TREATMENT RESPONSE TO IMATINIB AMONG CHRONIC MYELOID LEUKAEMIA PATIENTS AS SEEN IN NAIROBI

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STUDENT'S DECLARATION

I declare that this dissertation is my original work and has not been presented for the award of a degree in any other university.

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DEDICATION

To the memory of my late dad Mr Dickson Muli, I wish you were here to see that the seed of education you sowed germinated, and is still growing. You remain dear in my memory.

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LIST OF ABBREVIATIONS

ABC - ATP-binding cassette transporter family

ABL- Abelson

ADP - Adenosine Diphosphate

AP- Accelerated phase

ATP- Adenosine Triphosphate

BCR- Breakpoint Cluster Region

BMA- Bone marrow aspirate

BMI - Body Mass Index

BP- Blast phase

BSA - Body Surface area

CCyR – Complete Cytogenetic Response

CHR – Complete Hematologic Response

CML – Chronic Myeloid Leukaemia

CP – Chronic phase

ELN – European Leukaemia Net

ERC – Ethics and Research Committee

GIPAP – Glivec International Patient Assistance Programme

GIST - Gastro-intestinal stromal tumor

HCT- Hemopoietic Cell Transplant

hOCT - human organic cation transporter

IRIS – International Randomised Study of Interferon and STI-571(Imatinib)

KNH – Kenyatta National Hospital

MAPKs - Mitogen-activated protein kinases.

MDR-1 - multidrug resistance gene product P-glycoprotein (P-gp; ABCB1)

MMR – Major Molecular Response

NCCN – National Comprehensive Cancer Network

Ph – Philadelphia Chromosome

Ph +ve – Philadelphia Chromosome positive

RQ-PCR – Reverse Transcriptase quantitative Polymerase Chain Reaction

SCT- Stem Cell Transplant

SPSS- Statistical Package for Social Sciences

STAT - Signal transducer and activator of transcription

TKI – Tyrosine Kinase Inhibitors

UON – University of Nairobi

WBC – white blood cells

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ABSTRACT

Background: Imatinib (Glivec/Gleevec) therapy has improved outcomes in chronic myeloid leukaemia (CML) in the last 10 years. It is an expensive drug. In Kenya, over 250 patients with CML access the medication via GIPAP (Glivec International Assistance Programme), at no cost. Their response to imatinib was unknown. This study was carried out to determine patients' hematologic response to imatinib, proportion of patients with failure and the possible predictors of failure.

Methods: This was a cross-sectional descriptive study carried out at the Nairobi Hospital GIPAP clinic. Subjects studied were Philadelphia chromosome positive (Ph+ve) CML patients who had been on imatinib therapy for at least 3 months. The investigator reviewed patients and perused through their records, extracting social-demographic, clinical and laboratory data. Characteristics of patients with hematologic failure were recorded and compared with those of patients with good response.

Treatment failure was defined as lack of complete hematologic response after 3 months of imatinib treatment or relapse after initial response.

Results: A total of 206 patients were studied, 194 of whom were in chronic phase. Median period of clinic follow up was 26 months. Prevalence of hematologic failure was 20.9 %(95% CI: 15.5-26.7). The mean duration of illness was 27.5 months, mean duration on imatinib was 25.5 months, and mean duration to start of imatinib was 1 month. Heterogeneity was noted in following variables; the failing group had higher; BCR-ABL concentration (mean of 81% vs. 68.2%), non-adherence (60.5% vs. 37.4%), longer durations between diagnosis and start of imatinib treatment, prior therapy, prolonged illness as well as exposure to imatinib itself, these differences were all significant with p values<0.008. Prolonged

duration between diagnosis and start of imatinib was an independent predictor of failure, OR 2.69(1.03-7.03 95%CI), p-value 0.043. Kaplan Meier estimator of time in CHR; showed Mean Survival time of 67 months (95% CI: 59.3-74.7).

Conclusion: This study demonstrated that majority [163 (79.1%)] of the patients had sustained CHR. A fifth of the 43 patients with failure had primary failure. Prolonged duration between diagnosis and start of imatinib was an independent predictor of failure.

INTRODUCTION

Chronic myeloid leukaemia (CML) is a haematological malignancy associated with the Philadelphia chromosome. It accounts for about 3.6 % of all haematological malignancies worldwide.[1] In Kenya CML prevalence remains unknown but studies done in Nairobi give an incidence of 90.3 cases annually[2].

For many years the treatment for CML has included hydroxyurea, interferon alpha with or without cytarabine and busulfan, with hemopoietic cell transplant (HCT) therapy giving a potential of cure [3].

The introduction of targeted therapy using tyrosine kinase inhibitors has revolutionized the management of CML. During the IRIS trial, Imatinib was compared to treatment with combination treatment with interferon and cytarabine. Due to the superiority of Imatinib demonstrated by this study, it has now become the standard of care [4]. Cases of failure on treatment with imatinib have been documented, with therapeutic failure to imatinib seen in approximately 10% to 15% of patients and can be classified as primary or secondary depending on whether an initial decline in disease levels is observed or not[5,31.]

Risk factors for failure on imatinib are yet to be fully established with the Sokal and Hasford scores being used to predict cytogenetic response in some centres [6,7].

In Kenya, most of the patients on Imatinib receive the medication via the GIPAP, an international assistance programme which provides Philadelphia chromosome positive (Ph+ve) CML patients with imatinib mesylate (Glivec®) at no cost. There had been no studies done to determine; treatment response, the proportion of patients who fail on imatinib and to describe their clinical-pathologic characteristics.

1.1 LITERATURE REVIEW

1.1.1 Chronic myeloid leukaemia (CML)

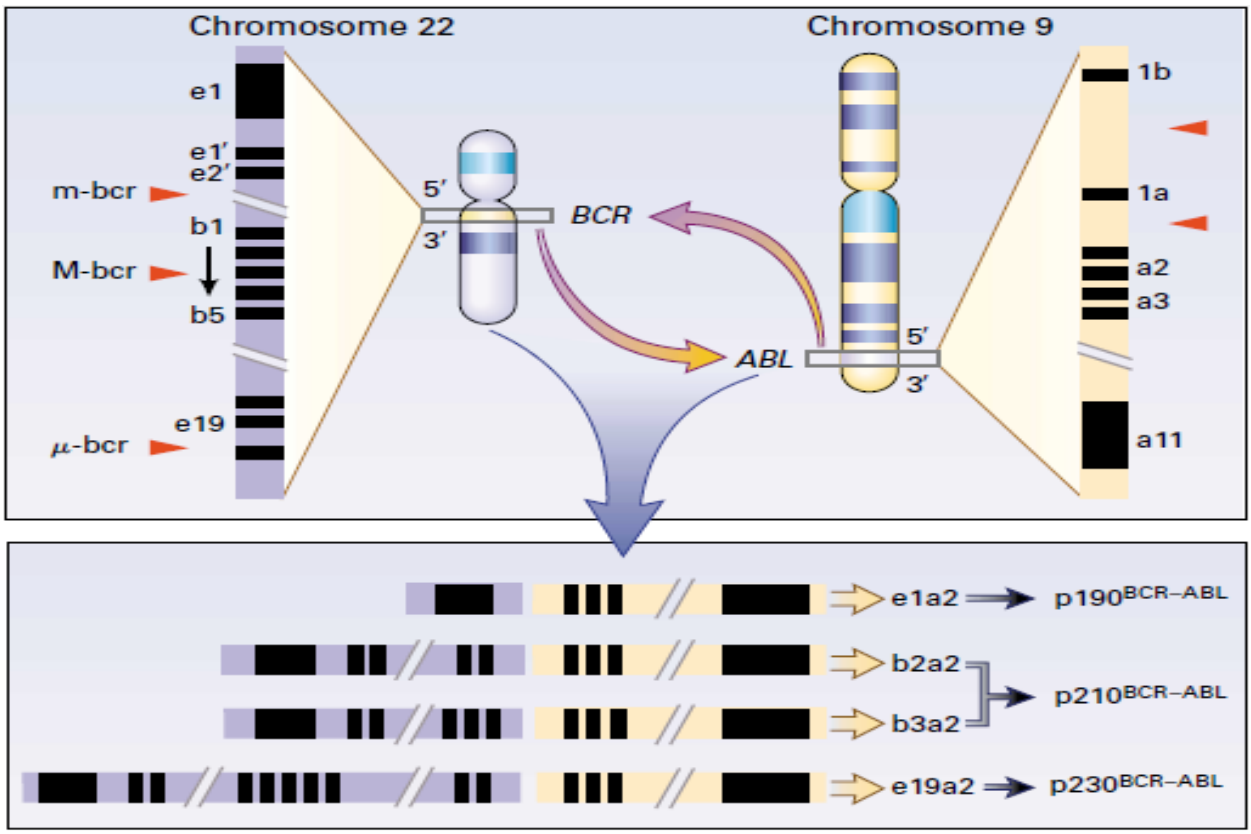
Chronic myeloid leukaemia (CML) is a clonal myeloproliferative disorder associated with the Philadelphia chromosome (Ph), a balanced translocation between the long arms of chromosomes 9 and 22, t(9;22)(q34;q11) producing BCR-ABL oncoprotein as illustrated in **figure 1**. It is characterized by proliferation of mature granulocytes (neutrophils, eosinophils, and basophils) and their precursors.

Peter C. Nowell and David Hungerford in 1960 at Philadelphia, Pennsylvania, noticed an abnormally small chromosome 22 in the cells of patients with CML, which got named Ph chromosome. This made CML the first cancer shown to be caused by an underlying genetic abnormality.[8] Later, in 1973, Janet Rowley reported that Ph chromosome represents a balanced reciprocal translocation between long arms of chromosomes 9 and 22 [9]

The chromosome is detected in about 95% of patients, [11] this genetic abnormality results in the formation of a unique gene product (BCR-ABL), which results in a constitutively active tyrosine kinase. It is this deregulated tyrosine kinase that is implicated in the development of CML and is the target of current therapies [12]

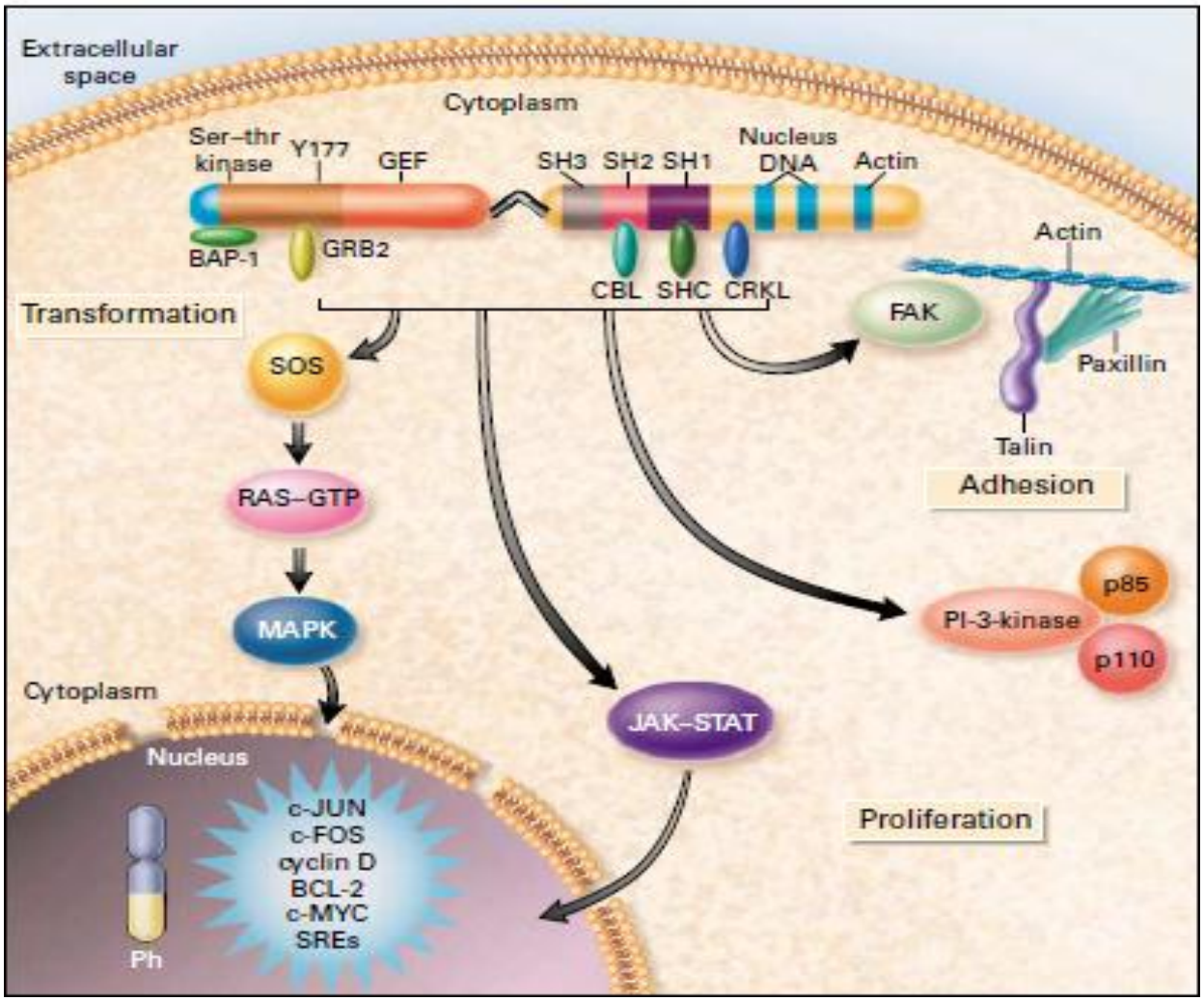
BCR-ABL oncogene mediates the development and maintenance of CML through interaction with multiple downstream signalling partners resulting in altered cellular adhesion, activation of mitogenic signalling, defective DNA repair, and inhibition of apoptosis: all these lead to transformation of hematopoietic stem cells.[11] The Signaling Pathways of BCR-ABL are illustrated in **figure 2**.

Fig 1: The Translocation of t(9;22)(q34;q11) in CML[10]



The Philadelphia (Ph) chromosome is a shortened chromosome 22 that results from the translocation of 3' *ABL* segments on chromosome 9 to 5' *BCR* segments on chromosome 22. Breakpoints (arrowheads) on the *ABL* gene are located 5' of exon a2 in most cases. Various breakpoint locations have been identified along the *BCR* gene on chromosome 22. Depending on which breakpoints are involved, different-sized segments from *BCR* are fused with the 3' sequences of the *ABL* gene. This results in fusion messenger RNA molecules (e1a2, b2a2, b3a2, and e19a2) of different lengths that are transcribed into chimeric protein products (p190, p210, and p230) with variable molecular weights and presumably variable function. The abbreviation m-bcr denotes minor breakpoint cluster region, M-bcr major breakpoint cluster region, and μ-bcr a third breakpoint location in the *BCR* gene that is downstream from the M-bcr region between exons e19 and e20.[10]

Figure 2: Signaling Pathways of p210 BCR–ABL.[10]



Several regions of BCR–ABL serve as important control elements for RAS, which is at the center of the most prominent signaling pathways in CML. Activation of RAS is mediated through a series of adapter proteins, such as GRB2, CBL, SHC, and CRKL. Adapter proteins also connect p210BCR–ABL to focal adhesion complexes, PI-3 kinase, and other messenger systems such as JAK–STAT kinases. Signaling events downstream of RAS are less well characterized. They appear to involve mainly mitogen-activated protein kinases (MAPKs), preferably the JUN kinase (JNK) pathway. BAP-1 denotes BCR-associated protein 1, GRB2 growth factor receptor–bound protein 2, CBL casitas B-lineage lymphoma protein, SHC SRC homology 2–containing protein, CRKL CRK-oncogene–like protein, JAK–STAT Janus kinase–signal transducers and activators of transcription, FAK focal adhesion kinase, SOS son-of-sevenless, GDP guanosine diphosphate, GTP guanosine triphosphate, SRE stimulated response element, Ser–thr serine–threonine, Y177 a conserved tyrosine residue, GEF GDP–GTP exchange factor, and SH SRC homology domain.[10]

Epidemiology

Aetiology: The only known risk factors for CML are high doses of ionizing radiation and occupational exposure to benzene as evidenced by 20-25 fold increase in the incidence of all leukaemia's among atomic bomb survivors.[13] CML accounts for 0.34% of all cancers, 3.6% of all haematological malignancies and 0.08% of all cancer mortalities. It also accounts for 15% of all adult leukaemias.[1, 14.] The median age of onset is 45 to 55 years.

In Kenya CML Prevalence remains unknown, Ministry of health records between 1998 and 2002 estimate a mean of 90.3 cases of CML per 100,000 people in Nairobi annually.[2] A retrospective study done on 104 patients treated for CML between April 1990 and August 2000, at Kenyatta National Hospital, Nairobi, Kenya showed age range of 10-72years with a median of 35 years which is a decade younger than the age of 45 years described among Whites.[15]

Apart from on average younger patient age group, the other differences in patient characteristics which have been noticed when African patients are compared with patients from other populations include high incidence of cytogenetic abnormalities and long duration of time between diagnosis and onset of treatment.[16]

1.1.2 Clinical features of CML

About 40% of CML patients are asymptomatic and therefore diagnosis is based on abnormal blood count.[10] The commonest physical finding is splenomegaly which occurs in about 50% of CML patients, followed by hepatomegaly. Fatigue, anorexia, and weight loss are the commonest symptoms.[11]

The disease is characterized by an overabundance of hematopoietic stem cells and progresses through Chronic, accelerated and blast phases with more than 80% of patients being diagnosed in the chronic phase.[11, 14] Median duration of chronic phase is 5-6 years[33].

WHO Criteria for diagnosis of accelerated and blastic phase[17]

The diagnosis of **accelerated phase** CML may be made when one or more of the following are present:

1. Blasts 10-19% of WBCs in peripheral blood and/or of nucleated bone marrow cells
2. Peripheral blood basophils ≥ 20%
3. Persistent thrombocytopenia (<100 x 10^{-9}/L) unrelated to therapy or persistent thrombocytosis (>1000 x 10^{-9}/l) unresponsive to therapy.
4. Increasing spleen size and increasing WBC unresponsive to therapy.
5. Cytogenetic evidence of clonal evolution.

Blast phase may be diagnosed if one or more of the following are present:

Blasts ≥ 20%

Extra medullary blast proliferation

Large foci or clusters of blasts in the bone marrow biopsy[17]

1.1.3 Laboratory features

Peripheral-blood findings include elevated white-cell count (usually greater than 25,000/mm^3) elevated platelet count in 30-50% of cases, basophilia and reduced leukocyte alkaline phosphatase activity.

Upon achievement of hematologic response the patient's WBC falls to the normal range (4-10,000/mm^3).

The *Leucocyte alkaline phosphatase (LAP)* score is based on a cytochemistry test that was once often used to test blood samples of patients who were suspected of having CML. Normally the LAP score goes up as the white blood cell (WBC) count goes up. However, people with CML, tend to have low LAP scores in spite of high WBC counts.[18]

All stages of granulocyte differentiation are visible on peripheral smear.

Most patients have a normochromic normocytic anaemia.

The bone marrow is markedly hyper-cellular, predominantly because of a proliferation of myeloid precursors from myeloblasts to segmented neutrophils, reduced fat content, with increased ratio of myeloid cells to erythroid cells.

Megakaryocytes are increased. Blasts and promyelocytes typically constitute less than 10% of all cells[14]

Vitamin B12 serum concentration in chronic myeloid leukaemia is approximately 15 times of the normal, it exists in the bound form, and the binding capacity for added B12 is increased.[19]

Chromosomal analyses are aimed at identifying the Ph chromosome t (9; 22) the hallmark of CML.[10]

These include cytogenetic detection, Fluorescence In Situ Hybridization (FISH) and molecular techniques. Cytogenetic detection entails Karyotypic analysis best performed from bone marrow material. The finding of a translocation between chromosome 9 and 22, generally the t (9; 22) (q34; q11), confirms the diagnosis. This chromosomal translocation may also be demonstrated by Southern blot analysis, or the Transcripted messenger RNA (mRNA) fusion product may be detected by reverse transcriptase polymerase chain reaction (RT-PCR).[20,]

Cytogenetic analysis is the gold standard diagnostic test in CML.

1.1.4 Treatment of CML

During the ancient times arsenicals were used for the treatment of CML.[26] In the first half of 20th century X-ray splenic irradiation for symptomatic relief was the mainstay of therapy.[26]

For many years busulfan, hydroxyurea, and interferon alpha were used for the treatment of CML. During the chronic phase of CML, cytoreductive therapy (with hydroxyurea or busulfan) is required in most patients to avoid thrombotic complications that can be due to high circulating levels of neutrophils. Hydroxyurea is preferred to busulfan primarily because of its favourable toxicity profile [3]. Treatment with either drug has no effect on the rate of progression to blast crisis; therefore, these treatments must be considered palliative[11]

Allogeneic hematopoietic stem-cell transplantation can cure CML in selected patients. Age <40 years, early disease in chronic phase, and HLA-identical sibling donor, confer better outcome post transplantation.[12, 22]

The IRIS trial (International Randomized study of Interferon and STI571) led to the introduction of targeted therapy using tyrosine kinase inhibitors, the first of which was imatinib mesylate (marketed as Gleevec or Glivec; previously known as STI-571).

1.1.5 Prognosis of CML

Two sets of prognostic factors can be established, namely those that can be identified prior to therapy (baseline factors) and those that can be employed during the treatment (time dependent or response related factors). Most important is accurate identification of the phase of the disease.

In early chronic phase important prognostic information is derived from clinical and laboratory features. Currently, the Hasford score is the best predictor of outcome for patients with CML treated with interferon alpha (IFN-α) [7, 23]

Kantarjian and colleagues in the Anderson Hospital & Tumor institute, Houston, Texas (1985); did a multivariate analysis of the associations of 303 Ph+ CP-CML patient characteristics and therapy with survival. They found that patient characteristics associated with shortened survival were age above 60 years, black race, and the presence of hepatomegaly, splenomegaly, symptoms, weight loss, and poor performance status.

Adverse blood and bone marrow parameters were anaemia, thrombocytosis or thrombocytopenia, a high proportion of peripheral blasts plus promyelocytes or of basophils, a high proportion of marrow blasts or basophils, decreased marrow megakaryocytes and cytogenetic abnormalities in addition to the Philadelphia chromosome [24].

The Sokal and Hasford Scores have been used for predicting response to therapy.

Hasford risk score[7] is a stratification that includes patients' age, spleen size, percentage of blasts, eosinophils and basophils in the peripheral blood and platelet count. It was initially used for patients treated with interferon.[7, 25] The scoring systems classify patients into three risk groups: low, intermediate, and high and is calculated using of the following equation:

(0.6666 × age [0 for <50 years; 1 for older age] +

0.0420 × spleen size [cm below costal margin] +

0.0584 × blasts [%] + 0.0413 × eosinophils [%] +

0.2039 × basophils [0 for <3%; 1 for higher value] +

1.0956 × platelet count [0 for $<1500\times10^9$/l; 1 for a higher value]) × 1000.

A score of less than 780 is considered to indicate low risk, a score of 780 to 1480, intermediate risk, and a score higher than 1480, high risk.

The Sokal score[6] is a prognostication score that in-cooperates patients age, spleen size, percentage of blasts in the peripheral blood and platelet count. It was developed when

busulfan was primarily used to treat CML and has been used to classify patients by risk in the imatinib clinical trials.

It is calculated as follows:

(0.0116 (age – 4.34)) +

0.0345 (spleen – 7.51) +

0.188 ((platelets/700)2 – 0.563)

0.0887 (% of blasts – 2.1)

< *0.8*= ***good*** prognosis, 0.8–1.2= ***moderate*** prognosis, > 1.2= ***poor*** prognosis.[6]

Both Hasford and Sokal scoring systems have been shown to be useful in predicting cytogenetic response to Imatinib[23].

1.1.6 Imatinib mesylate

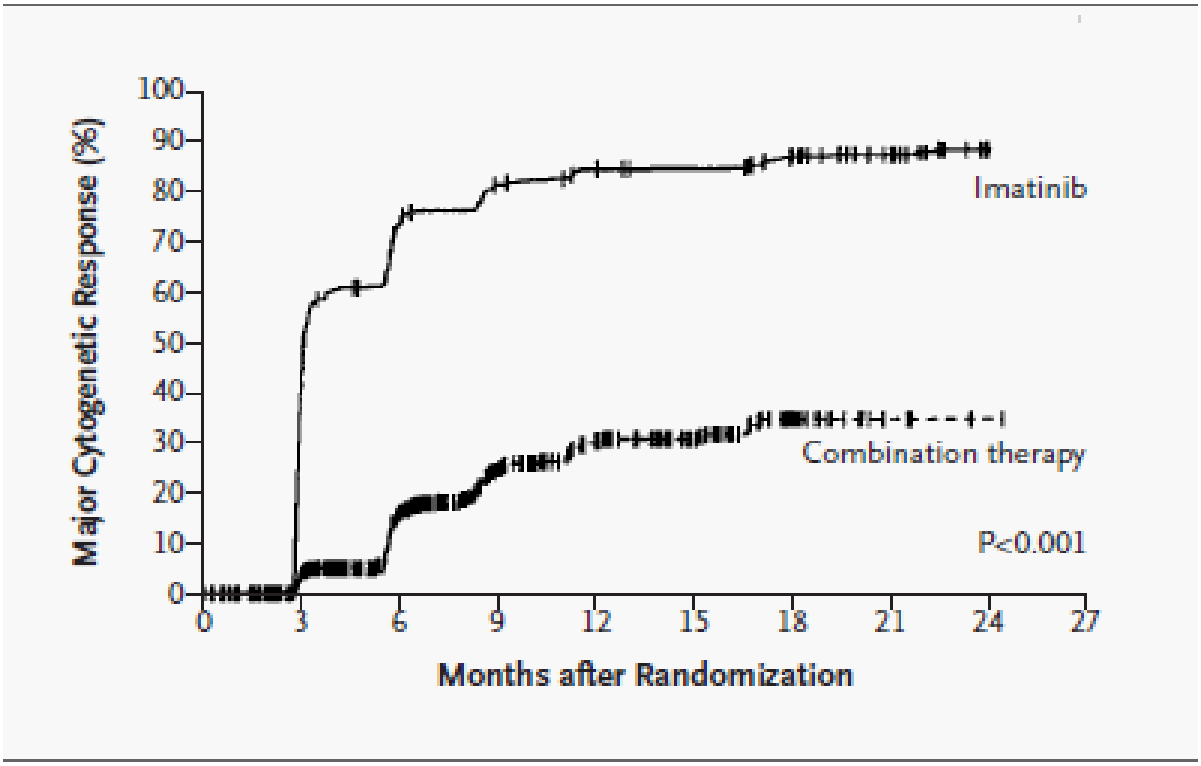
Imatinib Mesylate is a tyrosine kinase inhibitor administered orally is considered the first-line drug in treatment of Ph +ve CML following the IRIS trial.[4]

In 1992, Alexander Levitzki of Hebrew university, Tel Aviv suggested that inhibiting ABL with tyrphostins ("tyrosine phosphorylation inhibitor") might be useful to treat leukaemia's driven by ABL oncogenes.[26] At about the same time, Alois Matter, Jürg Zimmermann, and biochemist Nicholas Lydon, a former researcher for Novartis, at Ciba-Geigy had synthesized a compound termed GCP57148B (now known as imatinib) that inhibited ABL and several other tyrosine kinases at submicromolar concentrations.[26] Clinical trials initiated by Brian Druker, an oncologist at Oregon Health and Science University (OHSU) rapidly established the compound's activity in patients with CML and revolutionized CML therapy [26,28]. It was approved by the United States Food and Drug Administration (FDA) in 2001.

The IRIS trial (International Randomized study of Interferon and STI571)[4] was a phase III randomized, open-label, multicenter, crossover trial of Imatinib Mesylate (Glivec®) versus

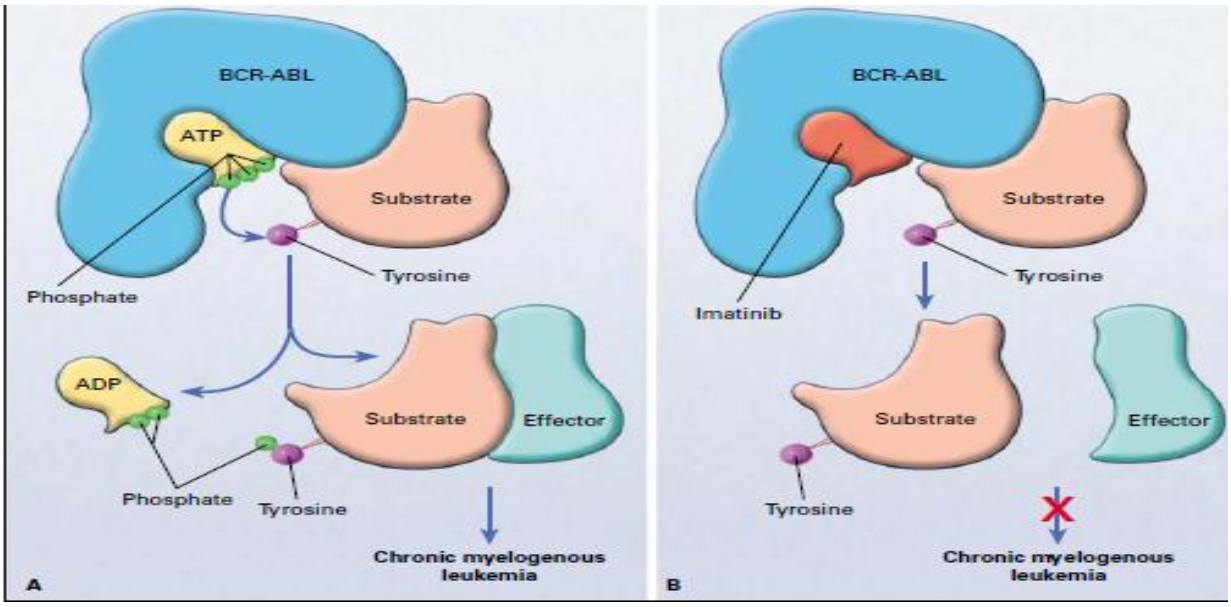
interferon plus cytarabine (Ara-C) in 1106 patients with newly diagnosed chronic phase CML. Data from the trial showed a definite superiority of Imatinib Mesylate over Interferon/Ara-C in terms of responses, overall survivals and freedom from progression. The Kaplan-Meier curve in figure 3 illustrates imatinib effectiveness over combination therapy (interferon and ara-C).

Figure 3 **Kaplan–Meier Estimate of the Time to a Major Cytogenetic Response.** [4]



Imatinib Mesylate is a (tyrosine kinase inhibitor), synthetic ATP analogue which occupies the ATP binding site of the ABL tyrosine kinase component of the BCR-ABL oncoprotein and therefore maintains it in an inactive conformation.[27] This mechanism of action is illustrated in figure 4.

Figure 4. Mechanism of Action of BCR-ABL and mechanism of Its Inhibition by Imatinib[27].



Both the National Comprehensive Cancer Network (NCCN) and European Leukaemia Net (ELN) recommend that patients with chronic-phase CML start with an oral imatinib dose of 400 mg/day. Advanced stages (accelerated and blast crisis) require at least 600mg/day. [28, 29]

Adverse effects of imatinib are classified as haematological or non-haematological. The haematological effects are; leukopenia, thrombocytopenia and anaemia whereas the non-haematological are oedema particularly infra-orbital, nausea as well as other gastro-intestinal symptoms, rashes, bone pains, and hepatic dysfunction.[4]

Costs

The cost of Gleevec (imatinib) for CML is $32,000 to $98,000 a year.

Prices for a 100 mg pill of Gleevec internationally range from $20 to $30.

1.1.7 CML response definitions and Imatinib failure/ resistance

Hematologic Response (HR)

The first treatment goal is the normalization of peripheral blood count which in most CP-CML patients occurs within 1-3 months after start of therapy. Failure to achieve this complete hematologic response (CHR) within the 3 months meets failure criteria as per ELN and NCCN guidelines and is associated with low success to therapy.[31] See table 1.

Cytogenetic Response (CCyR)

Cytogenetic analysis of Philadelphia chromosome-positive metaphase cells using bone marrow sample is the next level of response evaluation after blood count. The degree of cytogenetic response is based on the number of Ph+ metaphases. CCyR with no Philadelphia chromosome-positive metaphases is optimal, followed by partial cytogenetic response PCyR) with 1%-34% Ph+ metaphases, and minor response (35%-90% Ph+ metaphases). See table 1.

Table 1: Definitions of response[30]

Response	**Definition**
Complete ***hematologic***	WBC <10x 10^9 /l with normal differential; platelet count <450x 10^9/l; ≤1% circulating immature cells (only if consisting of metamyelocytes); disappearance of all signs and symptoms of disease including palpable splenomegaly
Partial ***hematologic***	WBC of 10–20x 10^9 /l; or normal WBC with >1% immature peripheral cells (blasts, promyelocytes, myelocytes, or metamyelocytes), or palpable splenomegaly, or the presence of other signs of disease.
Cytogenetic	At least 20 marrow metaphases must be examined
Major	Ph+ metaphases, 0% (complete)
	Ph+ metaphases, 1%–35% (partial)
Minor	Ph+ metaphases, 36%–95%
Molecular	
Bcr-Abl undetectable	No detectable Bcr-Abl transcripts by nested RT-PCR or Bcr-Abl/Abl ratio <0.001%
Major	Bcr-Abl/Abl ratio <0.10% or >3-log reduction from baseline

Molecular Response

Once CCyR is achieved, response can be monitored by molecular study using peripheral blood only. Molecular testing involves a polymerase chain reaction (PCR) for Bcr-Abl. The Recommendations are to conduct PCR every 3 months at the beginning of treatment until patient achieves a major molecular response (MMR), then it can be performed every 6 months.

PCR is a more sensitive assay compared to cytogenetic examination and is used to detect and quantify residual CML.

Imatinib failure/resistance

Therapeutic resistance to imatinib is seen in approximately 10% to 15% of patient[32]

Patients who do not achieve a complete hematologic response by 3 months or any cytogenetic response by 6 months of Imatinib therapy are considered to have failed. Failure is also diagnosed if there is no major cytogenetic response by 12 months or complete cytogenetic response within 18 months of the start of therapy. Resistance at the molecular level indicates a loss or lack of complete molecular response (CMR), such as undetectable BCR-ABL transcripts by either a real-time quantitative polymerase chain reaction, or the loss or lack of a MMR, These patients are considered for second-line treatment with second-generation tyrosine kinase inhibitors (TKIs).

Failure is classified as either primary or secondary. Primary (intrinsic) resistance is seen when a patient fails to achieve a desired response to initial treatment, whereas secondary (acquired) resistance occurs in patients who initially respond to imatinib but ultimately relapse. Failure is further sub-classified as hematologic (subdivided into chronic and advanced phases), cytogenetic and molecular**.**

In the chronic phase, hematologic resistance refers to a loss or lack of normalization of spleen size, peripheral blood counts, or differential WBC counts.

Accelerated or blast phase of hematologic resistance indicates a lack of return to the chronic phase or a hematologic relapse following an initial response to therapy [32]

A subset of patients who develop TKI resistance do so because of the presence of the T315I point mutation in the ABL1 kinase domain.[33] Detection and quantitation of the T315I point mutation is clinically useful in managing those patients who fail current front-line CML therapies.

1.1.8 Monitoring imatinib therapy

The recommendations of the National Comprehensive Cancer Network (NCCN)[29] and European LeukemiaNet (ELN)[30] are to do blood tests frequently(1-2 weeks) at the beginning of treatment; until patients are in complete hematologic response (CHR) or the count has normalized. Aftewards do it less often, usually every 3 months. Cytogenetics analysis of Philadelphia (Ph) chromosome in metaphase cells using a bone marrow sample should be done at 6, 12, and 18 months on treatment or until CCyR. Thereafter, response can be monitored by molecular study using peripheral blood only. Third molecular testing; PCR for Bcr-Abl, should be conducted every 3 months at the beginning of treatment until patients achieve a major molecular response (MMR), then it can be performed every 6 months.[28,29]

Measuring imatinib plasma concentrations may be useful in the case of resistance although this is not currently part of routine management.[34] The time based landmarks for determining response are as shown on table 2.

Table 2 **Time-based landmarks for evaluation of response** [32,35]

	3months	**6 months**	**12 months**	**18 months**
Failure	No hematologic response	>95% Ph+	>35% Ph+	>0% Ph+
Suboptimal response	No complete hematologic response	35-95% Ph+	1-35% Ph+	0% Ph+, <3log reduction in BCR-ABL transcripts
Optimal response	1-2 log ↓ in BCR-ABL transcripts	<35% Ph+	0% Ph+, ≥3 log ↓ in BCR-ABL transcripts	0% Ph+, ≥3 log ↓ in BCR-ABL transcripts

Various mechanisms of resistance to imatinib have been proposed. These include decreased intracellular drug levels which can be due to plasma binding by α-1 acid glycoprotein or drug efflux from P-glycoprotein (MDR-1) overexpression, increased expression of BCR-ABL kinase from genomic amplification or Clonal evolution. Mutations in ABL kinase of BCR-ABL can also cause resistance by affecting drug interaction or kinase activity.[36]

The association of primary imatinib resistance with higher transcript levels of the drug metabolism gene prostaglandin-endoperoxide synthase 1/cyclooxgenase1 (PTGS1/COX1) has been described by Gene expression profiling in newly diagnosed, imatinib-treated CML. However, secondary resistance is mainly due to acquired kinase domain point mutations and BCR-ABL gene amplification.[31, 37] Notably, mutations associated with secondary imatinib resistance occur more frequently in later stages of the disease and are associated with older age, prior interferon therapy, initiation of imatinib in the accelerated phase or blast crisis, development of clonal evolution, high-risk Sokal score at diagnosis, and failure to achieve CCyR by 12 months [38] A summary of the mechanisms of failure is shown on table 3.

Table 3: **Various mechanisms of resistance/failure**[37]

BCR-ABL independent	**BCR-ABL dependent**
Patient related - Poor compliance	Increased expression of BCR-ABL 1
Pharmacological	Mutations in the ABL-kinase domain
Poor intestinal absorption	
Drug interactions	
Binding with plasma components	
Leukaemia cell related	
Reduced levels of transporter (hoct 1)	
Increased levels of exporter (ABCB1, ABCG2	
QSCs (Quiescent stem cells)	
Clonal evolution	
SRC overexpression	

1.1.9 Treatment options for imatinib resistance/failure

The approved options are hematopoietic stem cell transplant, Dasatinib, Nilotinib and high dose imatinib where tolerable. Imatinib dose escalation, up to 800mg/day, induces a response in up to 40% of patients with CP-CML not responding to standard dose[40].

START-R Trial[46], was a phase 2, open-label study involving 150 CP-CML patients resistant to imatinib who were randomized (2:1) to receive either dasatinib 70 mg twice daily or high-dose imatinib 800 mg (400 mg twice daily), at a minimum follow-up of 2 years, dasatinib demonstrated higher rates of complete hematologic response (93% vs. 82%; P = .034), major cytogenetic response (MCyR) (53% vs. 33%; P = .017), and complete cytogenetic response (44% vs. 18%; P = .0025).

Nilotinib and dasatanib initially considered second-line therapy were approved by FDA for first-line therapy in 2010. They were developed to overcome imatinib resistance and to increase responsiveness of TK inhibitors. Dasatinib, a dual Bcr-Abl/Src kinase inhibitor, has shown efficacy against all imatinib-resistant Bcr-Abl mutations except for T315I (substitution of isoleucine for threonine) [33]

Novel therapeutic agents under active investigation for the treatment of CML include:

- Pegylated interferon (Polyethylene glycol IFN-α)
- Decitabine (cytidine analogue) -inhibits DNA methylation.
- Homoharringtonine (HHT/omacetaxine) is a plant alkaloid that inhibits CML progenitor cells in vitro.
- Histone Deacetylase Inhibitors
- Farnesyl transferase inhibitors.

2. JUSTIFICATION

CML is a relatively common haematological malignancy whose treatment has been revolutionised by imatinib mesylate. Local data on response to treatment and failure on imatinib was however lacking.

This study sought to shed light on treatment response, proportion of hematologic failure on imatinib and possible predictors of failure at initiation of therapy. This would be of great value to clinicians when making decisions on which subset of patients would benefit from imatinib and those who should be considered for second line treatment.

The Sokal and Hasford scores have been shown to have different levels of accuracy for different populations; this study sought to provide insight on the usefulness of these scoring systems locally.

3. RESEARCH QUESTION

What is the treatment response of Philadelphia chromosome positive CML patients to imatinib therapy, and what are the clinical and haematological characteristics of patients with imatinib failure?

4. OBJECTIVES

4.1 Broad objective

Broadly we were to determine the hematologic treatment response, proportion of CML patients with hematologic imatinib failure, and to describe the clinical and haematological characteristics of patients with imatinib failure.

4.1.1 Primary objectives

The primary objectives were to determine the hematologic treatment response of CML patients receiving imatinib therapy, the proportion of CML patients with hematologic failure and to describe clinical and haematological characteristics of patients with hematologic failure and compare this with those of patients with optimal response.

4.1.2 Secondary objectives

Our secondary objectives were to determine the possible predictors of imatinib failure in patients with Ph +ve CML and to determine the proportion of patients with imatinib failure in relation to the Sokal and Hasford scores.

5. MATERIALS AND METHODS

5.1 STUDY DESIGN

This was a cross-sectional descriptive study.

5.2 STUDY SETTING

The study was carried out at the GIPAP Clinic at the Nairobi Hospital. GIPAP is a Norvatis donation program for the treatment of Ph+ve CML and certain stages of gastrointestinal stromal tumors (GIST). The Max foundation (TMF) is a non-profit organization dedicated to supporting patients and is in charge of ensuring that patients meet the conditions to receive Glivec^{R} under the GIPAP program. Axios international approves the quantities to be shipped for approved patients and ensures drug delivery to the final recipient among coordination of other drug logistics like waiving of tax.

The GIPAP clinic has been operating in Kenya since January 2006.

The Nairobi Hospital is located in Nairobi, Kenya and is a leading private hospital in the East African region. The clinic runs fortnightly: on Saturdays and the patients are seen at no consultation fee. On average 50 patients are seen per clinic.

5.3 STUDY POPULATION

This constituted all Patients fitting the inclusion criteria who attending GIPAP clinic at Nairobi Hospital. Majority of the patients were referrals from the Kenyatta National Hospital, other hospitals and Private clinics in Kenya or at times a few from the general East African region; having tested positive for Philadelphia chromosome. The patients have to cater for their baseline analysis for Philadelphia chromosome which costs at least KShillings 17,000 plus other routine laboratory tests; however they receive Imatinib Mesylate and specialised consultation at no cost.

5.4 PATIENT SELECTION

5.4.1 Inclusion criteria

We included patients of both sexes aged 18 and above *(at the time of study regardless of their age at entry into the GIPAP)* with Ph+ve CML and had been on Imatinib for at least 3 months attending the GIPAP clinic, from whom written consent has been obtained.

5.4.2 Exclusion criteria

We excluded patients who declined consent, those who had been on treatment for less than 3 months and patients below 18 years at time of study

5.5 SAMPLE SIZE DETERMINATION

The sample size for this study was calculated using Fischer's formula as follows:

$$n = \frac{Z_{1-\alpha/2}{}^2 \, P \, (1-P)}{d^2}$$

{(L. Naing, T. Winn, B.N. Rusli: Practical Issues in Calculating the Sample Size for Prevalence Studies; Archives of Orofacial Sciences 2006; 1: 9-14)

This formula is used in prevalence studies where the target population is less than 10,000, thus it was appropriate for this study}.

n – Sample size

$Z_{1-\alpha/2}$ - Two-sided significance level (1-alpha)-95% = 1.96

P – Estimated proportion of patients with imatinib failure = 10-15% [32]

d – Precision error = ±5%

Substituting the prevalence of 10% and 15% into the formula **n** =

P	**N**
10%	**138**
15%	**196**

Thus, the minimum sample size calculated for the study was **138 patients**.
A total of 206 patients were recruited by consecutive sampling over a period of 4 months.

5.6 SAMPLING TECHNIQUE

All files of Ph+ve CML patients on follow-up at the GIPAP clinic were screened.
All those patients who fulfilled the inclusion criteria and gave informed consent were enrolled into the study through consecutive sampling technique.

5.7 PATIENT EVALUATION

5.7.1 Screening and Recruitment

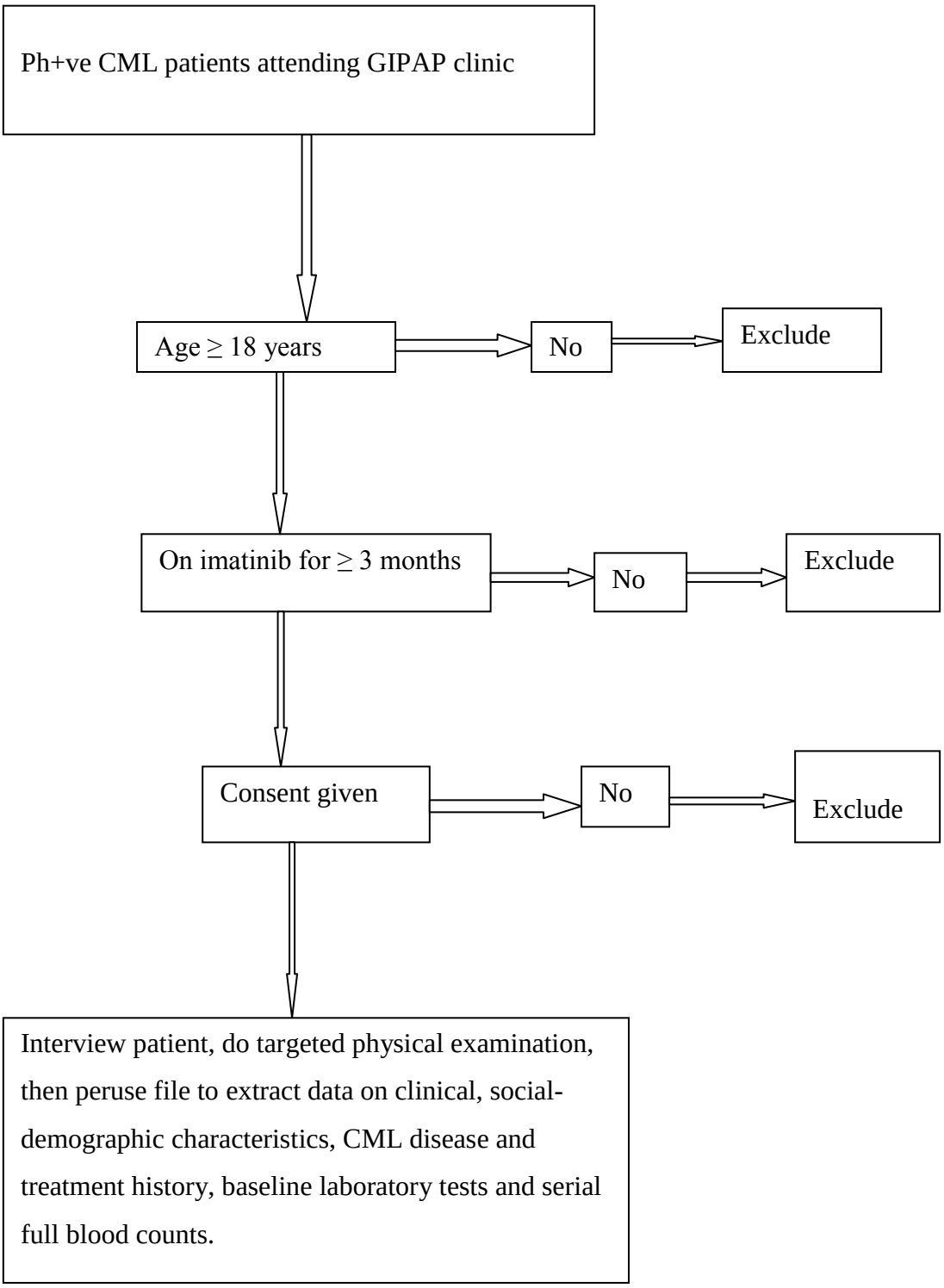
Patients referred into the GIPAP clinic had an initial evaluation for symptoms, and are examined particularly to check for pallor, splenomegaly or hepatomegaly.

At entry into the program the patient must have a full blood count, bone marrow aspirate and molecular analysis for confirmation of Philadelphia chromosome positivity.

Patients in chronic phase CML are commenced on 400mg of imatinib taken orally once a day, those in advanced phases (AP, BP) are started on 600mg of imatinib taken orally once a day those under 18yrs are started on 300mg –this is in line with NCCN/ELN guidelines and is GIPAP approved.

Upon commencement of treatment the patients are seen every 1-2weeks (at Physician's discretion) and reviewed with the latest blood count until he/she reaches complete hematologic response and is subsequently reviewed every 2-3 months.

FIGURE 5: Flow Chart of Patient Screening and Recruitment



The investigator screened files of patients who satisfy the inclusion criteria on any clinic day. Any eligible patient was informed about the study and requested to fill the informed consent

form before recruitment. Those recruited were interviewed briefly, examined to look for any signs of disease especially splenomegaly or hepatomegaly. The investigator subsequently perused through the patient's file/records and extracted data –on disease and treatment history, social-demographic, clinical and haematological using a pre-designed data extraction sheet. Data extracted included age, sex, anthropometric measurements, presence of splenomegaly or hepatomegaly, serial blood counts, results of cytogenetic or molecular studies, duration from diagnosis to start of imatinib, duration of any prior therapy, duration on imatinib itself and of CML illness. Time to complete hematologic response, response to treatment with imatinib and evidence of treatment failure were also extracted. Body surface area, Sokal and Hasford score (Appendix I & II), was calculated and recorded. Characteristics of patients with resistance to Imatinib were recorded and compared with those of patients with good response.

5.7.2 Clinical Methods

Once informed consent was given, the investigator entered demographic and clinical data into a pre-designed data sheet outlined in Appendix III. A complete medical history and full physical examination with targeted abdominal examination was undertaken. Body mass index and body surface area (The Mosteller formula: BSA (m^2) = [(Height (cm) x Weight (kg)]/ 3600)½ were carried out.[49]

Demographic data included, nationality, gender, age, and education level and employment status, or CML disease and treatment history variables (disease phase at diagnosis and initiation of imatinib, time to start imatinib, duration of prior CML therapy, duration on imatinib, and overall CML illness; all these in months). This study was conducted over 4 months between August and November 2012.

5.7.3 Outcome variables

5.7.3.1 Complete Hematologic response (CHR)

Complete hematologic response was defined as WBC <10x 10^9 /l with normal differential; platelet count <450x 10^9/l; ≤1% circulating immature cells; disappearance of all signs and symptoms of disease including palpable splenomegaly, as described by the NCCN and ELN.[29, 30]

5.7.3.2 Hematologic Treatment failure

Hematologic failure was defined as lack of complete hematologic response (CHR) after 3 months of imatinib treatment or loss of complete hematologic response (hematologic relapse following an initial response to therapy). The 3 month milestone was settled on, for operational definition based on the fact that, at this time the patient should have attained CHR as per the NCCN and ELN guidelines.

Loss of complete hematologic response was classified as secondary failure. Inability to achieve complete hematologic response throughout treatment was classified as primary failure.

6. DATA MANAGEMENT AND ANALYSIS

All participants' data bore a serial number and no names or unique identifiers of the participants. Data forms were kept in a secure lockable cabinet only accessible by the study investigator and the statistician. Data was entered into a password protected MS Access database prepared by the statistician. The investigator was able to verify the entered data against the hard copy data extraction forms and sorted out any inconsistencies**.**

The data from the pre-designed data sheet was entered into Microsoft Access database. Data entry was done continuously in the course of data collection. At the end of data entry, data was then cleaned, verified and imported to and analysed using statistical package for social scientists (SPSS) version 18.0. The study population was described by summarizing categorical data including sex, level of education and phase at diagnosis into proportions while continuous data such as age, height, weight, blood counts, durations of illness and treatment, spleen and liver were summarized into means or medians. Imatinib hematologic failure was presented as a proportion.

The factors associated with imatinib failure (such as BCR-ABL concentration, delay between diagnosis and initiation of imatinib, non-adherence on treatment) were analyzed using Student's t-test or Mann-Whitney U test for continuous data and Chi-square test for associations for categorical variables. Strength of association in for example non-adherence and treatment failure were expressed as odds ratios. Logistic regression was used to investigate factors independently associated with treatment failure. Precision was indicated by inclusion of 95% confidence limits. Correlations were deemed to be of statistical significance when the *P* value was less than 0.05. Socio-demographic, clinical and haematological characteristics, Sokal and Hasford scores at entry, treatment history, factors associated with failure and dose alteration findings are presented using tables. A scatter plot was used to illustrate the white blood cell and platelet counts at entry into the GIPAP program, pie charts were used to demonstrate the proportions of imatinib response. A line graph was used to show the WBC trends between the complete hematologic response and failing groups. Presenting clinical features and BMA findings were presented using graphs.

Survival analysis was performed using the Kaplan-Meier method. Duration of CHR was assessed for responding patients as the time from the first observation of response to disease relapse (failure).

7. ETHICAL CONSIDERATIONS

The study was carried out following approval by the department of Internal medicine (U.O.N) and KNH/UON- Ethics & Research Committee. Permission was also sought from and granted by the Education and Research committee and the Chief executive officer, Nairobi hospital.

Informed consent was obtained from the patient before enrolment into this study. Only patients who consented to the study were included. Patients' treatment was continued accordingly. The information collected was treated with utmost confidentiality and no patient names were included in the data entry sheet or any publications arising from the study.

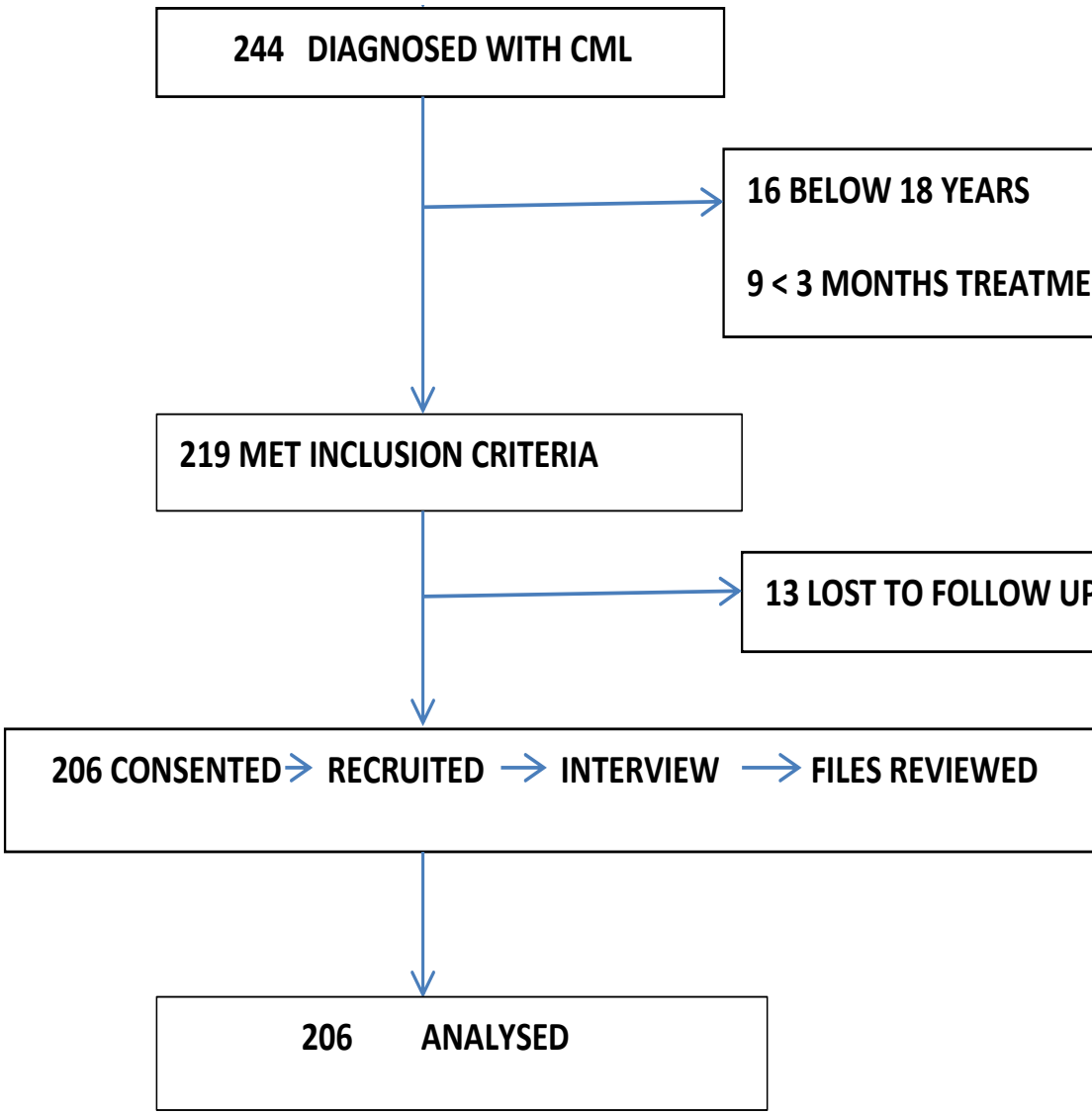
The patients did not bear any extra cost due to their participation in this study and neither did they travel to the clinic any other day besides that which was booked by the attending physician. Patients who did not consent to the study were not penalised and they continued to receive usual health care at the GIPAP clinic.

8. RESULTS

A total of 206 Philadelphia chromosome positive CML patients were studied. A total of 244 patient files were screened for patient's study eligibility, 16 of them were excluded due to age below 18 years at time of study and a further 9 excluded for having been on imatinib for less than the required 3 months. A total of 219 met the inclusion criteria but 13 of them never turned up for their clinic appointments, the remaining 206 who consented to the study were recruited.

The patient flow chart is as shown in figure 6.

FIGURE 6: Flow Chart Representing Participants Recruited



Socio-demographic characteristics

A total of 206 subjects (115 males and 91 females. The mean age was 38.6 ± 13.7 (range 14-85) years. Majority (197) were Kenyans with only 9 foreigners; one from India, one Malawian, two southern Sudanese and five Somalis. There were 53.9% employed vs. 46.1% unemployed.

Only 10(4.9%) patients had exposure to first hand smoking.

All the patients had some basic education with 81.6% having at least completed secondary school. Their socio-demographic characteristics are as shown in table 4.

Table 4: Socio-demographic Characteristics

Variable	**Frequency (%)**
Nationality	
Kenyan	197(95.6)
Foreign	9(4.4)
Mean **age** (SD)	38.6 (13.7)
Range	14-85
Sex	
Male	115 (55.8)
Female	91 (44.2)
Level of education	
Primary	38 (18.4%)
Secondary	110 (53.4%)
Tertiary	58 (28.2%)
Employment	
Employed	111(53.9)
Unemployed	95(46.1)
Marital status	
Single	58 (28.2%)
Married	146 (70.9%)
Separated	1 (0.5%)
Widowed	1 (0.5%)
Smoking	10 (4.9%)
Median p**ack years** (IQR)	10 (5-13.75)

Clinical characteristics

A total of 206 subjects of whom 194(94.2%) were in chronic phase CML (CP-CML), 10 in accelerated phase and only 2 in blast phase were studied The mean BMI was 22.6 with 47 (22.8%) being at least overweight and 21(10.2%) underweight. Majority; 197 (95.6%) of the

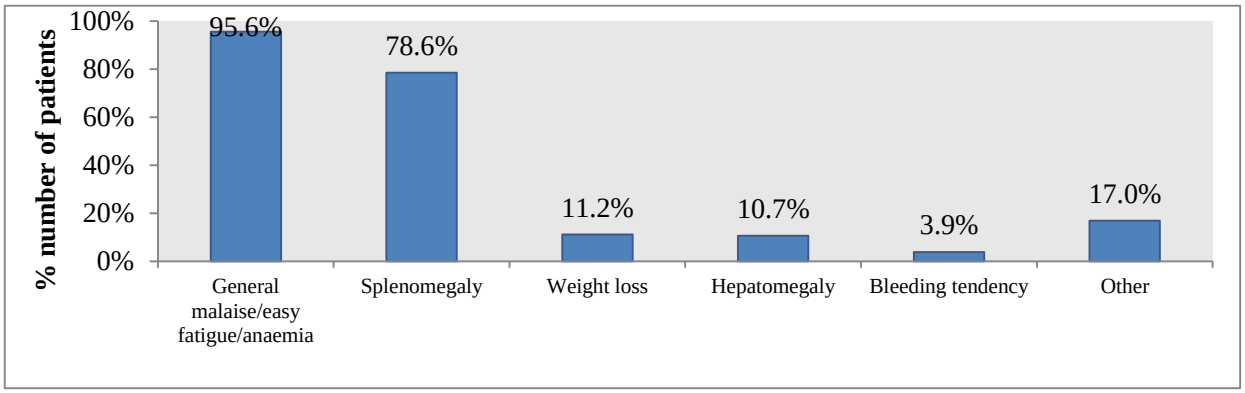
patients were symptomatic at diagnosis. The median spleen size at GIPAP entry was 12 cm below the costal margin along the mid clavicular line (interquartile range 8-18). The median liver size was 4cm (IQR; 0-8). See Table 5.

Table 5: Clinical Characteristics

Variable	**Frequency (%)**
Mean height (SD)	166.8 (7.9)
Mean weight (SD)	63.0 (12.1)
Mean BSA (SD)	1.7 (0.2)
Mean BMI (SD)	22.6 (3.9)
BMI category	
<18.5 Underweight	21 (10.2%)
18.5-24.9 Healthy	138 (67.0%)
25-29.9 Overweight	38 (18.4%)
>=30 Obese	9 (4.4%)
Presence of symptoms of diagnosis	197 (95.6%)
Phase at diagnosis	
CP	194 (94.1%)
AP	10 (4.9%)
BP	2 (1.0%)
Median **spleen size** (IQR)	12 (8-18)
Median **liver size** (IQR)	4 (0-8)

A majority of the patients; 197 (95.6%) complained of general malaise whereas splenomegaly was found in 162 out of the 206(78.6%) studied patients, 20 patients had hepatomegaly and 22 complained of unintentional weight loss. Less common presentations were priapism, stroke, loss of hearing and vision, bone pains and myalgia all together accounting for 17% as shown in figure 7.

Figure 7: Presenting Clinical Features



At entry into the GIPAP clinic, the median WBC at entry was 143 (IQR; 69-247) $x10^9$/l, whereas that of platelet was 387(IQR; 69-247) $x10^9$/l. Median hemoglobin concentration was 10.4g/dl. At 3 months the median WBC count had dropped to a median of 4.8(IQR; 3.8-6.4) $x10^9$/l, at 12 months it was 5.2(IQR; 4.1-7.0) $x10^9$/l with a similar count at 36 months and 5.6(IQR; 4.4-15.3) $x10^9$/l at 48 months. The median platelet count trends were as follows; 194(IQR; 127-275) $x10^9$/l at 3 months, 192(IQR; 136-272) $x10^9$/l, 191(IQR; 142-262) $x10^9$/l and 212(IQR; 160-306) $x10^9$/l at 48 months. The hemoglobin level rose to a median of 12.5(IQR; 11.4-13.7) g/dl at 3 months, 13.2(IQR; 11.7-14.5) g/dl at 12 months, and stagnated at 13.3 g/dl between 36 and 48 months. The patients' median BCR-ABL/ABL ratio was 71.0 (71%) with an inter quartile range of 28 at entry into GIPAP. (See table 6a)

Table 6 a: Hematological Characteristics

Variable	**Entry** Median (IQR)	**3 months** Median (IQR)	**12 months** Median (IQR)	**36 months** Median (IQR)	**48 months** Median (IQR)
WBC	**143 (69-247)**	**4.8 (3.8-6.4)**	5.2 (4.1-7.0)	5.2 (4.3-7.4)	5.6 (4.4-15.3)
Platelets	387 (243-585)	194 (127-275)	192 (136-272)	191 (142-262)	212 (160-306)
Hb	**10.4 (8.7-12.6)**	**12.5 (11.4-13.7)**	13.2 (11.7-14.5)	13.3 (11.9-14.3)	13.3 (12-14.5)
Eosinophils	1.9 (1-3)	1.6 (0.9-2.3)	1.9 (1.0-3.1)	2 (1-3.3)	1.2 (0.9-2.9)
Basophils	2 (1-5)	1.1 (0.8-1.7)	1.1 (0.7-1.8)	1.1 (0.6-1.5)	1.2 (0.6-1.8)
BCR ABL/ ABL ratio, Mean (SD)	**71.0 (28.0)**	-	-	-	-

●*WBC: white blood cells, Hb: hemoglobin concentration*

Leukocytosis was found in 157(76.2%) of the subjects which dropped to be found in only 11(5.3%) after 3 months of imatinib therapy, 27(13.1%) at 12 months, subsequently, the number of patients with leukocytosis gradually increased to 33(16%) at 36 months and 39(18.9%) at 48 months of treatment.

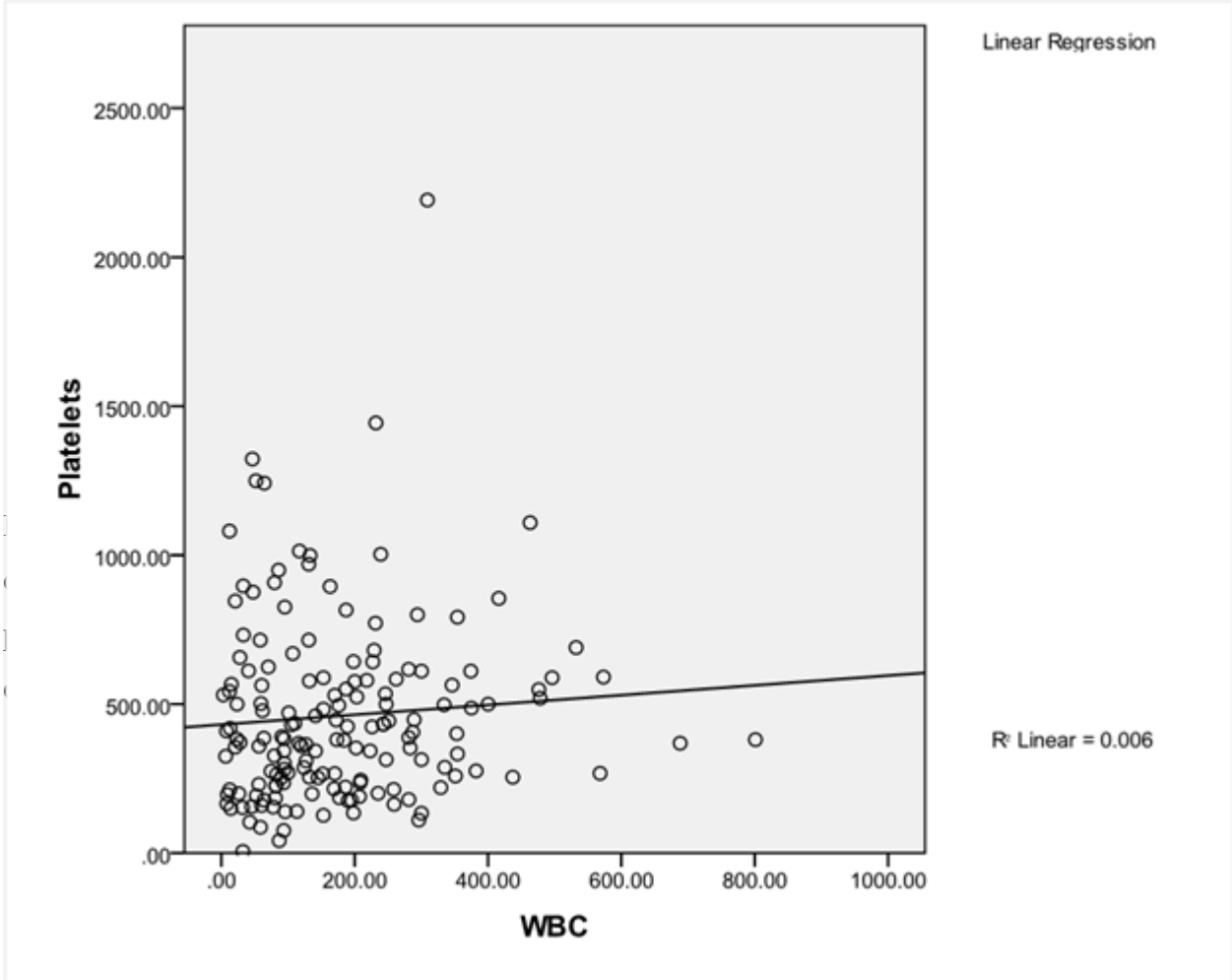
Thrombocytosis was found in 66(32%) subjects at entry, at 3 months only 15(7.3%) patients had thrombocytosis, 16 (7.8%) at 12 months and only 9(4.4%), but the number rose to 17(8.3%) at 48 months of clinic follow up. See table 6b.

Table 6 b: Hematological Characteristics

Variable	**Entry** n (%)	**3 months** n (%)	**12 months** n (%)	**36 months** n (%)	**48 months** n (%)
Leucocytosis	157 (76.2)	11 (5.3)	27 (13.1)	33 (16.0)	39 (18.9)
Thrombocytosis	66 (32.0)	15 (7.3)	16 (7.8)	9 (4.4)	17 (8.3)

The scatter plot shown in figure 8, illustrates white blood cells and platelet counts distribution amongst the subjects at GIPAP entry. It shows that some patients presented with either leukocytosis or both leukocytosis and thrombocytosis. There is a notable clustering at WBC counts of up to 40 x 10^9/l and platelet counts of up to 500 x 10^9/l.

Figure 8: A Scatter Plot Showing White Blood Cell and Platelet Counts at GIPAP entry

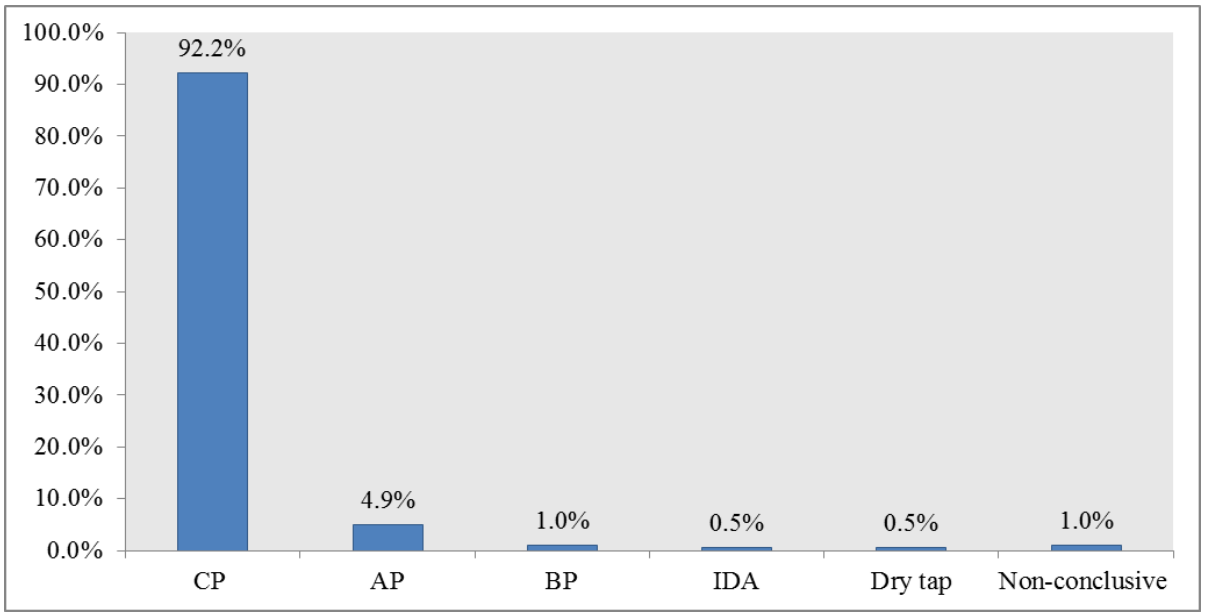


Bone marrow aspirate findings

The bone marrow aspirate findings were as shown in figure 9. BMA features of CML were hyper cellularity, with predominant myeloid precursors from blasts to mature neutrophils, with increased ratio of myeloid to erythroid cells. A hundred and ninety subjects (92.2%) had marrow features of chronic phase (CP) and therefore marrow blasts were less than 10%, 10 (4.9%) had features in keeping with accelerated phase (AP); about 10-19% blasts, and 2 subjects had marrow blasts of more than 20% and so diagnosed as blast crisis. One patient

had a dry marrow tap, 2 BMA findings were reported as non-conclusive, and one other was additionally reported to have no stainable of iron, suggestive of iron deficiency anemia.

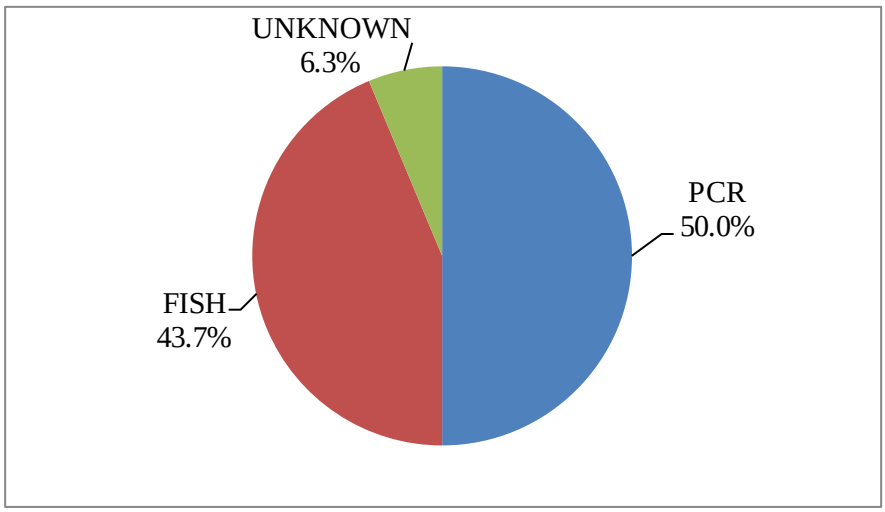
Figure 9: BMA Findings at Entry.



CP, AP and BP denote chronic, accelerated and blast phase of CML respectively. IDA denotes Iron deficiency anemia.

In testing for Philadelphia chromosome positivity, quantitative polymerase chain reaction (PCR) was used in 103 (50%) of the patients, fluorescence in situ hybridization (FISH) analysis in 90 (43.7%), with data missing for 13 (6.3%) subjects. See fig 10.

Figure 10: Analysis for Philadelphia Chromosome



FISH denotes, Fluoresence in-situ hybridization,

PCR; polymerase chain reaction.

Of the 13 (6.3%) subjects whose copies of the Philadelphia chromosome results were missing, Ph+ve status was derived from file notes but further details e.g. of method used could not be established.

Sokal and Hasford scores at entry to GIPAP

Several patients' data at diagnosis to aid in Sokal and Hasford scoring was missing 73(35.4%) for Sokal and 89(43.2%) for Hasford. Among the 133 whom the Sokal score could be determined, 69(33.5%) of them were stratified as poor prognosis, 45(21.8%) classified as moderate prognosis and only 19(9.2%) classified as good prognosis. Hasford scoring categorized 24(11.7%) patients as high risk, 58(28.2%) as intermediate risk and 35(17%) as low risk. See table 7

Table 7: Sokal and Hasford Scores at entry to GIPAP

Variable	**n (%)**
Sokal score	
Good prognosis	19 (9.2)
Moderate prognosis	45 (21.8)
Poor prognosis	69 (33.5)
Missing	73 (35.4)
Hasford score	
Low risk	35 (17.0)
Intermediate risk	58 (28.2)
High risk	24 (11.7)
Missing	89 (43.2)

Treatment history

One hundred and twelve (54.4%) of the subjects had exposure to hydroxyurea prior to commencing imatinib 2 patients had used busulphan prior and a further 2 had used cytosine arabinoside .One hundred and ninety four (94.2%) were started on 400mg with those in advanced disease (accelerated and blast phase) starting at 600mg.

Eighty seven (42.2%) of the study subjects, admitted to missing their imatinib dose on occasion(s). The duration of non-adherence varied from a few days to several months. We did not have a pre-specified definition of non-compliance but any self-report of not taking imatinib daily was documented as 'missed dose'. See table 8.

Table 8: Prior Treatment and CML Phase at Start of Imatinib

Variable	**Frequency (%)**
Prior treatment with other agents	
Hydroxyurea	112 (54.4)
Cytosar	2 (1.0)
Busulphan	2 (1.0)
Phase at initiation of imatinib	
AP	10 (4.8%)
BP	2 (1.0%)
CP	194 (94.2%)
Imatinib dose at commencement	
300mg	2 (1.0%)
400mg	194 (94.2%)
600mg	10 (4.8%)
Ever missed imatinib dose(s)	87 (42.2 %)

Imatinib complete hematologic response and failure

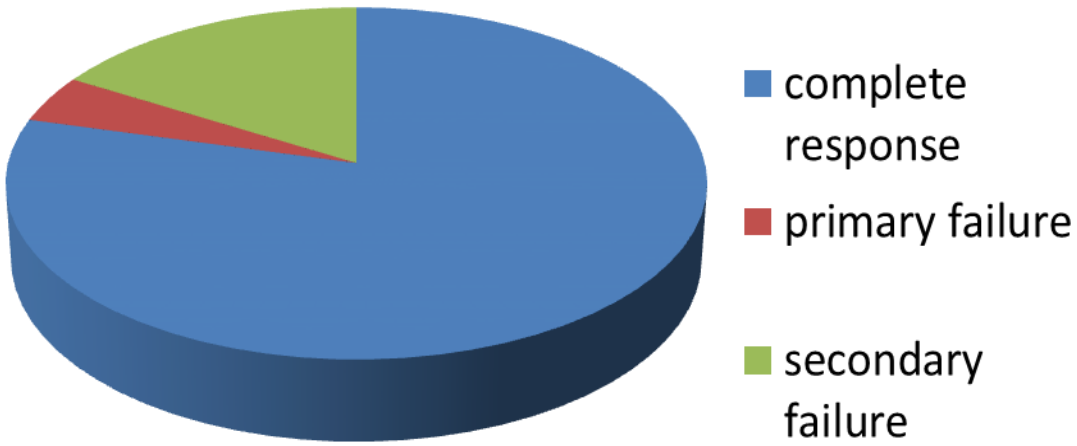
Sustained complete hematologic response was noted in 163/206 (79.1%) whereas 43/206 (20.9 %) (95%, CI: 15.5- 26.7), had hematologic failure on imatinib.

Primary hematologic failure (never achieved CHR throughout treatment with imatinib) occurred in 9 patients therefore contributing 21% of the overall treatment failure. Secondary failure was found in 34 of the 43 overall failure group (79%).See table 9 and figure 11.

Table 9: Imatinib Hematologic Failure vs. Response

Variable	n (%)	95% CI of %
Imatinib response Overall Failure • Primary • Secondary	**43/206 (20.9)** • 9/43 - 21% • 34/43 -79%	**15.5- 26.7**
Complete hematologic response	**163/206 (79.1)**	73.3- 84.5

Figure 11: A pie chart illustrating imatinib hematologic response



Factors associated with imatinib failure

High BCR-ABL concentration (median of 81% SD; 22.8 versus 68.2% SD; 28.6) (p-*value: 0.008*), and lower leukocytosis (WBC of 96.8 (32.9-247.0) x 10^9/l versus 161.0 (83.7-248.5) x 10^9/l) p=0.031 at GIPAP entry, non-compliance to treatment (60.5% vs37.4%, p=0.007) in the failing group were associated with imatinib failure significant. There were just a few patients in the AP and BP making a total of 12 and only one of them got hematologic failure.

The rest responded well. The comparisons between the 2 groups in relation to BMI, platelet counts, hemoglobin concentration, phase of disease, Sokal and Hasford scores at entry did not show any heterogeneity. See table 10.

Table 10: Comparison for Factors associated with Imatinib Failure

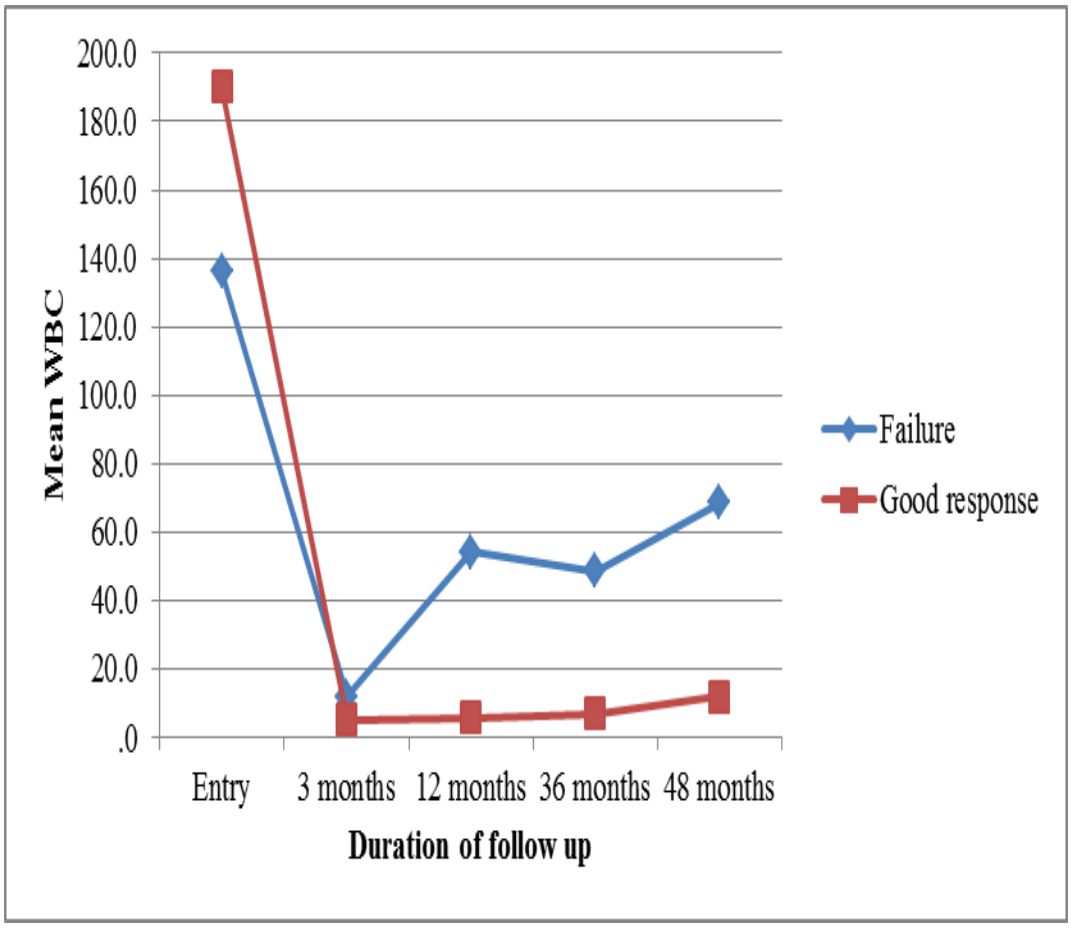
Variable	Failure	Good response	P value
BMI			
<18.5 Underweight	6 (14.0%)	15 (9.2%)	0.749
18.5-24.9 Healthy	28 (65.1%)	110 (67.5%)	
25-29.9 Overweight	8 (18.6%)	30 (18.4%)	
>=30 Obese	1 (2.3%)	8 (4.9%)	
WBC	96.8 (32.9-247.0)	161.0 (83.7-248.5)	0.031
Platelets	399.0 (197.0-563.0)	385.0 (255.0-586.5)	0.584
Hb	10.9 (3.4)	10.5 (2.6)	0.401
BCR_ABL	81.0 (22.8)	68.2 (28.6)	0.008
Sokal score	1.3 (1.0-2.5)	1.2 (0.9-1.8)	0.201
Hasford score	1131.4 (760.8-1504.0)	982.8 (666.6-1384.2)	0.157
Phase at diagnosis			
CP	40 (97.6%)	150 (93.2%)	0.804
AP	1 (2.4%)	9 (5.6%)	
BP	0 (0.0%)	2 (1.2%)	
Missed dose[a]			
Yes	26 (60.5%)	61 (37.4%)	0.007
No	17 (39.5%)	102 (62.6%)	

[a] OR 2.6 (95% CI 1.3-5.1)

Patterns of WBC drop between the 2 groups

Figure 12 illustrates the drop of WBC counts on imatinib. The patients in the failure group started with lower counts and their rate of WBC drop was less precipitous. Patients responding well hit a much lower minimum WBC count at three months as compared to the other group and the counts remained within normal range as opposed to the rise above normal in failure group.

Figure 12: A Line Graph Comparing WBC Trends between The 2 groups



The failing group had a longer duration of CML disease as compared to the good response group as shown in table 11. The mean duration from diagnosis to start of imatinib was 4(failure group) versus 1 month ($p=0.008$), mean period on imatinib was 42 versus 23 months in the good response group ($p<0.001$), the failure group had also been treated with other agents for longer: 3 versus 0.5 months ($p<0.001$).

The mean duration to complete hematologic response (CHR) was 3 months in the good response group versus 4.8 in the failing one with 9 out of the 43(21%) never attaining CHR therefore meeting the definition of primary failure. See table 11.

Table11: Comparison of CML Disease and Treatment History in Failure vs. CHR group

Variable (**mean duration**)	**Overall**	**Failure**	**Good response**	**P value**
Dx to imatinib (months)	1.0 (0.75-4.0)	4.0 (1.0-12.0)	1.0 (0.8-3.0)	**0.008**
Duration on imatinib (months)	25.5 (15.0-48.3)	42.0 (27.0-62.0)	23.0 (14.0-41.0)	**<0.001**
Duration of prior Rx (months)	0.9 (0.0-3.3)	3.0 (0.0-12.0)	0.5 (0.0-2.0)	**<0.001**
Duration of CML illness (months)	27.5 (17.0-57.0)	52.0 (28.0-72.0)	25.0 (15.0-49.0)	**<0.001**
Time to CHR (months) CHR never achieved	3.4	4.8 (3.0-12.0) **9/43(21%)**	3.0(3.0-3.0)	-
Time to failure (months)	-	21.0 (6.0-36.0)	-	-

Independent predictors of hematologic failure.

One of our secondary objectives was to document possible predictors of imatinib (hematologic) failure. Factors that were found to be associated with failure in the univariate analysis were subjected to logistic regression.

The only factor found to be independently associated with failure was duration from diagnosis to start of imatinib with an odds ratio of 2.69 (95% CI: 1.03-7.03) p=0.043. This implies that delayed commencement of imatinib regardless of prior therapy like hydroxyurea

was a predictor of failure albeit with a wide confidence interval, probably because this study was not primarily designed to detect predictors of failure.

See Table 12.

Table 12: Independent Predictors of failure on logistic regression

Variable	OR (95% CI)	P value
WBC	1.00 (0.998-1.01)	0.328
BCR ABL	1.00 (0.98-1.02)	0.926
Missed dose/non-adherence	1.96 (0.84-4.50)	0.121
Duration to imatinib	2.69 (1.03-7.03)	0.043
Duration on imatinib	2.28 (0.93 -5.61)	0.072
Duration of prior treatment	0.82 (0.60-1.11)	0.201
Duration of CML illness	0.43 (0.18-1.06)	0.067

Imatinib dose adjustments.

One hundred and seventeen (56.8%) of the patients required Imatinib dose adjustments either escalation or reduction, out of which 35 required further adjustments and 10 of them even a third dose adjustment. In the initial dose change, the majority (83/117; 71%) got 300mg followed by 23.1% of the 117 subjects getting 600mg. Of the 35 subjects who were subjected to a second dose change, 17(48.6%) 400mg of imatinib, 8(22.9%) got 800mg. the details on dose changes are as shown in table 13.

Table 13: Imatinib dose adjustments

Change of imatinib dose		New doses	
Change	**n (%)**	**Dose**	**n (%)**
First (n=206)	117 (56.8)	200mg	1 (0.9)
		300mg	83 (71.0)
		400mg	5 (4.3)
		600mg	27 (23.1)
		800mg	1 (0.9)
Second (n=117)	35 (29.9)	100mg	1 (2.9)
		200mg	4 (11.4)
		300mg	4 (11.4)
		400mg	17 (48.6)
		600mg	1 (2.9)
		800mg	8 (22.9)
Third change (n=35)	10 (28.6)	200mg	2 (20)
		300mg	3 (30)
		400mg	2 (20)
		600mg	2 (20)
		800mg	1 (10)

Reasons for dose changes

Cytopenias (thrombocytopenia, leucopenia especially neutropenia, and anemia) were the reason for imatinib dose reductions whereas leukocytosis and thrombocytosis called for dose escalation. The following were noted to be the various blood count cut offs that necessitated dose reductions: thrombocytopenia of less than 100x 10^9/l, leukopenia of less than 3000 x 10^9/l, or neutropenia (ANC-absolute neutrophil count) of less than1000 x 10^9/l, or anemia with hemoglobin level less than 8g/dl. Dose increments were necessitated by leukocytosis above 10,000 x 10^9/l, or thrombocytosis above 450 x 10^9/l.

The initial dose change was due to neutropenia/leucopenia in 51 out of the total 117 who got dose change (43.6%), thrombocytopenia in 29/117 (24.8%), leukocytosis in 27/117 (23.1%), thrombocytosis in 6 and anemia in 8 patients See table 14.

Table 14: Reasons for change of dose

	Initial change: n=117(%)	**Further change n=35 (%)**
Thrombocytopenia (plt < $100x10^9$/l)	29 (24.8)	5 (14.3)
Thrombocytosis (plt > $450x10^9$/l)	6 (5.1)	4 (11.4)
Leukopenia (WBC < $3000x10^9$/l) or Neutropenia (ANC < $1000x10^9$/l)	51 (43.6)	5 (14.3)
Leucocytosis (WBC >$10,000x10^9$/l)	27 (23.1)	19 (54.3)
Anemia (Hb < 8g/dl)	8 (6.8)	1 (2.9)

Key: *WBC denotes total white blood cell counts, ANC; absolute neutrophil count and Plt; platelet counts.*

Duration of clinic follow up

The study subjects had been followed up at the GIPAP for various durations with mean of 32.1 months with a standard deviation of 22.7 months. The median clinic follow up was 26 months (IQR: 14-51).

Management of the 43 failing patients: at the time of study.

Six out of the 43 failing patients failed on a maximum imatinib dose of 800mg and so imatinib was stopped and patients switched to hydroxyurea .Four other patients had their doses escalated to 800mg and a further 14 were on 600mg at the time of study.
Nineteen patients were still on the standard imatinib dose of 400mg, some as a restart dose after defaulting over several months and a few due to intolerance (mainly hematological) prohibiting dose escalation.

Imatinib was withheld for uncertain safety in pregnancy and in severe anemia until blood was transfused. See table 15.

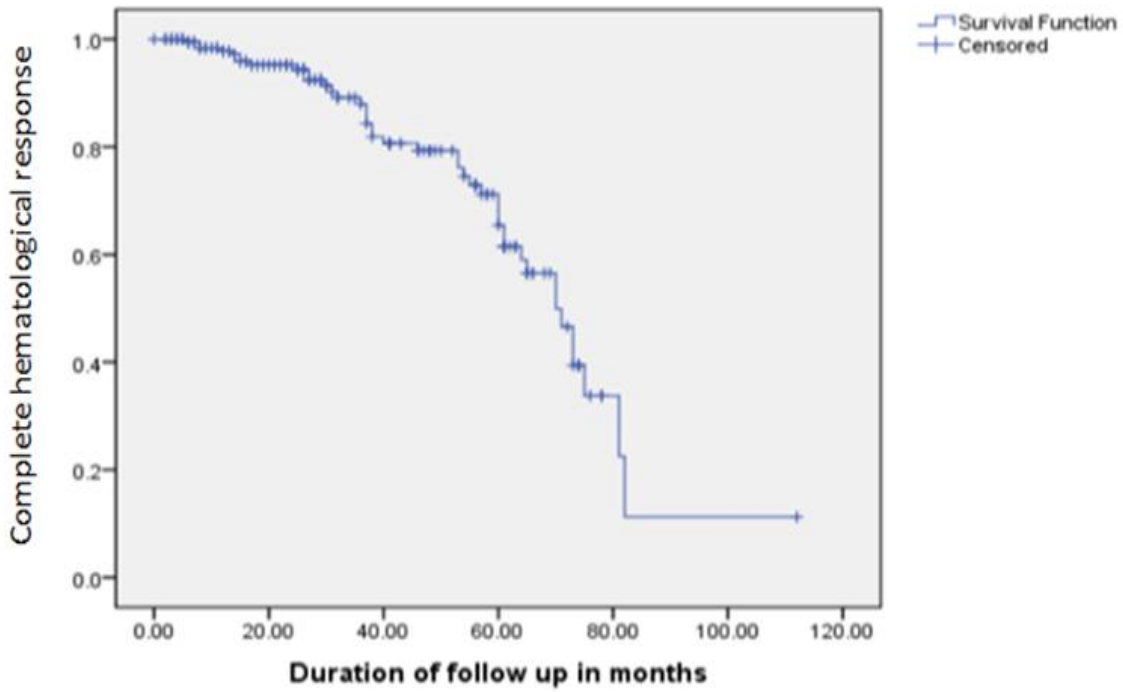
Table 15: Therapy and challenges in the failing patients

Variable	**Frequency: n (%)**
current Rx	
HU	6 (2.9)
IM-400mg	19 (9.2)
IM-600mg	14 (6.8)
IM-800mg	4 (1.9)
Not stated	*163 (79.1)*
Special issues	
Anemia	2 (1.0)
Cytopenia (besides anemia)	3(1.5)
Defaulter, Non-compliance	6 (2.9), 5 (2.4)
Defaulter, Pregnancy	4 (2.0)
Few serial FBCs	1 (0.5)
Menorrhagia	1 (0.5)
Vomiting	1 (0.5)

Key: *IM denotes imatinib, FBC; full blood count and HU denotes hydroxyurea.*

Survival analysis was performed using the Kaplan-Meier method. Duration of CHR was assessed for responding patients as the time from the first observation of hematologic response to disease relapse (hematologic failure). Mean Survival time was 67 months (95% CI: 59.3-74.7) with a median of 70 months (95% CI: 63.4-76.6). The curve is skewed to the left because more patients had a relatively shorter period of clinic follow up (joined the clinic closer to the study period) with only a few having been followed up at the GIPAP clinic for longer periods. See figure 13.

Figure 13: Kaplan-Meier Estimator of the Time in Complete Hematologic Response on Imatinib



10. DISCUSSION

In this study 206 patients above 18 years at time of study, with Philadelphia chromosome positive chronic myeloid leukemia regardless of the phase were studied.

Social-demographic characteristics

The mean age at diagnosis was 38.6 years with the youngest being 14 and the oldest 85 years. This is similar to a mean of 35 years found by Othieno-Abinya et al in a study done at Kenyatta National Hospital[15], and a median of 40 years found in a black African population having CML in a Cote d'Ivore study[16]. In contrast, a median age of 53 years has been described among Caucasians[11] which is at least 10 years older than the African CML patient. Out of the 206 studied patients, 115 (55.8%) were males the rest being females giving a male to female ratio of 1.26:1 in keeping with slight male predominance noted in other studies.[10]

Unemployment was found in 95 (46.1%) of the study population, these patients were therefore dependent on someone else for their provisions such as meeting cost for serial full blood counts, and travelling to the clinic.

One hundred and ninety seven (95.6%) were Kenyans versus 9(4.4%) foreigners. This is simply because the GIPAP clinic in located in Kenya's capital city. Majority of the foreigners were refugees from the troubled neighboring Somalia, the rest were one Indian, a Malawian and two southern Sudanese. Out of the 9 foreigners, 4 had hematologic failure but further analysis and comparisons were not done considering the small population.

Clinical characteristics

Majority of our patients; 197 (95.6%) were symptomatic at diagnosis with just a little over 4% being asymptomatic unlike the finding of about 40% of CML patients being asymptomatic in developed countries[10]. This suggests that our patients mostly present late in the course of the disease when it is more likely to be symptomatic[10] or that the disease in our

population has a tendency to early onset of symptoms this is especially plausible in that the disease tends to occur among much younger subjects in the African setting as alluded to earlier. CML disease among Africans seems more aggressive and some of the possible contributing factors are additional cytogenetic chromosomal abnormalities leading to poor prognosis[16]. Translating symptomatology to imply advanced disease, the 4% diagnosed whilst asymptomatic and therefore diagnosed following a routine full blood count, could imply early patient presentation possibly while attending routine medical check-up although this was not explored in our study. This finding of asymptomatic patients is in contrast to what was found in a KNH retrospective study of 117 CP-CML patients in whom all (100%) were symptomatic [15]. The commonest symptom amongst our subjects was general malaise. Splenomegaly was found in 162 (78.6%) patients, elsewhere, only about 50% have splenomegaly at diagnosis[11].

In this study, 194 (94.1%) of the patients were in chronic phase; which is in keeping with usually more than 80% of CML diagnosis in the chronic phase[11, 14]

One of our study's secondary objectives was to determine the proportion of patients with imatinib failure in relation to the Sokal and Hasford scores; however, the study did not demonstrate differences between the groups, probably because several patients' data at diagnosis to aid in Sokal and Hasford scoring was missing. This made it difficult to utilize the scores in predicting treatment outcomes. The main reasons that made the objective not fully achievable were that; a number of the initial blood counts and evaluation of peripheral blood films did not indicate the percentage of eosinophils and/or basophils or spleen size at entry into GIPAP clinic and these parameters are essential in calculating both scores. Of the 133 who were Sokal scored 69 of them had poor prognosis and of the 117 Hasford scored 24 were high risk. This suggested a tendency to adverse prognostication among our patient group; but as already stated, the numbers scored were too small for one to make a significant conclusion.

A little over half; (112)54.4% of the patients had been on hydroxyurea prior to starting imatinib most of them having used it for a week to a few months as they awaited molecular analysis for BCR-ABL. Only 2 had used Busulphan and 2 using Cytarabine. It was noted that longer exposure to other leukemia targeted therapy prior to imatinib was a factor associated with imatinib failure. Considering that the majority had been exposed to hydroxyurea, it raises a question of a remote possibility of imatinib-hydroxyurea cross resistance. Another explanation would be that the longer the patient has been on hydroxyurea, the more the disease has time to progress. This is especially plausible because hydroxyurea therapy is considered palliative and has no effect the rate of progression to blast crisis[11].

Patients who had lower WBC counts at commencement of imatinib were shown to be more likely to fail. This trend is illustrated in figure 12 and may be attributed to the fact that these same patients had a longer period of prior treatment with hydroxyurea which is cytoreductive (decreases production of white blood cells, platelets and also RBCs).

Long durations between diagnosis and start of imatinib were observed and this could be explained by either the patient's delay in meeting the cost to confirm Philadelphia chromosome positivity (and therefore meeting eligibility for imatinib) or lack of imatinib, for patients diagnosed before January 2006, before the GIPAP clinic was established.

Hematologic response to imatinib

Hematologic monitoring is the most affordable and available to our patient population and therefore this is what our study described.

One hundred and ninety four (94.2%) of the patients were commenced at 400mg of imatinib which is the standard dose recommended for chronic phase chronic myeloid leukemia. GIPAP approves starting doses of 300mg, for the pediatric (<18yrs) group only.

At a median clinic follow up of 26 months, complete hematologic response (CHR) as per NCCN/ELN guidelines [11,12] was attained in 163 [79.1% (95% CI: 73.3, 84.5] of the patients with 43[20.9 % (95% CI: 15.5, 26.7] classified as failure. This is comparable to a Cote d'Ivore prospective study, which showed CHR rate of 76% among 42 newly diagnosed chronic phase CML on 400mg imatinib, although their median follow-up time was 32 months, 6 months longer compared to our study. Median time to CHR in these African patients was 8 months[16] versus our mean of 3.4 months.

A Brazil hospital descriptive study based on institutional data of 70 CP-CML patients on 400mg mean imatinib dose, found CHR rate of 92.1% at 6 months[41]. However, unlike our study, they excluded pregnant women and patients previously exposed to hydroxyurea, which was a factor associated with failure in our study, (4 out of the 43 patients with hematologic failure had defaulted on treatment during follow-up due to pregnancy). Their definition of hematologic failure was lack of CHR at 6 months (or relapse) was more liberal compared to our failure definition hence may contribute to the different response outcomes.

Eighty seven (42.2%) patients reported having missed their drug at some point, mostly for at least a week before coming for refills others for prolonged durations. For instance in pregnancy, 4 patients were off imatinib for more than 9 months. A few were given 'drug holiday' due to anemia, neutropenia, leucopenia or thrombocytopenia. Looking at the pharmacokinetics of imatinib whose terminal elimination half-life is 18 hours, [51] one needs to consistently take their imatinib every 24 hours to maintain adequate plasma concentrations for tyrosine kinase inhibition. Among the failing patients, 4 out of the 43 interrupted their treatment due to pregnancy. Kuwabara et al studied seven pregnant women with CML who had been initiated on imatinib but got treatment interruption. He found that suboptimal responders to initial treatment with imatinib either demonstrated the same response after discontinuation or, deteriorated to imatinib failure[48].

The main reason patients gave for non-compliance to imatinib therapy was: that they had financial constraints limiting their travel to clinic for review and imatinib refill. The patient only access imatinib via the clinic and therefore a missed clinic attendance tends to imply missed treatment. Other reasons given were forgetfulness, and patient's travel out of the country coinciding with clinic appointment dates. Assessment of adherence to treatment was by patient interview (self-reports) and therefore there is a chance that the proportion of non-adherent subjects is understated because a patient may not want to admit such shortcomings for fear of being reprimanded or simply self-reports might be influenced by memory deficits of the missed doses. Medication event monitoring system is the gold standard of assessing adherence[50]. However, this option, as well as other objective measures of compliance such as pill counts, blood tests for plasma imatinib levels, and direct observation therapy was not feasible in our study due to unavailability, high cost, time consumption and the cross-sectional design of our study.

Factors associated with imatinib failure

Leucocytosis differed between the two groups (p=0.013) with the patients likely to fail having commenced on imatinib with a lower WBC count as compared to the optimal responders. This was probably because these particular patients were more likely to have been on hydroxyurea (cytoreductive) before starting imatinib, they had a less rapid drop in WBCs which never got as low a minimum as the good response group and after the lowest mean WBC count there was start to rise again whereas the good responders maintained their counts within normal range. Elevated BCR-ABL concentration at entry was also associated with failure (p=0.01).

Among the failure group, non-adherence was in 26/43(60.5%) versus 61/163 (37.4%) in optimal response group (p=0.011). In a Hammersmith Hospital, (London) prospective study

of 87 CP-CML patients adherence was assessed using a medication event monitoring system for 3-months then followed for a median of 19 months. Multivariate analysis identified an adherence rate of 85% or less (relative risk [RR] = 27.8, *P* = .002), as one of the independent predictors for loss of CCyR[43]. In our study non adherence based on patient self-report was associated with hematologic failure (odds ratio [OR] =2.6 [95% CI 1.3-5.1], p=0.007). However, on logistic regression of all the factors associated with failure, longer duration between diagnosis and start of imatinib was identified as the only independent predictor of failure OR 2.69 (95% CI 1.03-7.07) p=0.043. The determination of the predictors of hematologic failure was a secondary objective and therefore our study was probably not powered enough to demonstrate this.

The Sokal and Hasford risk score did not differ significantly between the failing and patients with complete hematologic response. Similar findings were noted by Silveira et al in a Brazil hospital descriptive study based on institutional data of 70 CP-CML patients[41]. This could be so, because the numbers scored for Hasford and Sokal in both studies were too small to show significance, but notably the Brazil study had a small sample size smaller than the number scored in our study (133 for Sokal and 117 for Hasford).

Cytopenias (leukopenia, neutropenia, thrombocytopenia and anaemia) which is a manifestation of haematological adverse imatinib effects were the reason for imatinib dose reductions. In the IRIS trial: at median follow-up of 4.5 years, 5% of patients discontinued imatinib due to adverse events (grade 3/4 cytopenias and elevated liver enzymes) [42].In our study there was no noted hepatotoxicity although liver enzyme tests were not performed routinely during GIPAP clinic follow up.

In a retrospective study of 116 Chinese patients who received imatinib between 2003 and 2008, found response rates for 102 patients in chronic phase were: complete hematologic,

94.1%; (complete cytogenetic, 69.6%; and complete molecular response, 54.9%). Imatinib related grade 3/4 leucopenia were associated with an inferior cytogenetic response. Skin hypopigmentation was the most common side effect (77.6%) [44], whereas in a Turkey prospective study of 31 CP-CML patients, the most commonly observed adverse event was edema (38.7%) especially of the face [45].

From the aforementioned studies, showing different adverse effects among different study settings, one could infer that there is a possibility of varied inter-patient exposure related to metabolizing enzyme activities and probably imatinib concentration. Adverse events such as fluid retention, rash, myalgia, and anaemia, have been shown to be more common at higher imatinib concentrations[47].

11. STUDY LIMITATIONS

This study had limitations in that hematologic response was the sole marker used to determine imatinib failure. It also relied on medical records which at times were missing.

Thirdly, the population studied was a biased sample since patients came only as referrals and had to afford the costly baseline tests.

12. STUDY STRENGTHS

This study is the first one of this kind, with an ample sample size done amongst CML patients in Kenya and therefore serves as a benchmark. It also highlights the need for further evaluation. It also points out issues that could be explored to improve response.

13. CONCLUSION

This study demonstrated that majority [163 (79.1%)] of the patients had sustained CHR. A quarter of the 43 patients with failure had primary failure.

Factors associated with failure were lower leukocytosis at entry, non-adherence on imatinib, high BCR-ABL concentration and longer periods of illness and treatment. Prolonged duration between diagnosis and start of imatinib was an independent predictor of failure. Cytopenias were the main adverse effects necessitating dose decrease.

14. RECOMMENDATIONS

Based on this study finding, it is recommended that imatinib should be 1st line therapy as soon as the diagnosis of CML is established. Studies should be carried out to explore and seek solutions to non-compliance on imatinib (especially given at no cost). A study to investigate the possibility of hydroxyurea-imatinib cross-resistance is recommended.

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APPENDICES

APPENDIX I: HASFORD SCORE[7]

Hasford score

0.6666 × age (0 when < 50 years, 1 otherwise)

+ 0.042 × spleen size (cm below costal margin)

+ 0.0584 × blasts (%)

+ 0.0413 × eosinophils (%)

+ 0.2039 × basophils (0 when < 3%, 1 otherwise)

+ 1.0956 × platelet count (0 when < 1500, 1 otherwise)

× 100

Interpretation of score:

≤ 780 low risk group

> 780 and ≤ 1480 intermediate risk group

> 1480 high risk group

APPENDIX II: SOKAL SCORE[6]

Sokal score

Exp. (0.0116 (age – 4.34))

+ 0.0345 (spleen – 7.51)

+ 0.188 ((platelets/700)2 – 0.563)

0.0887 (percentage of blasts – 2.1)

Interpretation of score:

< 0.8 good prognosis

0.8–1.2 moderate prognosis

> 1.2 poor prognosis

APPENDIX III: DATA ENTRY SHEET

NAME (initials only):_____

Date of birth**:** _____

Country of Birth: _____

Telephone number: _____

Date: _____ Study no: _____

Imatinib registration number: _____

PART A: social demographics

Age: _____

Sex (Tick) Male ☐ Female ☐

Height (cm) _____ Weight (Kg) _____ BSA (m^2) _____ BMI (Kg/m^2) _____

Level of education: primary ☐ secondary ☐ tertiary ☐

Occupation: _____

Marital status (tick) single_____ married _____ separated _____

Lifestyle: smoking: yes _____ no_____ If yes: pack years: _____

PART B: Clinical details

Date of CML diagnosis

Were there symptoms at diagnosis? _____ If yes list them_____

Phase at diagnosis (tick) CP: _____ AP: _____ BP: _____

Spleen size in cm below the costal margin: _____

Liver size in cm below the costal margin: _____

PART C: Lab findings

Full blood count at:

1 Entry: WBC(x10 ^{9} /l) _____ Platelet(x10 ^{9} /l) _____ Hb (g/dl)

Eosinophils (%) _____

Basophils (%) _____

1. 3 mo's: WBC(x10 ^{9} /l) _____ Platelet(x10 ^{9} /l) _____ Hb (g/dl)

Eosinophils (%) _____

Basophils (%) _____

2. 6 mo's: WBC(x10 ^{9} /l) _____ Platelet(x10 ^{9} /l) _____ Hb (g/dl)

Eosinophils (%) _____

Basophils (%) _____

3. 12 mo's: WBC(x10 ^{9} /l) _____ Platelet(x10 ^{9} /l) _____ Hb (g/dl)

Eosinophils (%) _____

Basophils (%) _____

4. 18 mo's: WBC(x10 ^{9} /l) _____ Platelet(x10 ^{9} /l) _____ Hb (g/dl) _____

Eosinophils (%) _____ Neutrophils(x10 ^{9} /l) _____

Basophils (%) _____ Neutrophils (%)

5. 24 mo's: WBC(x10 ^{9} /l) _____ Platelet(x10 ^{9} /l) _____ Hb (g/dl) _____

Eosinophils (%) _____ Neutrophils(x10 ^{9} /l) _____

Basophils (%) _____ Neutrophils (%)

6. 36mo's…….

BMA findings: at entry _____

Cytogenetic or molecular analysis: (tick method used)

Cytogenetic _____

PCR _____

BCR-ABL/ABL ratio at entry _____

PART D: treatment details

Prior treatment with other agents: 1_____ Duration: _____

2 _____ Duration: _____

Date of Imatinib commencement _____

Dose at commencement ____

Phase at initiation of imatinib ____

Any change in dose of imatinib? Yes____ No ____

- If yes, date ____ New dose ____Reason for change _____
- Further change ____ new dose____ reason _____
- Further change ____ new dose____ reason _____

Have you ever missed your dose? Yes____ No ____

- If yes how often _____
- List any other medicine you are taking _____

PART E: Sokal and Hasford scores

Sokal score at entry_____

- Age(yrs) _____
- Spleen(cm) _____
- Platelet(x10 ^{9} /l) _____
- Myeloblasts in blood (%) _____

Hasford score at entry _____

- Age(years) _____
- Spleen(cm) _____
- Platelets(x10 ^{9}/l) _____
- Blasts (%) _____
- Eosinophils (%) _____
- Basophils (%) _____

APPENDIX IV:

STUDY EXPLANATION

Study title

Treatment response to imatinib among chronic myeloid leukaemia patients as seen in Nairobi

Introduction

I am Dr. Esther Dindi from the department of Internal Medicine, University of Nairobi. I am carrying out a research titled "treatment response to imatinib among chronic myeloid leukaemia patients". This study will be looking at how patients with CML (a type of cancer involving the blood) respond to treatment with Imatinib. Your participation in this study is voluntary.

I wish to enrol you in my study, which will involve a brief interview, physical examination and perusing through your treatment records. Should you choose not to participate you will still receive all your treatment and benefits.

Perceived benefits

There will be no direct benefits to you but the results obtained from this study will provide information that could help in improving decision making for other patients diagnosed with the same illness.

Risks

There are no anticipated risks for participating in the study.

Costs and payments:

This study is strictly voluntary and no monetary compensation will be given.

Confidentiality:

All personal information will be kept strictly confidential. There will be no way of identifying participants in any presentations or publications from this study

Withdrawal privilege:

You may refuse to participate or withdraw from the study at any time without penalty or prejudice. If you do this, you will continue to receive health care at the GIPAP clinic as you would normally receive.

APPENDIX V

CONSENT FORM

Voluntary consent:

I …….certify that I have read all of this consent form or it has been read to me and that I understand it. Any questions pertaining to the research have been answered to my satisfaction and my rights have been assured. My signature below is an indication that I freely to consent to participate in this study.

Signature of participant …….. Date……

Investigators statement:

I certify that I have explained to the above individual the nature and purpose of this study, potential benefits and possible risks associated with participation in this study. I have answered any questions that have been raised. I have explained the above to the participant on the date on this consent from.

Signature of Investigator……Date……

APPENDIX VI:

MAELEZO YA UTAFITI

Kichwa cha Utafiti

Matokeo ya matibabu na imatinib katika wagonjwa wa CML

Ufunguzi

Jina langu ni Daktari Esther Dindi wa chuo kikuu cha Nairobi, kitengo cha matibabu. Ninafanya utafiti uitwao "matokeo ya matibabu na imatinib katika wagonjwa wa CML" Utafiti huu unachunguza jinsi wagonjwa wenye CML (aina ya saratani ya damu) wanavyoendelea baada ya kutibiwa na imatinib.

Ningependa kukuhusisha katika utafiti huu, ambao utahusisha maswali machache, upimaji na uchunguzi katika fomu zako za matibabu. Ukichagua kutoshiriki katika utafiti huu bado utapokea matibabu yako kama kawaida.

Manufaa yanayotarajiwa

Matokeo ya utafiti huu huenda yakatumika kutengeneza mpangilio utakaotumiwa kuwezesha kujulikana kwa mapema kwa wagonjwa wasiokuwa na matokeo mwafaka wanapotumia imatinib

Madhara

Hakuna madhara yoyote yanayotarajiwa unaposhiriki katika utafiti huu

Gharama na malipo

Utafiti huu ni kwa hiari na hakuna malipo yoyote yatatolewa

Faragha

Habari zote zitakazotolewa zitawekwa kwa faragha kuu. Watakaoshiriki katika utafiti huu hawawezi kutambulika katika njia yeyote

Kujiondoa kwa utafiti

Unaweza kukataa kushiriki katika utafiti huu wakati wowote bila ya kudhulumiwa kwa njia yoyote. Ukifanya hivyo matibabu yako yataendelea kwa kiliniki ya GIPAP kama kawaida

APPENDIX VII

FOMU YA KUKUBALI

Kukubali kwa hiari

Mimi …….Ninahakikisha ya kwamba nimesoma fomu ya kukubali na nimeielewa. Maswali yote kuhusu utafiti huu yamejibiwa na nimeridhika na nimehakikishiwa haki zangu. Sahihi yangu hapa chini yaonyesha kuwa nimekubali kwa hiari kushirikishwa katika utafiti huu.

Sahihi ya mshiriki …….. Tarehe……

Neno la mtafiti

Nimemuelezea mgonjwa huyu jinsi na sababu za kufanya utafiti huu, manufaa yanayotarajiwa na madhara yoyote yanayohusishwa na kushiriki katika utafiti huu. Nimejibu maswali yote yaliyoulizwa. Nimeyaeleza haya kwa tarehe iliyoonyeshwa kwenye fomu ya kukubali

Sahihi ya mtafiti……Tarehe……

APPENDIX VII: CONTACTS

In case you have any concerns about the study, you may contact the following:

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The Chairman,

KNH/UoN Ethics and Research Committee

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