University of Nairobi

College of Health Sciences

Department of Clinical Medicine and Therapeutics

EVALUATION OF RESPONSE TO IMATINIB MESYLATE THERAPY AMONG CHRONIC MYELOID LEUKAEMIA PATIENTS AS SEEN IN NAIROBI, KENYA

By

Yatich, Robert Kibet; (MBChB, M.Med)

H113/5855/17

A thesis submitted in partial fulfillment of the requirements for the award of Fellowship in Medical Oncology of the University of Nairobi.

© 2018

Dr**. Yatich, Robert Kibet;** MBChB, M.Med

H113/5855/17

Medical Oncology Fellow

Department of Clinical Medicine and Therapeutics

University of Nairobi

Signed…………………………….. 	 Date………………………….

Supervisors' declaration

This thesis has been submitted with our approval as the University supervisors.

1. **Prof. N.A. Othieno-Abinya;** MBChB, M.Med, FRCP

Professor, Department of Clinical Medicine and Therapeutics

School of Medicine; University of Nairobi

Signed…………………………………

Date……………………………….

2. Dr. Jamila Rajab; MBChB, M.Med, MPH, FCPath ECSA

Lecturer in Hematology and Blood Transfusion

Department of Human Pathology

School of Medicine; University of Nairobi

Signed…………………………………..

Date………………………………

Dedication

I dedicate this thesis to my family for the support they gave me while preparing it.

Acknowledgement

Table of contents

List of tables

List of figures

List of abbreviations

ABL: Abelson leukemia virus

Allo-SCT: Allogeneic Stem Cell Transplant

AP: Accelerated Phase

ATP: Adenosine Triphosphate

BCR: Breakpoint Cluster Region

BMA: Bone Marrow Aspirate

BP: Blast Phase

CCyR: Complete Cytogenetic Response

CHR: Complete Hematological Remission

CML: Chronic Myeloid Leukemia

CMR: Complete Molecular Response

COX1:   Cyclo-oxygenase 1

CP: Chronic Phase

DASISION: Dasatinib versus Imatinib Study in Treatment- Naive

DMR: Deep Molecular Response

DNA: Deoxyribonucleic Acid

EDTA: Ethylenediaminetetra-acetate

EFS: Event-Free Survival

ELN: European LeukemiaNet

ENESTnd: Evaluating Nilotinib Efficacy and Safety Trials in Newly Diagnosed

ERC: Ethics and Research Committee

EUTOS: European Treatment and Outcome Study

FDA: Food and Drug Administration

FISH: Flourescence In-Situ Hybridization

GIPAP:	Gleevec International Patient Assistance Program
IFNα:	Interferon alpha
IRIS:	International Randomized trial on Interferon alpha and STI571
IS:	International Scale
KNH:	Kenyatta National Hospital
LAP:	Leukocyte Alkaline Phosphatase
MDR1:	Multi-Drug Resistant 1
MMR:	Major Molecular Response
MOH:	Ministry of Health
MRD:	Minimal Residual Disease
NA:	Not Applicable
NCCN:	National Comprehensive Cancer Network
OS:	Overall Survival
PCC:	Probe Check Control
PCyR:	Partial Cytogenetic Response
PDGFR:	Platelet Derived Growth Factor Receptor
Ph:	Philadelphia chromosome
Ph+:	Philadelphia chromosome positive
PK:	Proteinase K
PTGS1:	Prostaglandin-endoperoxide Synthase 1
QPCR:	Qualitative Polymerase Chain Reaction
QRT-PCR:	Quantitative Reverse Transcriptase- Polymerase Chain Reaction
QSC:	Quiescent Stem Cells
RNA:	Ribonucleic Acid
SCT:	Stem Cell Transplantation

STIM: Stop Imatinib trial

TKI: Tyrosine Kinase Inhibitor

TOPS: Tyrosine Kinase Inhibitor Optimization and Selectivity

UoN: University of Nairobi

WBC: White Blood Cell

WHO: World Health Organization

ABSTRACT

Background: Without treatment, chronic myeloid leukemia (CML) progresses from a chronic phase (CP) followed by an accelerated phase (AP) and then to the blast phase (BP).The approval of imatinib mesylate by the Food and Drug Administration (FDA) in 2001 marked a change in paradigm CML therapy. Therapy with tyrosine kinase inhibitors (TKI) reduces the CML residual disease to levels which cannot be of detected by cytogenetic or hematologic testing but only by molecular testing using quantitative polymerase chain reaction(Qrt-PCR). Guidelines by both National Comprehensive Cancer Network (NCCN) and European LeukemiaNet (ELN) establish response milestones for TKI treatment. Early attainment of minimal residual disease in the treatment with TKIs is predictive of a favourable prognosis. There are 1200 CML patients currently enrolled in the Gleevec International Patient Assistance Programme (GIPAP) in Kenya. Their molecular response to imatinib therapy is unknown. This study aimed at determining the molecular response to imatinib among CML patients enrolled in the GIPAP programme in Nairobi.

Objective: To determine the response to imatinib mesylate among CML patients attending the GIPAP clinic at the Nairobi Hospital; Kenya.

Methods: This was a cross-sectional descriptive study carried out at the GIPAP clinic in Nairobi Hospital. Chronic myeloid leukaemia patients who had been on imatinib for not less than 18 months and gave consent were enrolled consecutively into the study until a sample size of 207 was attained. The investigator reviewed the patients and perused through their records to extract socio-demographic, clinical and laboratory data including breakpoint cluster region-Abelson leukemia virus (BCR-ABL) transcript level at baseline and after 18 months of imatinib therapy. Information was entered in a data entry form, transferred into a data base and analyzed using STATA version 13 SE.

Results: Out of 215 participants studied, 210 (97.7%) were in chronic phase. Most of the participants, 138 (64%) had a low EUTOS risk score at entry. The median period of clinic follow up was 4.7 years. Almost a half, 105 (49 %) of the participants had been exposed to hydroxyurea. The median duration to initiation of imatinib was 4.9 weeks. Majority of the participants, 180 (84%) achieved complete hematologic remission (CHR) at 3 months. More than a half of the participants reported having missed a dose. One third 74 (34%) of the participants had optimal response, 34 (20%) had treatment failure and 98 (46%) had suboptimal response. High EUTOS score (24% vs. 42%, p = 0.011), prior treatment with hydroxyurea (35% vs. 56%, p <0.001) and poor adherence (26% vs. 69%, p <0.001) were associated with suboptimal response and treatment failure.

Conclusion: This study demonstrated that a minority of the participants, 74 (34%) had an optimal treatment response and 34 (20%) had treatment failure. A high EUTOS score, prior treatment with hydroxyurea and poor adherence were associated with poor response to imatinib therapy.

Recommendation: Measures should be instituted to improve access and adherence to treatment with imatinib among CML patients attending the GIPAP clinic.

CHAPTER ONE: INTRODUCTION

1.1 Background

Chronic Myeloid Leukemia (CML); a myeloproliferative neoplasm, is characterized by uncontrolled proliferation of myeloid lineage cells in the bone marrow and subsequent pooling in the peripheral blood. It is caused by the aberrant expression of the fusion gene BCR-ABL that results from reciprocal translocation between chromosomes 9 and 22(1)(2).

CML accounts for 0.34 % of all cancers, 3.6 % of all hematological malignancies and contributes 15% in adult and 5% in children of the total leukemias(3)(4). The median age of occurrence is 45 years to 55 years. The global incidence of CML is estimated at 8-9 cases/100,000 annually(5). CML contributes to 0.08% of all cancer mortalities(4). Approximately 5,920 new cases were diagnosed and 610 people died from the disease in the United States of America in the year 2016 (5). As per Ministry of Health (MOH) records from 1998 to 2002, a mean of 90.3 cases of CML were reported in Nairobi annually(6). In a study done at Kenyatta National Hospital from 1990 to 2000, a total of 104 cases of CML were reported; 55 males and 49 females(7). There are 1,200 cases of CML currently enrolled in the GIPAP program in Kenya. If it is not treated, CML progresses from a stable CP to an AP within 60 months and then the BP within 12 months(8).

A paradigm shift has been witnessed in CML treatment in the last decade. The imatinib mesylate (IM) approval for CML treatment marked one of the first breakthroughs in molecularly targeted therapy(9). Tyrosine kinase inhibitors (nilotinib, imatinib, bosutinib, dasatinib,and ponatinib) are very effective in reducing the disease burden that to monitor the treatment responses adequately requires very

sensitive techniques. There is growing evidence showing early achievement of minimal residual disease is predictive of a better prognosis and treatment modifications for suboptimal responders is necessary(10).

Response to treatment in CML is measured in several levels: cytogenetic, hematologic and molecular response. Molecular testing using (qRT-PCR) detect minimal residual disease (MRD) better than cytogenetic and hematologic tests(11)(12). Monitoring of BCR-ABL1 transcript levels being reported on an International Scale (IS) is recommended by guidelines issued by the NCCN 2012 and ELN(13).

The front-line treatment for CML is TKI therapy (dasatinib, nilotinib or imatinib)(14). Tyrosine kinase inhibitor therapy elicits molecular responses through the inhibition of BCR-ABL1. Tyrosine kinase inhibitors reduce the frequency of CML progression. This is critical because the overall survival (OS) of patients on TKI therapy who progress to advanced stages of the disease is short (10.5 months);(15). Accelerated phase/blastic phase –CML is difficult to treat than CP-CML and limited treatment choices are available(14). Both ELN and ELN have established response milestones to be achieved within specific timeframes for first-line(16)(14)and second-line(16) TKI treatment. With respect to treatment-response milestones, both guidelines have considerable similarities and differences. Most CML treatment centres use ELN guidelines in the management of patients because the clinical end points and treatment response criteria used in contemporary studies are based on these guidelines(17). Achievement of an optimal molecular response within the first 18 months of treatment predicts a better long term outcome. Testing 3-monthly until a MMR is achieved and maintained and 6-monthly thereafter is recommended by ELN(16). The routine

monitoring of BCR-ABL transcripts provides patients and clinicians with prognostic information on CML control.

There are no published studies done in Kenya on molecular treatment response in CML patients on TKI. This study therefore aimed at evaluating the treatment response among CML patients on imatinib mesylate in Nairobi; Kenya.

1.2 Justification of the study

The treatment of CML has improved drastically since the introduction of imatinib mesylate. To rapidly assess treatment response and predict outcomes, regular molecular monitoring of BCR-ABL1 can be used. Better prognosis is seen in patients who achieve timely milestone responses. Optimal responders have lower risk of disease progression than suboptimal responders. Moreover, accelerated or blast phases of CML have poor treatment responses and associated with high mortality and morbidity rates.

Local data on molecular response/ failure to treatment is lacking. Previous studies were done in other populations (Caucasians, whites). The African population has unique differences (socioeconomic, poor understanding of CML disease and possible differences in drug metabolism) which may affect response rate. There was need to establish the molecular treatment response in the GIPAP program in Nairobi. The findings from this study will inform clinical decision making regarding dose adjustment and timely switching of therapy for CML patients.

Appropriate measures can be taken to improve treatment response hence favorable outcomes. Improvement of patient adherence to medication, development of new and updating of existing protocols, continuous medical education and reminders within

patients' medical records are some of the measures which can improve treatment response.

1.3 Research question

What is the molecular treatment response among chronic myeloid leukemia patients treated with imatinib in Nairobi?

1.4 Research objectives

1.4.1 Broad objective

To determine the treatment response in chronic myeloid leukemia patients treated with imatinib in Nairobi.

1.4.2 Specific objectives

1. To determine the molecular treatment response of chronic myeloid leukemia patients treated with imatinib in Nairobi.

2. To describe the hematological and clinical characteristics of the patients in the study.

3. To correlate patients' characteristics with response to imatinib therapy.

CHAPTER TWO: LITERATURE REVIEW

2.1 Disease overview.

Chronic myeloid leukemia (CML); a myeloproliferative neoplasm (MPN), is characterized by the Ph chromosome resulting from the t(9;22)(q34;q11) translocation producing the BCR-ABL oncoprotein(18). Proliferation of basophils, eosinophils and neutrophils and their precursors (complete maturation spectrum) is characteristic of CML. It contributes to approximately 15% the incidence of adult leukemia cases.

An abnormally small chromosome 22 in the white blood cells of CML patients was noticed by D. Hungerford and P. Nowell in 1960 at Philadelphia, Pennsylvania which got named Philadelphia chromosome. This made CML the first cancer shown to be caused by an underlying genetic abnormality(2). Janet Rowley reported in 1973 that the Philadelphia chromosome is a t(9;22) translocation(19) and is detectable in 95% of CML patients(18).

The resultant gene from this translocation is an oncoprotein called BCR–ABL1; a continuously activated protein kinase (figure 1)(18). This constitutively active protein kinase is central to the pathogenesis of CML and forms the basis for targeted CML therapy(20). The BCR-ABL oncogene by activates several intracellular signalling pathways resulting in altered adhesion, antiapoptotic signals and defective DNA repair: all these lead to hematopoietic stem cell transformation (Figure 2)(18).
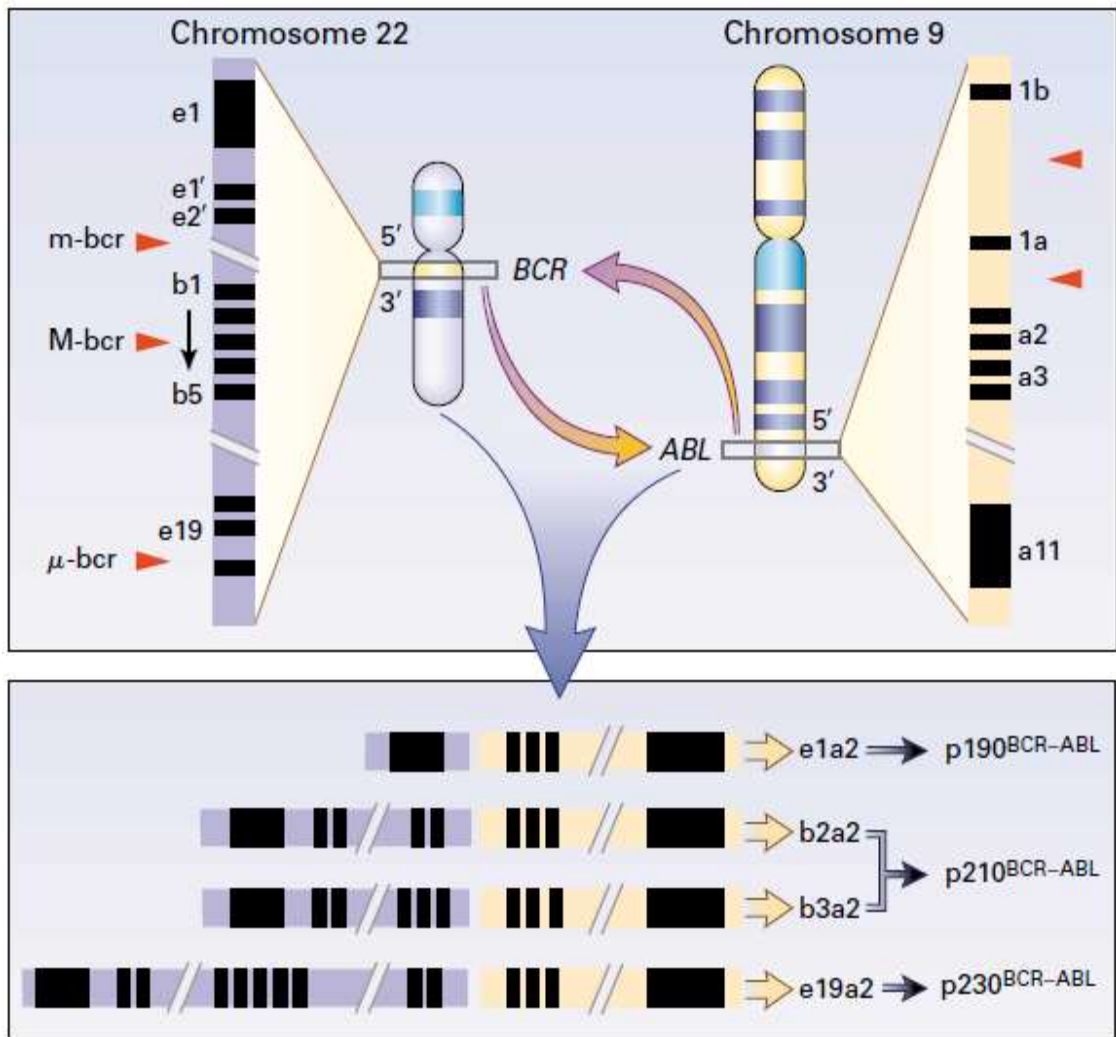
Figure 1: The t (9;22)(q34;q11) translocation in CML(18).
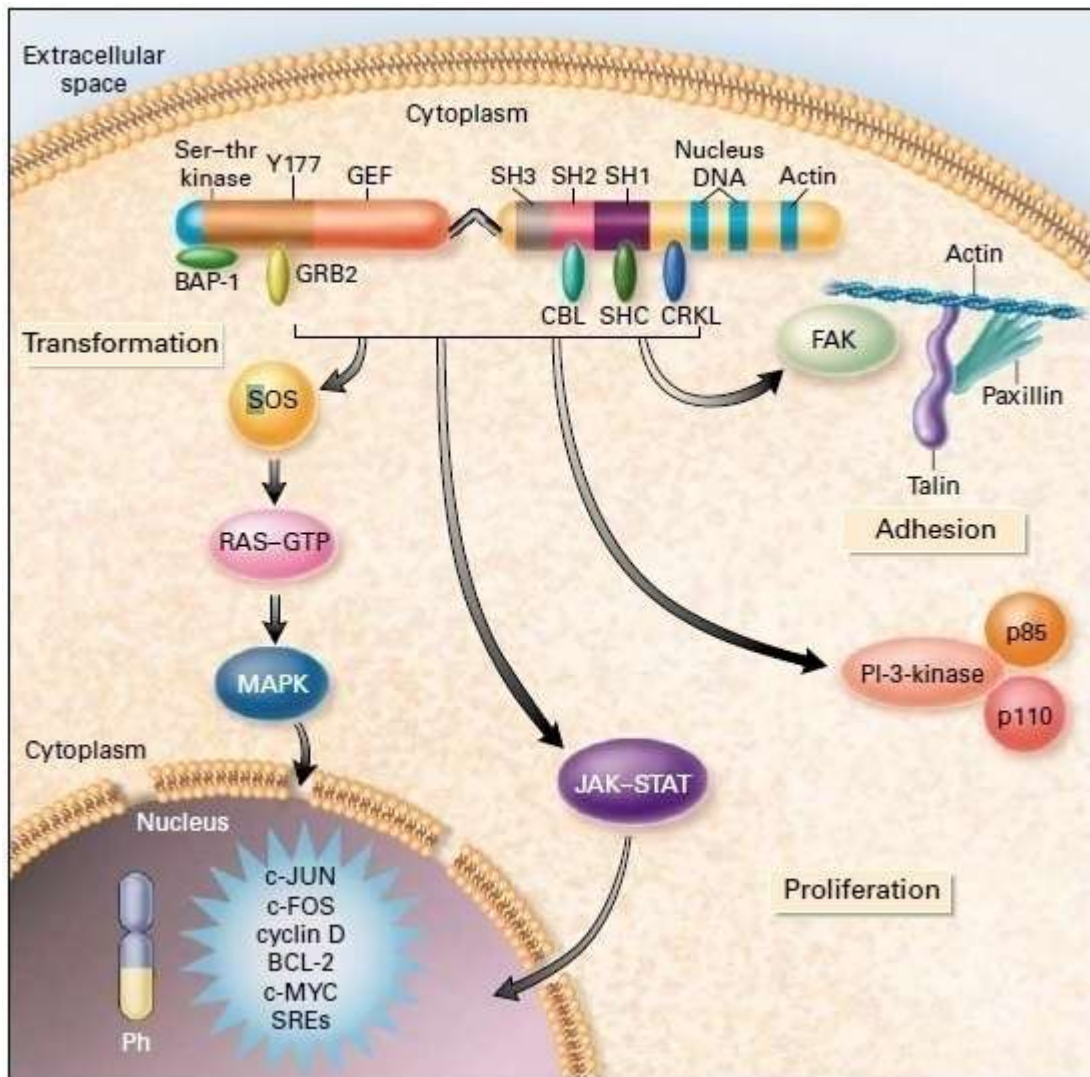
Figure 2: The p210^{BCR-ABL} signalling pathways(18)

Treatment of CML drastically changed a little more than 10 years ago(21). Although allo-SCT is associated with high risk of complications and death, it is curative. With the emergence of TKIs, the therapeutic landscape of CML changed drastically. The natural history of CML was altered by this approach increasing the 10-year OS by 60 – 70% (22).

2.2 Manifestations and staging of chronic myeloid leukemia

About 50% of CML patients have no symptoms at diagnosis. The disease is often diagnosed after a routine medical check-up(18). Chronic myeloid leukemia has three phases in the course of illness: chronic, accelerated and blast phases. Most (95%) of CML patients present in the chronic phase(23)(24). The median duration of chronic phase is 5-6years(25).

Common clinical features of the chronic phase are as a result of splenomegaly and anaemia. These are: easy fatigability, loss of weight, left upper quadrant pain or fullness and early satiety(23). Uncommon presentations include bleeding, gouty arthritis, venous and arterial thrombosis, retinal bleeds, priapism and upper gastrointestinal ulcerations and bleeding. Sludging of leukemic cells in the cerebral or pulmonary circulation can lead to leukostatic symptoms but these are not common in the chronic phase.

The most common physical sign (seen in >40% of cases) is splenomegaly. Hepatomegaly (<10%) and lymphadenopathy and infiltration soft tissues are infrequent; their presence favour accelerated or blast phases. CML transformation may present with bone pain, headache, fever, splenic infarction and arthralgias. Usually, CML progresses to the AP prior to BP transformation but some cases transition to the blastic phase without accelerated phase warning signs. Transformation into the accelerated phase might be gradual in onset or rapidly progressive presenting with worsening splenomegaly, anemia or infraction of visceral organs. Transformation into the blastic phase manifests as an acute leukemia (lymphoid in 30%, myeloid in 60% and undifferentiated or megakaryocytic in 10%) with infections, fever, bleeding tendency and constitutional symptoms.

2.3 Diagnostic evaluation of chronic CML.

The diagnosis of CML consists of detecting the presence of the Ph chromosome (t(9;22)(q34;q11)) or of BCR–ABL1 gene in the background of persistent leucocytosis and/or thrombocytosis.

Molecular testing (PCR) can be quantitative or qualitative. To monitor minimal residual disease, qualitative PCR is more sensitive. A high level of concordance is seen with concurrent peripheral blood and bone marrow QPCR. Both false–negative and false-positive results can be obtained with polymerase chain reaction. Failure of the reaction or poor quality of RNA can result in a false-negative test and a false-positive test can result from contamination. Depending on sample handling, procedures and staff experience, a difference of 0.5 – 1 log can occur

A negative FISH test is equivalent to a complete cytogenetic response (CCyR) which correlates with a BCR–ABL1 transcript level of <1%. A BCR–ABL1 transcripts levels of ≤10% (IS) is equivalent to a partial cytogenetic response.

The Ph- chromosome is often present as the only abnormality in all metaphases. Additional chromosomal changes involving isochromosome 17, trisomy 8, double Ph-/more loss of material from 22q or others are seen in 10-15% of patients.

All patients should have bone marrow aspiration done for confirming the diagnosis, staging and prognostication. Quantitative RT-PCR at baseline is mandatory to quantify BCR-ABL1 transcript level.

The diagnostic criteria for AP and BP of CML are as per the World Health Organization (WHO) are as follows(26):

Accelerated phase CML can be diagnosed when the following singly or in combination are present:

- Blast percentage of 10-19% of the total white cell count (WBC) in the PB and/or in the BM.

- Basophils percentage of ≥20% in the peripheral blood

- Platelet count $<100 \times 10^9$/L unrelated to treatment or (platelet count $>1000 \times 10^9$/L) refractory to therapy.

- Progressive splenomegaly and rising WBCt refractory to therapy.

- Of clone confirmed by cytogenetic studies

The diagnosis of blast phase may be made if the following are present singly or in combination:

- Blast percentage of ≥ 20%

- Extramedullary proliferation of blasts

- Bone marrow biopsy showing clusters or large foci of blasts

2.4 Differential diagnosis of CML

Leukemoid reactions which have a normal or elevated leukocyte alkaline phosphatase (LAP) levels must be differentiated from CML(27).

Other myeloproliferative neoplasms which mimick CML in presentation include: agnogenic myeloid metaplasia, myelofibrosis (frequently have neutrophilia, thrombocytosis and splenimegaly). A normal or elevated LAP score, a WBC <25 x 10^9/L and negative Ph- is seen in these conditions(27).

Patients with leukocytosis and an enlarged spleen but negative Ph- are a diagnostic challenge. However, the BCR − ABL1 gene is detectable in some inspite of the atypical cytogenetic pattern(27).

2.5 Prognosis of CML

The prognostication of CML can be made by using factors identified before treatment (baseline factors) and also by employing factors during treatment (treatment response-related or time dependent factors). The correct identification of the disease phase at diagnosis is of utmost prognostic significance. In the chronic phase of CML, the relevant prognostic information is derived from the laboratory and clinical features(28).

Kantarjian and colleagues in the Anderson Hospital and Tumor Institute, Houston, Texas (1985); did an analysis of the correlation of the characteristics of patients and therapy with survival for 303 Ph+ CP-CML patients. They found that black race, age above 60 years, splenomegaly, hepatomegaly, poor performance status and weight loss were associated with shortened survival. Thrombocytosis or thrombocytopenia, anemia, high peripheral blast counts, high basophil percentage in the PB, reduced BM megakaryocytes, a high BM blast or basophil percentage and additional cytogenetic abnormalities were also associated with adverse outcome(29).

The Sokal, Hasford and EUTOS scores have been used to predict response to therapy. The Hasford risk score is a stratification that includes spleen size, blast percentage, platelet count and eosinophils and basophils in the peripheral blood and patients' age(30). It was initially used for patients treated with interferon. This scoring system classifies patients into low, modertae and high risk groups (Appendix VII).

Sokal risk score is a prognostication score that incorporates patients' age, blast percentage, platelet count and spleen size(31). It was developed when busulfan was primarily used to treat CML was also used to classify patients by risk in the clinical trials on imatinib (Appendix VII).

The EUTOS score is a prognostic risk score that incorporates the spleen size and the percentage of basophils in the peripheral blood(32). It has a better prognostic index than the Hasford and Sokal scores. Moreover, it is specifically based on imatinib-treated patients and does not use variables not ascertained to have an effect on treatment response(32). This scoring system classifies patients with CML into high and low risk prognostic groups (Appendix VII). All the three (EUTOS, Hasford and Sokal) prognostic scoring are used to predict treatment response to imatinib.

Patients who achieve a molecular response early while on imatinib have a better long-term outcome(33). Attaining a BCR-ABL transcript level of <10% at 6 months and <1% at 12 months correlates with a better event-free survival and lower rate of disease progression. Achieving a major molecular response (MMR) by 18 months is also associated with more durable responses and very low rates of disease progression(34). Therefore, time-dependent measures should be used to determine the optimal treatment response.

2.6. Frontline therapeutic choices for CML

2.6.1 Imatinib mesylate

The FDA approved imatinib as the first TKI for the treatment of chronic phase CML patients in 2001. It competitively inhibits the ATP-binding site of the BCR − ABL1 oncoprotein stopping downstream transduction of signals (Figure 3)(9). It also inhibits C-KIT and platelet derived growth factor receptor (PDGFR).
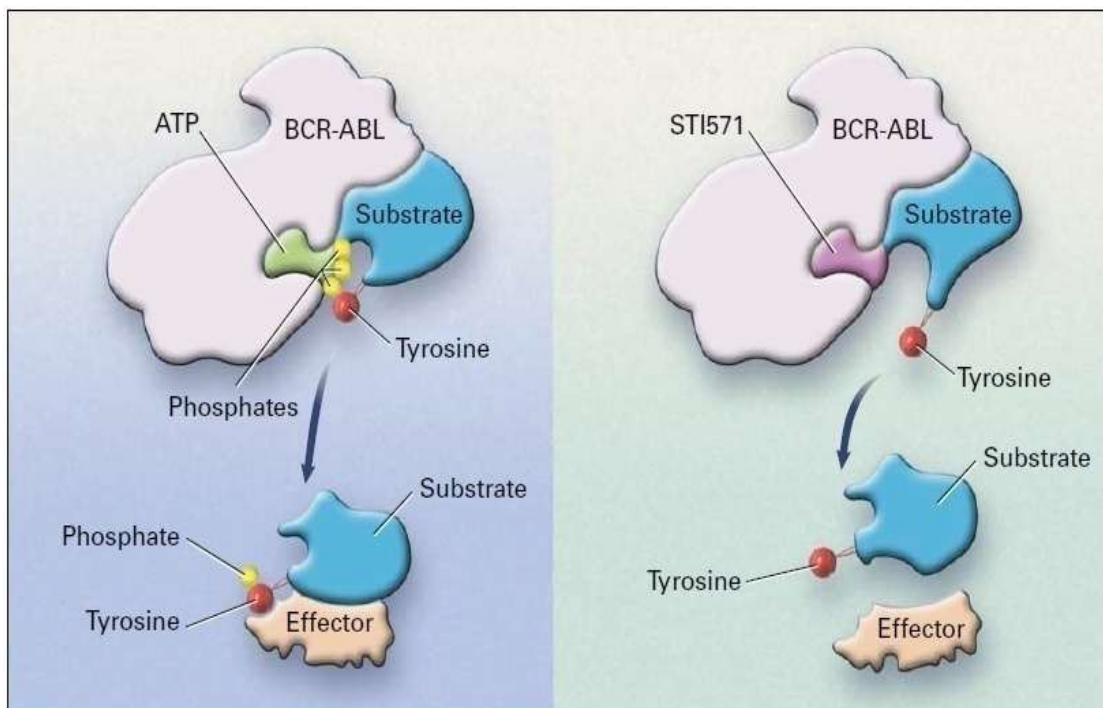
Figure 3: Mechanism of action of imatinib(9)

Although imatinib had impressive results in the landmark International Randomized study of Interferon and STI 571 (IRIS) study, after 8 years of follow-up, only 55% of patients remained on therapy. Additional treatment choices were therefore needed for patients who were intolerant to or failed imatinib. This necessitated the development of second-generation TKIs(35)(22).

2.6.2 Imatinib dose escalation and imatinib-based combinations.

Escalating the dose of imatinib or combination with other drugs are other strategies that can be used for frontline therapy. Patients were randomized to imatinib 400mg twice a day (800mg) or 400mg once a day in the Tyrosine Kinase Inhibitor Optimization and selectivity (TOPS) study(36). The study endpoint was major molecular response rate at 1 year and time to such responses and cytogenetic response were secondary end points. Faster MMR and CCyR were achieved in patients on high dose imatinib but the response rates were similar in the two groups at 1 year. Pegylated IFN-α, has also emerged as treatment choice in CML because it is administered less frequently and it is well tolerated(37).

2.6.3 Dasatinib

Dasatinib; a second-generation tyrosine kinase inhibitor, is about 350 times more active than imatinib(38). The Src family of kinases are critical in blocking cell signaling pathways and are also inhibited by dasatinib. Dasatinib was compared to imatinib in the firstline setting to determine whether the more potent tyrosine kinase inhibitors might improve outcomes in the randomized phase III DASASION trial(39). Attaining CCyR at 1 year was the primary outcome. In the 519 patients who were randomized, the imatinib group attained less CCyR at 1 year than the dasatinib group (66% versus 77%). The dasatinib arm also had favourable secondary endpoints. More rapid and deeper responses were also induced by dasatinib at early time points after a 5-year follow-up period(40). Moreover, a BCR-ABL1 transcripts (IS) ≤10% was achieved at 3 months in more of the patients assigned to dasatinib (80% vs 64%) which predicted for a better PFS and OS. Progression to accelerated or blastic phases was also lower in the dasatinib group (4.6% vs 7.3%).

2.6.4 Nilotinib

Nilotinib is 40 times more potent than imatinib(41). The two TKIs were compared in the ENESTnd trial. In this trial, nilotinib dose (400mg twice daily) was compared to imatinib 400mg once a day(42). The nilotinib arm achieved a higher rate of MMR at 1 year compared to the imatinib arm.

Better early results were observed in the nilotinib arm compared to imatinib after a 5-year follow-up(43). Achievement of MMR by 5 years in the two arms was 77% and 60% respectively.

2.6.5 Selecting a frontline tyrosine kinase inhibitor therapy.

Dasatinib, imatinib or nilotinib are recommended by current guidelines as frontline therapeutic options for treating chronic phase CML(14). More early optimal responses are produced by second-generation TKIs. They however have no long-term survival advantage. The advantage of second-generation TKIs is pronounced in high-risk patients with a significant reduction in the rate of disease progression is to accelerated and blast phases(27). Other issues to be considered when selecting an agent include: adverse event profile, patient's age, risk stratification score, comorbidities, BCR-ABL1 transcripts subtype and drug cost(27).

Because of the excellent responses and better long-term outcomes attained with the tyrosine kinase inhibitors, allogeneic-stem cell transplant and other chemotherapeutic agents are not recommended as up-front treatment options for chronic phase CML.

2.7 Milestones and surrogate endpoints for monitoring treatment response.

Surrogate markers of treatment outcome are crucial because of the long OS of patients with CML. Attaining a deeper response earlier is associated better outcomes. The use

of BMA for monitoring treatment has been obviated by technological advances which has made available molecular tests on peripheral blood(27).

2.7.1 Endpoints for monitoring treatment response and failure.

A BMA should be done at baseline to confirm the diagnosis, document blast and basophil percentage and do cytogenetics to rule out clonal evolution. Subsequent follow-up serial BMA examination after starting therapy is no longer recommended(13). Use of FISH or PCR on PB is an alternative method of determining cytogenetic response(44).

Regular molecular monitoring using qRT-PCR for patients who have achieved CCyR is useful and acceptable. Achievement of MMR is associated with modest improvement in EFS but no OS benefit(27). Achievement of CMR offers the consideration of discontinuing treatment(27). Several studies have shown that early molecular response with each of the three TKIs used in the frontline setting has prognostic value (Table 1)(40)(42)(45)(46).

Table 1: Important response categories in chronic myeloid leukemia(27)

Treatment response	Translation
BCR-ABL/ABL1 ≤10% (IS) at 6 months	Significant improvement in EFS and OS
Major molecular response	Slightly improved EFS; no OS benefit
Complete molecular response	Possible discontinuation of treatment

2.7.2 When to change tyrosine kinase inhibitor treatment

Achievement of major molecular response (MMR) within 18 months and sustaining MMR at any time beyond 18 months is the goal of therapy in CML. Major molecular response may need to be achieved sooner for second-generation tyrosine kinase inhibitors(47). Change in therapy should be considered for patients who fail to attain CHR by 3 months (Table 2).

Table 2: Chronic myeloid leukemia treatment milestones(13)

Evaluation time, months	Response			Warnings
	Optimal	**Suboptimal**	**Failure**	
Baseline	NA	NA	NA	High risk; CCA/Ph+
3	CHR and at least minor CyR (Ph+ ≤65 percent)	No CyR (Ph+ > 95 percent)	Less than CHR	NA
6	At least PCyR (Ph+ ≤35 percent)	Less than PCyR (Ph+ >35 percent)	No CyR (Ph+ >95 percent)	NA
12	CCyR	PCyR (Ph+ 1-35 percent)	Less than PCyR (Ph+ >35 percent)	Less than MMolR
18	MMolR	Less than MMolR	Less than CCyR	NA
Any time during treatment	Stable or improving MMolR	Loss of MMolR; Imatinib sensitive mutations	Loss of CHR; imatinib-resistant mutations; CCA/Ph+	Increase in transcript levels; CCA/Ph-

Treatment of patients failing to attain the 3-months BCR-ABL1 transcripts level is controversial. There are many options including switching TKIs early or doing a repeat measurement at 6 months which will identify patients requiring treatment change(48)(49). However, patients with BCR-ABL1 transcripts >10% at 3 months do not invariably have a worse outcome(49)(50).

Regular molecular monitoring is recommended for patients who meet all the treatment milestones within 12 months. A BMA with cytogenetics including mutational analysis should be done for patients with treatment failure and patients with cytogenetic relapse should have a change of treatment.

2.8 Management of tyrosine kinase inhibitor resistance

2.8.1 Overview

Increasing drug resistance is problem seen with all the availabe tyrosine kinase inhibitors. Approximately 10 to 15% of CML patients develop imatinib resistance(51). Treatment failure can either be primary or secondary. When a patient does not achieve a desired treatment response to initial therapy, it is called primary (intrinsic) resistance whereas secondary (acquired) resistance occurs in patients who relapse after initially responding to imatinib. Failure is further sub-classified as hematologic, molecular and cytogenetic.

The proposed mechanisms of imatinib resistance are shown in table 3 below. These include decreased intracellular drug levels which can be due to α-1 acid glycoprotein binding to imatinib in the plasma or over-expression of p-glycoprotein (MDR-1) causing drug efflux, BCR-ABL kinase over-expression and clonal evolution(52). Gene expression profiling on newly diagnosed imatinib-treated CML patients has shown that the prostaglandin–endoperoxide synthase 1/cyclooxygenase 1 (PTGS1/COX1) gene is associated with primary resistance. However, acquired ABL kinase point mutations and amplification are the main causes of secondary resistance(53)(54). Not achieving CCyR at 12 months, a high-risk Sokal score, clonal evolution, initiation of imatinib in the later stages of CML and older age are all associated with secondary imatinib resistance(55).

Table 3: Mechanisms of imatinib resistance/failure(54).

Independent of BCR-ABL	Dependent on BCR-ABL
poor compliance (patient factor)	BCR-ABL1 over-expression
Pharmacological: -Impaired intestinal absorption -Drug interactions -Binding with plasma component	Mutations in ABL-kinase domain
Leukemia cell related: -Low transporter (hoct1) levels -High exporter (ABCB1, ABCG2) levels	
Quiescence of cancer stem cells (QSCs)	
Evolution of leukemic clone	
SRC over-expression	

Mutation of BCR-ABL kinase is a common mechanism of resistance. Resistance to dasatinib and nilotinib has also emerged due to development of new mutations. Ponatinib is the only TKI which overcomes the T315I gatekeeper mutation(27).

Poor adherence to treatment and drug interactions should be ruled out before defining resistance to TKI therapy. Drug compliance rates of imatinib range from 75% - 90% and poor compliance is associated with worse (56)(57).

2.8.2 Second and new generation TKIs

The second-generation TKIs were first approved for use in the second-line treatment of CML before being moved to the first-line setting. Studies have shown that second-line TKIs yield high treatment response rates in suboptimal responders to imatinib(58)(59)(60)(61). Switching to second-line TKIs early in suboptimal

responders to imatinib gives better treatment outcomes than switching later (TIDEL-II study)(62).

Bosutinib is active against mutations conferring resistance to imatinib but not the T315I mutation. Ponatinib, a TKI that is several-fold more active than imatinib, is the only TKI that overcomes the T315I mutation(63)(64).

2.8.3 Selecting a second-line or third-line TKI

A bone marrow examination is mandatory when a patient develops failure to imatinib to determine the phase of CML and to rule out clonal evolution. Mutations of the ABL kinase domain should be tested in all CML patients to guide in selecting the TKI(65)(66)(67)(68). Bosutinib or dasatinib is preferred for the following mutations: E255K/V, Y253H or F359C/V. Nilotinib overcomes resistance due to F317L and V299L mutations. Cost of drug, toxicity profile and comorbidities are other considerations in selecting treatment. The toxicity profile of bosutinib is distinct; the common adverse effects being gastrointestinal complaints including diarrhea.

The adverse effects of ponatinib include pancreatitis, vaso-occlusive events, skin rash, hypertension and thrombosis.

2.8.4 Allo-SCT and its' role in TKI treatment failure.

The role of allo-SCT has diminished significantly since the introduction of TKIs. However, as the resistance to TKIs becomes more common its' use will increase. Patients who progress to AP/BP benefit more from allo-SCT(69). Previous exposure to TKIs has no negative impact on the outcome after transplant(70).

2.9 Duration of tyrosine kinase inhibitor therapy and discontinuation of treatment

The relapse rate in patients who had achieved CMR >2 years and had treatment discontinuation was investigated in the Stop Imatinib (STIM) trial(71)(72). In this trial, after a median follow-up of 4 years with close monitoring of 100 evaluable patients, 60% had molecular relapse with the majority (95%) of the relapses occurring within 7 months of discontinuing treatment. Complete molecular response was achieved by all patients once treatment was restarted. The findings of this study were replicated subsequent large CML studies. In the TWISTER study, 40 patients were followed up after stopping imatinib upon achieving undetectable minimal residual disease for >2 years (73). After a follow up period of 15 months, 50% of the patients had molecular relapse; majority (70%) of the relapses occurring early after treatment discontinuation.

Mixed patients groups including those exposed to interferon before imatinib era were included in early studies. The French group conducted a follow-up study to STIM (STIM2) was done by a study group from France who enrolled patients only with prior imatinib exposure (STIM2)(74). After a median follow-up of 1 year, 48 out of 124 patients had molecular relapse; majority (94%) of molecular relapses occurring within 6 months of treatment discontinuation. Deep molecular responses were however recaptured by all patients upon re-challenge(75).

The factors associated with durable DMR after discontinuing imatinib were defined by the European study; Stop Tyrosine Kinase Inhibitor trial(76). Out of a total of 200 enrolled patients, 123 remained relapse-free in the initial 6 months of follow up.

Molecular relapse was seen in 47% and 27% of patients on treatment for less than 8 years and more than 8 years respectively.

2.10 Treatment of advanced stage CML.

Treatment with the new generation TKIs followed by early allogeneic-SCT if the preferred option for advanced stage chronic myeloid leukemia(77)(78). When induction chemotherapy is combined with the tyrosine kinase inhibitors the response rates are 70-80% and 40% for lymphoid and non-lymphoid blastic phase CML respectively with survival times of 12 to 24 months and 6 to 12 months respectively. Response rates and survival times in blastic phase CML have significantly improved with the addition of tyrosine kinase inhibitors to induction chemotherapy..

Cure is achieved in 15-40% and 10-20% of accelerated and blastic phase CML respectively following allo-SCT(66)(67). Patients who progress from CP to AP CML have a worse outcome with tyrosine kinase inhibitor treatment than patients with de novo accelerated phase CML. With tyrosine kinase inhibitor treatment, the 8 year survival is 80% in de novo accelerated phase CML. Long-term tyrosine kinase inhibitor treatment should be continued in such patients if a MMR is achieved(79)

CHAPTER THREE: PARTCIPANTS, MATERIALS AND METHODS

3.1 Study site

Participants were drawn from the Gleevec International Patient Assistance Program (GIPAP) outpatient clinic at the Nairobi Hospital. The Nairobi Hospital is one of the biggest private hospitals in Kenya. It is located in Nairobi (the capital city of Kenya) and it offers both essential and specialized healthcare services. The GIPAP outpatient clinic is a two weekly clinic that offers free imatinib treatment to adult patients with gastrointestinal stromal tumours and chronic myeloid leukemia and it is the only referral centre for patients with these conditions requiring imatinib therapy in Kenya. This program was started in Kenya in 2002 as a partnership between the Nairobi Hospital, the Max Foundation and Norvatis Pharmaceuticals. There are 1,200 patients currently enrolled in the program. Approximately 10 newly diagnosed CML patients are enrolled in the program monthly.

3.2 Study population

The study population were adult chronic myeloid leukemia patients treated at the GIPAP clinic.

3.3 Study design

This was a cross-sectional descriptive study.

3.4 Eligibility

3.4.1 Inclusion criteria

Patients aged 18yrs and above diagnosed with CML and on imatinib therapy for not less than18 months.

3.4.2 Exclusion criteria

- Failure by the patient to give consent.

- Patients whose treatment had been interrupted by pregnancy

3.5 Operational definitions

3.5.1 Definitions of CML treatment response (ELNcriteria):

1. **CHR** was defined as complete normalization of PB counts and no sign or symptom of disease with disappearance of palpable splenomegaly.

2. MMR was defined as <0.1% ratio of BCR-ABL1 in the IS.

3. CMR was defined as undetectable BCR-ABL1 mRNA transcripts in two consecutive blood samples (sensitivity of more than 4 logs).

4. Disease progression was defined as transformation to AP or BP of CML.

3.5.2 Definitions of CML treatment response milestones as by European LeukemiaNet (ELN) criteria:

1. Optimal response was defined as: CHR at 3 months, MMR at 18 months **OR** stable or improving MMR at any time.

2. Suboptimal response was defined as: Less than MMR at 18 months **OR** loss of MMR.

3. Treatment failure was defined as: no CHR at 3 months, **OR** loss of CHR and emergence of imatinib-resistant mutations at any time.

3.6 Sample size calculation

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

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

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

n: Sample size

$Z_{1-\alpha/2}$: Two-sided significance level corresponding to 95% confidence interval= 1.96

P: Estimated proportion of patients with imatinib treatment failure = 16% (51)

∂: is the margin of error = 5%

Using a precision of 5% and an estimated proportion of patients with imatinib failure of 16% from an earlier study by Zhang WW, et al(11), a minimum sample size of **207**patients was obtained.

3.7 Sampling technique

All CML patients on follow-up at the GIPAP clinic were screened for eligibility. All the patients who fulfilled the eligibility criteria were consecutively enrolled into the study.

3.8 Patient evaluation

3.8.1 Patient screening and recruitment

Patients at first contact had an initial evaluation for symptoms, and were examined particularly to check for pallor, splenomegaly and/or hepatomegaly.

At entry into the GIPAP program the patient must have total blood count, bone marrow aspirate and molecular analysis for confirmation of BCR-ABL1 positivity. Patients in chronic phase CML are commenced on 400mg of imatinib taken orally once a day, those in advanced phase (AP, BP) are started on 600mg of imatinib taken

orally once a day; this is in line with NCCN/ELN guidelines and is GIPAP-approved. Upon commencement of treatment the patients are screened every 1-2 weeks and reviewed with the latest blood count until he/she reaches complete hematologic remission and is subsequently reviewed every 3 months. BCR-ABL1 transcript levels are done at baseline, at 3 months, at 6 months, at 12 months, at 18 months and yearly thereafter.

The investigator screened files of patients who met the eligibility 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 patients' files and extracted data on disease and treatment history, socio-demographic, clinical, hematological characteristics and molecular response status using a pre-designed data extraction sheet. The figure below shows the algorithm of the study procedure.

```
┌─────────────────────────┐
│ CML patient seen by the │
│ principal investigator  │
│ at the                  │
└─────────────────────────┘
            │
            ▼
┌─────────────────────────┐                    ┌──────┐
│ Patient eligible for    │───────────────────▶│  No  │
│ the study               │                    └──────┘
└─────────────────────────┘                        │
            │                                       │
         ┌──────┐                                   │
         │ Yes  │                                   │
         └──────┘                                   │
            │                                       │
            ▼              ┌──────┐                 ▼
┌─────────────────────────┐│  No  │     ┌──────────────────────────┐
│ Informed consent to     ││      │────▶│ Patient excluded from    │
│ participate in the      │└──────┘     │ the study                │
│ study obtained          │             └──────────────────────────┘
└─────────────────────────┘
            │
         ┌──────┐
         │ Yes  │
         └──────┘
            │
            ▼
┌─────────────────────────┐
│ Patient enrolled in the │
│ study                   │
└─────────────────────────┘
            │
            ▼
┌──────────────────────────────────────────────────────────────────┐
│ Patient interviewed, targeted physical examination done, file     │
│ perused to extract data on clinical, socio-demographic            │
│ characteristics, CML disease and treatment history, baseline      │
│ and serial full blood counts and BCR-ABL1 transcript levels at    │
│ baseline and after 18 months of imatinib therapy or time of       │
│ recruitment.                                                      │
└──────────────────────────────────────────────────────────────────┘
```

Figure 4: Schema showing the study procedure

3.8.2 Clinical methods

Once informed consent is given, the investigator entered demographic and clinical data into a pre-designed data sheet outlined in appendix VI. Medical history was taken and full examination with targeted abdominal examination was done.

Demographic data included nationality, gender, age, level of education, marital status smoking history and employment status. Chronic myeloid leukemia disease and treatment history variables included presenting symptoms at enrolment, comorbidities, disease phase, EUTOS score at diagnosis, time to initiation of imatinib, prior treatment, duration of prior CML therapy, duration on imatinib therapy,

adherence to imatinib and any change in dose of imatinib. Response to treatment with imatinib status and evidence of treatment failure was extracted.

3.8.3 Laboratory methods

Quantitative real-time PCR for BCR-ABL1 was done on blood samples of patients who had not done the test at the time of recruitment. For quality assurance purposes, the tests were done in one reference laboratory using standard operating procedures with adequate controls (Appendix I).

3.8.4 Outcome variables

The independent study variables included: age, gender, disease phase, European Treatment and Outcome Study (EUTOS) prognostic score, comorbidities, duration of illness, prior therapy, duration of any prior therapy, adherence to imatinib and duration of imatinib therapy. The dependent variable was the treatment response status.

3.9 Data management

3.9.1 Data collection

Data was collected on a data entry form and later transferred to a computer data base. Each entry form had a unique identifier which was the participant number. Data collected included socio-demographic characteristics, clinical characteristics and CML treatment history of each participant and treatment response status. Data was dually entered into Epidata software and validated.

3.9.2 Data analysis

Descriptive summary statistics such as median and the corresponding interquartile range (IQR) were used to summarize continuous variables such as age and duration. Frequencies and the corresponding percentages were used to summarize categorical variables such as gender, level of education, and marital status among others.

Categorical variables such as gender, missed dose of treatment, and disease phase among others were compared with the treatment response status using Pearson's Chi Square test.

Association between continuous variables and the outcome (treatment response) was assessed using Wilcoxon rank sum test. The factors associated with treatment response were studied using binary logistic regression. Odds ratios (OR) and the corresponding 95% confidence intervals (95% CI) were reported. Results were presented using tables and graphs. STATA version 13 SE was used to analyze data.

3.10 Ethical considerations

Approval was obtained from University of Nairobi/Kenyatta National Hospital Ethics and Research Committee and permission was obtained from Nairobi Hospital before the study commenced. All the study participants gave informed consent. Participants were informed about the discomfort which may be experienced while drawing blood. They were also told that the procedures and tests are not associated with any significant risks. Participants were not given any inducements. All the information obtained was handled confidentially. Only patient codes were used in the data entry form and no reference to their names was made.

CHAPTER FOUR: RESULTS

4.1 Patient enrolment

Nine hundred and sixty seven chronic myeloid leukemia patients were screened at the GIPAP clinic in Nairobi Hospital between May 2018 and August 2018 of whom 215 participants were enrolled into the study (Figure 5).
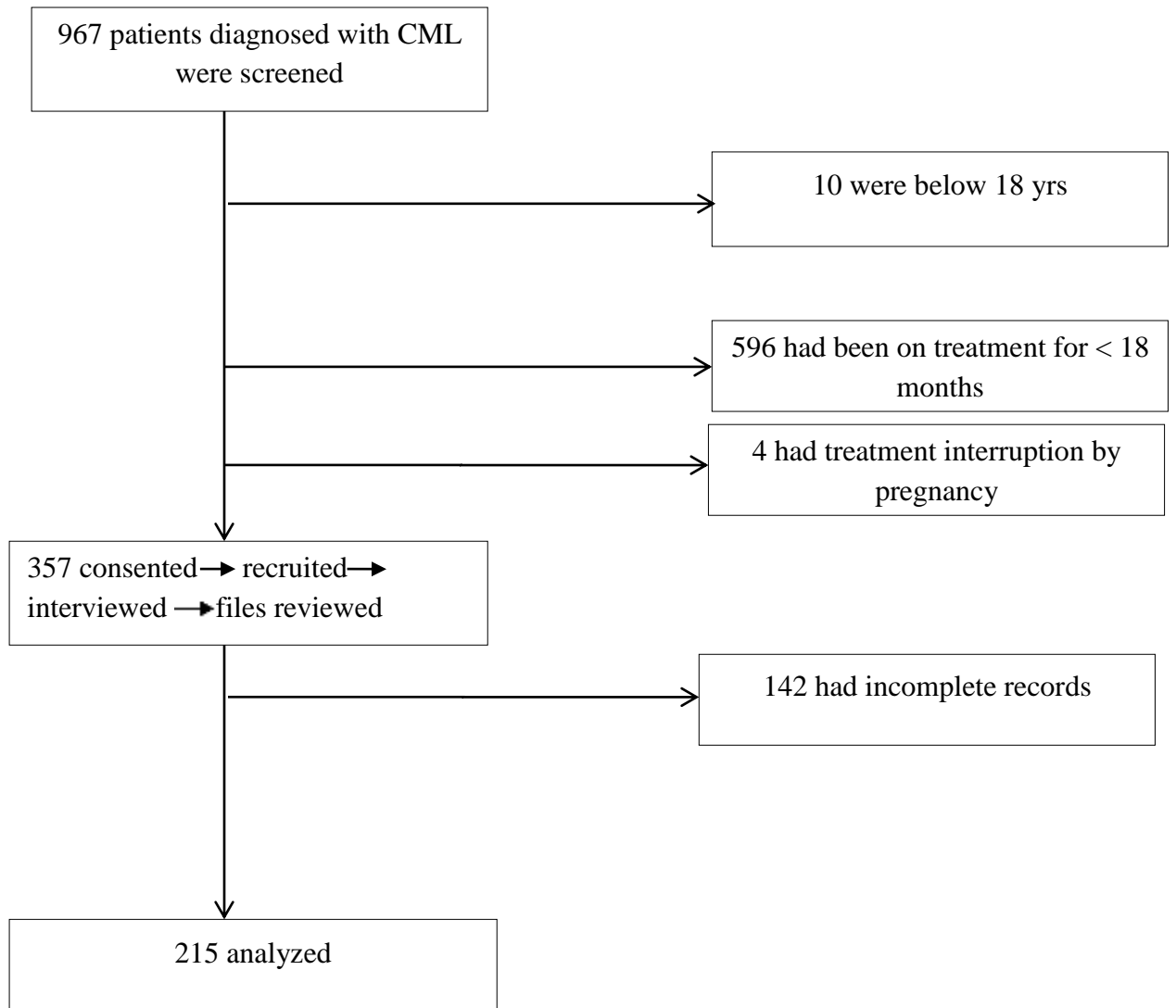
Figure 5: Schema showing patient screening and enrolment

4.2 Socio-demographic characteristics

Two hundred and fifteen patients aged 18 to 85 years were enrolled. The median age was 45.0 (IQR: 35.0, 56.0) years. Majority (93.5%) of the participants were Kenyans and the rest foreigners.

They constituted 55.8% male participants, and close to one third (31.6%) of the participants had formal employment. Majority (85.1%) had higher than secondary level of education.

Table 4: Socio-demographic characteristics

Variable	Median (IQR) or n (%)
Age (years), Median (IQR)	45 (35, 56)
Range (Min. - Max.)	18 – 85
Nationality, n (%)	
Kenyan	201 (93.5)
Non-Kenyan	14 (6.5)
Gender, n (%)	
Male	120 (55.8)
Female	95 (44.2)
Occupation, n (%)	
Formal employment	68 (31.6)
Self-employed	92 (42.8)
Unemployed	32 (14.9)
Retired	4 (1.9)
Student	19 (8.8)
Level of education, n (%)	
None	4 (1.9)
Primary	27 (12.6)
Secondary	80 (37.2)
Tertiary	103 (47.9)

4.3 Clinical characteristics of the study participants

Up to 88.4% of the participants were symptomatic at diagnosis and majority (97.7%) were in chronic phase of CML. More than a third (35.8%) of the participants had a high EUTOS risk score. The median baseline BCR-ABL/ABL1 was 77% (IQR: 25.0, 88.9) with a minimum and a maximum of 0.38% and 180% respectively.

Only 17.2% of the participants were on other drugs. Less than one fifth (17.2%) of the participants had comorbidities. The findings showed that one tenth (10.7%) of the participants had hypertension and 3.3% had diabetes. HIV and depression were reported in 1.4% and 0.9% of the participants respectively. One participant had sickle cell disease (SCD) (Table 5).

Table 5: Clinical characteristics

Variable	Median (IQR) or n (%)
Symptomatic at diagnosis, n (%)	190 (88.4)
Disease phase at diagnosis, n (%)	
CP	210 (97.7)
AP	5 (2.3)
EUTOS risk score, n (%)	
Low	138 (64.2)
High	77 (35.8)
Baseline BCR-ABL/ABL1 (%), Median (IQR)	57.7 (25, 88.9)
Range (Min. - Max.)	0.38 - 180
Used other drugs, n (%)	37 (17.2)
Comorbidities	
None	178 (82.8)
Hypertension	23 (10.7)
Diabetes	7 (3.3)
HIV	3 (1.4)
Depression	2 (0.9)
Sickle cell disease	1 (0.5)
Hypothyroidism	1 (0.5)
Others	8 (3.7)

Majority (88.4%) of the participants were symptomatic at the time of CML diagnosis. The main presenting clinical features were splenomegaly (45.1%), fatigue (26%), weight loss (14.9%), and abdominal pain (8.4%). Headache, symptoms of anemia and leg swelling were reported by 7%, 5.6% and 3.7% of the study participants respectively. Other presenting clinical features include hepatomegaly, lymphadenopathy, gouty arthritis and diarrhea.

4.4 Hematologic characteristics of the study participants

Leucocytosis, anemia, thrombocytosis, and basophilia were seen in 93%, 38.6%, 35.8% and 76.7% of the participants respectively (Table 6).

Table 6: Hematologic characteristics of the study participants

Variable	n (%)
Leucocytosis, n (%)	200 (93)
Anemia, n (%)	83 (38.6)
Thrombocytosis, n (%)	77 (35.8)
Basophilia, n (%)	165 (76.7)

4.5 Chronic myeloid leukemia disease treatment history of the participants

Majority (83.7%) of the participants had complete hematological remission (CHR) at 3 months and 48.8% of the participants were on prior treatment. Among those who got prior treatment all except one (99%) were on hydroxyurea. One participant had prior treatment with generic imatinib. The duration of prior treatment had a median of 2.0 (IQR: 1.0, 3.0) months with the shortest and the longest reported durations being 0.25 and 48.0 months respectively. The duration from diagnosis to imatinib initiation had a median of 4.0 (IQR: 2.3, 12.1) weeks with the shortest and the longest being 0.0 and 239 weeks respectively.

The median duration of follow up for the participants was 4.7 (IQR: 3.0, 7.1) years with a minimum and a maximum of 1.5 and 12.5 years respectively. The imatinib doses at commencement among the study participants were 400 mg (95.3%), 200 mg (0.5%) and 600 mg (2.3%). The imatinibdose was adjusted in 58.1% of the participants and the major reasons for the dose changes were cytopenias (52.8%) and resistance (40.8%). Majority (84.2%) of the patients were on imatinib of whom 2.8% were on 200 mg, 18.2% were on 300 mg, 64.6% were on 400 mg, 12.2% were on 600 mg and 2.2% were on 800 mg.

More than a half of the participants (54%) missed a dose at some point during the treatment period and 12.1% were on second-line TKI treatment. Among those on second-line treatment, 69.2% were on dasatinib, 15.4% were on nilotinib, 11.5% were on bosutinib and 3.7% were on ponatinib. Eight of the participants were not on TKI treatment of whom 4 were in treatment-free remission (TFR), 2 were on drug holiday due to severe cytopenias and 2 were on palliative hydroxyurea because they couldn't afford second-line TKIs (Table 7).

Table 7: Chronic myeloid leukemia disease treatment history of the participants

Variable	Median or n (%)
Complete hematological remission (CHR) at 3 months	180 (83.7)
Was on prior treatment	105 (48.8)
Prior treatment:	
Generic imatinib	1 (1)
Hydroxyurea	104 (99)
Duration of prior treatment (months), Median (IQR)	2 (1, 3)
Range (Min. – Max.)	0.25 – 48
Time from diagnosis to initiation of imatinib (weeks):	
Median (IQR)	4.9 (2.3, 12.1)
Range (Min. - Max.)	(0 - 239.4)
Duration of follow up (years), Median (IQR)	4.7 (3.0, 7.1)
Range (Min. - Max.)	1.1 - 12.5
Imatinib dose at commencement	
200 mg	1 (0.5)
400 mg	205 (95.3)
600 mg	5 (2.3)
Had imatinib dose adjustments	125 (58.1)
Reasons for dose change	
Resistance	51 (40.8)
Cytopenias	66 (52.8)
GI intolerance	6 (4.8)
Good response	2 (1.6)
Currently on imatinib	181 (84.2)
Current imatinib dose	
200 mg	5 (2.8)
300 mg	33 (18.2)
400 mg	117 (64.6)
600 mg	22 (12.2)
800 mg	4 (2.2)
Missed dose	
Yes	116 (54)
No	99 (46)
On second-line TKI:	26 (12.1)
Bosutinib	3 (11.5)
Dasatinib	18 (69.2)
Ponatinib	1 (3.8)
Nilotinib	4 (15.4)
Not on TKI	8 (3.7)
In treatment-free remission (TFR)	4
On drug holiday due to severe cytopenias	2
On palliative hydroxyurea	2

4.6 Treatment response among the study participants

One third of the participants (34%) had optimal treatment response status, 46% had suboptimal response and one fifth (20%) had treatment failure (Figure 6). Among the participants with optimal response, 4 were in treatment-free remission (TFR), 4 had complete molecular response (CMR), 1 had deep molecular response (DMR) and 65 had major molecular response (MMR).
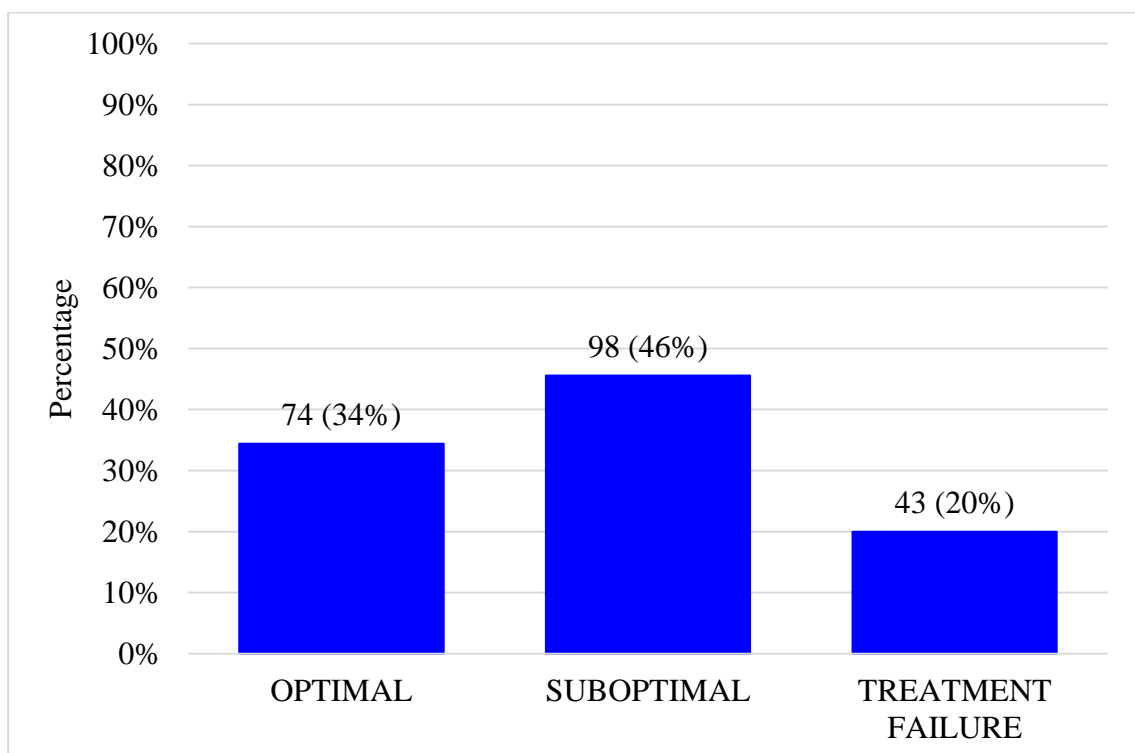
Figure 6: Bar graph showing the treatment response among the study participants

4.7 Comparison for factors associated with response to imatinib therapy.

There was no evidence of association between age and treatment response (p=0.959) as well as between gender and treatment response (0.285). The disease phase was not associated with treatment response (p = 0.492). Similarly, the baseline BCR-ABL/ABL1 transcript level did not demonstrate evidence of an association with the

treatment response (p = 0.597). However, there was a strong evidence of association between EUTOS risk score and treatment response. The results show that the participants who had high EUTOS risk score were less likely to have optimal response, 24.3% vs. 41.8%, p = 0.011.

Delay from diagnosis to imatinib initiation, duration of prior treatment, and the duration on imatinib were not associated with response to treatment (p>0.05).

Current dose of imatinib was not associated with response to treatment (p=0.847).

Participants who missed a dose were less likely to have optimal treatment response, 25.7% vs. 68.8%, p <0.001.

Use of other drugs and achieving CHR at three months were not associated with treatment response (p>0.05). Participants who were on prior treatment were less likely to attain an optimal treatment response, 35.1% vs. 56.0%, p < 0.001 (Table 8).

Table 8: Comparison for factors associated with response to imatinib therapy

Variable	Treatment response		P-value	Statistical Test
	Suboptimal/Treatment failure 141 (65.6%)	Optimal response 74 (34.4%)		
Age (years), median (IQR)	44.0 (35.0, 56.0)	45.5 (34.0, 56.0)	0.959	Wilcoxon ranksum test
Gender, n (%)				
Male	75 (53.2%)	45 (60.8%)		
Female	66 (46.8%)	29 (39.2%)	0.285	Chi Square
Disease phase				
AP	4 (2.8%)	1 (1.4%)		
CP	137 (97.2%)	73 (98.7%)	0.492	Chi Square
Baseline BCR-ABL/ABL1 (%), median (IQR)	61 (25, 90)	54 (25, 85)	0.597	Wilcoxon ranksum test
EUTOS risk score, n (%)				
High	59 (41.8%)	18 (24.3%)		
Low	82 (58.2%)	56 (75.7%)	0.011	Chi Square
Duration from diagnosis to imatinib initiation (months), median (IQR)	1.3 (0.5, 3.1)	1.0 (0.5, 2.3)	0.194	Wilcoxon ranksum test
On prior treatment				
Yes	79 (56.0%)	26 (35.1%)		
No	62 (44.0%)	48 (64.9%)	<0.001	Chi Square
Duration of prior treatment (months), median (IQR)	2.0 (1.0, 4.0)	1.0 (1.0, 3.0)	0.288	Wilcoxon ranksum test
Current imanitib dose, n (%)				
<400 mg	23 (20.5%)	15 (21.7%)		
≥400 mg	89 (79.5%)	54 (78.3%)	0.847	Chi Square
Missed dose				
Yes	97 (68.8%)	19 (25.7%)		
No	44 (31.2%)	55 (74.3%)	<0.001	Chi Square
Use of other drugs				
Yes	120 (85.1%)	58 (78.4%)		
No	21 (14.9%)	16 (21.6%)	0.214	Chi Square
CHR at 3 months				
Yes	114 (80.9%)	66 (89.2%)		
No	27 (19.2%)	8 (10.8%)	0.116	Chi Square

Adjusted for age, gender, baseline BCR-ABL/ABL1 and duration from diagnosis to start of imatinib, high EUTOS risk score was associated with up to 57 % reduced odds of attaining optimal treatment response, OR: 0.43 (95 % CI: 0.21, 0.88); missing a dose was associated with up to 83 % reduced odds of attaining optimal treatment response, OR: 0.17 (95% CI: 0.09, 0.32) and prior treatment with hydroxyurea was associated with up to 53% reduced odds of attaining optimal treatment response, OR: 0.47 (95% CI: 0.24, 0.92) (Table 9).

Table 9: Independent predictors of treatment response on logistic regression

Variable	Unadjusted OR (95 % CI)	Adjusted OR (95 % CI)
EUTOS risk score		
Low	Reference	Reference
High	0.45 (0.24, 0.84)	**0.43 (0.21, 0.88)**
Missed dose		
No	Reference	Reference
Yes	0.16 (0.08, 0.29)	**0.17 (0.09, 0.32)**
On prior treatment		
No	Reference	Reference
Yes	0.43 (0.24, 0.76)	**0.47 (0.24, 0.92)**
Age (years)	1.00 (0.98, 1.02)	0.99 (0.97, 1.01)
Gender		
Female	Reference	Reference
Male	1.37 (0.77, 2.42)	1.48(0.77, 2.84)
Baseline BCR-ABL/ABL1 (%)	1.01 (0.98, 1.05)	1.01 (0.96, 1.06)
Duration from diagnosis to imatinib initiation (months)	0.96 (0.90, 1.01)	0.99 (0.94, 1.05)

CHAPTER FIVE: DISCUSSION

In this study, 215 patients above 18 years with CML regardless of the phase were studied. The median age at diagnosis was 45 years with a range of 18 to 85 years. This is similar to a median age of 40 years found by Yanmin Zhao et al in a study done in a Chinese population(81) and also 40 years found in African patients having CML in a Cote d'Ivore study(82). Out of 215 studied patients; 120 (55.8%) were males giving a male to female ratio of 1.26:1 in keeping with slight male predominance noted in other studies(81).

Unemployment was found in 55 (25.6%) of the participants, these patients were therefore dependent on other people for their provisions such as meeting the costs for serial full blood counts, molecular tests and travelling to the clinic. Two hundred and one (93.5%) of the participants were Kenyans versus 14(6.5%) foreigners. This is simply because the GIPAP clinic is located in Kenya's capital city. Majority of the foreigners were refugees from the troubled neighbouring Somalia and Southern Sudan. Majority of the participants 183 (85.1%) had at least secondary level of education and higher meaning that they had fair understanding of their illness. Majority of our patients 190 (88.4%) were symptomatic at diagnosis with just a little over 11% being asymptomatic unlike the finding of about 40% of CML patients being asymptomatic in developed countries(18). This suggests that our patients mostly present late in the course of the disease when it is most likely to be symptomatic or that the disease in our population has a tendency to early onset of symptoms. Chronic myeloid leukemia among Africans seems more aggressive and some of the possible contributing factors are additional cytogenetic chromosomal abnormalities leading to poor prognosis(82).

Translating symptomatology to imply advanced diseases the 11% diagnosed while asymptomatic and therefore diagnosed following a routine blood count, could imply early patient presentation possibly while attending route 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(7). A possible reason for this is that some patients were already on hydroxyurea before enrolment into the GIPAP programme hence symptoms had resolved. The commonest symptom among our subjects was splenomegaly found in 102 (47.4%) of them; which is comparable to earlier studies(23). Fatigue was reported by 26% of the patients.

In this study, 210 (97.7%) of the patients were in the CP of CML; which is in keeping with usually >80% of CML patients diagnosed in the CP of disease(23)(24)(81). More than one third of our patients 77 (36%) had a high EUTOS prognostic score which is higher than that reported in other studies(81). This suggested a tendency to adverse prognostication among our patient group. A small proportion of the subjects (17%); had other comorbidities and therefore were on other medications which could have interacted with imatinib.

Majority of the patients had leucocytosis at presentation (93%) which is comparable to other studies(81). However, the proportion of patients who had thrombocytosis (36%) and basophilia(77%) were higher as compared to other studies(81). The proportion of patients who had anemia (39%) was comparable to other studies(81).

Majority; 180 (83.7%) of the patients achieved CHR at 3 months. This is comparable to an earlier study in the same patient population which showed a CHR rate of 79.1%(83). Almost a half 105 (48.8%) of the patients had been on hydroxyurea prior

to starting imatinib most of them having used it for a few months as they awaited molecular analysis for BCR-ABL. This is far much lower compared to other studies which found hydroxyurea exposure of up to 74%(81). Only one had used generic imatinib.

Longer durations between diagnosis and start of imatinib were observed and this could be explained by the patients' delay in meeting the cost to do molecular analysis for BCR-ABL (and therefore meeting eligibility for imatinib). The imatinib dose was adjusted in 125 (58.1%) of the patients and the main reasons for dose changes were cytopenias and resistance. The incidence of imatinib-induced cytopenias (31%) found in this study is comparable to that found in other studies (81). Among the participants who were on imatinib, majority (95.3%) were commenced on standard dose which is comparable to other studies(83).

More than a half of the participants (54%) missed a dose at some point during the treatment period. Although adherence to treatment was not measured using an objective tool, using this as a surrogate of adherence; it is low compared to other studies which have reported up to 97% adherence rates (56)(57). The main reasons patients gave for non-compliance to imatinib therapy was financial constraints limiting their travel to the clinic for drug refill and forgetfulness. 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. Medication event monitoring system (MEMS) is the gold standard for assessing adherence(56)(57). However, this option as well as other objective measures of compliance was not feasible in our study due to unavailability, high cost and time consumption.

The standard of care in evaluating treatment response to imatinib is molecular monitoring. Two hundred and five of the patients were commenced on 400mg of imatinib; which is the standard dose recommended for CP-CML.GIPAP approves a starting dose of 300mg for the paediatric group only. At a median clinic follow up of 4.7 years, optimal response as per ELN guidelines was attained in 74(34.4%) of the patients with 43(20%) classified as treatment failure. Ninety eight (45.6%) of the patients had suboptimal response. The optimal response rate found in our study is way below what is reported in developed countries(84), although their median follow-up was 10years, 6years longer compared to our study. Low socio-economic status, poor adherence to imatinib, prior exposure to hydroxyurea, possible inherent genetic differences in imatinib metabolism and poor risk profile of our patients may explain this difference. The failure rate of 20% found in this study is the same as that reported in an earlier study (83).

There was a strong association between EUTOS risk score and treatment response. Patients who had a high EUTOS risk score were less likely to have optimal response; 24.3% vs 41.8% p=0.011. This is in keeping with findings in other studies that a high prognostic risk score predicts a poor response to treatment with imatinib (81). Patients who were on prior treatment with hydroxyurea were less likely to attain an optimal response; 35.1% vs 56 % $p < 0.001$. Other studies have reported similar findings (81). Moreover, patients who had missed a dose were less likely to have optimal treatment response; 25.7% vs 68.8% $p < 0.001$; other studies have reported similar association(56)(57)(81).

Use of other drugs and achieving complete hematologic remission at 3 months was not associated with treatment response; $p > 0.05$. The duration from diagnosis to

imatinib initiation and duration of prior treatment with hydroxyurea were not associated with treatment response (p > 0.05). This is in contrast to other studies which reported association of these factors with treatment response (81)(83).

Study limitations

1. Some of the patients' medical records were incomplete and could not be included in the final analysis.

2. The molecular tests for BCR-ABL/ABL1 transcript levels were done at different times after 18 months of imatinib therapy.

3. Unmeasured confounders like drug non-adherence and interactions between other drugs and imatinib may have biased treatment response.

Study strengths

This study is the first one of this kind, with an ample sample size done amongst CML patients in Kenya. It points out issues that could be explored to improve response to imatinib therapy.

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Our study found an optimal molecular response to imatinib therapy in a third; 74 (34%) of the patients. One fifth (20%) of the patients had treatment failure.

Factors associated with treatment failure were high EUTOS prognostic risk score, non-adherence to imatinib and prior exposure to hydroxyurea.

6.2 Recommendations

Based on these findings, measures should be instituted to improve access and compliance to imatinib treatment among CML patients attending the GIPAP clinic. Imatinib should be the first therapy as soon as CML is diagnosed. Studies should be carried out to explore and seek solutions to non-adherence to imatinib therapy.

REFERENCES

1. Bernards A, Rubin CM, Westbrook CA, Paskind M, Baltimore D. The first intron in the human c-abl gene is at least 200 kilobases long and is a target for translocations in chronic myelogenous leukemia. MolCell Biol. 1987;7(9):3231–6.

2. Hungerford PCNDA. Chromosome studies on normal and leukemic human leukocytes. J Natl Cancer Inst. 1960;25(1):85–109.

3. Redaelli A, Bell C, Casagrande J, Stephens J, Botteman M, Laskin B, et al. Clinical and epidemiologic burden of chronic myelogenous leukemia. Expert Rev Anticancer Ther. 2004;4(1):85–96.

4. Siegel RL, Miller KD, Jemal A. Cancer statistics. CA Cancer J Clin. 2016;66(1):7–30.

5. American Cancer Society. Cancer Facts & Figures 2016. Cancer Facts Fig 2016. 2016;1–9.

6. Othieno-Abinya NA. The epidemiology of cancer in Kenys with special refence to chronic myeloid leukemia. Inst Natl du Cancer, Fr About Cancer Africa. 2006;1:293–300.

7. Othieno-Abinya NA, Nyabola LO, Kiarie GW, Ndege R, Maina JMD. Chronic myeloid leukaemia at the Kenyatta National Hospital, Nairobi. East Afr Med J. 2002;79(11):593–7.

8. M K. Chronic myelogenous leukemia. JAMA. 2001;286(8):895898.

9. Goldman JM, Melo J V. Targeting the BCR-ABL tyrosine kinase in chronic

myeloid leukemia. Vol. 344, The New England journal of medicine. 2001. p. 1084–6.

10. Hanfstein B, Muller MC, Hehlmann R, Erben P, Lauseker M, Fabarius A, et al. Early molecular and cytogenetic response is predictive for long-term progression-free and overall survival in chronic myeloid leukemia (CML). Leukemia. 2012;26(9):2096–102.

11. Jabbour E, Saglio G, Hughes TP, Kantarjian H, et al. Suboptimal Responses in Chronic Myeloid Leukemia. 2012;1181–91.

12. Radich JP. How I monitor residual disease in chronic myeloid leukemia. Blood. 2009;114(16):3376–81.

13. Baccarani M, Deininger MW, Rosti G, Hochhaus A, Soverini S, Apperley JF, et al. Review Article European LeukemiaNet recommendations for the management of chronic myeloid leukemia : 2013. Blood. 2013;122(6):872–84.

14. NCCN. National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology for Chronic Myelogenous Leukemia V.1.2013. 2013;

15. Larson RA, Hochhaus A, Hughes TP, Clark RE, Etienne G, Kim D-W, et al. Nilotinib vs imatinib in patients with newly diagnosed Philadelphia chromosome-positive chronic myeloid leukemia in chronic phase: ENESTnd 3-year follow-up. Leukemia. 2012;26(10):2197–203.

16. Baccarani M, Cortes J, Pane F, Niederwieser D, Saglio G, Apperley J, et al. Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet. J Clin Oncol. 2009;27(35):6041–51. PMC4979100

17. Guilhot J, Baccarani M, Clark RE, Cervantes F, Guilhot F, Hochhaus A, et al. Definitions, methodological and statistical issues for phase 3 clinical trials in chronic myeloid leukemia: A proposal by the European LeukemiaNet. Vol. 119, Blood. 2012. p. 5963–71.

18. Faderl S, Talpaz M, Estrov Z, O'Brien S, Kurzrock R, Kantarjian HM. The biology of chronic myeloid leukemia. N Engl J Med. 1999;341(3):164–72.

19. ROWLEY JD. A New Consistent Chromosomal Abnormality in Chronic Myelogenous Leukaemia identified by Quinacrine Fluorescence and Giemsa Staining. Nature. 1973;243(5405):290–3.

20. Goldman JM, Melo J V. Chronic Myeloid Leukemia — Advances in Biology and New Approaches to Treatment. 2003;1451–64.

21. Silver RT, Woolf SH, Hehlmann R, Appelbaum FR, Anderson J, Bennett C, et al. An evidence-based analysis of the effect of busulfan, hydroxyurea, interferon, and allogeneic bone marrow transplantation in treating the chronic phase of chronic myeloid leukemia. Blood. 1999;94(5):1517–36.

22. Hochhaus A, O'Brien SG, Guilhot F, Druker BJ, Branford S, Foroni L, et al. Six-year follow-up of patients receiving imatinib for the first-line treatment of chronic myeloid leukemia. Leukemia. 2009;23(6):1054–61.

23. Sawyers CL. Chronic Myeloid Leukemia. N Engl J Med. 1999;340(17):1330–40.

24. Redaelli A, Laskin BL, Stephens JM, Botteman MF, Pashos CL. The clinical and epidemiological burden of chronic lymphocytic leukaemia. Vol. 13, European Journal of Cancer Care. 2004. p. 279–87.

25. Quintás-Cardama A, Kantarjian HM, Cortes JE. Mechanisms of primary and secondary resistance to imatinib in chronic myeloid leukemia. Vol. 16, Cancer Control. 2009. p. 122–31.

26. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Vol. 127, Blood. 2016. p. 2391–405.

27. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2016 update on diagnosis, therapy, and monitoring. Am J Hematol. 2016;91(2):252–65.

28. Hochhaus A. Prognostic factors in chronic myeloid leukemia (CML). Onkologie. 2008;31(11):576–8.

29. Kantarjian HM, Smith TL, McCredie KB, Keating MJ, Walters RS, Talpaz M, et al. Chronic myelogenous leukemia: a multivariate analysis of the associations of patient characteristics and therapy with survival. Blood. 1985;66(6):1326–35.

30. Hasford J, Pfirrmann M, Hehlmann R, Allan NC, Baccarani M, Kluin-Nelemans JC, et al. A new prognostic score for survival of patients with chronic myeloid leukemia treated with interferon alfa. Writing Committee for the Collaborative CML Prognostic Factors Project Group. J Natl Cancer Inst. 1998;90(11):850–8.

31. Sokal JE. Evaluation of survival data for chronic myelocytic leukemia. Am J Hematol. 1976;1(4):493–500.

32. Uz B, Buyukasik Y, Atay H, Kelkitli E, Turgut M, Bektas O, et al. EUTOS CML prognostic scoring system predicts ELN-based "event-free survival"

better than Euro/Hasford and Sokal systems in CML patients receiving front-line imatinib mesylate. Hematology. 2013;18(5):247–52.

33. Hughes TP, Saglio G, Kantarjian HM, Guilhot F, Niederwieser D, Rosti G, et al. Early molecular response predicts outcomes in patients with chronic myeloid leukemia in chronic phase treated with frontline nilotinib or imatinib. Blood. 2014;123(9):1353–60.

34. Hanfstein B, Muller MC, Hochhaus A. Response-related predictors of survival in CML. Vol. 94, Annals of Hematology. 2015. p. 227–39.

35. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, et al. Imatinib Compared with Interferon and Low-Dose Cytarabine for Newly Diagnosed Chronic-Phase Chronic Myeloid Leukemia. N Engl J Med. 2003;348(11):994–1004.

36. Cortes JE, Baccarani M, Guilhot F, Druker BJ, Branford S, Kim DW, et al. Phase III, randomized, open-label study of daily imatinib mesylate 400 mg versus 800 mg in patients with newly diagnosed, previously untreated chronic myeloid leukemia in chronic phase using molecular end points: Tyrosine kinase inhibitor optimization and. J Clin Oncol. 2010;28(3):424–30.

37. Preudhomme C, Guilhot J, Nicolini FE, Guerci-Bresler A, Rigal-Huguet F, Maloisel F, et al. Imatinib plus Peginterferon Alfa-2a in Chronic Myeloid Leukemia. N Engl J Med. 2010;363(26):2511–21.

38. Tokarski JS, Newitt JA, Chang CYJ, Cheng JD, Wittekind M, Kiefer SE, et al. The structure of dasatinib (BMS-354825) bound to activated ABL kinase domain elucidates its inhibitory activity against imatinib-resistant ABL

mutants. Cancer Res. 2006;66(11):5790–7.

39. Kantarjian H, Shah NP, Hochhaus A, Cortes J, Shah S, Ayala M, et al. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2010;362(24):2260–70.

40. Cortes JE, Saglio G, Kantarjian HM, Baccarani M, Mayer J, Boqué C, et al. Final 5-year study results of DASISION: The dasatinib versus imatinib study in treatment-Naïve chronic myeloid leukemia patients trial. J Clin Oncol. 2016;34(20):2333–40.

41. Weisberg E, Manley PW, Breitenstein W, Brüggen J, Cowan-Jacob SW, Ray A, et al. Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. Cancer Cell. 2005;7(2):129–41.

42. Saglio G, Kim D-W, Issaragrisil S, le Coutre P, Etienne G, Lobo C, et al. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med. 2010;362(24):2251–9.

43. Larson RA, Kim D-W, Jootar S, Pasquini R, Clark RE, Lobo C, et al. ENESTnd 5-year (y) update: Long-term outcomes of patients (pts) with chronic myeloid leukemia in chronic phase (CML-CP) treated with frontline nilotinib (NIL) versus imatinib (IM). J Clin Oncol. 2014;32(5s):Abstr 7073.

44. Kantarjian H, Cortes J. Considerations in the management of patients with philadelphia chromosome-positive chronic myeloid leukemia receiving tyrosine kinase inhibitor therapy. Vol. 29, Journal of Clinical Oncology. 2011. p. 1512–6.

45. Marin D, Ibrahim AR, Lucas C, Gerrard G, Wang L, Szydlo RM, et al.

Assessment of BCR-ABL1 transcript levels at 3 months is the only requirement for predicting outcome for patients with chronic myeloid leukemia treated with tyrosine kinase inhibitors. J Clin Oncol. 2012;30(3):232–8.

46. Jain P, Kantarjian H, Nazha A, O'Brien S, Jabbour E, Romo CG, et al. Early responses predict better outcomes in patients with newly diagnosed chronic myeloid leukemia: Results with four tyrosine kinase inhibitor modalities. Blood. 2013;121(24):4867–74.

47. Jabbour E, Kantarjian HM, O'Brien S, Shan J, Quintás-Cardama A, Garcia-Manero G, et al. Front-line therapy with second-generation tyrosine kinase inhibitors in patients with early chronic phase chronic myeloid leukemia: what is the optimal response? J Clin Oncol. 2011;29(32):4260–5.

48. Jain P, Kantarjian H, Patel KP, Nogueras Gonzalez G, Luthra R, Kanagal Shamanna R, et al. Impact of BCR-ABL transcript type on response and survival in patients with chronic phase chronic myeloid leukemia treated with tyrosine kinase inhibitors. Blood. 2016;127(36):1269–76.

49. Roberts N, Yeung DT, FRCPA, Altamura H, Parker WT, Hughes TP. Any BCR-ABL Reduction Below 10% At 6 Months Of Therapy Significantly Improves Outcome For CML Patients With a Poor Response At 3 Months. Blood. 2013;122(21):254.

50. Nazha A, Kantarjian H, Jain P, Romo C, Jabbour E, Quintas-Cardama A, et al. Assessment at 6 months may be warranted for patients with chronic myeloid leukemia with no major cytogenetic response at 3 months. Haematologica. 2013;98(11):1686–8.

51. Zhang WW, Cortes JE, Yao H, Zhang L, Reddy NG, Jabbour E, et al. Predictors of primary imatinib resistance in chronic myelogenous leukemia are distinct from those in secondary imatinib resistance. J Clin Oncol. 2009;27(22):3642–9.

52. Mauro MJ. Defining and managing imatinib resistance. Hematology Am Soc Hematol Educ Program. 2006;219–25.

53. Ramirez P, DiPersio JF. Therapy options in imatinib failures. Oncologist. 2008;13(4):424–34.

54. Litzow MR. Imatinib resistance: Obstacles and opportunities. In: Archives of Pathology and Laboratory Medicine. 2006. p. 669–79.

55. Khorashad JS, De Lavallade H, Apperley JF, Milojkovic D, Reid AG, Bua M, et al. Finding of kinase domain mutations in patients with chronic phase chronic myeloid leukemia responding to imatinib may identify those at high risk of disease progression. J Clin Oncol. 2008;26(29).

56. Marin D, Bazeos A, Mahon FX, Eliasson L, Milojkovic D, Bua M, et al. Adherence is the critical factor for achieving molecular responses in patients with chronic myeloid leukemia who achieve complete cytogenetic responses on imatinib. J Clin Oncol. 2010;28(14):2381–8.

57. Noens L, van Lierde M-A, De Bock R, Verhoef G, Zachée P, Berneman Z, et al. Prevalence, determinants, and outcomes of nonadherence to imatinib therapy in patients with chronic myeloid leukemia: the ADAGIO study. Blood. 2009;113(22):5401–11.

58. Jabbour E, Kantarjian HM, Jones D, Shan J, O'Brien S, Reddy N, et al. Imatinib mesylate dose escalation is associated with durable responses in patients with chronic myeloid leukemia after cytogenetic failure on standard-dose imatinib therapy. Blood. 2009;113(10):2154–60.

59. Kantarjian H, Pasquini R, Lévy V, Jootar S, Holowiecki J, Hamerschlak N, et al. Dasatinib or high-dose imatinib for chronic-phase chronic myeloid leukemia resistant to imatinib at a dose of 400 to 600 milligrams daily: Two-year follow-up of a randomized phase 2 study (START-R). Cancer. 2009;115(18):4136–47.

60. Kantarjian HM, Baccarani M, Jabbour E, Saglio G, Cortes JE. Second-generation tyrosine kinase inhibitors: The future of frontline CML therapy. Vol. 17, Clinical Cancer Research. 2011. p. 1674–83.

61. H.G. G, S.Y. C, D. K, J. L, E.J. J, J.H. B, et al. Efficacy of nilotinib versus high-dose imatinib in early CP chronic myeloid leukemia patients who have suboptimal molecular responses to standard-dose imatinib (re-nice multicencenter study). Vol. 96, Haematologica. 2011. p. 292–3.

62. Yeung DT, Osborn MP, White DL, Branford S, Braley J, Herschtal A, et al. TIDEL-Ii: First-line use of imatinib in CML with early switch to nilotinib for failure to achieve time-dependent molecular targets. Blood. 2015;125(6):915–23.

63. O'Hare T, Shakespeare WC, Zhu X, Eide CA, Rivera VM, Wang F, et al. AP24534, a Pan-BCR-ABL Inhibitor for Chronic Myeloid Leukemia, Potently Inhibits the T315I Mutant and Overcomes Mutation-Based Resistance. Cancer Cell. 2009;16(5):401–12.

64.   Zhou T, Commodore L, Huang WS, Wang Y, Thomas M, Keats J, et al. Structural Mechanism of the Pan-BCR-ABL Inhibitor Ponatinib (AP24534): Lessons for Overcoming Kinase Inhibitor Resistance. Chem Biol Drug Des. 2011;77(1):1–11.

65.   Cortes J, Jabbour E, Kantarjian H, Yin CC, Shan J, O'Brien S, et al. Dynamics of BCR-ABL kinase domain mutations in chronic myeloid leukemia after sequential treatment with multiple tyrosine kinase inhibitors. Blood. 2007;110(12):4005–11.

66.   Jabbour E, Branford S, Saglio G, Jones D, Cortes JE, Kantarjian HM. Practical advice for determining the role of BCR-ABL mutations in guiding tyrosine kinase inhibitor therapy in patients with chronic myeloid leukemia. Vol. 117, Cancer. 2011. p. 1800–11.

67.   Müller MC, Cortes JE, Kim DW, Druker BJ, Erben P, Pasquini R, et al. Dasatinib treatment of chronic-phase chronic myeloid leukemia: Analysis of responses according to preexisting BCR-ABL mutations. Blood. 2009;114(24):4944–53.

68.   Hughes T, Saglio G, Branford S, Soverini S, Kim DW, Müller MC, et al. Impact of baseline BCR-ABL mutations on response to nilotinib in patients with chronic myeloid leukemia in chronic phase. J Clin Oncol. 2009;27(25):4204–10.

69.   Jabbour E, Cortes J, Santos FPS, Jones D, O'Brien S, Rondon G, et al. Results of allogeneic hematopoietic stem cell transplantation for chronic myelogenous leukemia patients who failed tyrosine kinase inhibitors after developing BCR-

ABL1 kinase domain mutations. Blood. 2011;117(13):3641–7.

70.   Lee SJ, Kukreja M, Wang T, Giralt SA, Szer J, Arora M, et al. Impact of prior imatinib mesylate on the outcome of hematopoietic cell transplantation for chronic myeloid leukemia. Blood. 2008;112(8):3500–7.

71.   Mahon FX, Réa D, Guilhot J, Guilhot F, Huguet F, Nicolini F, et al. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. Lancet Oncol. 2010;11(11):1029–35.

72.   Mahon F-X, Rea D, Guilhot J, Guilhot F, Huguet F, Nicolini FE, et al. Discontinuation of Imatinib in Patients with Chronic Myeloid Leukemia Who Have Maintained Complete Molecular Response: Update Results of the STIM Study. ASH Annu Meet Abstr. 2011;118(21):603.

73.   Ross DM, Branford S, Seymour JF, Schwarer AP, Arthur C, Yeung DT, et al. Safety and efficacy of imatinib cessation for CML patients with stable undetectable minimal residual disease: Results from the TWISTER study. Blood. 2013;122(4):515–22.

74.   Nicolini FE, Noël M-P, Escoffre M, Charbonnier A, Rea D, Dubruille V, et al. Preliminary Report Of The STIM2 Study: A Multicenter Stop Imatinib Trial For Chronic Phase Chronic Myeloid Leukemia De Novo Patients On Imatinib. Blood. 2013;122(21):654.

75.   Rousselot P, Charbonnier A, Cony-Makhoul P, Agape P, Nicolini FE, Varet B, et al. Loss of major molecular response as a trigger for restarting tyrosine

kinase inhibitor therapy in patients with chronic-phase chronic myelogenous leukemia who have stopped imatinib after durable undetectable disease. J Clin Oncol. 2014;32(5):424–30.

76.   Mahon F-X, Richter J, Guilhot J, Muller MC, Dietz C, Porkka K, et al. Interim Analysis of a Pan European Stop Tyrosine Kinase Inhibitor Trial in Chronic Myeloid Leukemia : The EURO-SKI study. Blood. 2014;124(21):151.

77.   Oehler VG, Gooley T, Snyder DS, Johnston L, Lin A, Cummings CC, et al. The effects of imatinib mesylate treatment before allogeneic transplantation for chronic myeloid leukemia. Blood. 2007;109(4):1782–9.

78.   Jabbour E, Cortes J, Kantarjian H, Giralt S, Andersson BS, Giles F, et al. Novel tyrosine kinase inhibitor therapy before allogeneic stem cell transplantation in patients with chronic myeloid leukemia: no evidence for increased transplant-related toxicity. Cancer. 2007;110(2):340–4.

79.   Ohanian M, Kantarjian HM, Quintas-Cardama A, Jabbour E, Abruzzo L, Verstovsek S, et al. Tyrosine kinase inhibitors as initial therapy for patients with chronic myeloid leukemia in accelerated phase. Clin Lymphoma Myeloma Leuk. 2014;14(2):155–162.e1.

80.   Expert BCR-ABL Ultra Kit GXBCR-CE-10.

81.   Zhao Y, Liu L, Wang Y, Wu G, Lai X, Cao W, et al. Efficacy and prognosis of chronic myeloid leukemia treated with imatinibmesylate in a Chinese population. Int. J Hematol. 2009; 89 (10): 445-451.

82. Koffi KG, Nanho DC, N'athz E, KouetrionP,Dissika R, Attia A, et al. The effect of imatinibmesylate for newly diagnosed Philadelphia chromosome-positive chronic-phase myeloid leukemia in Sub-Saharan Africa patients: The experience of Cote d' Vore.Advances in Hematology.2010; 1-6.

83. Dindi E, Othieno-Abinya N.A. Treatment response to imatinib among chronic myeloid leukemia patients as seen in Nairobi. Unpublished. 2013.

84. Hochhaus A, Larson RA, Guilhot F, Radich JP, Branford SB, Hughes TP, et al. Long-term outcomes of imatinibmesylate treatment for chronic myeloid leukemia. NEJM. 2017; 376 (10): 917-27.

APPENDICES

Appendix I: Quantitative RT-PCR for BCR-ABL transcripts.

<u>**Cepheid Xpert BCR-ABL Ultra Kit (Standard operating procedure)**</u>(80)

<u>Purpose</u>

The XpertBCR-ABL ultra is an automated test for quantifying the amount of BCR-ABL transcript as a ratio of BCR-ABL/ABL. The assay is performed on Cepheid Gene Xpert Instrument System automate and integrate sample purification, nucleic acid amplification, target sequence detection in simple or complex samples using real-time RT-PCR and nested PCR assays. The systems require the use of single-use disposable Gene Xpert cartridge that hold the RT-PCR and nest PCR reagents and host the RT-PCR and nest PCR processes.

The Xpert BCR-ABL Ultra includes reagents to detect BCR-ABL fusion gene resulting from two major breakpoints, translocation e13a2 (b2a2) e14a2 and ABL transcript as an endogenous control in peripheral blood specimens. The amount of BCR-ABL transcript is quantified as the ratio of BCR-ABL/ABL.

CML is part of a group of diseases called myeloproliferative neoplasms. More than 95% of CML patients have the distinctive Philadelphia chromosome that results from a reciprocal translocation between the long arms of chromosomes 9 and 22. The translocation involves the transfer of the Abelson (ABL) gene on chromosome 9 to the breakpoint cluster region (BCR) of chromosome 22, resulting in a fused BCR-ABL gene. The fusion gene produces a protein p210 (b2a2 and b3a2), a tyrosine kinase with deregulated activity that plays a key role in the development of CML.

Besides an endogenous control (ABL), a Probe Check Control (PCC) is also included in the cartridge. The endogenous control normalizes the BCR-ABL target and ensures

that sufficient sample is used in the assay. PCC verifies reagent rehydration, PCR tube filling the cartridge, probe integrity and dye stability.

<u>Scope</u>

The procedures for the automated extraction, amplification, detection and quantification of BCR/ABL translocations.

<u>Procedure</u>

i. <u>Sample collection and storage</u>

The sample may be run only on 4ml of peripheral blood collected inEDTA tubes. Blood samples may be stored at 2-8^0 C. Assay must be run within 72 hours of collection. Blood samples older than 72 hours must not be processed.

ii. <u>Specimens</u>

Before processing the specimen, the white blood cell count must be checked. In some instances the WBC count will be available on Meditech. If no WBC count has been performed, the technologist performing the test must take the sample to the hematology laboratory and request that a WBC count be run immediately.

iii. <u>Procedure for sample preparation</u>

Reagents and cartridges are packed in single use packs. When required, remove a single pack from the fridge 20 minutes before starting the procedure to make them to come to room temperature. Briefly spin down Proteinase K (PK) in a microcentrifuge.

- To the bottom of a new 50ml conical tube, add 100µl of Proteinase K.
- Ensure blood specimen is well-mixed by inverting the blood collection tube 8 times immediately before pipetting.
- Add 4ml of EDTA blood to the tube already containing Proteinase K.
- Mix the sample with a vortex mixer at maximum setting continuously for 3 seconds.

- Incubate at room temperature for 1 minute. To the same tube add 2.5ml of Lysis Reagent (Ly).

- Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds.

- Incubate at room temperature for 10 minutes.

- To the same conical tube, add 2ml of reagent grade absolute ethanol.

- Mix the sample with a vortex mixer at maximum setting continuously for 10 seconds. Set it at room temperature.

- Transfer the entire contents to the cartridge's sample chamber.

- Discard any remaining PK and Ly reagents.

iv. <u>Preparing the cartridge</u>

- Remove the cartridge from cardboard packaging. Inspect for any damage

- Open the cartridge lid and transfer the entire contents of washReagent;1 ampoule to the wash R   eagent chamber (small opening).

- Pipette the entire contents of the prepared sample into the sample chamber (large opening).

- Close the cartridge lid. Ensure the lid snaps firmly into place.

v. <u>Loading the GeneXpert and running the assay.</u>

- Switch on software and log in appropriately. Log is on the front of the platform in the Virology Laboratory. Create test-enter the sample ID and select the appropriate assay-Xpert BCR-ABL Ultra auto calc. Place the loaded cartridge in the GeneXpert module as specified by the software. Select Scan barcode and scan the barcode on the cartridge.

- Press start test. When the green light on the loaded module begins flashing, close the module door and hold closed for about 5 seconds.

- Additional tests may be loaded simultaneously into additional modules.

vi. Calculation of results

- Xpert BCR-ABL results are reported on the International Scale (IS). Determination and validation of an assay-specific conversion factor has been completed in the IRIS study.

- The test amplifies both the normal ABL gene and the BCR-ABL gene if it is present. The software then calculates the end points for both ABL and BCR-ABL and employs an efficiency value which is lit lot number-specific and a conversion factor, to calculate the BCR-ABL percentage.

- Result reporting will now include IS (International Scale)

- International Scale (IS) is a globally recognized standardization of BCR-ABL values and reporting BCR-ABL in IS is accepted as a global standard.

- The calculation is as follows: **% IS ratio BCR-ABL/ABL=$E_{DCT}{}^{DCT}$ x100 x 0.47**

vii. Manual resulting on Meditech

- Results are manually entered into Meditech system and will be signed out by senior staff or Molecular Pathology in that department

- Meditech calculates the BCR-ABL ratio and the BCR/ABL comment is added automatically by Meditech and is a retrospective archive search for previous results on the patient.

Appendix II: Study explanation (English version)

Study title

Evaluation of response to imatinib therapy among chronic myeloid leukaemia patients as seen in Nairobi; Kenya

Introduction

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

I wish to enroll you in my study, which will involve a brief interview, physical examination, perusing through your treatment records and a blood test to determine the level of CML treatment response. Should you choose not to participate, you will still receive all your treatment benefits.

Perceived benefits

The results obtained from this study will enable provide information that could help in improving clinical decision making for you and other patients diagnosed with the same illness as far as dose adjustment of imatinib or switching to other drugs is concerned.

Risks

There are no anticipated risks for participating in this 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 III: Consent form (English version)

<u>Voluntary consent</u>

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 do consent to participate in the study.

Signature of participant…………………………………Date…………………

<u>Investigator's statement</u>

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 in this consent form.

Signature of investigator…………………………………………Date………………

Appendix IV: Study explanation (Kiswahili version)

Maelezoyautafiti

<u>Kichwa cha utafiti</u>

Matokeoyamatibabunaimatinibkatikawagonjwawa CML.

<u>Ufunguzi</u>

JinalanguniDaktari Robert Yatichwachuokikuu cha Nairobi, kitengo cha matibabu.Ninafanyautafitiuitwao "matokeoyamatibabunaimatinibkatikawagonjwawa CML".Utafitihuuunachunguzajinsiwagonjwawenye CML (ainayasarataniyadamu) wanavyoendeleabaadayakutibiwanaimatinib.

Ningependakukuhusishakatikautafitihuu, ambaoutahusishamaswalimachache, upimajinauchunguzikatikafomuzakozamatibabu.Ukichaguakutoshirikikatikautafitihuu badoutapokeamatibabuyakokamakawaida.

<u>Manufaayanayotarajiwa</u>

Matokeoyautafitihuuhuendayakatumikakutengenezampangilioutakaotumiwakuwezesh akujulikanakwamapemakwawagonjwawasiokuwanamatokeomwafakawanapotumiaim anitib.

<u>Madhara</u>

Hakunamadharayoyoteyanayotarajiwaunaposhirikikatikautafitihuu.

<u>Gharamanamalipo</u>

Utafitihuunikwahiarinahakunamalipoyoyoteyatatolewa.

<u>Faragha</u>

Habarizotezitakazotolewazitawekwakwafaraghakuu.Watakaoshirikikatikautafitihuuha wawezikutambulikakatikanjiayeyote.

<u>Kujiondoakwautafiti</u>

Unawezakukataakushirikikatikautafitihuuwakatiwowotebilakudhulumiwakwanjiayoy

ote.Ukifanyahivyomatibabuyakoyataendeleakwakilinikiya GIPAP kamakawaida.

Appendix V: Consent form (Kiswahili version)

<u>**Fomuyakukubali**</u>

<u>Kukubalikwahiari</u>

Mimi…………………………………………………….Ninahakikishayakwambani

mesomafomuyakukubalinanimeielewa.Maswaliyotekuhusuutafitihuuyamejibiwanani

meridhikananimehakikishiwahakizangu.Sahihiyanguhapachiniyaonyeshakuwanimeku

balikwahiarikushirikishwakatikautafitihuu.

Sahihiyakushiriki………………………… Tarehe………………….

<u>Neno la mtafiti</u>

Nimemuelezeamgonjwahuyujinsinasababuzakufanyautafitihuu,

manufaayanayotarajiwanamadharayoyoteyanayohusishwanakushirikikatikautafitihuu.

Nimejibumaswaliyoteyaliyoulizwa.Nimeyaelezahayakwatareheiliyoonyeshwakwenye

fomuyakukubali.

Sahihiyamtafiti……………………………….Tarehe……………………

Appendix VI: Investigators' and ERC contact details

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

Dr. Robert K. Yatich (Principal investigator)

University of Nairobi, Department of Clinical Medicine and Therapeutics

P.O. Box 19676, Nairobi

Tel. 0724436176

Prof. N.A. Othieno-Abinya (Lead supervisor)

University of Nairobi, Department of Clinical Medicine and Therapeutics

P.O. BOX 19676, Nairobi

Tel. 020-2723127

The Chairman,

KNH/UoN Ethics and Research Committee

Tel. 020-2726300 Ext. 44355

Appendix VII: Data entry sheet

Name (initials only) ——————————

Date of birth ——————————

Country of birth ——————————— County of residence ———————————

Telephone number ——————————

Date ——————————— Study no: ——————————

Imatinib registration number: ———————————

PART A: Social demographics

Age: ———————————

Gender: Male ☐ Female ☐

Level of education: Primary ☐ Secondary ☐ Tertiary ☐ None ☐

Occupation: ———————————

Marital status: Single ☐ Married ☐ Separated ☐

Lifestyle: Smoking: Yes ☐ No ☐ If yes: pack years: ———————

PART B: Clinical details

Date of CML diagnosis ———————————

Were there symptoms at diagnosis? Yes ☐ No ☐ If yes:
Fatigue ☐ Weight loss ☐ Abdominal swelling ☐

CML phase at diagnosis: CP: ☐ AP: ☐ BP: ☐

Spleen size in cm below the costal margin: < 10 cm ☐ 10-20 cm ☐ > 20 ☐ cm

PART C: Laboratory findings

1. Full blood count at:

Entry: WBC(x10^9/L) _____ Platelet count (x10^9/L) _____ Hb(g/dl) _____

Eosinophils (%) _____ Basophils (%) _____

3 months: WBC (x10^9/L) _____ Platelet count (x10^9/L) _____ Hb (g/dl) _____

Eosinophils (%) _____ Basophils (%) _____

18 months or time of recruitment: WBC (x10^9/L) _____ Platelet count (x10^9/L) _____

Hb (g/dl) ____ Eosinophils(%) ____ Basophils (%) ____

2. Bone marrow aspirate findings at entry: CP [____] AP [____] BP [____]

3. BCR-ABL/ABL ratio at: Entry _____ 18 months or time of recruitment _____

PART D: Treatment details

Prior treatment with other agents: 1. _____ Duration _____

                                  2. _____ Duration _____

Date of imatinib commencement _____ Dose at Commencement _____

Any change in dose imatinib? Yes ____ No ____ Reason for change _____

Current dose of imatinib _____

Have you ever missed your dose? Yes ____ No ____

- If yes how often < 1 week [____]   2 weeks [____]   1 month [____]   >1 [____] month

List any other medicines you are taking _____

PART E: Prognostic score at entry

EUTOS score at entry _____

- Spleen size _____

- Basophil percentage _____

Appendix VIII: Hasford, Sokal and EUTOS prognostic scoring for CML

i. Hasford score

The Hasford scoring system classifies patients into three risk groups: low, intermediate, and high and is calculated using the following equation:

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

0.0420 x spleen size [cm below costal margin]+

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

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

1.0956 x platelet count [0 for <1500 x 10^9/1; 1 for a higher value]) x 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.

ii. Sokal score

The Sokal score 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)

A score of < 0.8= good prognosis, 0.8-1.2= moderate prognosis, > 1.2= poor prognosis

iii. The EUTOS score

The EUTOS score classifies patients into two risk groups: low risk and high risk. It is calculated as follows: (7 x basophils) + (4 x spleen size) where the spleen is measured in centimeters below the costal margin and basophils as a percentage at baseline.

A EUTOS score of > 87 indicates high-risk and ≤ 87 low-risk.

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/216

June 13, 2018

Dr. Robert Kibet Yatich
Reg. No.H113/5855/2017
Dept. of Clinical Medicine and Therapeutics
School of Medicine
College of Health Sciences
University of Nairobi

Dear Dr. Kibet

RESEARCH PROPOSAL – EVALUATION OF RESPONSE TO IMATINIB MESYLATE THERAPY AMONG
CHRONIC MYELOID LEUKEMIA PATIENTS AS SEEN IN NAIROBI, KENYA   (P61/02/2018)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above research proposal. The approval period is from 13th June 2018 – 12th June 2019.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
f) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
g) Submission of an *executive summary* report within 90 days upon completion of the study.
   This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

Protect to discover