An observational study of non-adherence in patients with chronic myeloid leukemia on treatment with Imatinib – prevalence, predictors and outcomes

A Dissertation submitted in partial fulfillment of D.M Branch X (Clinical Haematology) Examination of the Dr. M.G.R Medical University, Chennai, Tamil Nadu, to be held in August 2013

CERTIFICATE

This is to certify that the dissertation entitled "**An observational study of non-adherence in patients with chronic myeloid leukemia on treatment with Imatinib – prevalence, predictors and outcomes"** is a bonafide work done by Dr. Anu Korula in part fulfillment of the rules and regulations for D.M Branch X (Clinical Haematology) examination of the Tamil Nadu Dr. M.G.R Medical University, Chennai, to be held in August 2013.

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REVIEW OF LITERATURE

Keep a watch…on the faults of the patients, which often make them lie about the taking of things prescribed. For through not taking disagreeable drinks, purgative or other, they sometimes die.

Hippocrates, *Decorum*

1 Introduction:

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm that originates in an abnormal pluripotent bone marrow stem cell(1). It is consistently associated with a fusion gene, the BCR-ABL1, which is located on the Philadelphia chromosome(2). The Philadelphia chromosome is formed by the translocation t(9;22)(q34;11) – which results in juxtaposition of the ABL gene from Chromosome 9 to the BCR gene in Chromosome 22. This gene codes for a fusion protein (BCR-ABL) which has constitutive tyrosine kinase activity, resulting in uncontrolled myeloid proliferation. Although the major initial laboratory abnormality noted is usually neutrophilic leukocytosis, the BCR-ABL1 is found in all myeloid lineages as well as some lymphoid cells and endothelial cells(1). If untreated, CML will progress from an indolent chronic phase to a more aggressive accelerated phase or blast crisis.

Insights into the role of the aberrant BCR-ABL fusion protein in promoting uncontrolled proliferation of myeloid progenitors led to the discovery of molecules (tyrosine kinase inhibitors) which specifically target the abnormal protein(3), and these drugs have resulted in unprecedented

success in disease control, with complete cytogenetic response rates of 76% with imatinib, compared to 14% with Interferon-α, and 8-year overall survival rates of 86%(4).

The use of tyrosine kinase inhibitors which block the activity of the abnormal tyrosine kinase (but do not reverse the genetic change causing the disease) has changed CML from a life-threatening malignancy to a chronic disease in which lifespan may be normal.

Continuous and adequate imatinib dosing is paramount in achieving optimal therapeutic outcomes(5)(6). Non-adherence to medication is a significant problem in chronic diseases, and in CML, non-adherence to imatinib has been shown to adversely affect outcomes. Poor adherence is the leading cause of suboptimal response and loss of cytogenetic response in patients who previously responded optimally to Imatinib(7).

LITERATURE REVIEW - SECTION I

AN OVERVIEW OF CHRONIC MYELOID LEUKEMIA

2.1 Epidemiology:

CML is a rare disease, with an annual worldwide incidence of 1-2 cases per 100,000 population(1). The median age at diagnosis is the 5^{th} to 6^{th} decades of life in Western populations (8), however in one series of 430 patients, 65% of patients were between 20-40 years of age. There is a slight male predominance (9)(10). CML is extremely rare in childhood, and accounts for less than 2% of all paediatric leukaemias (11) with an annual incidence (0.6–0.8 per million in 0-14 age group). The incidence is higher in adolescents (1.2 per million per year) (12) than in children.

In India and other developing countries, CML is the commonest form of adult leukemia and the median age at presentation is 1-2 decades lower than in the western population (13)(14)(15). The incidence of CML in developing countries is low (Age-Adjusted Rate (AAR) = 0.71 in males and 0.53 per 100,000 in females) compared to USA and other developed countries (AAR=2.0 in males and 1.1 per 100,000 in females) (15).

2.2 Pathogenesis:

The reciprocal translocation t (9;22)(q34;q11.2) that results in the Philadelphia chromosome [der(22q)] was first described in 1960 (16), and at the time of diagnosis, 90-95% of patients with CML have this characteristic translocation. The genetic changes resulting from this translocation - with fusion of the BCR gene on chromosome 22 with the ABL gene on

chromosome 9 - result in an oncogene BCR-ABL which results in dysregulated tyrosine kinase activity and uncontrolled myeloid proliferation(2).

Variant translocations have been described, the commonest (6% of cases at diagnosis) of which is the variant t(v;22)(17). Additional cytogenetic abnormalities have been described in up to 7% of cases – of which the commonest is deletion Y (-Y) in 3.3%. Other additional cytogenetic abnormalities include presence of a second Philadelphia [Ph] chromosome, trisomy 8, isochromosome 17q, or trisomy 19(17). The prognostic impact of presence of additional cytogenetic abnormalities at the time of diagnosis is unknown (9).

A small percentage of patients will have cryptic translocations of 9q34 and 22q11.2 that cannot be identified by routine karyotyping, and in these cases the fusion gene is identified by FISH analysis, RT-PCR or Southern blot techniques. In a prospective study of 430 patients with CML, 15% lacked the Ph chromosome by cytogenetic analysis – of these patients, half had complex cytogenetic abnormalities which masked the underlying t(9;22) translocation, and others had evidence of bcr-abl-1 gene fusion by interphase FISH or RT-PCR(9).

2.3 Genetics:

The most common BCR-ABL1 fusion transcript produced is from a breakpoint in exon 13 or exon 14 (also called exon b2 or b3) in the BCR gene, which is fused to the ABL1 gene at exon a2 (e13a2, e14a2 or alternatively b2a2 or b3a2)(1). The resultant BCR-ABL1 protein has a 210 kilo Dalton molecular mass (the p210 BCR-ABL1 protein) – with constitutively activated tyrosine kinase activity. Less commonly, an alternative e19a2 fusion transcript is found,

producing a larger fusion protein with 230 kilo Dalton weight (p230 BCR-ABL1), seen in rare cases of CML (<1%), but more commonly associated with the rarer chronic neutrophilic leukemia. A smaller e1a2 fusion transcript – with the p190 BCR-ABL protein product - is also seen in a very small number of CML patients, but is more frequently associated with Ph-positive acute lymphoblastic leukemia(1).

2.4 Clinical features:

During the chronic phase, systemic symptoms such as fatigue (34%), weight loss (20%), diaphoresis (15%), abdominal fullness (15%), and bleeding episodes due to platelet dysfunction (21%) are common (9). Rarer symptoms at diagnosis include dyspnea, cough, arthralgia, visual disturbances and priapism. Between 20-50% of patients may be incidentally detected to have CML, during a routine medical examination (9)(10). Compared to patients who were asymptomatic, those who were incidentally detected to have CML have lower WHB and platelet counts, lower blast counts and higher hemoglobin(9). Men and women differ in clinical presentation, with significantly higher WBC counts, platelet counts, severity of anemia and number with palpable spleen(9), however the duration of symptoms did not differ. Younger patients (<40years) have been noted to have higher counts at presentation. Involvement of extra medullary tissues or lymph nodes is generally restricted to the accelerated phase and during blast crisis.

2.5 Diagnosis:

1.5.1 Peripheral blood and bone marrow findings: In the chronic phase there is peripheral blood leukocytosis (median WBC 100x10^9/L), neutrophils in different stages of

maturation, with a myelocyte and segmented neutrophil peak (18), and usually <2% blasts. There is no significant dysplasia. There is usually basophilia and eosinophilia (18). Accompanying anemia and/or thrombocytosis/thrombocytopenia is common, and are unrelated to the leucocyte count (18). Bone marrow aspiration shows increased cellularity, with granulocytic hyperplasia and a maturation pattern similar to that seen in the peripheral blood. Megakaryocytes are smaller than normal (dwarf megakaryocytes). Pseudo-Gaucher cells and sea-blue histiocytes are also a common feature(1).

A diagnosis of accelerated phase of CML requires any one of the following: (a) persistent or increasing WBC (>10 x 10^9/L) and/or persistent or increasing splenomegaly which is unresponsive to treatment (b) persistent thrombocytosis (>1000 x 10^9/L) uncontrolled by therapy (c) Persistent thrombocytopenia (<100x10^9/L) unresponsive to therapy (d) clonal cytogenetic evolution (after initial karyotype is established) (e) ≥20% basophils in the peripheral blood and (f) 10-19% blasts in the peripheral blood or bone marrow. Criteria 5 and 6 are frequently associated with a blast transformation(1).

The blast phase of CML is diagnosed when (a) blasts ≥20% in the peripheral blood or bone marrow **or** (b) when there is an extra medullary blast proliferation. In 70% of cases the blast transformation occurs in the myeloid lineage.

Though the diagnosis of CML is suspected on peripheral blood smear and can be confirmed by FISH on peripheral blood, a bone marrow aspiration and trephine biopsy, with sampling for cytogenetics (karyotyping) and real-time quantitative reverse transcriptase (RQ-

PCR) for *BCR-ABL* transcripts are recommended at diagnosis (19). Neutrophil alkaline phosphatase is no longer routinely measured.

2.5.2 Cytogenetics:

Karyotyping: Karyotyping on metaphase nuclei from bone marrow is recommended in all patients, at diagnosis and during follow-up to assess response to therapy(19). The majority of patients (90 to 95 percent) demonstrate the t(9;22)(q34;q11.2) reciprocal translocation that results in the Ph chromosome, but the remainder have variant translocations and complex translocations involving other chromosomes ((9)(17). The prognostic significance of additional cytogenetic abnormalities at disease onset was unclear (20), however a recent study on 1151 patients with CML (17) showed an adverse prognosis with certain 'major route' cytogenetic abnormalities - second Philadelphia [Ph] chromosome, trisomy 8, isochromosome 17q, or trisomy 19.

Fluorescence in-situ hybridization: Though bone marrow karyotyping (metaphase analysis) has conventionally been used for assessing cytogenetic remission, some studies have looked at the correlation between conventional karyotyping and FISH in the diagnosis and follow-up of CML. In a study of 65 patients with paired samples for bone marrow cytogenetics and FISH, and peripheral blood FISH using dual color, double fusion probes, a tight correlation was found between the 3 modalities ($P < 0.0001$ for each) (21). This data suggests that FISH on interphase nuclei in peripheral blood may be used as a surrogate for conventional metaphase cytogenetic studies in at diagnosis and for monitoring cytogenetic remission status.

In a comparison of I-FISH and karyotyping results in 537 patients on Imatinib, major molecular response rates were significantly higher in cases with complete cytogenetic response (CCgR) by karyotyping and I-FISH less than 1% than in cases with CCgR and I-FISH 1% to 5% (22), suggesting that FISH is a more sensitive indicator than karyotyping in the assessment of treatment response in CML.

2.5.3 Reverse-transcriptase polymerase chain reaction

Polymerase chain reaction (PCR) permits specific and sensitive detection of Ph-positive cells through the amplification of the BCR/abl fusion transcripts (23)(24). With the introduction of quantitative PCR techniques, RQ-PCR has taken a central role in the monitoring of response to therapy (25). As the fusion transcript may vary in some cases of CML (24), it is of paramount importance to document the fusion transcript at the time of diagnosis, to allow response assessment using RQ-PCR. Standardization of reporting BCR/abl transcript levels across countries may be achieved using a conversion factor, whereby individual laboratories can express transcript levels on an international scale(26). Current recommendations from EuropeanLeukemiaNet include monitoring molecular response to therapy at 3 monthly interval by RQ-PCR, after a complete cytogenetic response has been achieved (19). Molecular monitoring and quantification of BCR/abl can identify subsets of patients who have a functional cure, and in whom discontinuation of Imatinib may be considered(27)(28).

2.6 Treatment:

2.6.1 Treatment of CML in the pre-Imatinib era:

The treatment of chronic myeloid leukemia has undergone radical changes over the past 10 years. Prior to the discovery of Imatinib, the mainstay of treatment was either with chemotherapy, interferon-α or allogeneic stem cell transplant. Interferon alpha was shown to be superior to chemotherapy (hydroxyurea and busulphan) in the treatment of CML (29). The probability of complete hematological and cytogenetic remission following treatment with interferon was between 40-70% and 2-8% respectively(30)(31), with 5yr survival rates of just over 50%. Results with interferon varied widely due to varying regimens, doses, duration and dose changes due to toxicity (32). Interferon was also used in combination with hydroxyurea, busulphan and cytarabine, with marginal improvements in outcome at the cost of added toxicity.

Allogeneic bone marrow transplant offered a hope for cure, but was associated with considerable morbidity and mortality, with overall 5 year survival rates of approximately 50%, and relapse rates of 20% (32)(33). In recent years, due to changes in conditioning regimens and improved supportive care, 5-year overall survival rates have improved to 85-90% for patients in chronic phase (with Imatinib failure) and 60% for patients in advances phases of disease(34).

2.6.2 Treatment of CML with Tyrosine kinase inhibitors:

First generation tyrosine kinase inhibitors: A turning point in the treatment of CML came with the discovery of tyrosine kinase inhibitors. Imatinib, the first described tyrosine kinase inhibitor, is a synthetic small molecule (a 2-phenylaminopyrimidine derivative) designed to block the adenosine triphosphate (ATP)–binding site of the BCR-ABL tyrosine kinase with

high selectivity(3) potently inhibiting the ABL tyrosine kinase activity. It has been found effective in the chronic(35) and accelerated phases of CML(36)(37) as well as in blast crisis (38).

The first randomized trial comparing imatinib with interferon-alpha was published in 2003, and at a median follow-up of 19 months, estimated rates of major and complete cytogenetic response were far superior in the imatinib group (87% vs. 34% ($p<0.001$) and 76% vs. 14% ($p<0.001$) respectively). Long-term data from the IRIS trial shows an overall survival (including deaths from all causes) of 86% and the event free survival is 81% at seven years. The estimated freedom from progression to accelerated phase/blast crisis was 97% vs. 91% ($p<0.001$)(4). Imatinib also demonstrated a better toxicity profile. Long-term follow-up in patients in chronic phase treated with imatinib have shown low rates of transformation to accelerated phase/blast crisis - 7% of patients progressed to accelerated or blast phase, with the highest risk being in the second year of treatment (0.9% after the first 3 years), giving an event-free survival of 83% (O 'Brien et al Blood. 2008; 112: Abstract 186). Eighty-two percent of patients achieved complete cytogenetic remission and 83% of these patients maintain remission. In patients who responded to Imatinib outcomes were as described, however a high percentage of patients (45%) discontinued treatment in the long-term. The reasons for discontinuation of therapy were as follows: Adverse events (6%), unsatisfactory therapeutic outcome (16%), stem cell transplant (3%), death (3%), others ((including lack of renewal of consent) 17%) (Deininger et al; Blood 2009; Abstract 1126).

Based on safety and efficacy results from the phase 3 IRIS trial (4), Imatinib 400mg OD was the recommended dose for patients with newly diagnosed Ph+ Chronic myeloid leukemia in chronic phase, 600mg per day in accelerated phase/blast crisis and 260mg/m2 in children. The

dose of 400mg was chosen in the original phase I study as imatinib was biologically active at this dose; however a true maximum tolerated dose was not determined. Recent data suggests that higher doses of Imatinib may be more efficacious. The TOPS Trial (39) compared the outcomes in newly diagnosed patients received either 400 or 800 mg Imatinib as front line therapy. Major molecular remission (MMR) rates at 12 months, were similar in the two arms, but patients onimatinib800 mg daily achieved complete molecular remission(CMR) faster (8.4 months versus 13.6 months, respectively, p = 0.0038) than patients receiving 400 mg of Imatinib daily. Adverse events were more common; however no new toxicities were described. Based on the efficacy and toxicity data, the starting dose continues to be 400mg once daily in newly-diagnosed patients in chronic phase.

Imatinib has been found to be effective in the treatment of CML in children and adolescents. In a retrospective study of 43 patients aged 7-20years, Imatinib was well tolerated, with all patients achieving complete hematological response, 58.1% with CCR and 42% with a major molecular response (40). Significant growth reduction has been reported in pre-pubertal children on long-term treatment (41).

Response to imatinib is monitored by assessing the hematological response at 3months, cytogenetic response at 6months, 12months and 18months, and molecular response at 18months and 3monthly thereafter, based on which the patient has either an optimal response, suboptimal response or imatinib failure (Table 2), and can be considered for 2nd line TKI therapy if indicated.

Second generation tyrosine kinase inhibitors: Second-generation tyrosine kinase inhibitors (Nilotinib, Dasatinib), are more potent BCR-ABL inhibitors with efficacy in patients who are resistant to or intolerant of imatinib (42). Dasatinib and nilotinib are active against all

BCR-ABL mutations except T315I. They have compare favorably with imatinib in the upfront treatment of CML, and have been shown to induce earlier and higher rates of cytogenetic remission (complete cytogenetic response rates of 96% and 95% at 1 year, with Nilotinib and Dasatinib, compared with 65% with Imatinib) (43) (44).

Nilotinib is an imatinib analogue with more specific BCR/ABL binding, and Dasatinib is a dual SRC/ABL kinase inhibitor. With the notable exception of T315I mutation, they are both effective against a large number of BCR/ABL kinase mutations(45). Dasatinib toxicity includes pleural effusions (20%) and it also may result in hemorrhage even in the absence of thrombocytopenia. Decreasing the dose of dasatinib to 100mg has decreased the incidence of toxicity, with similar efficacy rates. While on nilotinib, monitoring of QTc, potassium and magnesium is recommended to prevent arrhythmias.

Third generation tyrosine kinase inhibitors: Ponatinib (AP24534) is a newly approved tyrosine kinase inhibitor that blocks native and mutated BCR-ABL, including those with the T315I mutation, which was thus far resistant to TKIs, with 100% had a complete hematologic response and 92% had a major cytogenetic response at 3 months and 12 months respectively (46). Elevated pancreatic enzymes and pancreatitis are dose limiting toxicities associated with ponatinib, and are not uncommon (13.5%)(46).

Treatment algorithm in use of tyrosine kinase inhibitors:

There are different approaches to the use of tyrosine kinase inhibitors, with some groups using second-generation TKIs as upfront therapy in view of superior outcomes (47), while others recommend the use of these drugs only in cases of imatinib resistance or imatinib failure (19). In

a resource constraint setting Imatinib continues to be the TKI of choice in newly diagnosed CML.

2.7 Response assessment

Monitoring response to TKI therapy in patients with CML is critical to good patient management and involves assessment of hematologic, cytogenetic, and molecular responses (19) and achievement of these responses by specific time-points.

Table 1. Definitions of response in CML

Remission status	Definition
Hematologic response	
Complete (CHR)	Platelet count < 450 x 10^9/L WBC count < 10 x 10^9/L Differential: no immature granulocytes, basophils < 5% Non palpable spleen
Cytogenetic response	
Complete (CCgR)	No Ph+ fusion positive signals
Partial (PCgR)	1-35% Ph+ fusion positive signals
Minor	36-65% Ph+ fusion positive signals
Minimal	66-95% Ph+ fusion positive signals
None	> 95% Ph+ fusion positive signals
Molecular response	
Complete (CMR)	Undetectable BCR-ABL mRNA transcripts by real time quantitative and/or nested PCR in two consecutive blood samples of adequate quality(sensitivity > 10^4)
Major (MMR)	Ratio of BCR-ABL to ABL ≤ 0.1% on the international scale(26)

Response assessment has been recommended at specific time points, based on which changes in treatment are recommended (19) (Table 2).

Time	Optimal response	Suboptimal response	Failure	Warnings
At diagnosis	NA	NA	NA	High risk cytogenetics
3mths	CHR, at least minor CgR	No CgR	Less than CHR	NA
6mths	At least PCgR	Less than PCgR	No CgR	NA
12mths	CCgR	PCgR	Less than PCgR	Less than MMR
18mths	MMR	Less than MMR	Less than CCgR	NA
At any time	Stable or improving MMR	Loss of MMR, mutations	Loss of CHR, CCgR, mutations	Increase in transcript levels, additional cytogenetic abn.

Table 2. Response assessment on Imatinib

Though bone marrow karyotyping (metaphase analysis) has conventionally been used for assessing cytogenetic remission, peripheral blood FISH is less invasive, more practical alternative method of assessing cytogenetic remission. Interphase FISH has been found to be more sensitive than karyotyping in assessment of cytogenetic remission(48). The FISH and karyotyping results of 537 patients on Imatinib were compared with the rates of molecular response, and major molecular response rates were significantly greater in cases with CCgR (complete cytogenetic response by karyotyping) and I-FISH less than 1% than in cases with CCgR and I-FISH 1% to 5% (22). In a study of 65 patients with paired samples for bone marrow

cytogenetics and FISH, and peripheral blood FISH using dual color, double fusion probes, a tight correlation was found between the 3 modalities ($P < 0.0001$ for each) (21). This data suggests that FISH on interphase nuclei in peripheral blood may be used as a surrogate for conventional metaphase cytogenetic studies in monitoring cytogenetic remission status.

2.8 Resistance to Tyrosine kinase inhibitors

Primary (intrinsic) or secondary (acquired) resistance occurs in some patients on Imatinib (49). Primary resistance is defined as the failure to achieve hematologic remission within 3 to 6 months of treatment initiation, lack of any level of cytogenetic response at 6 months, lack of a major cytogenetic response at 12 months, or lack of CCgR at 18 months (National Comprehensive Cancer Network (NCCN). Clinical practice guidelines in oncology. Chronic Myelogenous Leukemia. V.2.2010.) Primary resistance, defined as a lack of CCgR at 18 months, and occurs in up to 24% of patients (21).Secondary resistance is defined as disease progression/ loss of therapeutic effect while on an imatinib regimen that had previously resulted in optimal response. In a 5-year follow-up of the IRIS trial, the incidence of secondary resistance was 24% of patients (relapse rate, 17%; progression rate, 7%)(50).

2.8.1 Mechanisms of resistance:

Imatinib resistance is attributed to several mechanisms. The commonest cause of imatinib resistance is point mutations in the BCR-ABL oncogene, and these mutations occur at a frequency of 35-70% of patients with resistance, particularly secondary resistance (51).The conformation of the bcr-abl protein is changed, to a form where imatinib is unable to bind, and these conformational changes confer resistance to imatinib without interfering with the function

of the oncogene. The mutations occur either spontaneously or as a result of selective pressure, where imatinib treatment eliminates sensitive leukemic cells and selects for resistant mutant cells.

BCR-ABL gene amplification and alternative pathways of disease progression not targeted by imatinib are other mechanisms by which leukemic cells develop resistance to the drug.

2.8.2 Mutation analysis:

Indications for mutation analysis:

The EuropeanLeukemiaNet guidelines state that mutation analysis is indicated at diagnosis only in patients who present in accelerated phase or blast crisis(52). While on imatinib, mutation analysis is indicated in case of suboptimal response, imatinib failure or rising levels of the bcr-abl transcript (52), and while on 2nd line TKIs, mutation analysis is recommended in case of hematologic or cytogenetic failure.

***Methods to detect BCR/ABL mutations*:**

Direct gene sequencing is the recommended method for detection of BCR/ABL mutations, and can be used in combination with denaturing high performance liquid chromatography (D-HPLC) which is more sensitive to the presence of mutations, but does not allow characterization of a specific sequence(52). Using these methods together will decrease the number of samples requiring gene sequencing.

Choice of TKI based on mutation analysis:

The full range of mutations and their IC_{50} data has been published, and the choice of TKI is dependent on the specific mutation detected (52).

—V299L, T315A, or F317L/V/I/C mutations, nilotinib is the TKI of choice

—Y253H, E255K/V, or F359V/C/I mutations, dasatinib is the TKI of choice

—In case of a T315I mutation, highly resistant to imatinib, dasatinib, and nilotinib, ponatinib is effective

—In case of other mutations, dasatinib and nilotinib are similarly effective.

2.9 Discontinuation of TKIs:

The concern that indefinite use of Imatinib will eventually result in the evolution of resistant clones has led to studies focusing on outcomes following discontinuation of imatinib therapy. The multicenter STIM (Stop Imatinib) trial (53) evaluated the outcomes of 100 patients with CML in complete molecular response (undetectable bcr-abl transcripts) for at least 2 years, where imatinib was discontinued, of whom 42 patients (out of 62 with follow-up at least 12 months) had a molecular relapse, most within the first 7 months after cessation of therapy. All patients responded well to re-initiation of Imatinib therapy, with complete molecular remission re-achieved in 26/42 patients.

No consensus exists regarding a residual disease threshold at which TKI therapy should be re-initiated.

2.10 Outcome determinants in CML

2.10.1 ***Karyotyping:*** Cytogenetic analysis at diagnosis is recommended in all

patients. The development of new cytogenetic abnormalities (clonal evolution) is indicative of disease progression with a worse prognosis((54)(55)(56), however the prognostic significance of additional cytogenetic abnormalities at disease onset is not known (20). Bone marrow examination is recommended at diagnosis at 3 monthly until MMR is achieved (19), BCHS).Deletion of derivative chromosome 9 (der (9) deletion), known to be a poor prognostic factor in CML patients treated with hydroxyurea, interferon, or stem cell transplantation(57)(58), has now lost its prognostic significance in the era of treatment with TKIs(59)(60)(61). At 60 months, the cumulative incidence of CCR and MMR, EFS and OS in patients with and without deletions were not statistically different(60).

2.10.2 Sokal/Hasford prognostic scores: The Sokal prognostic score was developed for patients receiving conventional chemotherapy, and includes spleen size, percent blasts, and platelet count >700,000/mm^3 (62), with 10year overall survival rates of 8%, 28% and 34% in the low risk, intermediate risk and high risk groups respectively. The Hasford or Euro score, which adds eosinophilia and basophilia to the calculation, was developed for CML patients receiving treatment with interferon(63). The Sokal and Hasford prognostic scores were developed in the pre-imatinib era, but they continue to have value in predicting outcome even with Imatinib therapy(64). CCR and MMR are more frequently observed in low-risk Sokal patients (4)(64).

2.10.3 Intrinsic sensitivity to ABL-kinase inhibitors: The intrinsic sensitivity of CML cells to Bcr-Abl kinase inhibition by Imatinib is determined by finding the concentration of imatinib needed *in vitro* to inhibit Bcr-Abl kinase activity by 50% ($IC_{50}^{imatinib}$). There is considerable inter-patient variability in $IC_{50}^{imatinib}$ values and this sensitivity to imatinib is

biologically relevant because molecular responses achieved is superior in patients with high intrinsic sensitivity (low $IC_{50}^{imatinib}$)(65).

The differences in $IC_{50}^{imatinib}$ are due to varying efficiency of intracellular uptake and retention (IUR) of imatinib. Imatinib uptake is dependent on active influx mediated mainly by the OCT-1 pump – a organic cation transporter (OCT) influx protein belonging to the solute carrier (SLC) superfamily(66). Patients with higher expression of OCT-1 mRNA have been shown to have better cytogenetic responses following treatment with Imatinib (67). Uptake of other TKIs (notably Nilotinib) is Oct-1 independent. (66).

2.10.4 BCR-ABL mutations: Some patients on first-line Imatinib therapy show early progression to accelerated phase/blast crisis, and are detected to have mutant clones which have a low sensitivity or complete resistance to Imatinib. There is currently no evidence that the early emergence of an aggressive mutant clone can be predicted by high-sensitivity screening for mutations prior to commencing imatinib (68)(19).

Risk factors for emergence of mutations in include duration of disease prior to imatinib therapy, pre-therapy peripheral blood blast count and poor cytogenetic response at 6 months are all predictive of the risk of developing resistance associated with mutations (20).

LITERATURE REVIEW - SECTION II

COMPLIANCE IN CHRONIC DISEASE

3.1 Definitions of adherence

Adherence can be defined the extent to which a person's behavior is consistent with health care recommendations (69). Adherence rates are highest in HIV disease, arthritis, gastrointestinal disorders, and cancer, and lowest in pulmonary disease and diabetes (70).

Non-adherence to medications can be intentional or non-intentional (71). Intentional non-adherence is an active process where the patient deliberately chooses to deviate from the prescribed treatment regimen. Unintentional non-adherence is a more passive process wherein the patient is forgetful about adhering to the treatment regimen. Although often dismissed and trivialized as a problem occurring due to patient-related factors, adherence behavior is strongly influenced by the treating physician, social and economic factors.

3.2 Methods of assessing adherence to medication

Measurement of medication adherence is difficult because adherence is an individual patient behavior, and patients may lie about adherence to medication. In addition to questioning the patient about adherence behavior, the following methods of confirming adherence have been studied(69) – (a) subjective measurements - asking family members about the patient's medication use; (b) objective measurements through pill-counters or electronic medication event monitoring systems (c) biochemical measurements by measurement of serum drug levels. These methods may not be applicable in clinical practice in most situations.

One of the reasons that plasma trough levels may not correlate with outcome may be because of "white collar compliance" or better adherence to medication prior to hospital visit,

falsely giving the impression of adequate drug levels at the time of testing(72). In a study comparing trough levels with electronic pill monitoring(72), it was found that compliance was high (86%-88%) in the period prior to and after hospital visit, whereas one month later, it had dropped to 67%.

Electronic medication monitors have been used to assess adherence and have been shown to compare favorably with standard tools of adherence. Electronic monitoring has clarified the patterns of non-adherence seen in patients on chronic medication(73): (1) Close to perfect adherence; (2) take nearly all doses with some timing irregularity; (3) miss an occasional single day's dose; (4) take drug holidays occasionally (3 to 4 times per year); (5) take drug holidays monthly or more frequently (6) take few or no doses. Patients commonly improve adherence shortly before and after an appointment with a healthcare provider - termed "white-coat adherence" (74).

Medication adherence scales are simple and low-cost approaches to identifying medication non-adherence in clinical practice. Many adherence scales have been described in literature, including the Morisky scale, Basel adherence scale, Medication Adherence Questionnaire (MAQ)Brief Medication Questionnaire (BMQ), Hill-Bone Compliance Scale and Medication Adherence Rating Scale (MARS)(75). Some of these adherence scales are specific to certain diseases, for example, the Medication Adherence Rating Scale (MARS) is for use specifically in a psychiatric setting. Most studies have addressed adherence in chronic disease like hypertension, cardiac failure, tuberculosis, AIDS and asthma. No single assessment scale is appropriate for every scenario(75).

The modified Basel Adherence Assessment scale has been validated in assessment of non-adherence to Imatinib in patients with Chronic Myeloid leukemia, and in combination with

Visual analog scale and pill count, has been shown to be effective in identifying patients with poor adherence at risk for suboptimal outcomes with Imatinib(76).

3.3 Compliance with Imatinib in chronic myeloid leukemia:

Continuous and adequate imatinib dosing is paramount in achieving optimal therapeutic outcomes. Non-adherence in chronic disease is conventionally defined as intake of <80% of prescribed dose(77). In cancer, higher rates of adherence have been observed (80%)(70)(71), however a meta-analysis of studies looking at medication adherence rates in oral chemotherapy found non-adherence ranging from 16% to 100% depending on the drug, regimen and definition of adherence used in different studies(78).

3.4 Impact of non-adherence on outcome in CML

The first study which looked at compliance issues with Imatinib addressed a patient population with both CML and Gastrointestinal stromal tumours (GIST) over a period of 1 year. Showed mean and median persistence rates of 69.4% and 79.7%, respectively (no disease-specific rates were reported) (J ClinOncol. 2008; 26:5S. Abstract 6598).

In a study on non-adherence with Imatinib(76), out of 169 evaluable patients, 1/3rd were found to be non-adherent over a 3-month period, adherence being defined as taking >90% of prescribed doses. Only 14% of the studied population showed 100% adherence. Patients with suboptimal response had higher mean percentages of missed doses compared to patients with optimal response (23.2%vs 7.3%; p=0.005).

In a study on factors affecting major molecular response rates in patients who have attained a complete cytogenetic response(5), multivariate analysis identified adherence (relative risk [RR], 11.7; $P < .001$) and expression of Oct-1 (RR, 1.79; $P = .038$) as the only independent predictors for MMR. Adherence was the only independent predictor for complete molecular response (5). Non-compliance has also been identified as the single most important risk factor for loss of complete cytogenetic response and imatinib failure in patients treated with Imatinib long-term(7)(5).

In the Indian subcontinent, data on compliance in CML is sparse. In developing countries, where non-compliance to medication is intertwined with financial limitations, it is often not possible to separate the financial reasons for non-compliance from other causes. One study on compliance in 516 patients with CML, defining non-compliance as interruption of therapy for one week at any point during follow-up, found non-compliance rates of nearly 30% non-adherence rates. With a median follow-up of 39 months, estimated EFS rates were 70%. There was a significant adverse impact on cytogenetic responses at any time point (26% vs. 44% (p=0.004))(79).

3.5 Assessment of non-adherence in CML

Adherence studies in CML have used different methods of identifying non-adherence, and different cut-offs for defining poor adherence:

- Pill count converted to mg taken / mg prescribed x 100 (Blood. 2007; 110. Abstract 4553)
- Mean medication possession ratio (MPR, defined as total days' supply of imatinib divided by 365)(80)

- MEMS (microelectronic monitoring system). An adherence of at least 90% of prescribed medication was the level that best predicted clinical responses(5).
- Imatinib trough plasma levels. The clinical relevance of low serum levels of imatinib are not clear, because it has been shown that do not show a correlation with the response in a routine clinical setting(81). In a study comparing Imatinib trough concentrations (Cmin) with clinical response, Cmin correlated with neither the achievement of complete cytogenetic response (977 vs. 993 ng/ml, P = 0.48) nor a major molecular response (1,044 vs. 818 ng/ml, P = 0.17), and conclusions drawn were that adherence to the standard dose is the single most important factor for the achievement of molecular response(6).

3.6 Reasons for non-compliance in CML

Imatinib, with its once daily dosing schedule, good side-effect profile and remarkable efficacy, is an almost ideal drug to ensure good compliance. In a qualitative study examining the reasons for non-compliance with imatinib, it was found that the most common reason for intentional non-adherence was side-effect related, and the commonest cause for non-intentional non-adherence was forgetfulness(71).

An important cause for non-adherence stated by patients was an apparent miscommunication between the physician and patient – wherein it was not clear to the patients that strict adherence to therapy would result in superior outcomes. When physicians focused on giving positive feedback about disease control, without stressing on continued compliance or questioning the patients on non-adherence, patients tended to miss occasional doses(71).

Physicians also gave patients the impression that missing occasional doses of Imatinib would not affect their outcomes.

With long-term imatinib therapy, there are two different patterns of patient behavior with regard to adherence that have been described – some patients are initially strictly adherent to therapy and tend to become non-adherent as they begin to feel symptomatically better or as they believe their disease is under good control. Another group of patients tend to become more adherent to medication the longer they are on the drug, as it becomes part of a regular habit after a period of time(71).

Delay in renewing prescriptions was the major cause for non-compliance in a study of 267 patients on Imatinib(80). Thirty-one percent of patients were found to have treatment interruptions of at least 30 consecutive days due to delay in renewing prescriptions during a 365 day follow-up period.

Medication-related side effects have been consistently implicated in non-adherence, especially in chronic disease including tuberculosis, human immunodeficiency virus (HIV) infection, asthma, and hypertension. With 5 years of follow-up, 10% of patients in the IRIS study stopped imatinib because of intolerable toxicity(36). Though the rates of drug discontinuation due to intolerable toxicity is low, it is the chronic mild toxicities of tyrosine kinase inhibitors that lead to lower adherence (5).Chronic grade 1 toxicity such as diarrhea may significantly impair quality of life, and on occasion some patients choose not to take imatinib to avoid such toxicity(71).

The commonest toxicities (occurring in >10% patients) described with Imatinib at a dose of 400mg in newly diagnosed CML in chronic phase are as follows: Fluid retention, nausea, muscle cramps, diarrhea, skin rash, fatigue, joint pain, myalgia, weight gain, fever, constipation.

Most of these adverse effects are mild, with grade 3-4 toxicity <2%(82). Rarer adverse effects noted include secondary malignancies(83), blistering(84), photo toxicity (85) and hepatotoxicity(86).

Significant growth retardation has been described following the use of imatinib in pre-pubertal children(40).In the comparison of imatinib versus dasatinib as first-line therapy in chronic phase CML (DASISION), the most common non-hematologic adverse reactions were more frequent with imatinib(87).

It is important to understand the factors associated with non-adherence, particularly those which are modifiable, to design effective interventions that will lead to better adherence among patients.

3.7 Methods to improve compliance

Minimizing the frequency of dosing has been shown to improve adherence (88). In a meta-analysis, adherence ± SD with once daily dosing was 79%± 14%; to twice-daily dosing, 69%± 15%; and to (*P*=.008 vs. once-daily); and to dosing 4 times per day,51%± 20% (*P*<.001 vs. once-daily; *P*=.001 vs. twice-daily dosing) (89). This suggests that there is approximately a 10% decrease in adherence which occurs with each additional daily dose (90).

Counseling patients on the importance of good compliance with Imatinib has been shown to improve compliance rates (91). Overall compliance was better in patients who were regularly counseled compared to those who were not (93.0 ± 2.3% vs. 76.2 ± 7.4%, $P = 0.001$). The counseling was especially effect in patients on higher doses of imatinib (>400 mg/day) (87.8 ± 6.0% vs. 65.5 ± 16.1%). This study did not address differences in clinical response in the two groups.

Unintentional non-adherence, most commonly due to forgetfulness, may be addressed in several ways: The use of 'prompts' to take medication – either by relatives or by taking medicines according to a regular routine, for example, with a meal, have been found to be useful in ensuring almost perfect adherence(71). In some chronic diseases like tuberculosis and AIDS, the use of short-messaging-service (SMS) to mobile phones has been effectively in improving adherence.

Intentional non-adherence (deliberate medication default) – is either due to excess or troublesome side effects, or because of significant improvement in symptoms or because of a conscious decision to stop medication for other reasons. Understanding the reasons for non-compliance will help in delineating specific strategies to compliance.

Each of these should be addressed in by different strategies – improving patients' knowledge of disease and increasing awareness of consequences of default, or alleviating seemingly trivial side-effects with simple symptomatic therapy.

The current study attempts to address the issue of non-compliance with Imatinib in patients with chronic myeloid leukemia, ascertain whether non-compliance may be gauged by a simple questionnaire, assess the factors contributing to non-compliance and whether non-compliance (defined as <90% of medication taken in the previous month) has an effect on outcome.

AIMS AND OBJECTIVES

4 AIMS AND OBJECTIVES

Objective 1: Prevalence Estimation – To determine the prevalence and severity of non-adherence to Imatinib among CML patients in the month prior to interview; provided that these patients have been on treatment for at least 6 months.

Objective 2: Identification of Predictors of Non-Adherence – To identify those variables which are predictive of patient non-adherence.

Objective 3: Non-Adherer Analysis: To assess impact of non-adherence on outcome.

Objective 5: To assess patient-perceived adverse effects with Imatinib

Objective 6: To document degree of knowledge of disease.

Objective 7: To document the toxicity profile of imatinib in patients with CML

METHODOLOGY

5 Methodology

5.1 Study design and inclusion/exclusion criteria

The study is designed as an observational study in patients with chronic myeloid leukemia, who are currently under follow-up in the department of Hematology, CMC Hospital Vellore, during the period May 1st 2012 – July 31st 2012

All patients with CML on Imatinib Mesylate (Glivec / Veenat) for at least 6 months were included in the study.

All patients were counseled about the study and informed consent was taken from all patients prior to enrollment. In case of minors (age <18years), assent forms were signed by one parent prior to enrollment.

5.2 Data Collection

5.2.1 Patient interview

During the routine follow-up visit, all patients consenting to enrollment were interviewed by one of the following health care professionals - a doctor, pharmacist or nurse.

They were questioned regarding the compliance behavior in the preceding one month, guided by 4 questions as described in the Modified Basel Adherence assessment scale (BAAS) (Appendix).

In a form provided in English and 4 regional languages (Tamil Telugu, Hindi and Bengali) the patients were asked to describe the perceived adverse effects of treatment. They were also administered a questionnaire (multiple choice format) to assess their knowledge of disease. For patients who were illiterate, assistance in filling the forms was provided by health care professionals.

The following information was thus collected from patients either by interview or by questionnaire:

1) Demographic details
2) Basel Adherence Assessment Scale (BAAS) for compliance over the past one month (Annexure 1)
3) Adverse effects form
4) Questionnaire on CML to assess knowledge of disease (Annexure 2).

The treating physician was requested to provide information regarding concomitant medication and adverse effects noted by the physician during the course of treatment.

5.2.2 Treatment outcome

Patients' records were reviewed for information on peripheral blood counts, cytogenetics (PB-FISH) and real-time quantitative polymerase chain reaction (RQ-PCR).

5.3 Techniques used for response assessment:

5.3.1 Interphase FISH analysis

Interphase FISH analysis was performed using fixed cell suspensions obtained by direct or unstimulated overnight cultures of peripheral blood using standard protocols and dual colour, dual fusion BCR/ABL1 translocation probes (Abbott Molecular-Vysis Inc., Des Plaines, IL, USA) as previously described(92).

5.3.2 Qualitative and quantitative RT- PCR assays:

Peripheral blood samples collected in EDTA tubes were subjected to total RNA extraction and cDNA synthesis, followed by qualitiative and quantitive PCR assays (details in Annexure 3).

5.4 Definition of optimal and suboptimal outcome:

Outcomes were divided into optimal and suboptimal as defined by standard guidelines for response assessment at 3 months, 1year, 18 months(19) as previously described (Table 1 and 2).

Molecular response was also assessed at 24 months and 30 months following initiation of therapy.

At a time-point of 3 months following initiation of therapy, anything less than a complete hematological remission was considered a suboptimal outcome. Complete hematological remission was defined normalization of blood counts (total WBC count <10,000/mm3 and

platelet count less than 4, 50,000/mm3), without any immature granulocytes in the peripheral blood.

At a time point of 1year following initiation of therapy, anything less than a complete cytogenetic response with fluorescence in-situ hybridization (FISH) on peripheral blood was considered a poor outcome. Complete cytogenetic response was defined as <1% fusion positivity.

At a time point of 18months following initiation of therapy, anything less than a major molecular response with quantitative polymerase chain reaction on peripheral blood was considered a suboptimal outcome. A major molecular response was defined as a PCR product of ≤0.1 on the international standard scale.

5.5 Statistical analysis

Descriptive analyses was done for all raw data, and for purposes of comparing outcome with discrete and continuous variables, the Chi square test and Fisher's Exact test (or its non-parametric variant when appropriate) were used. Variables affecting compliance were tested for correlation. Correlation between outcome and compliance was calculated. P values of <0.05 were considered significant.

Other parameters predicted to affect outcome were also tested for correlation. Cytogenetic outcomes at 12 months and 18 months were correlated with molecular responses at 18mths and 24 months.

RESULTS

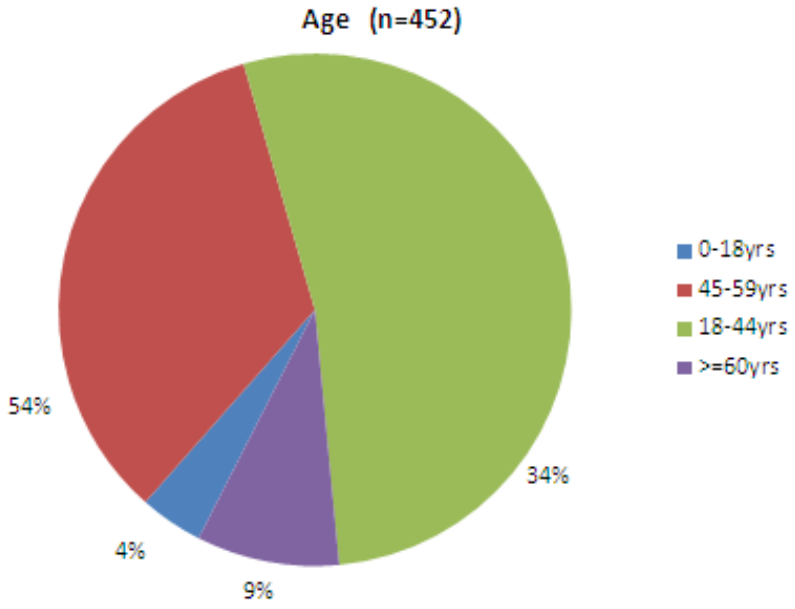
6 RESULTS

6.1 Demographics:

There were 454 patients eligible for study, of which 2 patients refused consent for participation, who did not wish to divulge information on compliance, despite assurances that it would have no bearing on the cost subsidy for therapy. Data was collected from the 452 patients who gave consent.

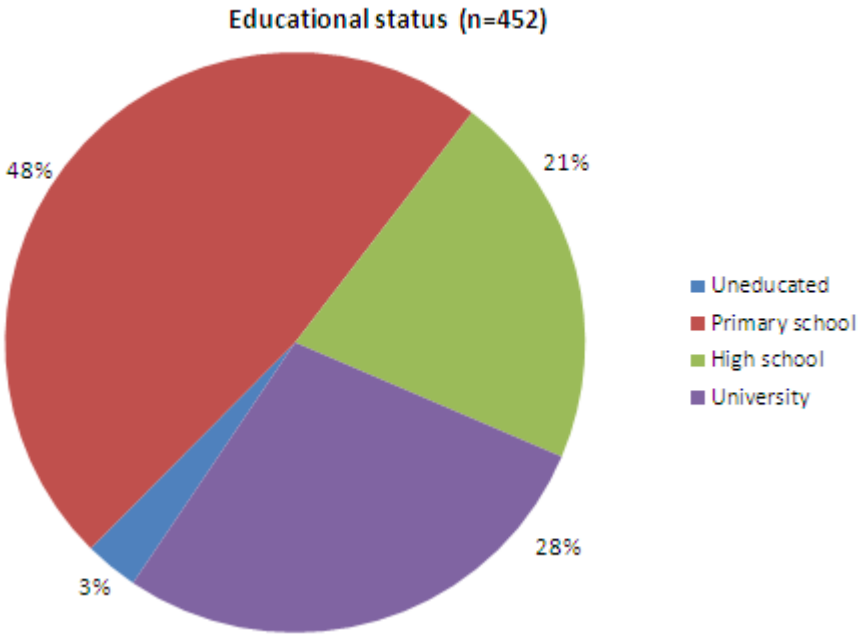
The median age of presentation in this study was 42 years (range: 4-81). Children and adolescents comprised 3.7% of the study population, and patients above 60yrs of age comprised 8.5% of the population under study (Figure 1).

Figure 1: Age distribution



There was a striking male predominance (67.5% versus 32.5%). The majority of patients had received only primary school education (47.5%), 21.4% had high school education and 27.6% were university educated. Fifteen patients (3.3%) were uneducated (Figure 2).

Figure 2: Educational status among patients



Only 18 patients (3.9%) paid for the medicine, and 96% of patients received imatinib free of cost on the GIPAP (Glivec International Patient Assistance programme) scheme.

The baseline characteristics of the patients are given in Table 3.

Table 3: Patient characteristics – clinical and haematological

Baseline characteristics (n=452)	
Age (years)	Median 42 (4-181)
No: patients per age group	
0-12yrs	5
13-17yrs	13
18-44yrs	244
45-59yrs	151
>=60yrs	39
Sex (%)	
Male	305 (67.5%)
Female	147 (32.5%)
Hematological parameters at diagnosis	Median (Range)
Hemoglobin (g %)	10.2g% (4.1-13.2)
WBC count (/mm^3)	186,000/mm^3(1400/mm^3-7,50,000)
Platelet count (/mm^3)	357,000/mm^3(26000-1647000)
Disease phase at diagnosis	
Chronic phase	390/426 (91.5%)
Accelerated phase	29/426 (6.8%)
Blast crisis	3/426 (0.7%)
Fusion transcript	
b2a2	74
b3a2	114
b3a3	1
e1a2	1
unknown	262

Symptoms at diagnosis: A total of 431 out-patient records were available, out of which 376 patients had information on symptoms at diagnosis. The majority of patients (283/376) were symptomatic at presentation; Ninety three patients were incidentally detected to have high WBC counts on routine medical check-up, amongst who were 7 pregnant women, detected on a routine antenatal checkup. Symptoms at diagnosis are tabulated in Table 4.

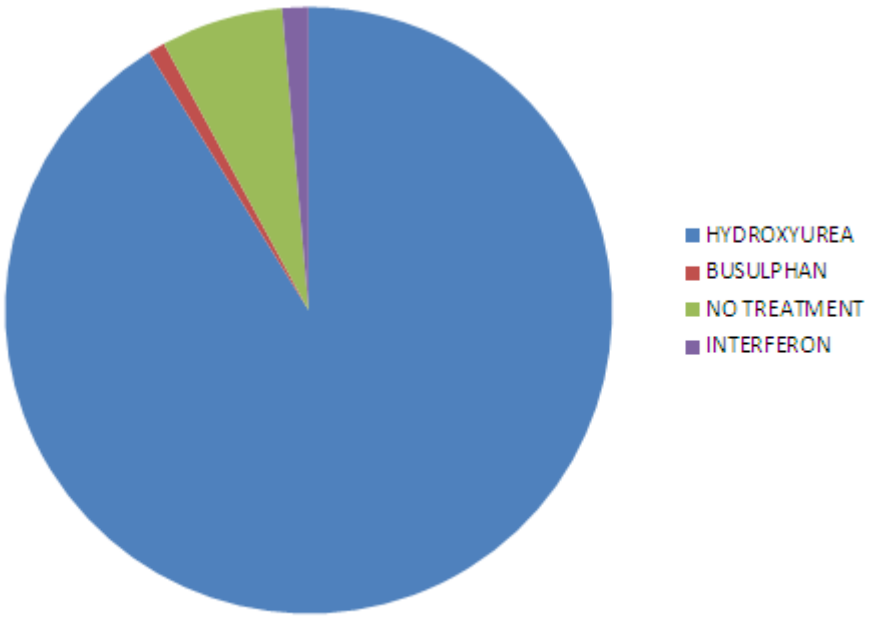
Table 4: Symptoms at diagnosis

SYMPTOMS AT DIAGNOSIS (n=376)	No: (%)
No symptoms	93 (24.7%)
Abdominal pain	141 (38.6%)
Fatigue	102 (35.2%)
Loss of weight	81 (27.5)
Fever	73 (23.2%)
Loss of weight	24 (5.3%)
Bleeding	6 (2.1%)
Priapism	2 (0.77%)

Therapy prior to Imatinib:

The commonest therapy received prior to initiation of imatinib was hydroxyurea (91%). A few patients had been treated with Interferon (6 patients) or Busulphan (4 patients), prior to the introduction of Imatinib therapy (Figure 3).

Figure 3. Therapy prior to initiation of Imatinib



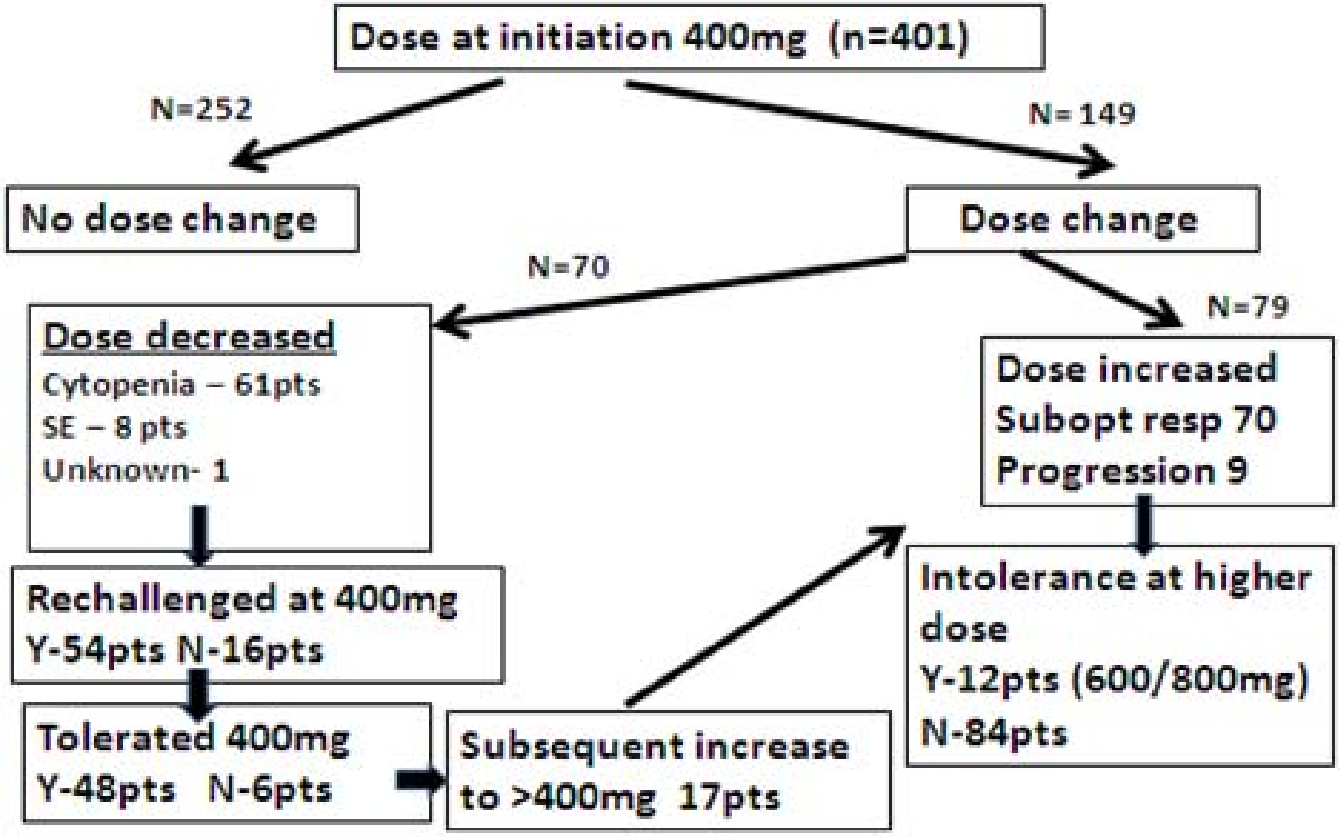
The median duration of time to initiation of Imatinib therapy was 1 month (range: 0-62), and the median duration of follow-up was 56 months (range: 11-178).

6.2 Dose changes during the course of therapy in adult patients:

Dose adjustments in 431 adult patients were reviewed from the available out-patient charts. Out of the 401 patients who were started on a dose of 400mg once daily, 252 did not have any dose change at any time during the entire course of therapy. At the time of the study, 318 patients were still on 400mg once daily, and 58 and 35 had dose increases to 600mg and 800mg respectively, while 20 patients were on reduced doses (<400mg) . The dose changes during course of therapy, in adult patients who were initiated at 400mg are depicted in Figure 4. Doses were decreased to lower than 400mg in 70 patients because of cytopenia (61 cases) or side effects (8 cases). Of these, fifty-four patients were re-challenged at 400mg, and significantly, 48 out of 54 patients tolerated re-challenge, and 6 patients required dose reduction due to persistent intolerance.

Doses were increased in 96 patients due to suboptimal response (n= 87) and disease progression (n= 9). Intolerance to the increased dose was noted in 12/96 patients at doses of 600mg and 800mg.

Figure 4: Dose changes in adult patients initiated on 400mg



6.3 Adverse effects

6.3.1 *Adverse effects noted by patients*

According to the adverse effects forms filled by the patients, 82 out of 452 patients (18%) did not report any adverse effects. Adverse effects noted by the other 370 patients (82%) are shown in Table 3. The commonest adverse effect noted was muscle cramps (n=220 (49%)), tiredness (n=204(45.7%)), oedema (n=140 (31%)) and gastritis (n=107 (31%)) (Table 5).

6.3.2 *Drug toxicity as assessed by physicians*

Drug toxicity sheets were provided to the treating physicians. Toxicity was noted in 272/452 patients. The commonest toxicity noted was hypopigmentation (51%), followed by oedema (10%), cytopenia (6%), skin rash (4%) and muscle cramps (4%) (Table 6).

Table 5. Adverse effects described by patients (n=452)

ADVERSE EFFECTS	**No: patients (%)**
Cramps	220 (49%)
Tiredness	204 (45.7%)
Oedema	140 (31%)
Abdominal pain/gastritis	107 (23.8%)
Nausea	92 (20.5%)
Decreased sleep	92 (20.5%)
Vomiting	81 (18%)
Dizziness	79 (17.6%)
Loss of appetite	69 (15.4%)
Taste change	68 (15.1%)
Constipation	55 (12.9%)
Diarrhoea	55 (12.9%)

Table 6. Drug toxicity noted by physicians = (n=452)

DRUG TOXICITY	**No: patients (%)**
Hypopigmentation	234 (51.7%)
Oedema/fluid retention	45 (10%)
Cytopenia	26 (5.7%)
Skin rash	20 (4.4%)
Muscle cramps	20 (4.4%)
Dyspepsia	20 (4.4%)
Weight gain	5(1.1%)

6.4 Concomitant medication

Physicians were asked to state any concomitant drugs the patient might be taking, to look for any concomitant medication which might have had an effect on adverse events (Table 7).

Table 7: Concomitant medication (n=452)

Concomitant medication	No: patients
Calcium channel blockers	4
Rifampicin	1
HMG CoA inhibitors	3
Warfarin	1
Phenytoin	1
Acetaminophen	3

6.5 Knowledge of disease

The results of the questionnaire (Format: True/False/Don't know) were analyzed in 445 patients. Seven patients did not fill out the required information. One hundred and twenty-three patients had given the answer "Don't know" to all the 14 questions. There were 4 questions directly pertaining to compliance and these questions were given taken into account when determining the patient's knowledge of disease (Figure 5):

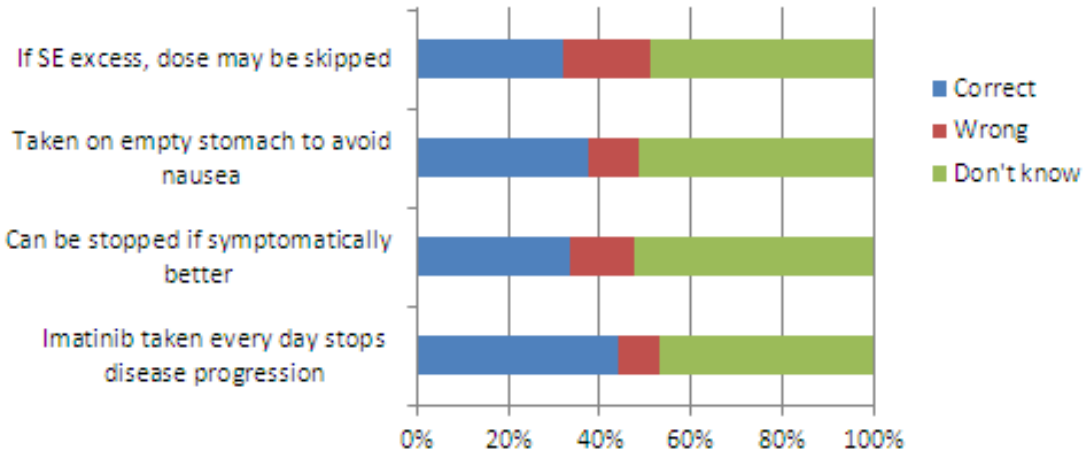
QUESTION 1 : Does taking Imatinib daily prevent progression of disease?

QUESTION 2: If you have side effects, is it alright to skip doses of Imatinib?

QUESTION 3: Does nausea associated with Imatinib decrease if taken on an empty stomach?

QUESTION 4: If you feel better, is it alright to stop taking Imatinib?

Figure 5. Knowledge of disease.



6.6 Compliance

5.6.1 <u>Prevalence of poor compliance:</u> The frequency of skipped doses and past documentation of poor compliance are given in Table 8 and 9 respectively. None of the patients who were non-compliant on interview were found to be non-compliant by chart review, and vice versa, therefore patients were included in the non-compliant group if they fulfilled either criteria i.e. compliance <90% via interview or non-compliance with medication previously documented on out-patient medical records. As the definition of non-compliance also included a review of out-patient records (retrospective data), they may not have been uniformity in the labeling of patients as non-compliant.

Using these definitions of poor compliance, 36 out of 452 (8%) patients were poorly compliant with imatinib (Table 10).

Table 8: Prevalence of non-compliance by patient interview.

Non-compliance was defined as skipping ≥4 doses of Imatinib in the past one month. A total of 23 patients were non-compliant based on this definition.

No: Days missed	No: Patients (%)	Compliance
0	325 (71.9%)	≥90%
1-3	104 (23%)	≥90%
4-7	10 (2.2%)	<90%
≥7	13 (2.9%)	<90%

Table 9: Non-compliance by default noted in out-patient medical records

Non-compliance note in medical records	**No: patients (%)**
Yes	13 (2.9%)
No	418 (97.1%)

Table 10: Prevalence of non-compliance (composite)

	No: patients (%)
Non-compliance by interview	23 (5.1%)
Non-compliance by medical records	13 (2.9%)
Total	36 (8%)

6.6.2 Reasons for non-compliance:

During the interview, patients were asked the reasons for having missed doses in the past. One hundred and thirty seven patients gave one or more reasons for missing doses of Imatinib (Table 11).

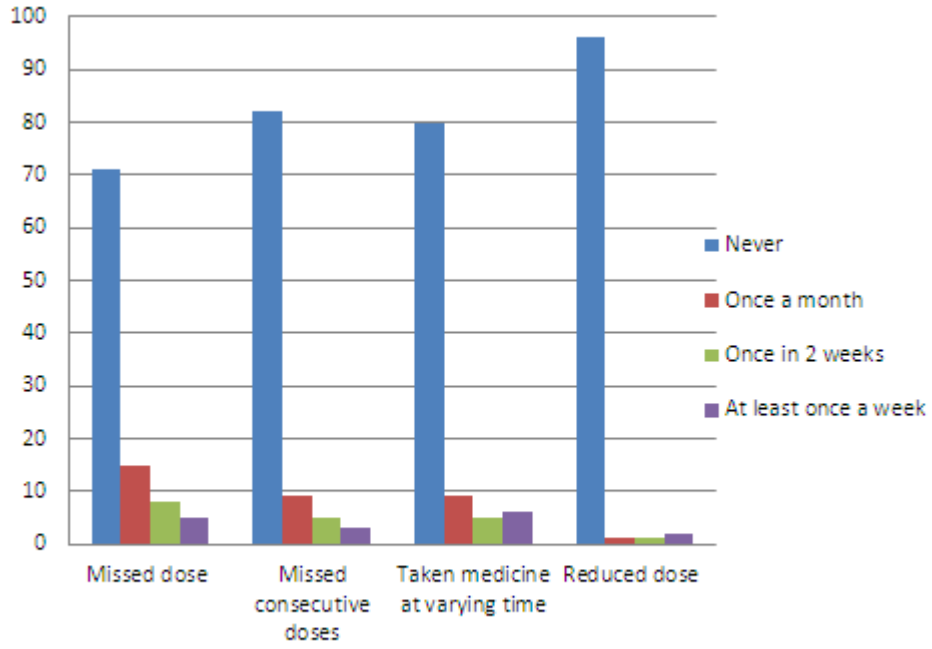
Table 11. Reasons for non-compliance

Reason for missing dose	**No: patients (%)**
Travelling	56 (12%)
Forgot	36 (8%)
Illness	26 (6%)
Side-effects	19 (4%)
Late appointment	9 (2%)
Late from work	6 (1.3%)
Family function/festival	5 (1.1%)
Weekly fasting	1 (0.22%)

6.6.3 Assessment of regularity of medication intake

Patients were interviewed by a doctor, nurse or pharmacist regarding compliance over the past one month. The Modified Basel Adherence Assessment scale (BAAS) was also used to document regularity of medication intake - whether they had skipped a dose or not, whether they had skipped consecutive doses, how often had they skipped a dose and whether they took the medication at a regular time or at different times in the day (Figure 6).

Figure 6. Frequencies of missing medication and irregularities in dosing (n=452)



6.6.4 Modifiers of non-compliance

Parameters tested for association with non-compliance were age, educational status, cost of therapy, dose, and knowledge of disease, side effects and interviewer. The parameters were grouped as follows (Table 12). It was found that only educational status (p=0.05) and dose >400mg (p=0.02) showed a statistically significant association with poor compliance (Figures7 & 8). The association of compliance with other variables - age, education, cost of therapy, knowledge of disease, any adverse effects - was not statistically significant. Although all patients who paid for the drug (n=18) and all patients less than 18 years of age (n=18) were compliant with therapy, these groups had too few patients to attain statistical significance.

Table 12: Variables tested as possible modifiers of compliance

Variable tested	**Groups**	**Poor Compliance (<90%) n=36**	**Good Compliance (≥ 90%)n=416**	**P value**
Age (n=452)	0-12 12-17 18-44 45-59 ≥60	0 0 23 10 3	5 17 217 145 32	0.288
Educational status (n=452)	University Any other	5 31	120 296	**0.05**
Dose of Imatinib (n=452)	>400mg ≤400mg	13 21	80 317	**0.027**
Adverse effects (n=452)	No adverse effects Any one adverse effect	5 31	80 336	0.596
Knowledge of disease (n=445)	Composite score 50% Composite score <=50%	10 26	106 303	0.471

6.7 Outcomes

Hematological response assessment:

Out of the 452 patients, 449 patients were eligible for assessment of hematological response. The remaining 3 patients did not have hematological assessment done at this hospital, as they were under follow-up elsewhere during the first year of therapy.

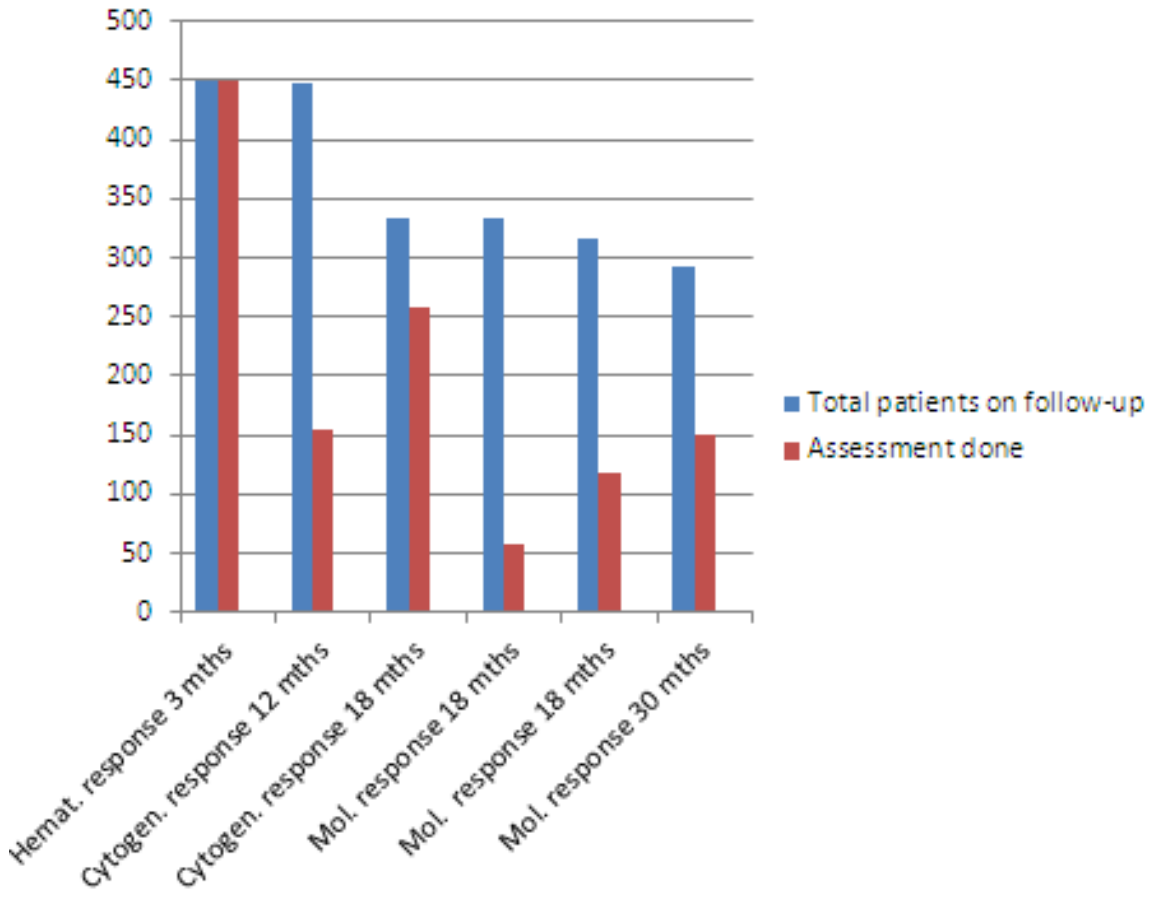
Cytogenetic response assessment:

Cytogenetic response was assessed by peripheral blood interphase FISH. There were 447 and 334 patients who were under follow-up for at least 12 months and 18 months respectively and therefore eligible for a FISH analysis at these time points. Of these, 155 patients had a FISH done at 12 months, and 258 patients had a FISH done by 18months.

Molecular response assessment:

Molecular response was assessed by RQ-PCR on PB at the time-points of 18 months, 24 months and 30 months post-initiation of Imatinib. There were 334 patients who had a follow-up of at least 18 months, and were therefore eligible for evaluation of molecular response at 18 months, however only 58 patients had the test done. There were 316 patients with follow-up of at least 24 months, of whom 118 patients had the test done, and there were 292 patients who had a follow-up of at least 30 months, of whom 150 patients had the test done (Figure 9.)

Figure 9.Proportion of patients on follow-up who were assessed at each time point.



6.7.1 Response rates (Table 13)

Hematological response:

Of the 449 patients assessed, 389 (86.6%) were in hematological remission, with normalization of peripheral blood counts at 3months after initiation of Imatinib. Of the 396 patients in chronic phase at the time of diagnosis, 89% were in hematological remission at 3months.

Cytogenetic response:

Of the 155 patients who had cytogenetic response assessed at 12 months, a complete cytogenetic response was seen in FISH was <1% in 99 patients (63.8%). A partial cytogenetic response (FISH 1%-35%) was seen in 25.1%, a minor cytogenetic response (FISH 36%-65%) in 5.1%, a minimal response in 4.8% and in 1.2% there was no response (FISH >95%).

By 18 months, there were 258 patients who had a FISH done (inclusive of patients who had a FISH done at 12months), and the rates of complete cytogenetic response had dropped to 57%, partial cytogenetic response 35%, minor cytogenetic response 4.6%, minimal cytogenetic response 9.3% and no cytogenetic response 0.77%.

Molecular response:

53% of patients who were assessed at 18 months had a major molecular response (RQ-PCR ≤0.1% on the International Scale), with MMR rates of 48% and 50% at 24 months and 30 months respectively. Undetectable transcript levels were noted in 7/58 patients (12%) at 18months, 18/118 patients (15.25%) at 24 months and 24/150 (16%) patients at 30 months.

Table 13 Response rates

Assessment	Time point	No: patients assessed	Response rates	
			Optimal	Suboptimal
Hematological response	3mths	449/452	86.6%	13.4%
Cytogenetic response	12mths	155/447	63.8%	36.2%
	18mths	258/334	57%	43%
Molecular response	18mths	58/334	53%	47%
	24mths	118/316	48%	52%

6.7.2 Modifiers of outcome:

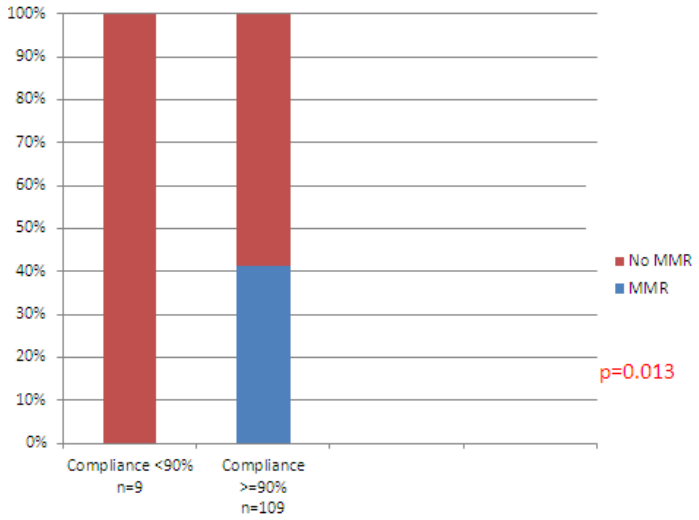
The following variables were tested for association with outcomes (as assessed by FISH at 12 months and 18 months and RQ-PCR at 18 months, 24 months and 30 months): age, phase of disease at diagnosis, compliance, current dose <400mg, educational status, knowledge of disease, and hematological response at 3 months (Table 14).

Table 14. Modifiers of cytogenetic response at 12 months.

Variable	Groups	CCR 12mths	No CCR 12mths	P value
Age	0-18 18-44 45-59 ≥60	5 57 24 13	3 27 24 2	0.09
Phase at diagnosis	CP AP/BC	91 5	45 7	0.113
Compliance	<90% ≥90%	2 97	5 51	0.09
Current dose	<400mg 400mg	3 86	2 34	0.625
Hematological response at 3mths	CHR No CHR	92 46	7 10	0.05
Educational status	University Other	30 69	9 47	0.02

A complete hematological response at 3months (p=0.05) and educational status (p=0.02) were found to be significantly associated with complete cytogenetic response at 12mths. There was a trend towards suboptimal cytogenetic response at 12months in patients who were in the poor compliance group (p=0.09) and at 24 months this association was statistically significant in predicting a poor molecular response (p=0.01) (Figure 18).

Figure 18. Association between compliance and molecular response at 24months.



6.7.4 Correlation between hematological, cytogenetic and molecular response:

There was a modest correlation between a complete hematological response at 3months and complete cytogenetic response at 12 months (R^2 0.178, p=0.03) and at 18 months (R^2 0.228, p=0.000). The cytogenetic response by FISH for bcr-abl at 12 months and 18 months strongly correlated with the molecular response at 18 months (R^2 0.542, p=0.003), 24 months (R^2 0.553, p=0.000) and 30 months (R^2 0.541, p=0.000). A strong correlation between molecular response at 18 months and 24 months was also seen (R^2 0.930, p=0.000).

DISCUSSION

7 DISCUSSION

7.1 Demographics

Chronic myeloid leukemia is one of the commonest haematological malignancies in the adult population, with a median age of presentation in the 6th decade. The median age of presentation in this study was 42 years, which concurs with data from the Indian subcontinent, where CML appears to present 1-2 decades earlier than described in Western populations(14)(13). The male predominance is also exaggerated in this study, with a male to female ratio of 3:2. Though similar gender differences are described in many diseases in the Indian population (97)(98), there have been no genetic causes described for this gender skew probably reflecting patterns of health-seeking behavior and social practices. CML is very rare in children, and there were 18 children and adolescents in this study (3.7% of overall patient population).

The majority of patients - 390/422 (92%) - presented while in chronic phase, with 29/322 (9%) in accelerated phase and 3/422 in blast crisis (0.7%). The majority of patients were symptomatic at presentation, and the most common symptoms at presentation were dragging abdominal pain, weight loss and fever, and rarely patients presented with bleeding and priapism. Only 33% of patients were asymptomatic at presentation. There were 7 patients who presented with high blood counts, incidentally detected during antenatal check-up.

7.2 Compliance

One of the challenges of treating chronic diseases is ensuring adequate patient compliance with medication. Compliance studies in chronic disease like hypertension, diabetes, asthma and schizophrenia have demonstrated varying degrees of non-compliance with medication, ranging

from 29% to 59% (74)(77). These high rates of non-compliance with medication result in long-term increase in health care costs, increased hospitalization, morbidity and mortality.

A meta-analysis of studies looking at medication adherence rates in oral chemotherapy found non-adherence ranging from 16% to 100% depending on the drug, regimen and definition of adherence used in different studies (78). Non-compliance in malignancies may be more fatal than in other chronic diseases - with increased risks of relapse and resistance.

By the end of the 1990s, median survival in chronic myeloid leukemia when treated with interferon-α was 5-6years. With the introduction of Imatinib, chronic myeloid leukemia was transformed into a more chronic disease, with over 90% of patients on therapy attaining hematological remission by 3 months (compared to between 40-70% with Interferon-α) and over 75% in cytogenetic remission by one year (compared to 8-10% with Interferon-α).

The new challenge in treating imatinib was to ensure good compliance with medication. Though fairly well-tolerated with relatively few side effects and serious adverse events, studies have shown that many patients are non-compliant with daily dosing of Imatinib, and this level of non-compliance ranges from 10% to 60%, depending on the definition of non-compliance used(5)(99)(100)(101). This non-compliance has been shown to adversely affect rates of cytogenetic response and molecular response. Non-compliance has also been shown to increase rates of loss of cytogenetic response and rates of molecular response in patients who had attained a complete cytogenetic response (5)(7).

Financial burden and costs incurred due to chronic disease leads to intentional non-adherence, and with consistent links between medication non-adherence and high medication

costs (102)(103)(104). Up to 32% of older patients with chronic diseases exhibit intentional non-adherence to cut costs. For a 10% increase in cost, adherence to prescription refill decreases by 2% to 6%, depending on drug prescribed and condition of the patient (105). The implications of poor compliance due to inability to purchase medicines cannot be under-estimated in a developing country such as India. The presence of a well-functioning patient assistance programme for Imatinib in CML (GIPAP – Glivec ® International Patient Assistance Programme) has allowed us to study the causes of poor compliance without a monetary bias. In fact, in the patients studied, it appeared that compliance may improve if the patient bears a small part of the cost of therapy, as opposed to totally free therapy.

7.2.1 Assessment of non-compliance

Non-compliance is difficult to quantify as it is not easily measured in the routine clinical setting. Trials using electronic pill monitoring and plasma imatinib trough levels are some of the more accurate measures of compliance; however these are not applicable to routine clinical work. Questionnaires and patient interviews, are less accurate in assessing compliance compared to more objective methods, but these are still of practical value in the out-patient clinic where it may not be possible to used electronic monitors or plasma drug levels. The more elaborate the questionnaire, the higher the degree of non-compliance detected. Elaborate questionnaires have the advantage of giving a good overall understanding of the depth and reasons for non-compliance, however it is not always practical to use these lengthy interviews in a busy out-patients setting. In addition, the advanced statistical analyses required to weight and interpret these questions are not designed for use on a day-to-day basis.

In this study we have chosen to define non-compliance based on one question – the number of days Imatinib was skipped in the preceding month – to see if this simple indicator of compliance would be able to predicting outcomes and help to identify patients who require counseling to improve compliance. Out of the 452 patients interviewed, 26 patients had issues with compliance, having skipped ≥4 doses of Imatinib in the preceding month, thereby exhibiting compliance of less than 90%.

On review of the out-patient records, it was noted that some patients who were apparently compliant during patient interview, had been noted in the past to be non-compliant. These additional 13 patients were also included in the non-compliant group – giving a total non-compliance rate of 8%. This rate of non-compliance is low compared to those other studies. There are several possible reasons for this: (a) as the majority if patients received the drug free of cost, there is a possibility that they were afraid to divulge details of non-compliance for fear of reversal of sanction (b) the simplicity of the stratification into the two groups could have under-estimated the degree of non-compliance in the study population and (c) the retrospective nature of the chart review data may not have identified all patients previously non-compliant with therapy. Though non-compliance rates could be increased to between 20-30% with incorporation of other aspects of the patient interview – such as irregular dosing, missing consecutive doses and reducing dose of the drug – we analyzed the effect of non-compliance with the narrow definition of number of doses missed in the past one month, to see if this simple stratification would delineate a group of patients who had adverse outcomes.

The possibility that the levels of compliance at our center are higher than those described in literature was also considered. This may be due to the fact that over 95% of patients receive Imatinib on a patient assistance programme, where at a subsidized cost the drug is provided

every 3 months, and this is dispensed by a single designated pharmacist, who functions from within the out-patient clinic itself. As patients received the drug in a constant manner from the same pharmacist who maintains records on compliance, pill-counts and follow-up visits, compliance rates are likely to be higher than if the drug was dispensed at a counter with an anonymous dispenser. The involvement of a pharmacist or health care provider who re-enforces the doctors instructions, has been shown to increase compliance rates in chronic disease, as the sense of personalized service increases (106)(107)(108). To prove that the compliance rates are truly high, we would require electronic pill monitoring systems which are out of the scope of this study, and are not relevant in a resource-constraint setting such as ours.

Studies on compliance with TKIs have used various definitions of poor compliance - <90% of medication, prescription refill, medication. Compliance is difficult to ascertain in a uniform manner, and possibly a definition of compliance should center around the impact that non-compliance has on outcome, and the ease of assessing non-compliance in a routine clinical setting. With the end-point of complete cytogenetic response at 12 months and molecular responses at 18mths ($p=0.09$), we found that the non-compliant group showed trend towards suboptimal outcomes, with significantly poorer molecular responses at 24 months ($p=0.013$).

7.2.2 Modifiers of compliance

The effect of educational status of non-compliance has been addressed in in chronic disease like AIDS(109), tuberculosis(110) and diabetes mellitus (111). Adherence studies in chronic myeloid leukemia (76)(5)(112) have been carried out predominantly in developed countries, and the focus on modifiers of compliance and outcome did not include the educational

status of patients. In this study, the effect of educational status on compliance was apparent despite the low numbers of patients in the non-compliant group, with patients having a university education showing significantly higher rates of good compliance compared to patients with any other educational status (p=0.05). The impact of educational status on outcome was also apparent, with a significantly higher number of optimal responses in the group with university education versus those with lower education (cytogenetic response at 12 months76% vs. 59 (p=0.021).

The recommended dose of imatinib in chronic phase is 400mg once daily. The recommendations in patients with suboptimal response/loss of response include increasing the dose of imatinib or changing to a second-line tyrosine kinase inhibitor (19). As most of our patients receive imatinib on a patient assistance programme at no or subsidized costs, changing to a second-line TKI is not an option in most cases. As part of routine practice, doses are increased in these patients, and if they continue to have suboptimal responses, are considered candidates for allogeneic transplant.

The increase in dose to 600mg or 800mg is associated with an increase in adverse effects, as at these doses imatinib is less well tolerated. In our study, patients on higher doses of Imatinib showed significantly higher rates of poor compliance. Causality could not be established as this was an observational study – i.e. - the question of whether the higher doses caused poor compliance, or the poor compliance resulted in poorer outcomes necessitating higher doses.

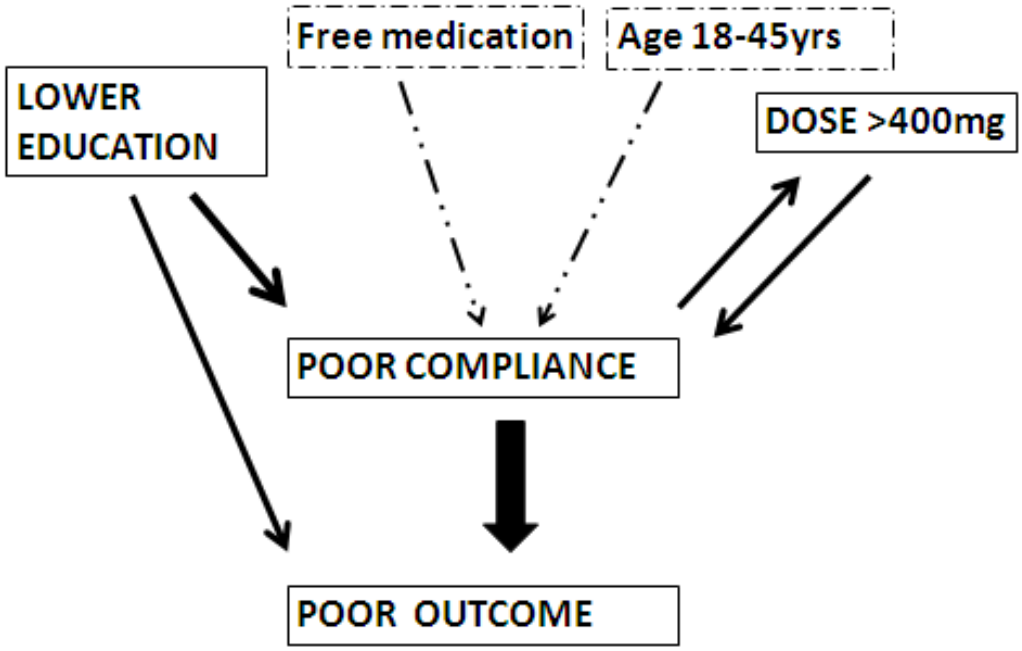


Figure 20. Factors affecting compliance and outcome. Poor compliance has an adverse effect on outcome in CML. A lower educational status contributes to poor compliance. Other possible modifiers of poor compliance include higher drug doses, free medication and age of the patient.

7.2.3 Reasons for non-compliance

Qualitative data on reasons for non-compliance were also collected. The commonest reason for skipping doses of imatinib was forgetfulness. It has been shown that daily reminders or prompts are extremely helpful in decreasing the number of missed doses due to this unintentional non-compliance. Patients also stated that they tended to skip doses during travel.

In a study exploring reasons of non-compliance, patients who were strictly adherent to imatinib planned ahead prior to travel, to ensure that doses were not skipped. Most patients tend to take the medicine with an evening meal, and coming home late from work or from social occasions was another reason cited for non-compliance. In the behavioural study on adherence in CML, on analyzing behavior of a group of patients who exhibited good adherence, it was noted that these patients tended to take the medicine in the morning, as evening schedules are frequently disrupted, whereas morning schedules tend to be fairly constant.

Several factors contribute to improving compliance – repeated patient education on consequences of non-compliance, regular feed-back on disease status, positive re-enforcement of good compliance, a non-judgmental approach while assessing adherence, attention to minor toxicity and symptomatic therapy, and special counseling for patients who are at risk for non-compliance patients (Table 16).

Table 16. Common reasons for non-adherence and possible corrective measures

Reason for skipping dose	Percentage in this study	Suggested corrective measures	Reference
Travel	12%	Plan ahead with a travel kit	(71)
Forgot	8%	Take the tablet with a meal Daily reminder/alarm on clock or cell phone	(71)(115)(116)
Nausea	4%	Take tablet with a meal	(82)
Late from work Festival/special occasions	1.3% 1.1%	Take tablet in the morning	(71)
Side effects	1.1%	Symptomatic therapy E.g. Diuretics, anti-emetics	(82)
Felt better	0.22%	Repeated counseling	(117)(91)

7.3 Outcomes

7.3.1 Response rates

A complete hematological response was seen in 87% of the total population under study, and 89% of patients who presented while in the chronic phase. Complete hematological response rates were higher in the IRIS trial with 95% patients in CHR at 3months(4).

Cytogenetic responses at 12 months and 18 months were 64% and 57% respectively. The rates of cytogenetic response are lower than those described in literature (Table 15) and may be

partly due to the fact that these assessments were done by interphase FISH on peripheral blood, (not on metaphases by conventional karyotyping) on bone marrow samples. Interphase FISH was chosen for assessment because of the convenience of testing peripheral blood over bone marrow. Interphase FISH is more sensitive than conventional karyotyping in detecting Ph+ve fusion signals (22), and MMR rates are significantly higher in cases with I-FISH <1% than in patients with I-FISH 1-5% (66.8% vs. 51.6%, $P < .001$). MMR rates are also higher in and in cases with CCgR and I-FISH less than 1% than in cases with CCgR and I-FISH 1% to 5% (66.1% vs. 49.4%, $P = .004$).

In this study, molecular responses were assessed at 18months, 24 months and 30months, and at these time-points, the rates of MMR were 53%, 48% and 50% respectively, comparable to other studies (Table 15).

7.3.2 Correlation between MMR and CCgR using interphase FISH

There was a good correlation between cytogenetic response at 12 months and molecular responses at 18mths (R=0.542, p=0.003), 24 months (R=0.553, p=0.000) and 30 months (R=0.541, p=0.000), and a good correlation between cytogenetic response at 18months with molecular responses at 24months (R=0.639, p=0.000) and 30months (R=0.627, p=0.000).

The time point at which cytogenetic and molecular response is documented varies in different studies. Table 15 compares the cytogenetic response and molecular response rates achieved in our population with that in various studies. In comparison, only one study (113) demonstrates community-based data – and the cytogenetic and molecular responses are inferior

to data from randomized trials, probably because of the inherent bias involved in conducting a trials with better compliance and follow-up.

Table 15. Comparison between outcomes in different studies

No: pts.	CCR12mths	CCR 18mths	MMR18mths	MMR24mths	Ref.
O'Brien et al (2003) n=456	**69%**	89	NA	NA	(4)(64)
Kantarijan et al (2011) n=283	**65%**	NA	NA	**53%**	(44)
Kantarijan et al (2010) n=260	**75%**	NA	NA	**46%**	(87)
Lucas et al (2011) n=68	**41%**	**49%**	**26%**	NA	(113)
Current study N=452	**64%**	**57%**	**53%**	**50%**	

The superior outcomes reported in clinical trials compared to community-based outcomes is well-described. In chronic myeloid leukemia, community-based results have been are inferior to previously published trial data from the IRIS trial (4), with overall CCR rates of 41% at 12mths and MMR rates of 31 % at 24 months (113), and CCR of 53% at 12 months(114). These results were published from specialist hematology units in developed countries. We would

expect similar rates to these, if compliance rates were comparable, however in the current study CCR rates of over 60% at 1 year and MMR of 48% by 2 years (in the patients who were assessed at these time-points) suggests that the results may be better than other community-based results, in part due to better compliance rates.

One of the drawbacks of the study was the relatively small proportion of patients who were assessed for cytogenetic and molecular response at specific time-points. The highest proportion of patients tested was at the FISH at 18mths (77%) and RQ-PCR at 30 months (51%).

7.3.3 Modifiers of outcome: A complete hematological response at 3months (p=0.05) and educational status (p=0.02) were found to be significantly associated with complete cytogenetic response at 12mths. There was a trend towards suboptimal cytogenetic response at 12months in patients who were in the poor compliance group (p=0.09) and at 24 months this association was statistically significant in predicting a poor molecular response (p=0.01) (Figure 18).

While outcomes with therapy are dependent on various variables – phase at diagnosis, primary and secondary drug resistance, disease progression with acquisition of additional cytogenetic abnormalities - compliance with medication is one factor that is not given due importance. This potentially modifiable variable may be easily targeted by simple techniques that do not take time or money, but result in a huge potential benefit to some patients who are otherwise at risk for a poor outcome.

CONCLUSIONS

8 CONCLUSIONS:

1. Non-compliance (<90% of recommended dose or >3 missed doses per month) is seen in 8% of the population under study

2. Non-compliance is a complex issue with some society-specific causes – such as frequent festivals, and practices of fasting.

3. University education is associated with higher rates of compliance.

4. Minor toxicities are commonly seen with imatinib, and in a small proportion of patients will translate to non-compliance with medication.

5. Rates of compliance may be improved by utilizing involving pharmacist/nurse involvement in patient care.

6. Non-compliance with daily dosing of Imatinib (>3 doses missed per month) is associated with a suboptimal cytogenetic response at 18months and suboptimal molecular responses at 24months.

7. Interphase FISH on peripheral blood is a practical method to assess cytogenetic response, and it correlates well with molecular response at 18months and 24months.

ANNEXURES

Annexure 1 : *Qualitative and quantitative RT- PCR assays:*

Peripheral blood samples collected in EDTA tubes were subjected to total RNA extraction (QIAamp RNA blood mini kit, Qiagen) or RNA stabilization in Trizol LS reagent (Invitrogen, Calif., USA) within 1 h of sample collection. The quality and quantity of RNA were assessed by electrophoresis and spectrophotometer. cDNA was synthesized using random hexamers and reverse transcriptase (Superscript II first strand cDNA synthesis system, Invitrogen, Carlsbad, Calif., USA). The type of *BCRABL1* transcript was determined in patients' samples by nested qualitative RT-PCR based on the previously published Biomed Concerted Action Protocol (93)]. The sensitivity of this technique in our laboratory which was established using a previously described method (94) was found to be 10^{-3} after the first 30 cycles of amplification with external primers and 10^{-5} after the second round of amplification with nested primers.

RQ-PCR for *BCR-ABL1* transcripts was done using the Taqman principle and the standard Europe Against Cancer (EAC) protocol(95) on an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, Calif., USA). All samples were tested in duplicates and a no-template control was included in each assay. The copy numbers of the target and control gene were calculated by comparison with the standard curve generated from serial dilutions of in-house plasmids. *ABL1* was used as the control gene. Results are reported according to EAC guidelines as the normalized copy number, derived by multiplying the *BCR-ABL1* copy number/ *ABL1* copy number by 100, and expressed as a percentage. The sensitivity of this technique as established in our laboratory was 10^{-5} using plasmid serial dilutions.

Conversion of *BCR-ABL* values to the International Scale (IS) is achieved by the application of laboratory-specific conversion factors. The conversion factor is derived from the

value that is equivalent to the MMR value as established in the IRIS trial (64). The formula for conversion to the IS is: *BCR-ABL1* $^{\text{lab specific}}$ X conversion factor = *BCR-ABL1* $^{\text{IS}}$

The results are expressed in IS(96) using our lab-specific conversion factor of 0.46. A value of <0.1 in the IS is interpreted as a major molecular response (MMR).

Annexure 2: The BASEL ASSESSMENT OF ADHERENCE WITH IMATINIB SCALE

Intro for Interview:
Taking medications for diseases such as cancer can be difficult for many patients. We would like to explore with you how you manage your medications in daily life.

1. Do you recall not having taken your Imatinib sometimes in the past 4 weeks?

   yes ☐ no ☐

   If yes, could you tell me how often this happened: _____
   Once a month

   Every two weeks

   Every week

   More than once a week

2. Have you skipped several consecutive doses of Imatinib in the past 4 weeks?

   yes ☐ no ☐

   If yes, could you tell me how often this happened: _____
   Once a month

   Every two weeks

   Every week

   More than once a week

3. Do you recall to have sometimes taken Imatinib medication with more than 2 hours time difference from the prescribed dosing time in the past 4 weeks?

yes ☐ no ☐

If yes, could you tell me how often this happened: _____

Once a month

Every two weeks

Every week

More than once a week

4. Have you reduced the prescribed amount of Imatinib medication during the past 4 weeks?

yes ☐ no ☐

If yes, could you tell me how often this happened: _____

Once a month

Every two weeks

Every week

More than once a week

Annexure 3: Assessment of patients' knowledge of disease

For the following questions, *mark only one answer for each question*.

	True	False	Don't Know
CML is a very common disease.	○	○	○
In CML, there are too many red blood cells produced in the bone marrow.	○	○	○
An abnormal chromosome (the Philadelphia chromosome) causes overproduction of white blood cells.	○	○	○
In the early phase of CML, patients often have no symptoms.	○	○	○
There are 3 phases of CML: the chronic phase, the accelerated phase, and blast crisis phase.	○	○	○
The worst phase of CML is the chronic phase.	○	○	○
Early CML is often diagnosed by chance based upon routine lab tests.	○	○	○
People with CML may have an enlarged spleen.	○	○	○
Bone marrow transplant (BMT) is a very common and safe treatment for CML.	○	○	○
Only a small number of CML patients are good candidates for BMT	○	○	○
Imatinib works by stopping the overproduction of abnormal white blood cells.	○	○	○
In order to keep your CML from progressing, it is important to take your Imatinib every day even if you don't have symptoms of CML.	○	○	○
It's OK to skip doses of your Imatinib medication if you have medication side effects.	○	○	○
To reduce nausea, Imatinib should be taken on an empty stomach.	○	○	○
Imatinib inhibits the mechanism of CML.	○	○	○
It's OK to stop taking Imatinib once your CML symptoms go away.	○	○	○
In some patients, Imatinib will actually eliminate the presence of the abnormal Philadelphia chromosome.	○	○	○

Annexures 2 & 3 Reproduced with permission from Noens et al Blood 2009 113:5401-5411

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BIBLIOGRAPHY

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