Glivec). The median duration of treatment prior to initiating Imatinib was 2 months.
5. Complete haematological response (CHR) at 3 months was observed in 92% of patients in early chronic phase and 87% in late chronic phase (overall 91%). It was 69% and 54% in accelerated phase and blast crisis respectively.
6. Major cytogenetic remission (MCR) was observed in 77.5% of patients in early chronic phase and 46.4 % in late chronic phase (overall 70%). The difference was statistically significant ($p < 0.001$). MCR was seen in 33.3% of accelerated phase and 28.5% of blast crisis patients.
7. Complete cytogenetic remission (CCR) was observed in 56% of patients in early chronic phase and 35.7% in late chronic phase (overall 51.2%). The difference was statistically not significant. CCR was seen in 33.3% of accelerated phase and 14.2%

of blast crisis patients.

8. Major molecular remission was observed in 13.6% of early chronic patients in CCR and 12.5% in late chronic phase (overall 13%). Complete molecular remission (CMR) was observed in only one patient (4.5%) in early chronic phase.
9. Primary haematological resistance (PHR) was noted in 8% of early chronic phase and 13 % in late chronic phase (overall 9.3%). The same was 25% and 50% in accelerated phase and blast crisis respectively.
10. Primary cytogenetic resistance (PCR) was observed in 12.5% of early chronic phase CML and 46% of late chronic phase CML (overall 21.34%). The difference is statistically significant. ($p < 0.001$). PCR was 50% in the accelerated phase and 57% in the blast crisis.
11. A total of 57% of patients underwent peripheral blood FISH examination. 72% of them underwent 2nd FISH examination between 3 and 12 months. There was no difference in the cytogenetic response rates in chronic phase CML at various time intervals.
12. The overall survival (OS) in chronic phase was 98% at a mean follow up of 46 months. The OS in accelerated phase was 54% at a mean follow up of 28 months and in blast crisis it was 51% at 24 months.
13. The progression free survival (PFS) in chronic phase was 81%, in accelerated phase it was 48% and for blast crises it was 64%. The Mean follow ups were 42 months, 28 months and 26, months respectively.
14. The progression free survival in chronic phase patients in CML was 85% in early CP at 42 months and 81% in late CP at 39 months. The difference was not statistically significant.
15. PFS in early and late chronic phase patients in CCR were 100%, 84% with Minimal/Minor CR, and 78% with Partial CR.

16. There was no correlation with Hasford score at diagnosis and overall survival in patients on Glivec.
17. Haematological toxicity was noted in 14% of patients in chronic phase, 23% in accelerated phase and 50% of patients in blast transformation.
18. The overall non- haematological toxicity were noted in 17% of patients in chronic phase and 30% of patients in accelerated phase and blast transformation.

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ABSTRACT

Title: Response to Imatinib Mesylate In Patients With Chronic Myeloid Leukemia

Department: Clinical Haematology
Name of the candidate: Dr M Joseph John
Degree and Subject: DM – Clinical Haematology.
Name of the guide: Dr Mammen Chandy.

Objective: To assess the response to imatinib mesylate in patients with chronic myeloid leukemia in chronic and advanced phase who came to our institution from January 2002 to December 2005.

Methods: All Chronic Myeloid Leukemia patients who were eligible to enroll into GIPAP (Glivec International Patient Assistance Program) for imatinib were included for analysis. Response to treatment was analyzed by peripheral blood counts and peripheral blood FISH analysis for t(9;22) at various intervals. This was subsequently correlated with overall survival and progression free survival using log-rank test.

Results: A total of 243 patients were enrolled into the study (CP-202, AP – 13, BT – 28). Complete haematological remission was achieved in 91% of patients in CP, 69% in AP and 57% in BT. Major cytogenetic remission was observed in 70% of patients in CP, 33.3% in AP and 28.5% in BT. Complete cytogenetic remission was observed in 51.2% of patients in CP, 33.3% in AP and 14.2% in BT. The overall survival in CP was 98%, and for AP and BT, it was 54% and 51%. The Progression Free Survival in CP was 81% and for accelerated and blast crisis, it was 48% and 64%.

Conclusion: There is significant hematological and cytogenetic response to Imatinib Mesylate in patients with chronic myeloid leukemia.