RETROSPECTIVE ANALYSIS OF PATIENTS WITH CHRONIC MYELOID LEUKAEMIA (CML) AT THE CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL (CMJAH) MEDICAL ONCOLOGY UNIT (2002- 2015)

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Declaration

I, Dr Dineo Tshabalala declare that this dissertation is my own work. It is being submitted for the degree of Master of Medicine in the clinical disciplines in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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..........6th.....day of September, 2017

Dedicated to my mom

Phindile Cynthia Tshabalala

"Blesssed"

Born 08-06-1949

ABSTRACT

Background: The objective of this study is to explore the use of bcr-abl Tyrosine Kinase Inhibitors in the treatment of chronic myeloid leukemia (CML) patients at the CMJAH Medical Oncology Unit in Johannesburg, South Africa, and to confirm the evidence from bodies such as the European LeukaemiaNet (ELN) that the era of bcr-abl TKIs has significantly advanced the treatment of CML, with affected patients living normal lives in their chronic phase, therefore making allogeneic stem cell transplantation no longer an essential part of therapy.

Method: A cohort of 101 adult patients diagnosed with CML, 48% males and 52% females, with a median age of 40 years, were retrospectively analysed using data from their clinic files. The Sokal score could be evaluated as a pretreatment prognostic tool in 55% of the patients. Molecular responses to three sequential TKIs ie. Imatinib followed by dasatinib and nilotinib, were sought by the monitoring of serial RQ-PCRs. Adverse effects and mutational analyses were also analysed.

Results: Once patients were started on bcr-abl TKI therapy (post the interferon-α era), better treatment responses were seen and better overall survival achieved without progression to advanced stages of CML. In addition, second line TKI therapy showed a benefit following the first line TKI imatinib. TKIs were generally well tolerated with 63 of the 101 patients experiencing grade 3/ 4 AEs mainly due to haematological toxicity. A low number of documented mutations (3 out of 101) also suggest that TKI therapy is very effective in treating CML.

Conclusion: There seems to be an improved outcome with TKI therapy compared to the older interferon alpha based therapy; as well as a treatment response with second generation TKI therapy in patients at the CMJAH Medical Oncology unit treated for CML. Patients on TKI therapy remained in CP-1 of CML for longer periods without transformation to advanced stages of CML, with improved PFS and 5 year OS, as long as they were compliant on treatment. Hematological adverse effects were observed due to both dasatinib and imatinib therapy.

Key words: Chronic myeloid leukaemia; Tyrosine kinase inhibitor; Molecular response; Overall survival; Progression free survival.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure	Page

LIST OF ABBREVIATIONS

CML - Chronic myeloid leukaemia

ALL - Acute lymphoblastic leukaemia

CP- 1 - First chronic phase

CP- 2 - Second chronic phase

AP - Accelerated phase

BC - Blast crisis

TKI - Tyrosine kinase inhibitors

Ph - Philadelphia chromosome

BCR - Breakpoint cluster region

ABL/ BCR1 - Abelson murine leukaemia

PB - Peripheral blood

BM - Bone marrow

WBC - White blood cell

WCC - White cell count

FISH - Flourescence-in-situ-hybridaization

ELN - European LeukaemiaNet

CHR - Complete haematological response

CCyR - Complete cytogenetic response

PCyR - Partial cytogenetic response

RT-PCR - Real-time quantitative polymerase chain reaction

MMR - Major molecular response

MR - Deep molecular response

USA FDA - United States of America Food and Drug Administration

SA MCC - South African Medicines Control Council

CMJAH - Charlotte Maxeke Johannesburg Academic hospital

PFS - Progression free survival

EFS - Event free survival

OS - Overall survival

AlloSCT - Allogeneic stem cell transplant

TRM - Treatment related mortality

IS - International scale

ATP – Adenosine triphosphate

GISTs - Gastrointestinal stromal tumours

PDGFR - Platelet-derived growth factor

KD - Kinase domain

IC50 - Half-maximal inhibitory concentration

CCA - Clonal chromosome abnormalities

EC - Endogenous control

LTF - Lost to follow-up

nLTF - Not lost to follow-up

HIV - Human immunodeficiency virus

HAART - Highly active antiretroviral therapy

TB - Tuberculosis

TTP - Time to Progression

EAP - Expanded Access Programme

TED - Thromboembolic disease

Chapter 1

Background, Rationale and Motivation for this Study

1. Introduction

1.1 Chronic Myeloid leukaemia (CML)

Chronic myeloid leukaemia (CML) is a chronic myeloproliferative disorder which accounts for 15 to 20 percent of all adult leukaemias (Walz et al., 2008). The median age of occurrence is about 50 years for patients enrolled in clinical studies with an incidence of 2 per 100,000/year with a slight male predominance (Louw et al., 2012); (Walz et al., 2008). South African leukaemia incidence data from the South African National Cancer Registry, for 2011 reported a leukaemia incidence with a total of 750 new cases of leukaemia (437 male, ASR 2.33 per 100,000 population and 313 female, ASR 1.33 per 100,000) reported, with no specific classification of the type of leukaemia mentioned (Singh et al., 2013). With the increased use and availability of bcr-abl tyrosine kinase inhibitors, the incidence of CML is steadily increasing worldwide due to more patients surviving and living longer (Louw et al., 2012); (Walz et al., 2008). It is important to note that although CML can occur in any age group; this research will focus on the adult population ($\geq$18 years of age).

CML is characterized by a clonal expansion of the haematopoietic stem cells associated with a translocation between the long arms of chromosomes 9 and 22 {t(9;22)} which results to the formation of the Philadelphia chromosome (Ph) (Louw et al., 2012); (Wetzler M et al., 2008). The t(9;22) translocation results in fusion of the breakpoint cluster region (BCR) gene on chromosome 22 at band q11 with the Abelson murine leukaemia (ABL) gene located on chromosome 9 at band q34 (O'Brien et al., 2003); (Wetzler M et al., 2008). This results in the BCR-ABL fusion gene which codes for a 210 kilodalton fusion protein, p210, which plays a central role in the initial development of CML with has deregulated tyrosine kinase activity. The p190 BCR-ABL fusion protein is usually associated with Ph+ acute lymphoblastic leukaemia (ALL). (O'Brien et al., 2003).
Pathogenesis of CML involves p210 being constitutively activated leading to increased proliferation of myeloid cells, enhanced cell adhesion, decreased apoptosis and genetic instability of the leukaemic cells. It is this genetic instability that forms the basis for

resistance to treatment and progression of disease to an acute leukaemic or blastic phase (Louw et al., 2012); (Wetzler M et al., 2008).

CML is characterised by the uncontrolled production of mature and maturing granulocytes which are predominantly of the neutrophil series, but can also have increased basophils and eosinophils (Walz et al., 2008). CML has a triphasic clinical course namely a chronic phase (CP-CML), which is present at the time of diagnosis in approximately 85% of patients followed by an advanced phase, which includes the accelerated phase (AP-CML) and the blast crisis (BC-CML) (Walz et al., 2008). (See Table 1.1).

Table 1.1: Definition of phases of CML (Baccarani et al., 2013); (Louw et al., 2012).

ELN criteria	WHO criteria
Chronic phase (CP)	
None of the criteria for AP or BP met	
Accelerated phase (AP)	
Blast cells in PB or BM 15-29%,	Blasts in PB or BM 10-19%
Blasts + promyelocytes in PB or	Basophils in PB ≥ 20%
BM> 30%, with blasts < 30%	Persistent thrombocytopenia
Basophils in PB ≥ 20%	($<100 \times 10^9$/L) unrelated to
Persistent thrombocytopenia	therapy
Unrelated to therapy	CCA/Ph+ on treatment
Clonal chromosome abnormalities	Thrombocytosis ($>1000 \times 10^9$/L)
in Ph+ cells (CCA/Ph), major route,	unresponsive to therapy
on treatment	Increasing spleen size and
	Increasing WBC count
	Unresponsive to therapy
Blast phase (BP)	
Blasts in PB or BM ≥30%	Blasts in PB or BM ≥20%
Extramedullary blast proliferation,	extramedullary blast proliferation,
apart from spleen	apart from spleen
	Large foci or clusters of blasts in
	BM biopsy
PB= peripheral blood; BM= bone marrow	

If untreated early in the course of the disease or if drug resistance occurs, CML will progress to an advanced phase within 3-5 years.(O'Brien et al., 2014) In the accelerated phase neutrophil differentiation becomes progressively impaired and leukocyte count more difficult to control with treatment associated with an increase in basophils and a decrease in platelets. The blast crisis resembles an acute leukaemia and can either have myeloid (75%) or lymphoid (25%) blasts proliferating in an uncontrolled manner (Etten et al., 2016).

Patients are usually asymptomatic at diagnosis with an incidental finding of a raised white blood cell count (WBC) on peripheral blood. Symptoms include fatigue, malaise, weight loss, left sided abdominal fullness and early satiety (secondary to splenomegaly), and less commonly bleeding due to platelet dysfunction (Louw et al., 2012). Clinically splenomegaly is the most common finding, there may also be hepatomegaly, gouty arthritis, pallor, features of hyperviscosity related to high WBC count (e.g. headache, visual and hearing disturbances, angina, dyspnoea, bone pain and priapism) (Louw et al., 2012). Commonly in the blast phase of the disease there may be extramedullary involvement beyond the spleen including lymph nodes, skin, soft tissue and liver, which may have prognostic and staging implications (Etten et al., 2016).

The work-up of CML therefore entails a good history and physical examination. The following laboratory tests are required to confirm the diagnosis:

 A full blood count with a peripheral smear demonstrates a leukocytosis with a median WBC of approximately 100,000/µl (normal range 12 to 100/µl), but may exceed 1,000,000/µl (Etten et al., 2016). The WBC differential count typically shows virtually all cells of the neutrophil differentiation, from myeloblasts to mature neutrophils with a peak in the percentage of myelocytes and segmented neutrophils (Etten et al., 2016). A "myelocytic bulge" which is the presence of a greater percent of myelocytes than mature metamyelocytes is one of the classic findings in CML. Absolute basophilia is universally found in the blood smear of CML patients with absolute eosinophilia also seen in 90% of cases. A normocytic anaemia is seen in 45 to 60 percent of patients with the platelet count can either normal or, in most cases elevated (Etten et al., 2016).

Bone marrow aspirate and trephine biopsy shows a granulocyte hyperplasia with a maturation pattern similar to that seen on peripheral blood smear. Both bone marrow aspirate and peripheral blood smear are key components in determining disease stage (Etten et al., 2016). Testing for Ph chromosome is demonstrated by conventional cytogenetic analysis (karyotyping), while BCR-ABL1 fusion gene is demonstrated by fluorescence-in-situ-hybridization (FISH) analysis or by real-time quantitative polymerase chain reaction (RQ-PCR) (Etten et al., 2016). All patients with CML have evidence of either the Ph+ chromosome, the BCR-ABL1 fusion gene or its product the BCR-ABL1 fusion mRNA (Etten et al., 2016).

Three prognostic systems: Sokal, Euro (Hasford), and EUTOS (European Treatment and Outcome Study Score); based on simple clinical and haematological data, have been shown to be of prognostic value in treatment naïve CP-CML. (Baccarani et al., 2013); (Etten et al., 2016).

For the Sokal score to be calculated, the following variables are required: age, spleen size in centimetres, platelet count and blast count. (See Table 1.2)

 For the Hasford score to be calculated variables needed are age, spleen size, blast count, basophil count and eosinophil count. (See Table 1.2).

The latest (2013) guidelines for CML management by the European LeukaemiaNet (ELN) suggest the use of risk stratification systems to influence overall clinical outcome.(Baccarani et al., 2013).

Table 1.2: Prognostic scores in CML (Etten et al., 2016); [7];(Hasford et al., 1998); (Hasford et al., 2011); (Sokal et al., 1984).

Study	Calculation	Risk calculation by definition
Sokal et al. 1984[6]	Exp 0.0116 x (age - 43.4) + 0.0345 x (spleen – 7.51) + 0.188 x[(platelet count ÷ 700)^2 – 0.563] + 0.0887 x (blast cells – 210)	Low risk: < 0.8 Intermediate risk: 0.8 - 1.2 High risk: >1.2
Euro Hashford et al. 1998[7]	0.666 when age ≥ 50y + (0.042 x spleen) + 1.0956 when platelet count > 1500 x 10^{9}L + (0.0584 X blast cells) + 0. .20399 when basophils > 3% + (0.0413 x oesinophils) x 100	Low risk: ≤ 780 Intermediate risk: 781-1480 High risk: > 1480
EUTOS Hashford et al. 2011[8]	Spleen x 4 + basophils x 7	Low risk: ≤ 87 High risk: > 87

Note: Spleen size in centimetres

Before the era of BCR-ABL TKIs, the treatment of CML included busulphan, hydroxyurea, interferon-α ± low dose cytarabine , and allogeneic haematopoietic stem cell transplantation (Louw et al., 2012). Since the introduction of BCR-ABL1 TKIs, which specifically target the non-receptor tyrosine kinase activity of the oncogenic protein encoded by BCR-ABL fusion gene, the management and outcome of CML has significantly improved (Louw et al., 2012). The first-generation BCR-ABL1 TKI, imatinib was the first to be approved by the US FDA (United States Food and Drug Administration) and South African MCC (Medicines Control Council) as first-line therapy for patients with CML after the IRIS study showed that after a median of 19 months, imatinib was significantly better than interferon-α ± cytarabine based treatment, as shown by the rates

of complete haematological response (CHR) of (95% vs 56%), and MCyR (≤ 35% Ph+ cells in metaphase); 85% vs 22%, and CCyR of 68% versus 8%. MMR rates at 12 months of (40% vs 2%) and CML Progression Free Survival (PFS) was also shown to be superior with imatinib (Baccarani et al., 2013); (O'Brien et al., 2003).

1.2 BCR-ABL1 Tyrosine Kinase Inhibitors (TKIs)

Tyrosine Kinases are enzymes responsible for the phosphorylation of tyrosine amino-acids resulting in the activation of various proteins involved in signal transduction cascades. The activation of these proteins is by the addition of a phosphate group to tyrosine amino-acids in the tyrosine kinase residue protein, ie. phosphorylation (Schiffer, 2007). BCR-ABL TKIs block the initiation of the BCR-ABL1 pathway by blocking its ATP binding site and thereby inhibiting phosphorylation and preventing a conformational switch to the active form, inhibiting signal transduction and thereby cellular proliferation and tumour formation without inducing apoptosis, thus producing a 92-98% decrease in CML growth *in vitro* without inhibiting normal cellular growth (Schiffer, 2007); (Jabbour et al., 2007).

It should be noted that BCR-ABL TKIs may also block other signalling pathways, making them effective in other haematological as well as solid tumours, e.g. imatinib also inhibits mutant platelet-derived growth factor α and β (PDGFR-α and β) and *c-Kit* (CD117, Stem Cell Factor Receptor) in Gastrointestinal Stromal Tumours (GISTs); (Giles et al., 2009); (Schiffer, 2007).

In CML however, imatinib is shown to specifically inhibit proliferating myeloid cell lines containing the BCR-ABL1 fusion gene without affecting or destroying normal cells of the granulocyte series. (Jabbour et al., 2007).

1.3 Response to BCR-ABL1 TKIs

Therapeutic responses are usually assessed with standardized real-time quantitative polymerase chain reaction (RQ-PCR) and/or cytogenetics (See Table 1.3); With 3, 6, 12, and 18 month intervals used as indicators of adequate response to treatment (Baccarani et al., 2013). With regards to RQ-PCR, a BCR-ABL1 transcription of <10% (1 log reduction) at 3 months, <1% (2 log reduction) at 6 months, and <0.1% (3 log reduction) at 12 months onwards defines an optimal response (Baccarani et al., 2013). Whereas a >10% BCR-ABL1 at 6 months and >1% at 12 months and onwards defines treatment

failure, requiring consideration of a change to second-line treatment. In the same token, with regards to cytogenetic response, partial cytogenetic response (PCyR) (<35% Ph+) at 3 months and complete cytogenetic response (CCyR) from 6 months onwards defines optimal treatment response. Failure is defined by a Ph+ chromosome of >95% at 3 months, less than PCyR at 6 months, and less than CCyR from 12 months onwards. Important to note is that treatment failure is confirmed when two or more samples show an increase in BCR-ABL transcripts (Baccarani et al., 2013); (Louw et al., 2012).

1.4 Adverse effect of BCR-ABL1 TKIs

The development of BCR-ABL1 tyrosine kinase inhibitors for the treatment of CML over the past twenty years has increasingly improved outcomes and management of chronic myeloid leukaemia (Larson, 2015). Worldwide oncologists and haematologists have access to 5 oral agents for treating CML. Three of these are available in South Africa; namely imatinib, nilotinib and dasatinib which are all approved as first line therapy and are generally well tolerated and very effective in CML management. (Larson, 2015). With current practice it seems that patients with CML are remaining on BCR-ABL TKIs therapy indefinitely, therefore clinicians need to familiarise themselves with the early and late toxicities associated with BCR-ABL TKI use (Larson, 2015). Efficacy is important; so choosing the appropriate medicine will be guided by understanding each agent with regards to its benefits and risks and patient-specific factors such as risk status, age, and comorbidities. (Larson, 2015).

Adverse effects (AEs) of BCR-ABL TKI therapy are rarely severe (grade 3/ 4). It is therefore important to recognise and treat these toxicities early as low grade toxicities may over a long period of time impact on compliance and eventually on overall survival (Giles et al., 2009); (Larson, 2015).

There seems to be a relationship between kinase inhibitor mechanism of action of BCR-ABL TKI therapy and safety profile(Giles et al., 2009).

i. Inhibition of ABL kinase. ABL is a key mediator of normal cardiac function and is expressed in cardiac cells. There is therefore an increased incidence of cardiac adverse events on second generation BCR-ABL1 TKI, dasatinib and nilotinib compared to imatinib. These cardiac AEs include QT prolongation, arrhythmias, left ventricular dysfunction and rarely present as

grade 3/4 AEs. They also more commonly occur with dasatinib use than with nilotinib (Giles et al., 2009).

ii. SRC kinase inhibition. SRC is important in signalling for normal haematopoiesis. SRC family members HCK, LYN, FGR, LCK, and, BLK are expressed only in haematopoeitic cells. With HCK critical in development and survival of myeloid and B lymphocytes. LYN is an important modulator of erythropoiesis, modulating erythroid progenitor cell expansion to promote erythroid survival. Out of the three BCR-ABL TKIs, dasatinib strongly inhibits SRC kinases with myelosuppression more common on patients being treated with dasatinib than with the other two BCR-ABL TKIs (Giles et al., 2009).

iii. Platelet- derived growth factor receptor (PDGFR) affects fluid retention and serosal inflammation. PDGFR is expressed in pericytes and lung tissue, and is also involved in angiogenesis regulation. Its inhibition therefore causes changes in interstitial pressures and fluid haemostasis in vascular compartments and extracellular compartments. This results in the development of oedema and third spacing of fluids. Other factors also influence pleural and pulmonary parenchymal fluid retention, this occurs when SRC inhibition affect vascular permeability (Giles et al., 2009).

iv. *C-kit* receptor is normally expressed in skin, basal cells, melanocytes, epithelial cells of the breast, mast cells and intestinal pacemaker cells of Cajal. Imatinib inhibits *C-kit*, causing patients to develop rashes and other cutaneous reactions (Giles et al., 2009).

C-kit is also important in the development of normal blood cells. It therefore explains why some patients on imatinib and less so on nilotinib develop cytopenias due to myelosuppressive effects of therapy(Giles et al., 2009).

Overall imatinib is well tolerated and effective in treatment of CP-CML. The common documented non- haematological AEs on imatinib are rashes, fatigue, headache, nausea, diarrhoea, muscle pains as well as haematological AEs reported above, especially myelosuppression (Giles et al., 2009).

Dasatinib was initially developed as a SRC inhibitor and subsequently found to be a powerful BCR-ABL1 inhibitor with 300 times the potency of imatinib *in-vitro*. While nilotinib was developed as an analogue of imatinib, with 30 times the potency of imatinib *in-vitro* (Kantarjian et al., 2010); (Saglio et al., 2010).

Nilotinib and dasatinib are both highly active in treating those CML patients who have failed imatinib because of resistance or intolerance (Giles et al., 2009). Common non-haematological AEs of nilotinib although not usually clinically significant are arrhythmias due to QTc prolongation, elevations in bilirubin and lipase levels, pancreatitis and hyperglycaemia, which rarely require directed therapy (Giles et al., 2009). The non-haematological AEs for dasatinib are headache, fluid retention including pleural effusion, pulmonary arterial hypertension, QTc interval prolongation and other cardiac events. (Giles et al., 2009).

Data on both safety and efficacy is now available for imatinib after 10 years for initial therapy and 5-6 years for dasatinib and nilotinib therapy. (Larson, 2015).

1.5   Monitoring in CML

1.5.1 Cytogenetics
Conventional cytogenetics have been the gold standard for decades, since the description of the Ph chromosome by Nowell and Hungerford in 1961, in the diagnosis and monitoring in CML. Cytogenetic responses are defined in terms of the percentage of cells that are in metaphase existing within the bone marrow that are Ph chromosome positive. This response is based upon a usual sample size of twenty cells in metaphase (Hughes et al., 2003); (Hughes et al., 2006). There are limitations to karyotyping as there is a high rate of failure due to lack of metaphase especially in patients with low WBC counts. In addition high costs, delayed results and invasive bone marrow procedures limit this procedure. Reference ranges for cytogenetic response are complete cytogenetic response (CCyR)- (no Ph+ metaphases - 0%), partial cytogenetic response (PCyR) – (1-

35% Ph+ metaphases), major cytogenetic response (complete and partial) – (0- 35% Ph+ metaphases), minor response- (>35% but <90% Ph+ metaphases). (See Table 1.3) (Baccarani et al., 2013); (Hughes et al., 2006).

1.5.2 Fluorescence-in-situ-hybridization (FISH)

FISH is done on peripheral blood (BP) or bone marrow looking at approximately 200 interphase cells with BCR-ABL1 translocation in myeloid cells. It may be less invasive, faster and less costly to carry out (Hughes et al., 2006). However it is an inferior method of quantifying and thereby monitoring disease response on both interferon-α therapy and BCR-ABL1 TKI therapy, being only semi-quantitative. It is therefore only useful for diagnosing CML, as an alternative measure when conventional cytogenetics are unavailable or inadequate (Hughes et al., 2006).

1.5.3 Molecular (RQ-PCR)

Molecular response in CML is measured using the percentage of BCR-ABL1 fusion transcripts (Hughes et al., 2003). This transcript is a marker of the presence and amount of transcriptionally active Ph chromosome positive leukaemia cells in CML patients. mRNA is extracted and purified from leukocytes, then reverse transcribed (RT) and the cDNA product is then quantified by fluorescent real-time quantitative polymerase chain reaction (RQ-PCR) (Branford et al., 2008); (Hughes et al., 2003); (Hughes et al., 2006). The RQ-PCR test must also analyse an endogenous control transcript (EC), usually either ABL or GUS to assess the quality and quantity of RNA and to normalize the potential differences between tests (Hughes et al., 2003); (Branford et al., 2008). Therefore monitoring of response to BCR-ABL1 TKI therapy is based on a variation of BCR-ABL1 expression levels over time, measured in fold change or more commonly log reduction change. RQ-PCR reference ranges are MMR3 (log 3 reduction)- ≤ 0.1%, MR4 (log 4 reduction)- < 0.01%, MR4,5 (log 4,5% reduction)- < 0.0032%), and MR5 (log 5 reduction)- < 0.001%. (Baccarani et al., 2013). (See Table 1.3).

1.5.4 International Scale (IS)

The International Scale (IS) was established in 2005 to standardize quantitative BCR-ABL1 measurements across tests and laboratories (Branford et al., 2008). The (IS) is anchored to the baseline BCR-ABL1 expression level from the International Randomized Study of Interferon vs STI571 (IRIS) Trial (100% IS) with a major molecular response

(MMR) corresponding to 0.1% IS. (Branford et al., 2008). The IRIS Trial and follow-up studies have demonstrated that achieving MMR3, or 3-log reduction in BCR-ABL1 expression from standardized baseline level is a key outcome in the treatment of CML. (Branford et al., 2008). ELN recommendations state that it is not possible to assess achievement of MMR3 if the (IS) is not available (Baccarani et al., 2013). The importance of the (IS) is that it standardises quantitative BCR-ABL1 measurements across test laboratories, facilitating inter-laboratory studies, patient portability, and harmonized definition of treatment response across the board (Hughes et al., 2003); (Branford et al., 2008). This scale was consolidated at the consensus meeting on October 2005 at the NIH in Bethesda, Maryland, making sure that an establishment of an International Scale that can be applied at individual centres be set in place (Hughes et al., 2003). For any local laboratory to adhere to the (IS) involves;

i) Adoption of the consensus principles established by the Bethesda group

ii) Testing a set of reference standards to establish a laboratory specific conversion factor and;

iii) Multiply all local BCR-ABL1 values by the conversion factor to express the results according to the (IS). All validated laboratories worldwide use one of three reference laboratories based in Adelaide, London and Seattle (Hughes et al., 2003).

Once patients are on BCR-ABL1 TKIs it is important to monitor cytogenetic and molecular response and development of mutations so as to identify early the subgroup of patients that will benefit from early intervention options (Baccarani et al., 2013). With regards to response to treatment, the ELN guidelines have maintained definitions of complete haematological response (CHR) and complete cytogenetic response (CCyR) (Baccarani et al., 2013). Changes were made with regards to Molecular Response, which should be corrected according to the International Scale (IS) as the ratio of BCR-ABL1 transcripts as a percentage on a log scale (Baccarani et al., 2013). (See Table 1.3).

Table 1.3: Definition of Response (Baccarani et al., 2013); (Louw et al., 2012).

<u>Complete haematological (CHR)</u>

- WBC < 10 x 10^9/L
- Basophils < 5%
- No myelocytes, promyelocytes, myeloblasts in the differential count
- Platelet count < 450 x 10^9/L
- Spleen not palpable

<u>Cytogenetic response</u>

- Complete (CCyR) - no Ph+ metaphases
- Partial (PCyR) – 1-35% Ph+ metaphases
- Major – 0-35% Ph+ metaphases (complete + partial)
- Minor - >35% Ph+ metaphases

<u>Molecular</u>

- Deep molecular response (MR)- BCR-ABL1 transcript < 0.01%, 0.0032%, 0.001% by QPCR (IS) or 4, 4.5, 5 log reduction respectively in BCR-ABL1 mRNA from standardized baseline
- Major molecular response (MMR)- BCR-ABL1 transcripts ≤ 0.1% or 3 log reduction in BCR-ABL1 mRNA from standardized baseline

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1.6 Clinical Studies with BCR-ABL1 TKIs

1.6.1 IRIS Trial (International Randomized Study of Interferon and STI571 Trial)

A 5 year update of the landmark IRIS study (O'Brien et al, 2003) continued to show positive results for imatinib (Gleevec®). A total of 382 from the initial 553 assigned patients remained in front-line imatinib therapy.(Druker et al., 2006). The cumulative best CHR, MMR, and CCyR rate were 98%, 92%, and 87% respectively, with the estimated EFS (Event Free Survival) at 5 years being 83% with only 6% of patients progressing to advanced stage CML (O'Brien et al., 2003); (Druker et al., 2006). The 5 year OS was 85%, and with exclusion of non-CML deaths was 95% (O'Brien et al., 2003). Also shown was that depth of cytogenetic and molecular responses after 12 months and 18 months on imatinib therapy, has important implications regarding survival without transformation (O'Brien et al., 2003); (Druker et al., 2006).

Eight year follow-up in the phase III IRIS trial on adult patients with newly diagnosed CP-CML. This trial reported a cumulative best CCyR of 85% and an estimated OS of 93% (in CML-related deaths with evidence of low progression rates to AP and BC (Kantarjian et al., 2006); (Sacha, 2013). However 17% of imatinib treated patients did not achieve a CCyR and 10% who did achieve CCyR relapsed. An additional 8% of patients were intolerant of imatinib (Sacha, 2013).

1.6.2 Dasatinib - DASISION Trial (Dasatinib versus Imatinib Study in treatment-Naïve CML patients) Trial

Dasatinib (Sprycel®, Bristol-Myers Squibb), a second generation BCR-ABL TKI is approved as second-line therapy for patients with CML following imatinib. It is approximately 300 times as potent as imatinib in inhibiting unmutated BCR- ABL1 kinase *in-vitro* (Kantarjian et al., 2010).

The DASISION Trial compared dasatinib versus imatinib in treatment naive CML patients, looking at efficacy and safety of dasatinib (Kantarjian et al., 2010). Dasatinib was administered in a dose of 100mg once daily and imatinib at 400mg once daily, in patients with newly diagnosed CML (Kantarjian et al., 2010). The primary objective was to determine whether patients who received dasatinib had a higher rate of confirmed complete cytogenetic response (CCyR) by 12 months after initiating treatment (Kantarjian et al., 2010). 30- 40% of CML patients on imatinib failed to reach CCyR by 12 months,

the long term outcome being less favourable with an increased risk of progression to the advanced stages of CML at 5 years. (Kantarjian et al., 2010). This lead to the hypothesis that initial therapy with more potent BCR-ABL TKIs that improve the rate of CCyR early after diagnosis of CML could improve the long term outcomes (Baccarani et al., 2013); (Hughes et al., 2006).

Results of this study show that 519 patients were enrolled, with 259 patients started on dasatinib and 260 patients started on imatinib, with a minimum follow-up of 12 months. (Kantarjian et al., 2010) [20] The Hasford risk score was used to stratify patients in this study (See Table 1.2). The rate of MMR by 12 months was also higher with dasatinib than with imatinib treatment (46% vs 28%).(Kantarjian et al., 2010). Also in patients in whom CCyR was achieved by 12 months, the rate of MMR was higher among the dasatinib group than the imatinib group (54% vs 39%). The rate of CCyR by 12 months in the dasatinib groups were 94% for low risk, 78% for intermediate risk, and 78% for high risk scores, when using the Hasford risk score. The corresponding imatinib CCyR by 12 months were 76%, 72%, and 64%; for low risk, intermediate risk, and high risk scores respectively (Kantarjian et al., 2010). The rate of MMR by 12 months for the dasatinib were 56% in the low risk, 45% in the intermediate risk, and 31% in the high risk group (Baccarani et al., 2013); (Kantarjian et al., 2010) compared to the corresponding imatinib MMR by 12 months of 36%, 28%, and 16%. This therefore showed that cytogenetic and molecular responses were achieved quicker with dasatinib than with imatinib, with time to CCyR and MMR being significantly shorter with dasatinib (Baccarani et al., 2013); (Kantarjian et al., 2010). Rate of CML PFS at 12 months was similar at 96% for dasatinib and 97% for imatinib (Kantarjian et al., 2010).

1.6.3 Nilotinib- ENESTnd Trial (Evaluating Nilotinib Efficacy and Safety in Clinical Trials- Newly Diagnosed Patients) Study

Nilotinib(Tasigna®,Novartis Pharmaceuticals) is another second generation BCR-ABL TKI that has approximately 30 times greater potency and selectivity for BCR-ABL tyrosine kinase than imatinib *in-vitro*. (Saglio et al., 2010); (Kantarjian et al., 2006). In the phase III ENESTnd Trial, comparison of efficacy and safety of nilotinib (300mg or 400mg twice daily) with that of imatinib 400mg once daily in patients with newly diagnosed Ph+ CML in CP was studied (Baccarani et al., 2013); (Kantarjian et al., 2006). The end point was the rate of MMR ($\leq$ 0.1% or log 3 reduction or better) at 12 months (Baccarani et al., 2013);

(Kantarjian et al., 2006). For this study the Sokal prognostic risk score was used to stratify patients accordingly, with low risk, intermediate risk, and high risk scores. (See Table 1.2). Results were as follows, with a total of 846 patients enrolled with a new diagnosis of CP-CML. (Kantarjian et al., 2006). 282 patients received nilotinib 300mg twice daily (group A), 281 patients receiving 400mg twice daily (group B)and 283 patients received imatinib 400mg once daily (group C). Those that achieved MMR at 12 months were 51% for group A, 50% for group B, and 27% for group C (Baccarani et al., 2013); (Kantarjian et al., 2006). Among the patients with high Sokal scores, the rate of MMR at 12 months were 41% group A, 32% group B, and 17% for group C (Kantarjian et al., 2006). At the time of data cutoff, the BCR-ABL1 transcript level was at 0.0032% (log 4.5 reduction) or less on the IS in 13 % of the group A patients, 12% of the group B patients, and 4% of the group C patients, proving that nilotinib on either dose had a shorter time to MMR than imatinib (Kantarjian et al., 2006).

1.6.4 Bosutinib - BELA (Phase III Bosutinib Efficacy and Safety in Newly Diagnosed Chronic Myeloid Leukaemia) Trial

Other second generation BCR-ABL TKIs, like bosutinib (Bosulif®, Pfizer) which is not registered by MCC in South Africa can also be used as second-line or third-line therapy for CML (Cortes et al., 2012). The BELA Trial also showed superiority of bosutinib compared to imatinib (Baccarani et al., 2013); (Cortes et al., 2012). This trial was a phase III study on adults with newly diagnosed CP-CML. This analysis was stratified using the Sokal risk score. A total of 502 patients were enrolled with 250 patients on bosutinib and 252 on imatinib, at doses of 500mg once daily for bosutinib and 400mg once daily for imatinib respectively (Baccarani et al., 2013); (Cortes et al., 2012). It was shown in this study that bosutinib did not have a superior CCyR rate at 12 months when compared to imatinib although the MCyR at 12 months was 73% and the MMR rate at 12 months was higher with bosutinib than with imatinib. (Cortes et al., 2012). The influence of the Sokal risk score on treatment affect for CCyR and MMR at 12 months was also shown to demonstrate no differences (Baccarani et al., 2013); (Cortes et al., 2012).

1.6.5 Ponatinib - EPIC (Evaluation of Ponatinib vs Imatinib in CML) Trial

Ponatinib (Iclusig®, Ariad Pharmaceuticals) a pan-TKI has shown to be the most active of the tyrosine kinase inhibitors, also inhibiting the T315I BCR-ABL1 mutation which confers resistance to all other BCR-ABL1 TKIs, was approved by the USFDA (not SAMCC) only

for treatment of patients who failed therapy with other BCR-ABL TKIs (Baccarani et al., 2013). Recently the use of ponatinib was suspended by the FDA due to the findings of adverse effects of arterial thromboembolic events in the EPIC Trial, which follows on the findings of the phase II PACE Trial. The suspension has subsequently been lifted by the FDA and a **"Black Box Warning"** re risk of thromboembolic disease (TED) added to the Package Insert. The EPIC trial showed that ponatinib offers improved efficacy over imatinib in patients with newly diagnosed CP-CML although that improvement comes at an expense of greater adverse events (Cortes et al., 2013).

The trial included 307 patients, but data was only available for 306 patients (154 on ponatinib and 152 on imatinib), with a median follow-up of 5.1 months. Patients were stratified using the Sokal risk score. Doses used were ponatinib 45mg once daily and imatinib 400mg once daily. Overall Molecular response rates for ponatinib were uniformly higher compared with imatinib for all response measures and at all time points, with MMR at 12 months of 41% for ponatinib and 18% for imatinib. (Cortes et al., 2013). Furthermore at 3 months patients that had achieved MMR3 or log 3 reduction in BCR-ABL1 transcripts was 94% for ponatinib and 68% for imatinib. (Cortes et al., 2013). When divided into low risk, intermediate risk, and high risk according to Sokal risk scores, the achievement of MMR3 was 98%, 96%, and 85% respectively for the ponatinib group of patients; and 76%, 69%, and 42% respectively for the imatinib group (Cortes et al., 2013). These results were analysed despite early termination of the trial at 5 months due to the serious arterial thromboembolic events, but clearly showed that ponatinib is a potent BCR-ABL TKI, active against native and mutated forms of BCR-ABL1, including T315I (Baccarani et al., 2013); (Cortes et al., 2013).

1.7 Mutations

1.7.1 BCR-ABL1 Kinase Domain (KD) Point Mutations
BCR-ABL1 KD mutations contribute and cause resistance to BCR-ABL1 TKIs (Soverini et al., 2011). These point mutations are detectable in roughly 50% of CML patients with treatment failure and disease progression on BCR-ABL TKI therapy. (Baccarani et al., 2013). More than 100 amino acid substitutions have been reported in association with resistance to Imatinib, while Dasatinib and Nilotinib have smaller spectra of resistant mutations. (Baccarani et al., 2013); (Soverini et al., 2011).

In general patients have many BCR-ABL1 Kinase Domain (KD) mutations that are sensitive *in-vitro* to all the BCR-ABL TKIs, and they are expressed as a half- maximal inhibitory concentration [IC50] (Baccarani et al., 2013). In patents in the chronic phase (CP) of CML, there is a relationship between the IC50 value for a specific mutation *in-vitro* and the patient's clinical response to TKIs when harbouring the same point mutation *in-vivo*. If a patient has higher IC50 levels they will have lower haematological and cytogenetic response rates compared to those patients harbouring mutations with lower IC50 values (Baccarani et al., 2013).

BCR-ABL1 point mutations in CML are important for optimal treatment. There are different kinds of KD point mutations of which two will be discussed here. The P-loop region KD mutations on exon 4 are insensitive to imatinib and nilotinib. Other KD mutations on exon 6 are insensitive to imatinib, dasatinib, and in some cases bosutinib. (Gorre et al., 2001); (Soverini et al., 2011). The T315I mutation results in an amino acid substitution at position 315 in the BCR-ABL1 from threonine to an isoleucine. The T315I mutation is the most implicated BCR-ABL1 point mutation described for development of pan-TKI; imatinib, dasatinib, nilotinib, and bosutinib resistance. The only drug that has retained sensitivity to T315I mutation is Ponatinib (Gorre et al., 2001).

Imatinib which was first approved by the FDA in 2001 and by the MCC in 2002 with an indication for use in diagnosed CML patients after interferon-α treatment failure. Imatinib has reduced sensitivity to almost all the common mutations, both P-loop (exon 4) and KD (exon 6) mutations. The list of mutations responsible for imatinib resistance include T315I, T315A, G250E, Y253H, E255K/V, C276G, M351T, L387M, F317L/C/V, F359C/I/V (Soverini et al., 2011).

Dasatinib a second generation BCR-ABL1 TKI developed for patients proven to be imatinib resistant or have had failure of disease response to prior therapy, in most instances imatinib.(Soverini et al., 2007). Dasatinib has decreased sensitivity to the KD (exon 6) mutations namely; T315I/A, F317/L/C/V/I. Dasatinib also shows decreased sensitivity to V299L mutation, which is in a SH3 (SRC- homology 3) contact region mutation of the KD on exon 5 (Baccarani et al., 2013); (Soverini et al., 2007).

Nilotinib another second generation BCR-ABL TKI developed for imatinib intolerance, resistance or treatment failure. Its range of resistant mutations besides the T315I, are

divided between P-loop mutations; Y153H, E255K/V, and the KD mutations are F359C, F359I, and F359V. If any of the above mutations are expressed on a mutational analysis, then the recommendation is to rather use dasatinib instead of nilotinib (Soverini et al., 2011).

Therefore, the use of BCR-ABL1 P-loop and KD mutational analysis plays a pivotal role in the decision making aimed at tailoring the best therapeutic profile for each patient (Soverini et al., 2011). There is however, a need to clarify on when to do the mutational analysis. This is because some data has shown that in some patients more especially those presenting in advanced stages of CML, may already have preexisting genetic instability and harbour some *in-vivo* mutations before initiation of a BCR-ABL TKI therapy (Soverini et al., 2011).

This raises the question of the need to do mutational studies prior to initiation of BCR-ABL TKI therapy in advanced phase CML patients (Soverini et al., 2011).

As it stands, there is no evidence for testing for point mutations in CP- CML patients prior to starting a BCR-ABL1 TKI, but there possibly could be a role especially in those patients presenting in advanced phases of CML (Soverini et al., 2011).

Therefore there should be appropriate labeling of resistance, namely primary and acquired resistance (Soverini et al., 2011).

It then seems that the ideal time for performing mutational analysis is on those patients showing treatment failure and when there is suboptimal response to BCR-ABL TKI therapy. There could also be a role as mentioned above in those patients presenting in advanced phases of CML (e.g. AP-CML, BC-CML) (Soverini et al., 2011).

1.8 Stem Cell Transplants

1.8.1 Allogeneic Haematopoietic Stem Cell Transplant

Allogeneic haematopoietic stem cell transplantation (AlloSCT) is no longer considered as standard first-line treatment or included in any guideline for CML, but it remains the only potentially curative treatment option which can render patients durably molecularly negative (Louw et al., 2012). It is however associated with a high incidence of procedure-related morbidity and mortality especially in the elderly (Baccarani et al., 2013). However there are a few studies that incorporate this treatment modality.(Grigg and Hughes, 2006).

The European Group for Blood and Marrow Transplantation (EBMT) risk score provides a simple tool to assess the outcome and risk of stem cell transplantation (AlloSCT) (Gratwohl, 2012). CML is the foundation from which AlloSCT was established as a treatment modality in other haematological malignancies (Forrest et al., 2009). Five factors are assessed with the EBMT score to give a clue on transplantation outcome namely; age, disease stage, time interval (from diagnosis to AlloSCT), donor type, and donor recipient tissue type. Each risk factor is individually important but all add to the overall cumulative risk (Gratwohl, 2012); (Grigg and Hughes, 2006). (Gupta and Khattry, 2014). With this tool, reasonable accuracy can be applied as to what the outcome after AlloSCT will be, with transplant related mortality (TRM) increasing in a stepwise pattern as the risk score increases, and survival decreasing correspondingly (Gratwohl, 2012).

Initial data comes from comparing baseline therapy between AlloSCT and systemic treatment with Inteferon-α and hydroxyurea (Gratwohl, 2012). The decision making in this regard involves a risk benefit assessment, with allotransplantation having a known high risk of early mortality, notwithstanding the prospect of a cure (Grigg and Hughes, 2006). On the other hand systemic chemotherapy renders minimal early morbidity and mortality. This EBMT risk score was used in CML patients who were treated with either of two pre-transplant modalities, myeloablative or reduced intensity conditioning followed by either transplantation with bone marrow stem cells or peripheral blood stem cells (Gratwohl, 2012); (Grigg and Hughes, 2006). This score still holds true today in the era of BCR-ABL1 TKI's.

In the IRIS Trial and follow-up studies imatinib was shown to be superior in the short and medium term when looking at PFS and OS, therefore replacing earlier therapies, more especially AlloSCT as initial treatment of choice in CP-CML (Grigg and Hughes, 2006). AlloSCT is no longer the preferred first-line therapy but possibly third-line after giving other more potent second generation BCR-ABL TKIs. To date in all review studies, disease stage and timing of treatment have shown to play a critical role in deciding which first-line therapy is more suitable (imatinib vs AlloSCT). CP1-CML has been suggested as a possible suitable time to make the decision of transplantation over a BCR-ABL1 TKI, although not currently standard use or part of any guideline. Factors that can assist in this decision making are the prognostic Sokal score and the EBMT score (Grigg and Hughes, 2006). There is a suggestion that individuals with high Sokal risk scores and low EBMT

scores have unsatisfactory CCyR and MMR while on imatinib at 12 months of follow-up, 49% and 18% respectively (Grigg and Hughes, 2006). Therefore these are the patients that may benefit from a decision to treat with AlloSCT. Arguments have however been raised to rather increase imatinib dose or switching to a second generation BCR-ABL TKI instead in cases of poor response, improving CCyR and MMR thus achieving longer PFS and OS (Grigg and Hughes, 2006).

The recommendation therefore is that imatinib and possibly nilotinib or dasatinib be the treatment of choice for first-line therapy in newly diagnosed CML patients (Baccarani et al., 2013); (Grigg and Hughes, 2006). AlloSCT can be reserved for patients who fail to respond to TKIs or have disease progression on BCR-ABL TKIs, or in patients developing resistant mutations (Baccarani et al., 2013); (Gratwohl, 2012); (Grigg and Hughes, 2006).

Almost 50% of patients who are started on BCR-ABL TKI therapy for CML will either develop treatment failure to one or all the BCR-ABL1 TKIs or have disease progression depending on the BCR-ABL1 kinase point mutation formed. As mentioned, more than 100 mutations have been reported in association with imatinib resistance, most of them can be overcome by the use of dasatinib or nilotinib which have a narrower spectrum of resistant mutations (Baccarani et al., 2013).

1.9 HIV and CML

1.9.1 CML and Human Immunodeficiency virus (HIV)

In a review of a paper by Patel et al, the finding of CML and HIV was coincidental with no link between the two pathologies. (Patel et al., 2012). Indeed there have been very few cases reported of the HIV and CML occurring in the same patient. (Schlaberg et al., 2008). Patel and his colleagues also pointed out in their paper that HIV infected patients tend to present with a more aggressive clinical picture instead of presenting in the chronic phase. (Patel et al., 2012). Their cohort of HIV infected patients at Chris Hani Baragwanath Academic Hospital (CHBAH) in Soweto, Johannesburg consisted of 18 out of the 240 patients known and treated for CML. (Patel et al., 2012). They also looked at was the concomitant use of highly active antiretroviral therapy (HAART) and BCR-ABL1 TKI therapy. Their view was that there were no significant drug interactions with a good outcome reported with regards to controlling both CML and HIV. (Patel et al., 2012). They also looked at the concomitant use of highly active antiretroviral therapy (HAART) and

BCR-ABL TKI therapy. Their view was that there were no significant drug interactions with a good outcome reported with regards to controlling both CML and HIV. (Patel et al., 2012). In addition the long-term survival achieved by these patients was similar to HIV-negative patients. Tolerability to BCR-ABL1 TKIs was equally good and similar to non-infected patients. (Patel et al., 2012). Of notice, the only adverse effect reported in this cohort was a tendency of the CD4 count to drop without the viral load being affected. (Patel et al., 2012).

In another paper by Schlaberg and colleagues, it was also shown that there was no real association of concurrent HIV and CML and that the only reasonable explanation of CML occurring in HIV infected patients or visa- versa was due to the long term survival of HIV patients on HAART (Schlaberg et al., 2008). Only 6 patients had been found to have these two pathologies concurrently, with 3 of them formally reported. (Schlaberg et al., 2008). These three patients were put onto imatinib and HAART, with the HAART regimen not specified. (Schlaberg et al., 2008).

They reported that therapy was generally well tolerated with cytogenetic response achieved in all 3 patients, with complete cytogenetic response achieved in 2 out of 3 of the patients after a follow up of 3 to 69 months. (Schlaberg et al., 2008).

In this paper, in contrast to the one by Patel and colleagues, it was mentioned that the viral loads and CD4 counts were stable during therapy and that concurrent HAART and imatinib resulted in appropriate control of both HIV infection and CML. Therefore there were no major drug interactions reported. (Schlaberg et al., 2008).

In our South African context, many of our HIV infected patients are at increased risk of opportunistic infections like tuberculosis (TB). (Schlaberg et al., 2008). Drug interactions are well documented for TB drugs especially rifampicin, which has a bactericidal antibacterial role in the therapy of TB, and forms the framework of TB therapy. (Schlaberg et al., 2008); (Haouala et al., 2010). Rifampicin induces CYP3A4, therefore causing decreased exposure of all 3 BCR-ABL TKI's available in our setting for treatment of CML namely; imatinib, nilotinib, and dasatinib (Schlaberg et al., 2008). This the means that while on TB therapy, CML patients on BCR-ABL TKI's will require a dosage increase of the BCR-ABL TKI therapy till completion of TB treatment (Schlaberg et al., 2008).

Chapter 2

Patients and methods

2.1   Aim and Objectives

To review all patients treated for CML at the CMJAH Medical Oncology unit from 2002 to 2015 to acertain:

1. Clinical presentation and stage of disease at time of diagnosis

2. Response to initial BCR-ABL1 TKI therapy

3. Disease progression and reason for change to second-line or third-line BCR-ABL1 TKI therapy

4. Adverse events on BCR-ABL1 TKI therapy

5. Development of BCR-ABL1 mutations

2.2   Methodology

2.2.1 Study type

This is a retrospective study involving a cohort of 101 patients with CML treated at the Medical Oncology Unit at CMJAH from 2002 to 2015.

Permission was obtained through the CEO at the Charlotte Maxeke Johannesburg Academic Hospital, Ms G Bogosi and through the Head of Medical Oncology at University of Witwatersrand Faculty of Health Sciences. Ethics approval was obtained from the University of Witwatersrand Human Research Ethics Committee (Medical) (Ethics Number: M140255) on the 03/03/2014.

2.2.2 Study population

This study looks at patients treated for CML with BCR-ABL1 TKIs and to see how the management of CML has advanced since the advent of BCR-ABL1 TKI's compared to the older treatment modalities, namely interferon-α +/- cytarabine, hydroxyurea and allogeneic stem cell transplantation.

2.2.2.1 Inclusion criteria

Patients with BCR-ABL1 positive CML

2.2.2.2 Exclusion criteria

BCR-ABL1 negative Patients

2.2.3 Patient clinical and molecular evaluation

Response to BCR-ABL TKIs was assessed clinically and by evaluating molecular response using reverse transcriptase real-time quantitative PCR (RQ-PCR) while on BCR-ABL TKI therapy, RQ-PCR values were referenced from the ELN guidelines. (See Table 1.3) RQ-PCR was in most instances done at 3 to 6 monthly intervals with regards to monitoring. A shortfall arose as some patients did not have complete monitoring of disease response to BCR-ABL1 TKI therapy recorded in the files studied.

2.2.4 Data collection and analysis

The initial data was collected using a data collection sheet that comprised several variables. The variables were tabulated allowing for correct insertion of information required for analysis.

The headings were as follows; age gender, family history, clinical features in the form of symptoms and signs, haematological features, date of death if death occurred, and adverse event profile. A modification was then made with molecular response by RQ-PCR being manually added to the data collection sheet.

Response to treatment in CML is assessed by evaluating molecular response, using standardized real time quantitative polymerase chain reaction (RQ-PCR), as well as cytogenetics although the first corrective measure when treating CML however is to achieve a complete haematological response.

As described above, a data collection sheet with variables that had to be answered was used to look at clinical features at diagnosis as well as at the effectiveness of BCR-ABL1 TKIs in the management of CP-CML. Secondary tasks were to see if patients developed mutations to BCR-ABL TKI therapy followed by evaluating the overall survival. The

following results were found to be true at this particular oncology unit with regards to the above hypotheses together with other variables such as demographics and presentation of CML.

2.2.4.1 Sokal score

The Sokal score was also one of the variables on the data collection sheet. This score is of prognostic significance as it assists with predicting the possible response and outcome of a particular individual to therapy prior to starting treatment. For this study only the Sokal score was used as a a prognostic index.

For the Sokal prognostic score to be calculated, certain variables had to be obtained. This was at times difficult as not all patient files had hard copies of the initial results or proper documentation of such results.

The Sokal score is calculated by the following formula:

"Exp 0.016 x (age- 43.3) + 0.0345 x (spleen- 7.51) + 0.188 x [(platelet count / 700) – 0.563] + 0.0887 x (blast cells – 2.10)" [1];[23]

However for this study, a computer generated App was used. The final calculation was then placed accordingly on a risk definition scale; with low risk Sokal score being < 0.8, intermediate risk 0.8– 1.2, and high risk >1.2.

A descriptive analysis format with tables was the most used format of analysis of age at presentation, family history of CML, whether the presentation was incidental or not, treatment response, mutational analysis and patient outcome. Graphs were used for presentation with symptoms and signs plotted respectively while pie charts were used to show the role of gender in the prevalence of CML, and disease stage at presentation. (See Figure 3.1).

2.3   Sample Size

The sample size includes 101 patients treated for CML with TKIs at CMJAH from 2002 to 2015.

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Chapter 3

Results
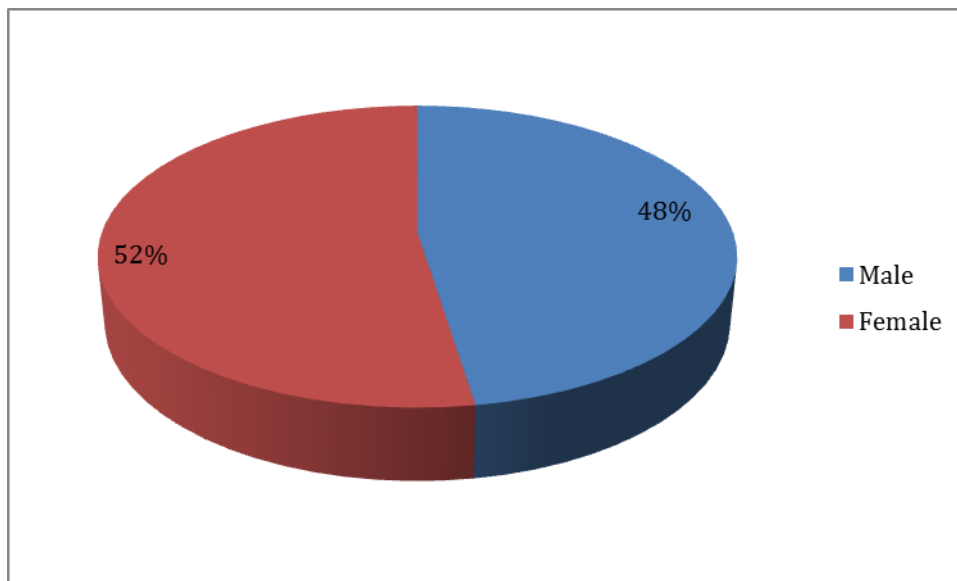
Age and Gender:

101 patients with CML were treated at the CMJAH Medical Oncology unit between 2002 and 2015 of whom 48% (number) were male and 52% (number) were female, with a median age of 40 years (Range)

Table 3.1: Age at Presentation

N	Median age	Percentiles		
		25	50	75
101	40.00	27.00	40.00	53.00

Figure 3.1: Gender

Presentation

CML Phase at presentation

89 (88%) of the patients presented with CP-CML with 1% in accelerated Phase and 11% phase unknown.
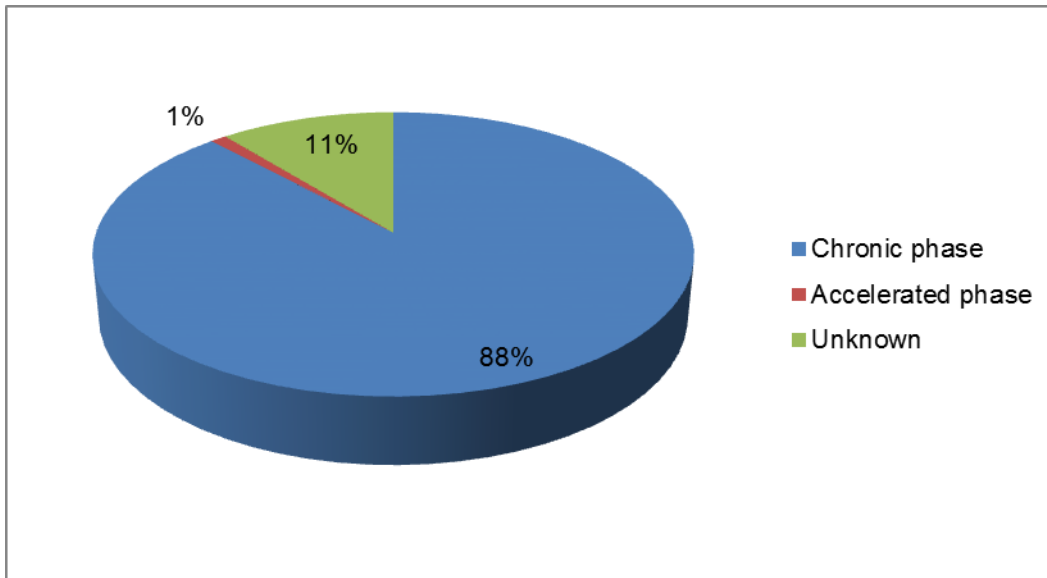
Figure 3.2: Disease phase at initial presentation

Clinical Presentation

92 (91%) of patients reported clinical symptoms and/or had documented signs on initial presentation while 9% presented incidentally. There was no familial link in our CML patients.

Table 3.2: Family History and Clinical Presentation

	Frequency	Percent
Family History	0	0%
No Family History	101	100%
Incidental finding	9	9%
Clinical signs and symptoms	92	91%

26

Symptoms and signs

Common symptoms at diagnosis were early satiety, fatigue and weight loss, and the commonest clinical sign found was splenomegaly.
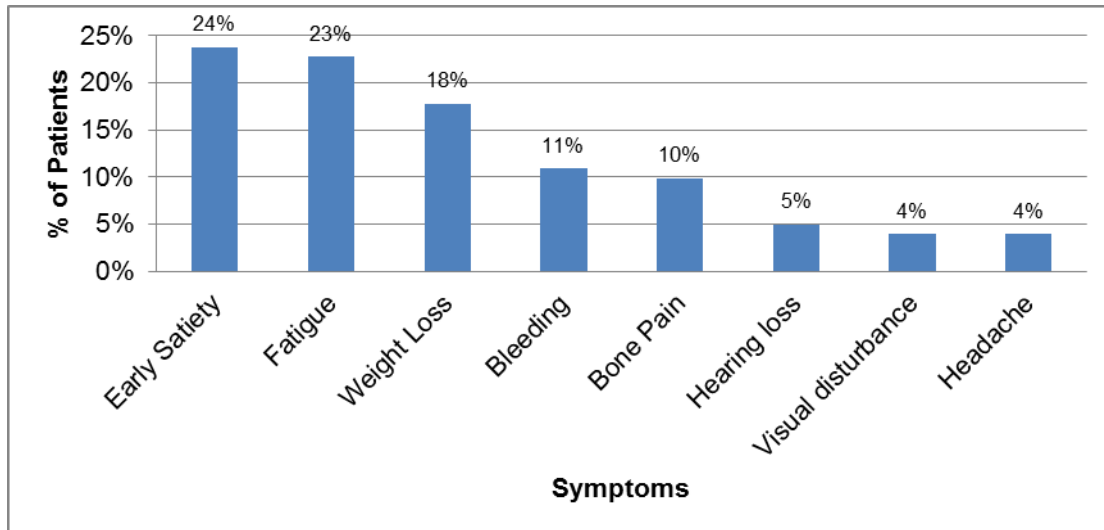
Figure 3.3: Clinical Symptoms
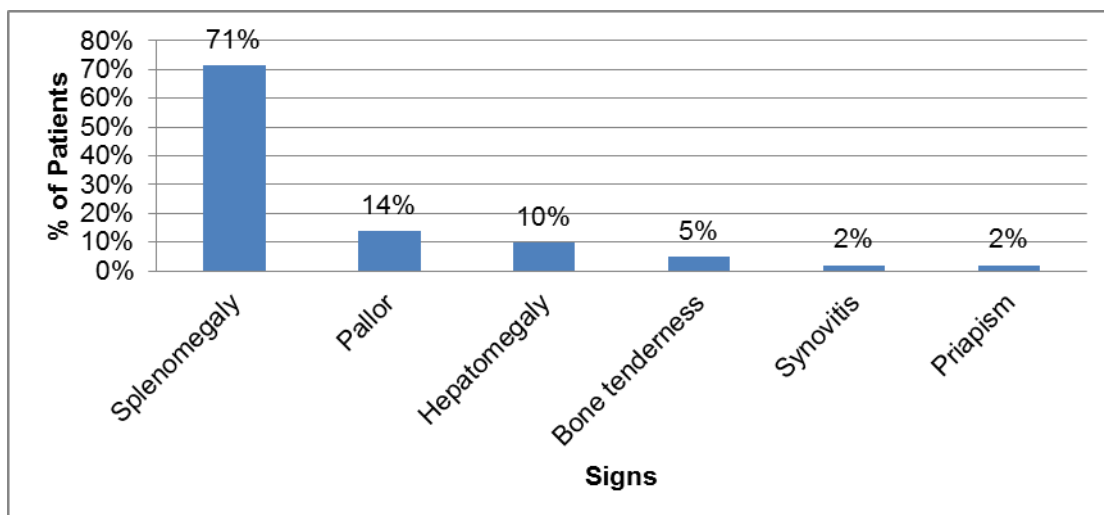
Figure 3.4: Clinical Signs

Prior therapy

Patients who were started therapy before the introduction of BCR-ABL TKI therapy, were treated with interferon-α therapy with or without cytarabine. 15 (15%) of patients in this cohort were initially on interferon-α while 85% had no prior exposure to interferon-α and started on imatinib as the 1st line therapy. 86 (85%) patients were diagnosed during or after the year 2002 when BCR-ABL TKI therapy started being available at the CMJAH Medical Oncology

Table 3.3: Prior Interferon-α Therapy

		Frequency	Percent
Interferon alpha	Yes	15	15%
	No	86	85%

Outcome

First line BCR-ABL1 TKI:

Of the 15 patients post interferon-α only 8 (53%) responded to imatinib, with 7 of these having a documented mean response time of 65.30 months. Seven (47%) patients in this cross-over group didn't respond to imatinib.

Of a total of 86 treatment-naïve patients only 85 (98.8%) were started on first line imatinib, with one (1.2%) patient never starting on BCR-ABL1 TKI therapy. 35% (30) of the 85 patients showed molecular response to first line imatinib therapy in a mean response time of 26.36 months, which was documented in 28 patients only. 64% of patients did not respond to first line imatinib therapy. When looking at both arms totaling 100 patients who received imatinib either as a cross-over from interferon based therapy or as first line therapy, 38% (n= 38) of these patients responded to first line imatinib at a mean time of 35.75 months.

Table 3.4: Prior interferon-α versus 1st line BCR-ABL TKIs.

First BCR-ABL TKI		Prior Interferon (n=15)	Treatment Naïve (n=86)	Total (n=101)
	Yes	100.0%	98.8%	99.1%
	No	0.0%	1.2%	0.9%
TKI MMol Response (MMR)		**n=15**	**n=85**	**n=100**
	Yes	53% (8)	35% (30)	38% (38)
	No	47% (7)	64% (54)	60% (61)
	Unknown	0% (0)	1% (1)	1% (1)
Mean Response Time(Months)		**n=7**	**n=28**	**n=100**
		65.30 months (SD =14.944)	26.36 months (SD =19.320)	35.75 months (SD =25.727)

Second line BCR-ABL1 TKI:

Seven patients from the initial interferon cohort not responding to imatinib after cross-over were then switched to a second line BCR-ABL TKI either nilotinib or dasatinib. Five patients went on to respond to 2nd line BCR-ABL TKI therapy but only 3 had a documented time of response with a mean of 28.97 months.

The interferon naïve cohort had a total of 54 patients who responded inadequately to first line imatinib. 35 (64.8%) of the 54 patients were switched to second line BCR-ABL TKI therapy. 12 out of 35 (34%) of these patients were shown to respond to 2nd line BCR-ABL TKI within mean a time of 8.65 months while 23 of the 35 (66%) patients never responded to second line BCR-ABL TKI therapy. One patient had inadequate documentation of response in both arms.

Overall, a total of 40 patients (of the 61 imatinib failures) were switched to second line BCR-ABL TKI therapy. 17 of the 40 (43%) responded to second line BCR-ABL TKI therapy. Only 13 patients of the 17 (76%) had a documented time for response with a mean time of 13.34 months while 23 patients (57%) responded inadequately to 2nd line BCR-ABL TKIs and one patient having inadequate documentation of response.

Table 3.5: 2nd line BCR-ABL TKI: Reasons for Switching and Molecular Response

		Prior Interferon (n=7)	Interferon-Naive (n=54)	Total (n=61)
Second BCR-ABL TKI	Yes	71.4% (5)	64.8% (35)	66.0% (40)
	No	28.6%	35.2%	34.0%
Reason for switch		n=5	n=35	n=40
	Disease Progression	100% (5)	89% (31)	90% (36)
	Intolerance or A/E to imatninb	0% (0)	11% (4)	10% (4)
TKI MMol Response (MMR)		n=5	n=35	n=40
	Yes	100% (5)	34% (12)	43% (17)
	No	0% (0)	63% (22)	55% (22)
	Unknown	0% (0)	3% (1)	3% (1)
Mean Response Time (Months)		n=3	n=10	n=13
		28.97 months (SD =16.928)	8.65 months (SD =6.799)	13.339 months (SD =12.721)

Third line BCR-ABL1 TKI:

Of the 22 patients who failed a second line BCR-ABL TKI, only 6 (27.3%) went on to third line BCR-ABL TKI therapy, either nilotinib or dasatinib leaving the remainder of the patients 16 (72.7%) not switching to any 3rd line therapy. Of the 6 patients who went on to 3rd line therapy, only one (16.7%) responded to therapy at a mean time of 18.3 months, while 5 patients did not respond to therapy.

Table 3.6: 3rd line BCR-ABL TKI: Reasons for Switching and Molecular Response

		Did not Start on Interferon (n=22)
Third BCR-ABL TKI	Yes	27.3% (6)
	No	72.7% (16)
Reason		n=6
	Disease Progression	66.7% (4)
	Intolerance to TKI or side effects	33.3% (2)
TKI MMol Response		n=6
	Yes	16.7% (1)
	No	83.3% (5)
Response Time (Months)		n=1
		18.30 months

Mutational analysis

BCR-ABL1 Mutational Analysis was performed on only 3 (3%) of the patients who either lost their MMR or didn't respond at all (never reached MMR) to BCR-ABL TKI therapy. Two patients had a pan-resistant T315I mutation, and were therefore resistant to imatinib, dasatinib, and nilotinib while one patient had a p-loop Y253H mutation, showing resistance to imatinib and to some extent nilotinib but not to dasatinib.

Table 3.7: Mutations analysed

		Frequency	Percent
Mutations	Yes	3	3%
	No	98	97%

## Follow up status

64% of patients are still attending the Medical Oncology clinic, while 36% have been lost to follow up.

Table 3.8: Follow-up Status

		Frequency	Percent
Status	Lost to Follow Up	36	36%
	On Follow Up	64	64%

Adverse Events

The adverse effects most experienced by the patients on BCR-ABL1 TKI therapy were cytopenias (39%), muscle cramps (25%), superficial oedema (9%), with rash, nausea and vomiting occurring less frequently. Of the 101 patients, only sixty three (63) had documented A/Es (in the above bar graph). Cytopenias were seen mainly in those patients on imatinib or dasatinib therapy especially neutropenia and/or thrombocytopenia, with a few patients had anaemia. Neutropenias or thrombocytopenias rarely were grade 3/4 (9%), although the 9 patients who had grade 3/4 cytopenias had to be switched to another BCR-ABL TKI.
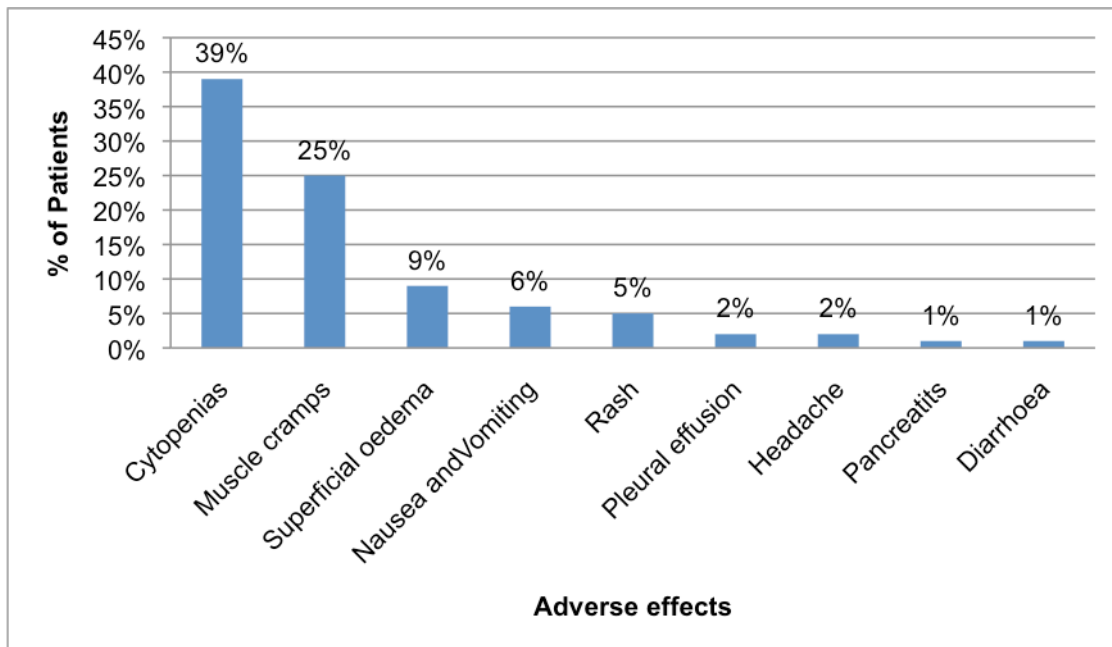
Figure3.5: Adverse Effects of BCR-ABL1 TKIs

Chapter 4

Discussion

CML has historically been shown to be potentially cured by performing an AlloSCT, with the best timing being in the CP1-CML. However since the advent of BCR-ABL1 TKIs, AlloSCT has been superseded as 1st line treatment of choice for newly diagnosed CP-CML. BCR-ABL TKIs have a favourable toxicity profile with deep molecular responses and prolonged PFS. This study initially looked at a cohort of 150 patients receiving BCR-ABL TKIs at the CMJAH Oncology unit, however only 101 patient files with CML were analysed as there were files of patients receiving imatinib for diagnoses other than CML, including gastrointestinal stromal tumours (GISTs).

Evidence shows that there is a male preponderance as compared to female patients that present with CML worldwide (Louw et al., 2012), however in this study there were slightly more females presenting with CML than males i.e. 53 (52%) females and 48 (48%) males. The median age of patients with CML in this cohort was 40 years, with the youngest patient being 16 years old and the most elderly being 80 years of age. In the literature review the median age was 50 years (Louw et al., 2012); (Walz et al., 2008), suggesting the possibility of a younger age of presentation in low middles income countries (LMICs) including South Africa.

Another interesting finding is that only 9 percent of patients presented incidentally with a raised WBC, with the remaining patients having documentation of clinical symptom/s or sign/s at presentation. This also varied from the literature review where most patients diagnosed with CML were said to present incidentally with a finding in the blood of a high WBC count without any presenting clinical features (Louw et al., 2012).

The most common symptoms reported was early satiety (24%), fatigue (23%), and weight loss (18%), demonstrated in the bar graph in the results section (Figures 3.3 and 3.4). Clinically the most common clinical sign was splenomegaly, with 71% of patients having this sign at presentation, which is not too varied from the evidence shown in the literature review (Louw et al., 2012). The size of the spleen was not uniformly recorded across the board although for the purpose of this study spleen size was required due to its importance as a variable in the calculation of the prognostic Sokal score (Sokal et al.,

1984). Another reason spleen size is important is to monitor disease response to BCR-ABL TKI therapy. However this was not feasible for this study as there was no continuity in objective documentation of spleen size at every doctor's visit in the files studied.

When looking at CML phase at presentation, the literature review and the study results were similar with most of the patients (88%) presenting in the CP-CML (Walz et al., 2008); (O'Brien et al., 2003). Only one patient presented in the accelerated phase while none of the patients presented *de novo* in the blast phase (BP). In 11% of patients the phase was not known at presentation, due to paucity in documentation in the patient files.

Some clinic patients came from an a STI-571 Expanded Access Programme (EAP), which was conducted before MCC registration of imatinib (Gleevec®) and the commencement of the GIPAP at the Medical Oncology Clinic in 2002/2003. Hence these patients' original clinical data was not in the clinic files and was not available for review.
Only 55% of the patients had all the variables available to calculate the Sokal prognostic score. This score is of significance as it makes for an important prognostic tool for newly diagnosed CML patients independent of response to BCR-ABL1 TKI therapy and is also useful in the early recognition of those patients who are likely to achieve favourable outcomes of OS and PFS. Of these patients; 20% had a low risk, 41.8% a moderate risk and 38.2% a high risk Sokal score.

Treatment response was analysed for 100 patients in total as one patient who presented to the clinic with a diagnosis of CML had a head injury resulting in an intracranial bleed. It was then decided by the attending physician at the time of presentation that this particular individual wasn't a candidate to receive BCR-ABL1 TKIs.
A molecular response was assessed as achieving at least MMR 3 (> 3 Log Reduction) in response to imatinib. Of the patients on the interferon-α group that crossed over to imatinib, 53% responded to their imatinib at a mean time of 65 months. In the 1st line imatinib cohort (interferon naive) 35% of the 85 patients responded to first line BCR-ABL TKI therapy at a mean duration of 26 months, while of the overall 100 patients, 38% responded to imatinib at a mean duration of 36 months.

The prior interferon group responded at a mean time of 28 months to second line BCR-ABL TKI therapy (nilotinib or dasatinib) compared to a mean time of 65 months it took for response to first line imatinib to occur, while the interferon naïve cohort took a mean time of 9 months to respond to second line BCR-ABL1 TKI therapy in comparison to a mean time of 26 months while on first line (imatinib) therapy.

Of the 6 patients switched to third line therapy (again either dasatinib or nilotinib) because of treatment failure or intolerance of second line BCR-ABL1 TKI therapy, only one patient responded at a mean time of 18 months.

Results reported in this study show that, as in most trials mentioned in the literature review comparing earlier treatment modalities and the newer BCR-ABL TKIs, there is a definite prolonged progression free survival and overall survival benefit and less transformation to advanced stage CML (Louw et al., 2012); (O'Brien et al., 2003). Those not responding to first line BCR-ABL1 TKI therapy or progressing to advanced stages of CML have an encouraging outlook as they can be switched to a second generation BCR-ABL1 TKI and achieve good disease response, including deep molecular responses. Also evident is the superiority of the second generation BCR-ABL1 TKIs in terms of shorter time to response achieved with dasatinib and/or nilotinib than with imatinib. Second generation BCR-ABL1 TKIs appear to be more efficacious, and have a comparatively better CML PFS than imatinib although in terms of OS there isn't yet enough data to compare outcomes.

Of the total 101 patients, only 3 (3%) were documented to have developed resistant mutations to the TKIs. Two of these patients had a pan-resistant T315I mutation, making them resistant to all three available TKIs in our setting possibly requiring the pan-TKI ponatinib which is not yet available in South Africa. One patient had a p-loop Y253H mutation which results in complete resistance to imatinib and partial resistance to nilotinib but sensitivity to dasatinib.

AlloSCT still remains the only treatment modality that can possibly cure CML patients but its role in therapy has been largely replaced by highly efficacious BCR-ABL1 TKI therapy. AlloSCT should therefore probably be reserved for patients who develop mutations, those who do not tolerate BCR-ABL TKIs and those who have disease progression or develop

accelerated or blast crises on available BCR-ABL TKI therapy although these patients need to be in a second CP (CP-2) CML to receive an allotransplant.

36 (36%) of our patients were lost to follow up, while 64 (64%) are still attending the Medical Oncology clinic to date with the majority having achieved a favourable response to 1st or 2nd line BCR-ABL1 TKIs of a MMR3 (>3 Log Reduction) or more. They also have achieved a prolonged OS and PFS and still remain on BCR-ABL1 TKI therapy.

The adverse effects most frequently reported in this cohort of patients on BCR-ABL1 TKI therapy were cytopenias, muscle cramps and superficial oedema. Very few patients experienced grade 3/4 A/Es including neutropenia and thrombocytopenia which were due mainly to dasatinib and imatinib, and required a switch to nilotinib which proved to be more tolerable with regards to their cytopenia.

Chapter 5

Conclusion

Patients treated at the CMJAH Medical Oncology Unit with BCR-ABL TKIs for CML generally show a moderate molecular response to both the first generation BCR-ABL1 TKI imatinib and to the second generation BCR-ABL TKIs, dasatinib and nilotinib, with at least an MMR3 (>3 Log Reduction) being achieved in 38% of first line and 43% of second line cohort of patients. However there is evidence to show that a quicker response time was achieved on a 2nd line BCR-ABL TKI (either dasatinib or nilotinib) than with 1st line imatinib therapy.

Where comparing time to molecular response, patients who received prior interferon, responded to a first line BCR-ABL1 TKI with a mean time to MMR of 65 months, while those who received a 2nd line BCR-ABL TKI responded with a mean time to MMR of 28 months. Also the interferon naïve group took a mean of 26 months to respond to 1st line imatinib compared to a mean of 9 months to respond to a 2nd line BCR-ABL1 TKI.

With both the interferon and imatinib group combined, the joint time to major molecular response to 2nd line BCR-ABL1 TKI therapy was 13 months compared to 35 months with first line imatinib.

The adverse event profile observed was mainly haematological with cytopenias especially neutropenia and/or thrombocytopenia being frequently observed. The culprit drug/s were more often dasatinib or imatinib, with a benefit achieved by switching to nilotinib.

At the time of data collection only three patients were found to have developed BCR-ABL1 mutations, two with a pan-resistant T315I mutation and one with a p-loop Y253H mutation. Patients who develop T315I mutations have no other treatment options as we do not have ponatinib available. These patients will eventually progress to advanced phases of CML and demise. Also AlloSCT is not possible in the event of progression to blast crisis as CP-2 CML is impossible to achieve without the necessary therapy.

However for the patients whose molecular responses have been favourable on BCR-ABL1 TKI therapy, whether 1st or 2nd line, prolonged OS and PFS have been achieved, with CP-1 of CML maintained without the need for salvage therapy including allogeneic stem cell transplantation. Indeed 64 (64%) patients remain on ongoing follow-up in chronic phase CML in our Medical Oncology clinic.

References

Baccarani, M., Deininger, M. W., Rosti, G., Hochhaus, A., Soverini, S., Apperley, J. F., et al., 2013. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. *Blood,* 122**,** 872-884.

Branford, S., Fletcher, L., Cross, N. C., Müller, M. C., Hochhaus, A., Kim, D.-W., et al., 2008. Desirable performance characteristics for BCR-ABL measurement on an international reporting scale to allow consistent interpretation of individual patient response and comparison of response rates between clinical trials. *Blood,* 112**,** 3330-3338.

Cortes, J. E., Kim, D.-W., Kantarjian, H. M., Brümmendorf, T. H., Dyagil, I., Griskevicus, L., et al., 2012. Bosutinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia: results from the BELA trial. *Journal of Clinical Oncology*, JCO. 2011.38. 7522.

Cortes, J. E., Kim, D.-W., Pinilla-Ibarz, J., Le Coutre, P., Paquette, R., Chuah, C., et al., 2013. A phase 2 trial of ponatinib in Philadelphia chromosome–positive leukemias. *New England Journal of Medicine,* 369**,** 1783-1796.

Druker, B. J., Guilhot, F., O'Brien, S. G., Gathmann, I., Kantarjian, H., Gattermann, N., et al., 2006. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *New England Journal of Medicine,* 355**,** 2408-2417.

Forrest, D. L., Trainor, S., Brinkman, R. R., Barnett, M. J., Hogge, D. E., Nevill, T. J., et al., 2009. Cytogenetic and molecular responses to standard-dose imatinib in chronic myeloid leukemia are correlated with Sokal risk scores and duration of therapy but not trough imatinib plasma levels. *Leukemia research,* 33**,** 271-275.

Giles, F., O'Dwyer, M. & Swords, R. 2009. Class effects of tyrosine kinase inhibitors in the treatment of chronic myeloid leukemia. *Leukemia,* 23**,** 1698-1707.

Gorre, M. E., Mohammed, M., Ellwood, K., Hsu, N., Paquette, R., Rao, P. N., et al., 2001. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science,* 293**,** 876-880.

Gratwohl, A. 2012. The EBMT risk score. *Bone marrow transplantation,* 47**,** 749-756.

Grigg, A. & Hughes, T. 2006. Role of allogeneic stem cell transplantation for adult chronic myeloid leukemia in the imatinib era. *Biology of Blood and Marrow Transplantation,* 12**,** 795-807.

Gupta, A. & Khattry, N. 2014. Current status of hematopoietic stem cell transplant in chronic myeloid leukemia. *Indian journal of medical and paediatric oncology: official journal of Indian Society of Medical & Paediatric Oncology,* 35**,** 207.

Haouala, A., Widmer, N., Duchosal, M. A., Montemurro, M., Buclin, T. & Decosterd, L. A., 2010. Drug interactions with the tyrosine kinase inhibitors imatinib, dasatinib, and nilotinib. *Blood***,** blood-2010-07-294330.

Hasford, J., Baccarani, M., Hoffmann, V., Guilhot, J., Saussele, S., Rosti, G., 2011. Predicting complete cytogenetic response and subsequent progression-free survival in 2060 patients with CML on imatinib treatment: the EUTOS score. *Blood,* 118**,** 686-692.

Hasford, J., Pfirrmann, M., Hehlmann, R., Allan, N. C., Baccarani, M., Kluin-Nelemans, J. C., 1998. A New Prognostic Score for Survival of Patients With Chronic Myeloid Leukemia Treated With Interferon AlfaWriting Committee for the Collaborative CML Prognostic Factors Project Group. *Journal of the National Cancer Institute,* 90**,** 850-859.

Hughes, T., Deininger, M., Hochhaus, A., Branford, S., Radich, J., Kaeda, J., 2006. Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. *Blood,* 108**,** 28-37.

Hughes, T. P., Kaeda, J., Branford, S., Rudzki, Z., Hochhaus, A., Hensley, M. L., et al., . 2003. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *New England Journal of Medicine,* 349**,** 1423-1432.

Jabbour, E., Cortes, J. & Kantarjian, H. 2007. Optimal first-line treatment of chronic myeloid leukemia. How to use imatinib and what role for newer drugs? *Oncology (Williston Park, NY),* 21**,** 653-62; discussion 663-4, 667-8.

Kantarjian, H., Giles, F., Wunderle, L., Bhalla, K., O'Brien, S., Wassmann, B., et al., 2006. Nilotinib in imatinib-resistant CML and Philadelphia chromosome–positive ALL. *New England Journal of Medicine,* 354**,** 2542-2551.

Kantarjian, H., Shah, N. P., Hochhaus, A., Cortes, J., SHAH, S., Ayala, M., et al., 2010. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. *New England Journal of Medicine,* 362**,** 2260-2270.

Larson, R. A., 2015. Is there a best TKI for chronic phase CML? *Blood,* 126**,** 2370-2375.

Louw, V., Dreosti, L., Ruff, P., Jogessar, V., Moodley, D., Novitzky, N., et al., 2012. Recommendations for the management of adult chronic myeloid leukaemia in South Africa: guideline, South African Medical Journal, 101 (11) 2011: pp. 840-846: erratum. *South African Medical Journal.*

NCCN Clinical Practice Guidelines in Oncology. Chronic Myelogenous Leukaemia. Version 4.2015. www.nccn.org. (Accessed October 15, 2016)- O'BRIEN

O'Brien, S. G., Guilhot, F., Larson, R. A., Gathmann, I., Baccarani, M., Cervantes, F., 2003. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *New England Journal of Medicine,* 348**,** 994-1004.

Patel, M., Philip, V., Fazel, F., Lakha, A., Vorog, A., Ali, N., 2012. Human immunodeficiency virus infection and chronic myeloid leukemia. *Leukemia research,* 36**,** 1334-1338.

Sacha, T., 2013. Imatinib in chronic myeloid leukemia: an overview. *Mediterranean journal of hematology and infectious diseases,* 6**,** 2014007.

Saglio, G., Kim, D.-W., Issaragrisil, S., Le Coutre, P., Etienne, G., Lobo, C., 2010. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. *New England Journal of Medicine,* 362**,** 2251-2259.

Schiffer, C. A. 2007. BCR-ABL tyrosine kinase inhibitors for chronic myelogenous leukemia. *New England Journal of Medicine,* 357**,** 258-265.

Schlaberg, R., Fisher, J. G., Flamm, M. J., Murty, V. V., Bhagat, G., Alobeid, B. 2008. Chronic myeloid leukemia and HIV-infection. *Leukemia & lymphoma,* 49**,** 1155-1160.

Singh E, Sengayi M, Urban M Babb, C., Kellett, P., Ruff, P., 2013. The South African National Cancer Registry: An update. Lancet Oncol 2014; 15: 363 - 364. www.ncr.ac.za (Accessed October 20, 2016)

Sokal, J. E., Cox, E. B., Baccarani, M., Tura, S., Gomez, G. A., Robertson, J. E., 1984. Prognostic discrimination in" good-risk" chronic granulocytic leukemia. *Blood,* 63**,** 789-799.

Soverini, S., Colarossi, S., Gnani, A., Castagnetti, F., Rosti, G., Bosi, C., 2007. Resistance to dasatinib in Philadelphia-positive leukemia patients and the presence or the selection of mutations at residues 315 and 317 in the BCR-ABL kinase domain. *Haematologica,* 92**,** 401-404.

Soverini, S., Hochhaus, A., Nicolini, F. E., Gruber, F., Lange, T., Saglio, G., 2011. BCR-ABL kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: recommendations from an expert panel on behalf of European LeukemiaNet. *Blood,* 118**,** 1208-1215.

Uptodate 2015. Clinical use of tyrosine kinase inhibitors for chronic myeloid leukaemia: Peer review. www.uptodate.com (Accessed October 15, 2016).

Van Etten RA. Uptodate 2016. Clinical manifestations and diagnosis of Chronic Myeloid Leukaemia: Peer review. www.uptodate.com. (Accessed October 20, 2016).

Walz, C., Cross, N. C., Van etten, R. A. & Reiter, A. 2008. Comparison of mutated ABL1 and JAK2 as oncogenes and drug targets in myeloproliferative disorders. *Leukemia,* 22**,** 1320-1334.