The Screening for Single Nucleotide Polymorphisms of *CYP3A4* in Chronic Myelogenous Leukemia Patients Receiving Imatinib

By

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DECLARATION

I certify that the dissertation hereby submitted by me for the Masters in Medical Science (M.Med.Sc) degree at the University of the Free State, is my independent work and has not previously been submitted by me for a degree at another University/Faculty. I furthermore waive copyright of the dissertation in favour of the University of the Free State.

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GA Lamprecht

We shall not cease from exploration! And the end of all our exploring will be to arrive where we started and know the place for the first time.

~ TS Eliot ~

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Abbreviations

3’	3 prime end
5’	5 prime end
℃	Degrees Celsius
3D	Three dimensional
A	Adenine
A1AGP	Alpha 1 or α1 – acid glycoprotein
ABL	Abelson
ADR	Adverse drug reaction
ALL	Acute lymphoblastic leukaemia
ATP	Adenosine tri-phosphate
BCR	Breakpoint cluster region
bp	base pairs
C	Cytosine
c-abl	Normal *ABL* gene
c-kit	Cytokine receptor (CD117, KIT)
CETP	Colesterol ester transfer protein
CHR	Complete haematologic remission
CI	Confidence interval
CML	Chronic myeloid leukaemia
CO	Carbon monoxide
CYP	Cytochrome
CYP450	Cytochrome P450 enzyme
CYP3A4	Cytochrome 3A4 enzyme
de novo	New
dNTP	Deoxyribonucleotide triphosphate (adenine, thymine, guanine, cytosine)
Cytochrome b5	CYP450 Co-enzyme
DNA	Deoxyribonucleic acid
e^-	Electron
EDTA	Ethylenediaminetetraacetic acid
ETOVS	Ethics committee of the Faculty of Health Sciences of the Free State
f	Frequency

F	Forward primer
FDA	Food and drug administration
Fe	Iron (Fe^{2+} and Fe^{3+})
G	Guanine
g	Gram
in vitro	Outside the living organism
in vivo	Inside living organism
kDa	kiloDalton
kV	kilo volt
M	Mole
mA	mili amp
mg/day	milligram per day
$MgCl_2$	Magnesium chloride
min	minute
ml	millilitre
mM	millimolar
mol	mole
mRNA	Messenger ribonucleic acid
NADPH	Nicotinamide adenine dinucleotide phosphate
ng	nano gram
N	Unknown nucleotide
NHS	National Health Service
NH_4CL	Ammonium chloride
NH_4HCO_3	Ammonium bicarbonate
OCT-1	Organic cation transporter -1
PAH	Polycyclic aromatic hydrocarbons
PCR	Polymerase chain reaction
PDB	Protein data bank
PDGF	Platelet derived growth factor
pmol	Pico mol
P-Fisher	Fisher’s exact probability test
P-Value	Probability value
Ph	Philadelphia
pH	Potential of hydrogen
POP-6	Performance optimized polymer no. 6

R	Reverse primer
RH	Substrate
RNA	Ribonucleic acid
ROH	Product of biosynthesis plus one electron
rpm	revolutions per minute
sec	Seconds
SCT	Stem cell transplantation
SNP	Single nucleotide polymorphism
SNPs	Single nucleotide polymorphisms
STI571	Signal transduction inhibitor
T	thymine
TAE	Tris-acetate-EDTA
TD	Tardive dyskinesia
TK	Tyrosine Kinase
Tris	Tris (hydroxymethyl) aminomethane
Tris-HCl	Tris (hydroxymethyl) aminomethane hydrochloride
tRNA	Transfer ribonucleic acid
U	Unit(s)
µA	Micro amp
µl	Micro litre
µg	Micro gram
UK	United Kingdom
USA	United States of America
UTR	Untranslated region
UV	Ultra violet
v	Version
V	Volt

Preface

Imatinib mesylate (Gleevec®) has become the gold standard for treating chronic myeloid leukaemia (CML). This drug restores crucial cell processes including apoptosis, by inhibiting the BCR-ABL tyrosine kinase responsible for the disease. CML patients generally respond well to imatinib treatment with approximately 70% of these achieving progression free survival. However, there are reports that some patients experience adverse drug reactions (ADRs) to imatinib. In the event of ADRs developing to a drug, it is common practice to reduce the dose, resort to a different drug or terminate treatment. Due to the concern that lowering the dose of imatinib could increase chances of developing resistance to imatinib, the current recommendation is that treatment be stopped, rather than reduced. In theory, if ADRs are a result of over exposure to imatinib, the dose could be lowered as long as the required levels of imatinib in the plasma were maintained without exposing the patient to risk of developing resistance.

The CYP3A4 enzyme is responsible for the metabolism of imatinib. Reduced activity of CYP3A4 can lead to an overexposure of the body to imatinib and result in an ADR. Conversely, an over active CYP3A4 can result in reduced efficacy of the drug. Thus the aim of this study was to screen the exons of *CYP3A4* for single nucleotide polymorphisms (SNPs) and determine whether any of these are associated with the presence of ADRs. The rationale for investigating SNPs, is that they can result in a change in amino acid sequence of the CYP3A4 enzyme, responsible for the break down of imatinib, that impact on the metabolic rate of the enzyme. Decreased metabolism could lead to the development of ADRs, while an increased metabolic rate could result in inefficient treatment.

This dissertation consists of 3 chapters, including a literature review (chapter 1), research aim and methodology (chapter 2), as well as results and discussion (chapter 3). The literature review presents a summary of the literature regarding CML disease, its treatment with imatinib and the CYP3A4 enzyme responsible for its metabolism. Chapter 2 includes the research aim and the methods used. The results and interpretation thereof are discussed in the final chapter. Throughout this dissertation, reference is made to specific genes and their protein products. Gene

names are referred to in the *italic* form and the protein/enzyme in normal text. Capital letters may be used to denote either a gene or its corresponding protein, according to the convention used in published literature.

Chapter 1: Literature Review

1.1 Chronic Myeloid Leukaemia (CML)

Chronic myeloid leukaemia (CML) is a myeloproliferative neoplastic disorder of haematopoietic stem cells, characterised by an increase in myeloid cells, predominantly granulocytes, in peripheral blood (Johnson *et al.* 2003; Gupta and Prasad 2007). CML occurs with a frequency of approximately one to two in every 100,000 individuals per annum and accounts for approximately 15% of newly diagnosed cases of adult leukaemia (Johnson *et al.* 2003). The median age at diagnosis is 53 years, but this disease affects all age groups, including children (Sawyers 1999).

CML is characterized by progressive granulocytosis, marked bone marrow hyperplasia and splenomegaly (Iqbal *et al.* 2004). Typical symptoms of CML at presentation include thrombocytosis, fatigue, weight loss, fever, anaemia and weakness (Sawyers 1999; Iqbal *et al.* 2004). Approximately 40% of CML patients at diagnosis are asymptomatic and are diagnosed as a result of atypical blood counts (Sawyers 1999). The disease progresses in three phases, from a benign chronic phase, to an accelerated phase and ending in the fatal blast crises, within three to five years from onset (Johnson *et al.* 2003). During the chronic phase, white blood cells continue to mature, whereas during the accelerated phase and blast crises, immature white blood cells (blasts) accumulate in peripheral blood (Sawyers 1999).

The genetic basis of CML, is the presence of the Philadelphia (Ph) chromosome (Nowell and Hungerford 1960). The Ph-chromosome is the result of a reciprocal translocation between the long arms of chromosomes 9 and 22, giving rise to the juxtaposition of *BCR* (breakpoint cluster region) and *ABL* (Abelson) genes on a shortened chromosome 22 (Figure 1) (Mauro and Druker 2001). The Ph chromosome is present in approximately 95% of CML patients. The other 5% have complex or alternative translocations involving additional chromosomes that may also result in the fusion of the *BCR* and *ABL* genes (Sawyers 1999).

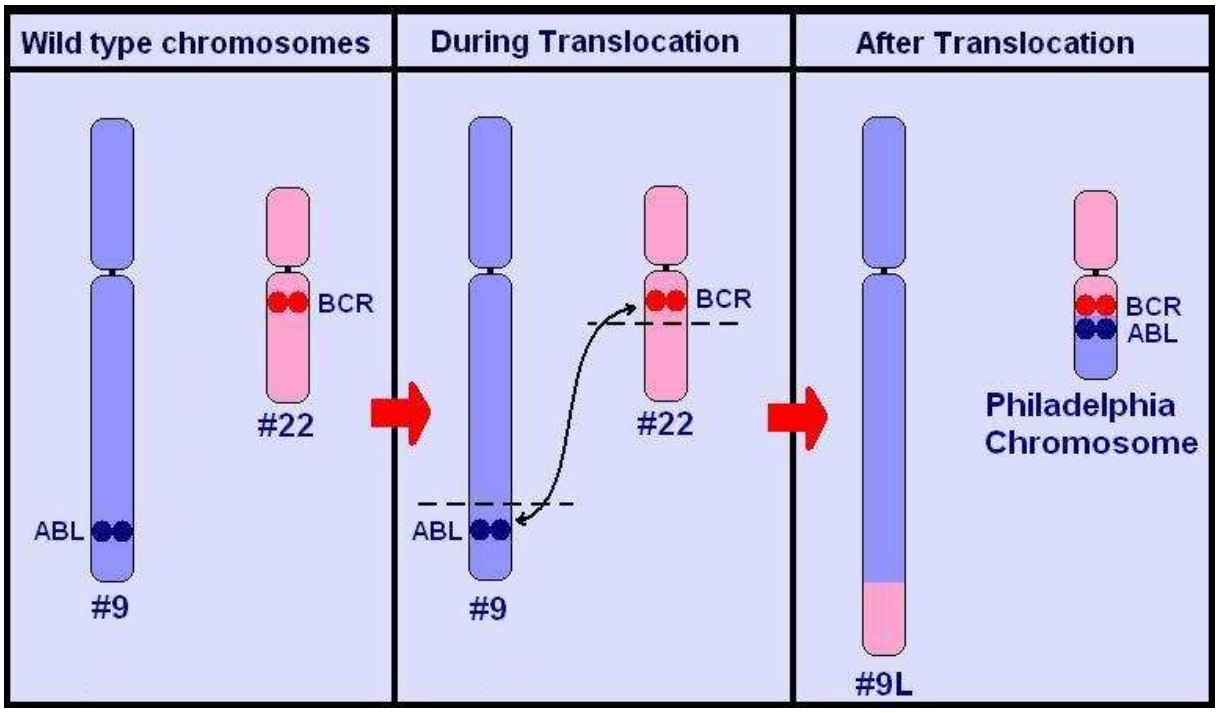


Figure 1: Schematic representation of the reciprocal translocation between the long arms of chromosome 9 and 22, resulting in a longer chromosome 9 and a shortened chromosome 22, known as the Philadelphia (Ph) chromosome. The Ph chromosome harbours the *BCR-ABL* oncogene (Figure adapted from http://www.budapeststudent.com/notes/pphys/hematology3.htm).

The BCR-ABL oncoprotein is a constitutively active tyrosine kinase, which interferes with multiple signal transduction pathways, which in turn affect cell growth, differentiation and death (Ottmann and Wassmann 2003; Iqbal *et al.* 2004). The BCR-ABL oncoprotein can vary in size, i.e. 185 kDa (p185), 210 kDa (p210) or 230 kDa (p230), depending on the breakpoint site in the *BCR* gene (Figure 2) (Mauro and Druker 2001). The majority (95%) of all CML patients express the p210 BCR-ABL protein, whereas patients with Ph positive ALL (acute lymphoblastic leukaemia) express either the p190 or p210 BCR-ABL protein. The rate of disease development for patients with the p230 BCR-ABL protein appears to be at a slower compared to those with the p210 or p190 BCR-ABL protein (Sawyers 1999). Each of the BCR-ABL fusion variants contains the ABL tyrosine kinase that is characteristic of CML (Sawyers 1999).

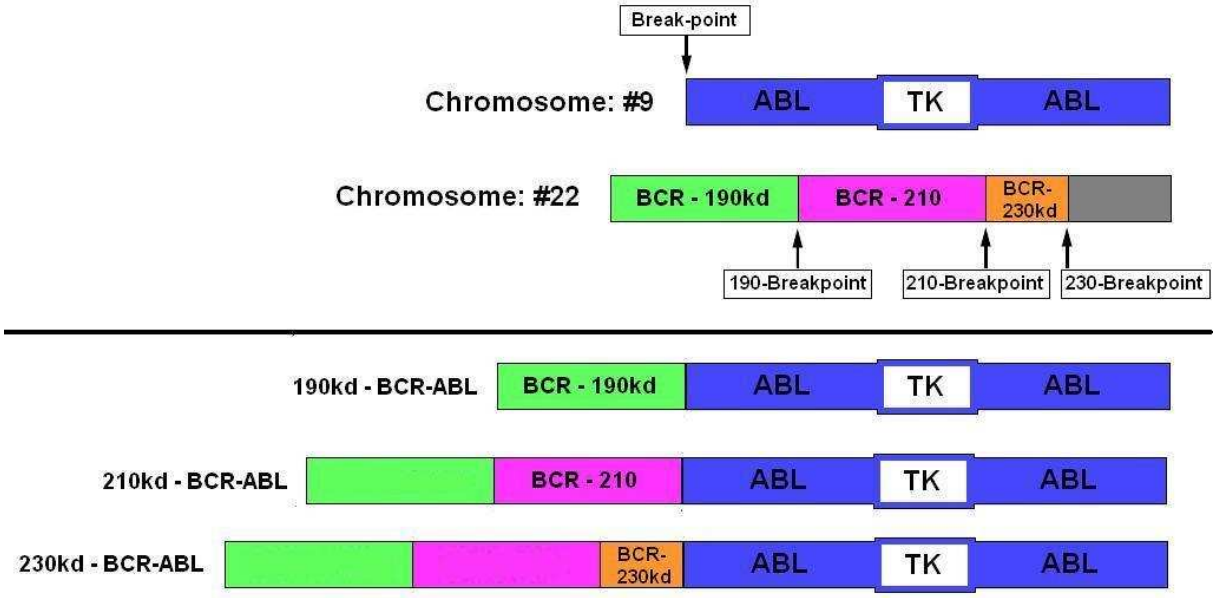


Figure 2: The three different BCR-ABL fusion proteins in CML. The size of the BCR-ABL protein depends on the breakpoint in the *BCR* gene. TK denotes the tyrosine kinase domain, which is located in ABL (Figure was adapted from Sawyers (1999)).

1.2 Tyrosine kinase and signal transduction

In general terms, a kinase enzyme transfers a phosphate group from a donor molecule (e.g. ATP) to a substrate molecule, which has a lower energy potential than the donor, by phosphorylation. Tyrosine kinase specifically transfers a phosphate group from ATP to tyrosine amino acids. Tyrosine kinase enzymes form a subgroup of a larger protein kinase group, which all play a vital role in normal cellular signal transduction (Tang *et al.* 2007).

Signal transduction is the process whereby a signal or stimulus is converted to a form that can be recognized within the cell. The transduction process incorporates a series of biochemical reactions carried out by specific enzymes and forms an integrated mechanism to relay information within and between cells (Mukherjee *et al.* 2006; Shenoy 2007). The activation of genes, altered metabolism, cell proliferation, cell division, differentiation and cell death (apoptosis) are typical cellular responses associated with the signal transduction pathways (Lalli and Sassone-Corsi 1994; Tang *et al.* 2007). A deregulation of any of these cellular responses, apoptosis in particular, can lead to any number of diseases. For example, apoptosis is overactive in Alzheimer's, Huntington's and Parkinson's disease, and infrequent in CML and

ALL (Robertson *et al.* 2000; Blume-Jensen and Hunter 2001). Over active signalling can result due to mutations, gene amplification or oncogenic fusion such as *BCR-ABL* in the case of CML (Li and Hristova 2006).

1.3 Treatment of CML

Options for CML treatment include bone marrow transplantation (allogeneic stem cell transplantation), cytoreductive chemotherapeutic drugs, Interferon-α (alpha) and tyrosine kinase inhibitors (Johnson *et al.* 2003).

1.3.1 Cytoreductive chemotherapy

CML cells are sensitive to several oral chemotherapeutic drugs including busulfan and hydroxyurea (Sawyers 1999). Approximately 90% of CML patients treated with these chemotherapeutic drugs achieve a complete haematologic remission (CHR)[1] (Golas *et al.* 2003). The use of busulfan, to treat CML, started in 1953 and at that time was regarded as the treatment of choice, since it produced better treatment results than radiation therapy (Henkes *et al.* 2008). However, busulfan is associated with a number of adverse drug reactions (ADRs) such as the risk of bone marrow aplasia, infertility, pulmonary, hepatic and cardiac fibrosis (Silver *et al.* 1999). As a result of ADRs associated with busulfan, hydroxyurea came into use during the 1960's (Silver *et al.* 1999). Compared to busulfan, Hydroxyurea has fewer reports of ADRs (Sawyers 1999). However, although both busulfan and hydroxyurea induce CHR, the majority of CML patients do not achieve a cytogenetic response[2] and none a molecular response[3] (Henkes *et al.* 2008). Thus, bone marrow transplantation is still preferred over chemotherapeutic treatment, due to the greater chance of curing the disease.

[1] A complete haematologic remission (CHR) is characterized by an absence of immature cells in peripheral blood, a white blood cell count less than 10 x 10^9/L, a platelet count less than 450 x 10^9/L and no obvious signs of splenomegaly. Furthermore this treatment response has to be maintained for at least four weeks.

[2] A cytogenetic response is characterized in four stages, namely complete, partial, minor or minimal. A complete cytogenetic response is characterized by the absence of the Ph-chromosome. For partial cytogenetic response the Ph chromosome is present at 1 to 35%, minor response at 36 to 55% and minimal response at 66 tot 95%.

[3] A molecular response is defined as a decrease in BCR-ABL transcript levels, with a complete response when transcript level is below the level of detection (0.01%) and a major molecular response is when the levels of BCR-ABL transcripts are lower than 0.1%.

1.3.2 Allogeneic stem cell transplantation (Bone marrow transplantation)

Stem cell transplantation (SCT) is currently the only curative approach for CML (Henkes *et al.* 2008). However, SCT in conjunction with chemotherapy has proven to be more successful than SCT alone. The success rate of SCT is 50 to 60% among CML patients in the chronic phase (Sawyers 1999). The disadvantage of SCT is finding a suitable bone marrow donor, as a result only 15 to 20% of patients are potential candidates. In addition, SCT has a high probability of side effects such as immunodeficiency, infection, organ toxicity and acute as well as chronic graft versus host disease (Sawyers 1999; Mauro and Druker 2001; Johnson *et al.* 2003; ISSCR: http://www.isscr.org/clinical_trans/pdfs/ISSCRPatientHandbook.pdf).

1.3.3 Interferon-α

In comparison to busulfan and hydroxyurea, interferon-α has been shown to induce complete haematologic and cytogenetic remission, with a lower incidence of ADRs in CML patients (Sawyers 1999; Silver *et al.* 1999; Johnson *et al.* 2003; Silver *et al.* 2003; Wang and Seed 2003). However, ADRs such as fatigue, arthralgias, weight loss, myalgias, headaches, depression, diarrhoea, neurological symptoms, memory changes, hair thinning and cardiomyopathy have been reported to hamper the efficacy of interferon-α treatment (Talpaz *et al.* 1991; Sacchi *et al.* 1995; Wetzler *et al.* 1995; O'Brien *et al.* 1996). In some cases, resistance to interferon-α has been shown to develop in CML patients (Johnson *et al.* 2003; Kühr *et al.* 2003).

1.3.4 Imatinib mesylate (Gleevec®)

1.3.4.1 Background

The development of the tyrosine kinase inhibitor, imatinib mesylate, took the treatment of CML into a new chapter. Imatinib, also known as Gleevec, formerly known as STI571 (Signal transduction inhibitor – 571), is a potent tyrosine kinase inhibitor, which is considered to be reasonably non-toxic, and able to induce a molecular response in CML patients in the chronic phase (Gambacorti-Passerini *et al.* 2003). Imatinib was developed by Novartis in 1990 and approved by the FDA in 2001 for the treatment of chronic phase CML patients (Hamada *et al.* 2003; Johnson *et al.* 2003; Monroy *et al.* 2007; Henkes *et al.* 2008).

A series of trials determined that imatinib is superior to interferon-α with regards to treatment efficacy and drug safety (Hamada *et al.* 2003; Johnson *et al.* 2003). The first trial of imatinib included 83 CML patients who had failed on interferon-α or who could not tolerate the drug (Henkes *et al.* 2008). According to Novartis, no ADRs hampering the treatment were encountered by patients participating in the trial. However, patients did experience toxicity at doses higher than 750 mg/day (Henkes *et al.* 2008). Approximately 98% of trial patients on imatinib experienced a complete haematological response, with 31% of these achieving a major cytogenetic response (Druker *et al.* 2001; Gupta and Prasad 2007). Recent results have shown that up to 70% of CML patients on imatinib achieve a complete cytogenetic response after 12 months of therapy, and 89% after 60 months (Druker *et al.* 2006).

To date, numerous trials and studies have focussed on the efficacy and safety of imatinib. However, only a few studies have reported on the pharmacokinetic characteristics of imatinib (le Coutre *et al.* 2004). Pharmacokinetics focuses on the absorption, metabolism, distribution and excretion of a drug, which all have an imperative role in treatment outcome. Although ADRs related to treatment with imatinib have been described, the pharmacokinetic link with these ADRs has not been investigated thoroughly.

1.3.4.2 Functioning of imatinib mesylate

At first it was thought that imatinib inhibits the BCR-ABL tyrosine kinase activity by acting as a competitive inhibitor through binding to the ATP-binding pocket, thus preventing ATP from binding (Gambacorti-Passerini *et al.* 2003). However, recent studies have shown that imatinib only occupies a part of the ATP binding domain of the tyrosine kinase resulting in the kinase to be stabilised in its inactive, non-ATP binding form (Schindler *et al.* 2000; Gambacorti-Passerini *et al.* 2003). According to the manufacturer, Novartis, imatinib produces comparable inhibition for different forms of tyrosine kinase such as abl (Abelson), c-abl (normal ABL), PDGF-R (platelet derived growth factor receptor) and c-kit (cytokine receptor) tyrosine kinases (le Coutre *et al.* 2004). However, these findings are subject to change, as the different factors involved with pharmacokinetics can be altered.

1.3.4.3 Pharmacokinetics of imatinib mesylate

Pharmacokinetic studies focus on the factors and time taken to transform the parent compound, such as imatinib, to its active metabolite as well as the time taken to further break down the compound or excretion. The active metabolite of imatinib (CGP74588) is present in the body for between 30 to 50 hours. Imatinib (Figure 3) has a half life of approximately 19 hours with a range of between 14 hours to 23 hours (de Kogel and Schellens 2007). However, recent research has suggested that the prolonged exposure to imatinib may be the cause for the development of ADRs, especially if a patient is sensitive to the active metabolite (Lin *et al.* 2003; Peng *et al.* 2005).

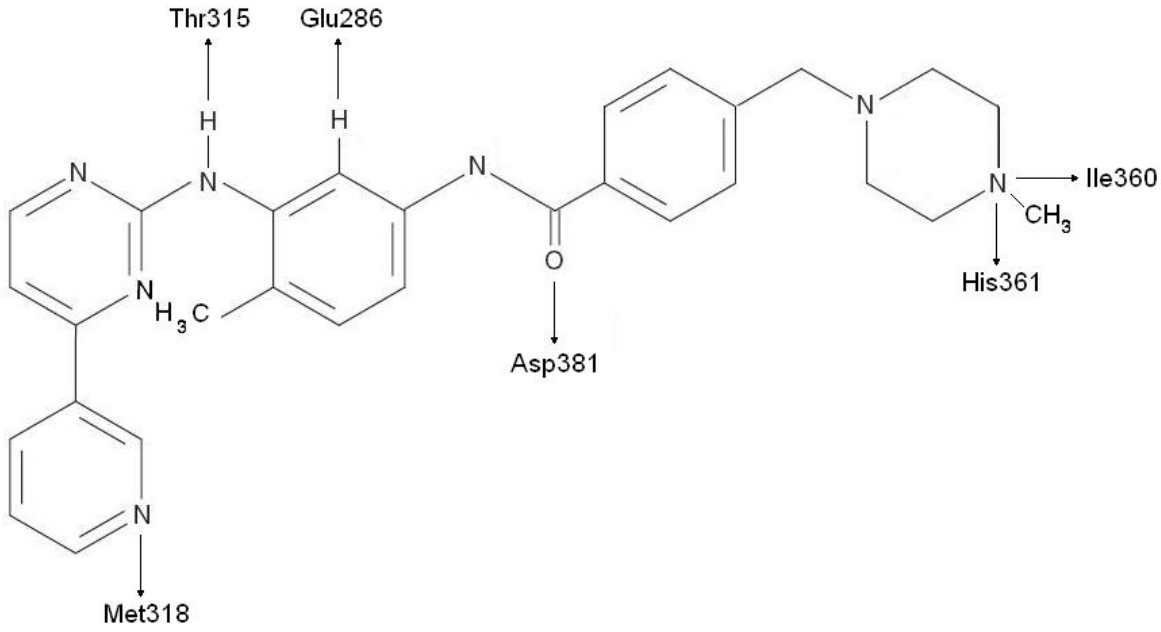


Figure 3: Structural formula of imatinib mesylate (4-[(4-methyl-1-piperazinyl1)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl] benzamide methanesulfonate) ($C_{29}H_{31}N_7O.CH_4SO_3$). The main metabolite of imatinib is CGP74588 ($C_{29}H_{33}N_7SO_4$), also known as N-desmethyl-imatinib (the structural formula of imatinib was copied from Peng *et al.* (2005) and Henkes *et al.* (2008).

1.3.4.4 Adverse drug reactions (ADRs) associated with imatinib treatment

Imatinib is prescribed at an initial dose of 400 mg/day for patients in the chronic phase and 600 mg/day for patients in the accelerated phase or blast crises of CML (Mauro and Druker 2001). The dose is adjusted according to treatment response as well as ADRs. The dose of imatinib can be increased when response to treatment is

not favourable (e.g. 800 mg/day) or decreased when ADRs develop (e.g. 300 mg/day) (Johnson *et al.* 2003; le Coutre *et al.* 2004; Cohen and Tang 2006). The ADRs experienced from imatinib can range from mild to severe.

The FDA accelerated the approval of imatinib during 2001 due to its treatment efficacy and favourable toxicity profile over interferon-α (Johnson *et al.* 2003; Henkes *et al.* 2008). The approval was based on a short follow-up period and as a result, not all the long-term ADRs were determined. However, a longer post release follow-up identified additional ADRs not reported initially. The ADRs from imatinib can be grouped into serious and less serious events. Less serious ADRs are usually managed with medication to provide symptomatic relief and the major ADRs on the other hand, are dealt with a temporary cessation in treatment (Table 1). According to Henkes *et al.* (2008) and Johnson *et al.* (2003), when a patient experiences ADRs, it is better to cease treatment rather than decrease dosage. Dose reduction is not considered due to the possible development of resistance to imatinib. However, if the ADRs are a result of prolonged exposure, it may be possible to reduce the dosage without compromising plasma drug levels and treatment efficacy.

Table 1: Major and minor ADRs experienced from imatinib treatment.

Major ADR	Reference	Minor ADR	Reference
Haematological cytopenias	Henkes *et al.* 2008; Hughes *et al.* 2003; O'Brien *et al.* 2003a; Sneed *et al.* 2003; Sawyers *et al.* 2002; Talpaz *et al.* 2002; Pestina *et al.* 2001; Simmons *et al.* 1990	Gastrointestinal side effects	Monroe *et al.* 2007; Johnson *et al.* 2003; Veronese *et al.* 2003; Cohen *et al.* 2002; Deininger *et al.* 2003
Severe skin rashes	Deininger *et al.* 2003; Rule *et al.* 2002	Musculoskeletal side effects	Henkes *et al.* 2008; Deininger *et al.* 2003
Fluid retention	Cohen *et al.* 2002; Ebnoether *et al.* 2002	Cutaneous side effects	Deininger *et al.* 2003; Rule *et al.* 2002
Cardiotoxicity	Henkes *et al.* 2008; Deininger *et al.* 2003; Cohen *et al.* 2002	Renal dysfunction	Henkes *et al.* 2008
Hepatictoxicity	Ridruejo *et al.* 2007; Deininger *et al.* 2003		
Teratogenic and embryonic side effects	Henkes *et al.* 2008; Huang *et al.* 2008 Choudhary *et al.* 2006; Ali *et al.* 2004; Deininger *et al.* 2003; Lee *et al.* 2003		
Pulmonary toxicity	Henkes *et al.* 2008; Lee *et al.* 2003;		

1.3.4.5 Absorption, distribution, metabolism and excretion of imatinib

It is generally accepted that understanding the pharmacokinetics of a drug assists in ensuring its efficacy (Lin *et al.* 2003; Ekins *et al.* 2005). This is achieved through studies focussing on the toxicogenomics, proteomics, metabolomics and pharmacogenomics of a drug (Ekins *et al.* 2005). Drug efficacy relies on a coordinated system of transporters, channels, receptors, gatekeepers and enzymes that can all in turn affect the absorption, distribution, metabolism, excretion and toxicity of a drug (Ekins *et al.* 2005).

1.3.4.5.1 Absorption of imatinib

Imatinib is an orally administered drug and is absorbed by the digestive system into the bloodstream. Ineffective absorption may influence drug-plasma levels of imatinib. However, this is not considered a problem with imatinib, since very few cases of poor absorption have been reported (Peng *et al.* 2005).

1.3.4.5.2 Distribution of imatinib

The efficient distribution of imatinib involves the transport of the active metabolite to the target cells via blood plasma and the uptake into the cell via transport proteins. The distribution of imatinib is generally regarded to be extensive, however, there are factors that can influence its distribution negatively (Hamada *et al.* 2003; Peng *et al.* 2004; Peng *et al.* 2005). Imatinib has been found to bind to plasma proteins such as albumin and α1 – acid glycoprotein (A1AGP). This binding to plasma proteins can reduce the exposure of free imatinib and it is hypothesised that this may indirectly contribute to resistance against imatinib (Gschwind *et al.* 2005; Peng *et al.* 2005). In addition, transport protein OCT-1 (organic cation transporter - 1) is responsible for cell influx and P-glycoprotein for efflux. Thus a decrease in influx or efflux may lead to inefficient treatment or toxicity, respectively (Hamada *et al.* 2003; Peng *et al.* 2005).

1.3.4.5.3 Metabolism of imatinib

The metabolism of a drug is hypothesised to be the main factor affecting drug efficacy after absorption, distribution and excretion (Evans and Relling 1999; Wolf and Smith 1999). Drug metabolism is primarily controlled by the cytochrome P450 (CYP450) enzyme system and the metabolism of imatinib is mainly controlled by the CYP3A4 enzyme (Schmidli *et al.* 2004; de Kogel and Schellens 2007). The CYP450 enzymes are mainly located in the liver and small intestine. Their function is to increase the water solubility of their substrates through oxidative metabolism, on order to facilitate their excretion from the body (Hashimoto *et al.* 1993; Sata *et al.* 2000). A decrease or increase in the metabolic rate of a CYP450 enzyme can lead to a drug being metabolised too quickly or too slowly and can ultimately result in treatment failure or ADRs (Wolf *et al.* 2000).

1.3.4.5.4 Excretion of imatinib

Excretion of imatinib relies on efflux proteins. This system needs to function at an optimal rate to ensure that the body is not exposed to imatinib for too long, as ADRs could develop. Imatinib is mainly excreted through faeces and urine as a metabolite.

1.4 Pharmacogenetics

Pharmacogenetics is the study of the genetic basis to drug metabolism and its impact on treatment response. Drug therapy is often based on the assumption that all individuals respond similarly to drugs (Innocenti *et al.* 2000). However, drug efficacy has been reported to vary considerably between individuals (Garcia-Martin *et al.* 2002; de Jong *et al.* 2006). This can impact treatment outcome as well as result in additional medical cost in treating ADRs (van Schaik 2004). In the United Kingdom (UK), ADRs were reported to occur at an incidence of 20,000 cases per year in 1999, compared to 250,000 cases in 2006. The estimated cost of this in 2006 was approximately £466 million to the National Health Service (NHS) (Wolf and Smith 1999; Hitchen 2006). In the United States of America (USA), ADRs were reported to be the 5th leading cause of death in 2006 (Nakamura 2008). It is not known whether the rise in the incidence of ADRs is a result of poor drug development or whether the reporting of ADRs has improved (Veltmann 2005; Ushma *et al.* 2007). What is evident is that patients do not respond uniformly to drug treatment and this has to be considered an important factor in the development of ADRs.

Differences in drug response between individuals are either a result of a lack of therapeutic effect, due to a too rapid drug clearance (rapid metabolisers), or ADRs due to impaired drug clearance (poor metabolizers) (Wolf *et al.* 2000; Murray 2006). There are several factors that can impact drug efficacy. These include dietary intake, age, health, gender, concurrent drug therapy and genetic variation (McKinnon and Evans 2000; Leeder 2001). Of these, genetic variation is considered to be the main factor contributing to altered drug efficacy and ADRs, with altered catalytic activity of CYP450 enzymes hypothesised to be the main cause (Evans and Relling 1999; Wolf *et al.* 2000; Innocenti *et al.* 2002).

CYP450 enzymes are responsible for the metabolic breakdown of any foreign compound that enters the body, including drugs. Variation in DNA that results in altered activity of CYP450 enzymes can be due to single nucleotide polymorphisms (SNPs), insertions, deletions, inversions or translocations. SNPs are the most common cause of change in the CYP450 protein sequence (van Schaik *et al.* 2001). SNPs either result in non-synonymous or synonymous changes in DNA. Non-synonymous SNPs result in a change in protein sequence by altering the codon for a specific amino acid. Synonymous SNPs can result in a translational pause during protein synthesis and this is hypothesised to have an impact on protein structure and function. Changes in CYP450 protein structure can alter the metabolic rate of the enzyme, which if decreased can all lead to ADRs, and if increased, will result in an increased clearance of a drug and possibly inefficient treatment (Sachese *et al.* 1999; van Schaik *et al.* 2001; Hirota *et al.* 2004; Mathijssen and van Schaik 2006; Schirmer *et al.* 2007).

In addition to genetic contribution, concomitant therapy is also an important factor in the development of ADRs or inefficient drug treatment. Drug interaction becomes problematic where more than one drug is metabolised by the same CYP450 enzyme (Murray 2006; www.healthanddna.com/Druglist.pdf). For example, the same enzyme that metabolises imatinib, CYP3A4, is also responsible for the metabolism of ketoconazole, indinavir, voriconazole, clarithromycin, delavirdine, itraconazole and simvastatin, to name a few. If imatinib and a drug such as ketoconazole, both shown to have an inhibitory effect on the CYP3A4 enzyme, are taken concomitantly, the enzyme CYP3A4 would metabolise these drugs at a slower rate than normal and result in an extended exposure to these drugs, and possibly lead to the development of ADRs. Furthermore, inhibition or induction of CYP3A4 does not only occur with drugs, but also with food. For example, CML patients being treated with imatinib are discouraged from ingesting grapefruit whilst on imatinib treatment, as grapefruit contains the furanocoumarin, epoxybergamottin and the flavinoid, naringenin, all of which are substrates of CYP3A4 and inhibit CYP3A4 activity (Wolf and Smith 1999; Veronese *et al.* 2003; Monroe *et al.* 2007).

1.5 Cytochrome P450 enzyme system (CYP450)

1.5.1 Background on cytochrome P450

Currently there are over 400 known members of the CYP450 family in diverse organisms such as mammals, fish, insects, nematodes, plants, yeast, fungi and bacteria (Wauthier *et al.* 2007; Sim *et al.* 2008). Eukaryotes require CYP450 enzymes for biotransformation, such as sterol biosynthesis for plasma membranes and metabolism of all compounds that enter the body. Although previously it was thought that these enzymes were only located in the liver, it was later shown to also be present in the intestines, skin, brain, kidneys, prostate and lungs (Hellmold *et al.* 1998). However, the liver harbours the majority of CYP450 enzymes, especially the enzymes responsible for drug metabolism.

CYP450 enzymes are a super family of haeme-containing proteins that are crucial for the oxidative, peroxidative and reductive metabolism of a diverse group of compounds, including endobiotics, such as steroids, bile acids, fatty acids, prostaglandins, leukotrienes and xenobiotics including most therapeutic drugs and environmental toxins (Ingelman-Sundberg *et al.* 1999; Rogers *et al.* 2002; Xu *et al.* 2002; Sim *et al.* 2008). These enzymes display inter-individual variation in activity as a result of SNPs, making CYP450 enzymes the focus of pharmacogenetics.

1.5.2 Cytochrome P450 nomenclature and classification

The CYP450 enzymes are denoted as CYP which is an abbreviation for the word cytochrome. A characteristic of all CYP450 proteins is that when these proteins are in the reduced state, the haeme iron (Fe^{3+}) pigment binds to carbon monoxide (CO), giving the CYP450 protein a unique absorption at 450 nm (Omura and Sato 1964; Garfinkel 2003). The cytochrome P450 (CYP450) name was derived due to this absorption wavelength (Omura 1999). The Arabic number following “CYP” is used to describe the specific family (e.g. CYP3). This is followed by a letter that denotes the sub-family (e.g. CYP3A) and a number that identifies the specific enzyme (e.g. CYP3A4). Different allelic variants, due to an alteration in DNA sequence, are denoted with “*“ followed by a number (e.g. CYP3A4*2) (Nebert *et al.* 1987).

In addition to the standard CYP nomenclature for allelic variants, different SNPs located in intron regions as well as non-synonymous and synonymous SNPs have

their own unique denotation, prior to naming by the CYP450 nomenclature committee (CYP450 Nomenclature Committee: http://www.cypalleles.ki.se/). A non-synonymous exon SNP is denoted by the original amino acid abbreviation, followed by the amino acid number and the subsequent amino acid abbreviation (e.g. M123V). Synonymous SNPs are denoted in a similar fashion, only with no substitution of the amino acid (e.g. M123M). Intron SNPs are denoted with the original base, followed by the nucleotide base location and the subsequent nucleotide substitution (e.g. G20228A).

To date, 265 CYP450 families have been identified, of which 18 families are found in humans and consists of 43 subfamilies (Sim *et al.* 2008). The system for cytochrome P450 nomenclature was initially proposed by Nebert *et al.* (1987). *CYP450* genes in a particular family have a 40% sequence similarity (e.g. *CYP2C9* and *CYP2B6*) and those with a 55% sequence similarity are placed within a subfamily (e.g. *CYP2C9* and *CYP2C19*) (Nebert *et al.* 1987; Coon *et al.* 1992; Hashimoto *et al.* 1993).

1.5.3 Cytochrome P450 function and mechanism

Different CYP450 enzymes are responsible for the metabolism of different compounds (Table 2). These enzymes can also catalyze a variety of compounds concurrently (Figure 4) (Frye 2004). For example, CYP3A4 is involved in steroid biosynthesis and oxidative drug metabolism (Table 2) (Frye 2004). Compounds are metabolised through oxidation which converts them from a hydrophobic to a hydrophilic form, which allows for excretion from the body (Ekins *et al.* 2005). This is achieved by introducing a polar hydroxyl group to the compound.

Table 2: Different human cytochrome P450 (CYP450) families and their major functions. Data was adapted from the Cytochrome P450 Homepage (http://drnelson.utmem.edu/CytochromeP450.html) (White *et al.* 1997; Lund *et al.* 1999).

Cytochrome P450 Family	Main Function
CYP 1	Xenobiotic metabolism
CYP 2	Xenobiotic metabolism, Arachidonic acid metabolism an Steroid Metabolism
CYP 3	Xenobiotic metabolism and Steroid metabolism
CYP 4	Arachidonic acid metabolism and Fatty Acid metabolism
CYP 5	Thromboxane synthesis
CYP 7	Cholesterol 7α –hydroxylation
CYP 8	Prostacyclin Synthesis and Bile acid biosynthesis
CYP 11	Steroid biosynthesis and Aldosterone synthesis
CYP 17	Steroid 17α – hydroxylation
CYP 19	Androgen aromatization
CYP 20	Unknown Function
CYP 21	Steroid biosynthesis
CYP 24	Steroid biosynthesis and Vitamin-D degradation
CYP 26	Retinoic Acid hydroxylation
CYP 27	Steroid hydroxylation and Bile acid biosynthesis
CYP 39	7α –hydroxylation of 24-hydroxy cholesterol
CYP 46	Cholesterol 24-hydroxylation
CYP 51	Cholesterol biosynthesis

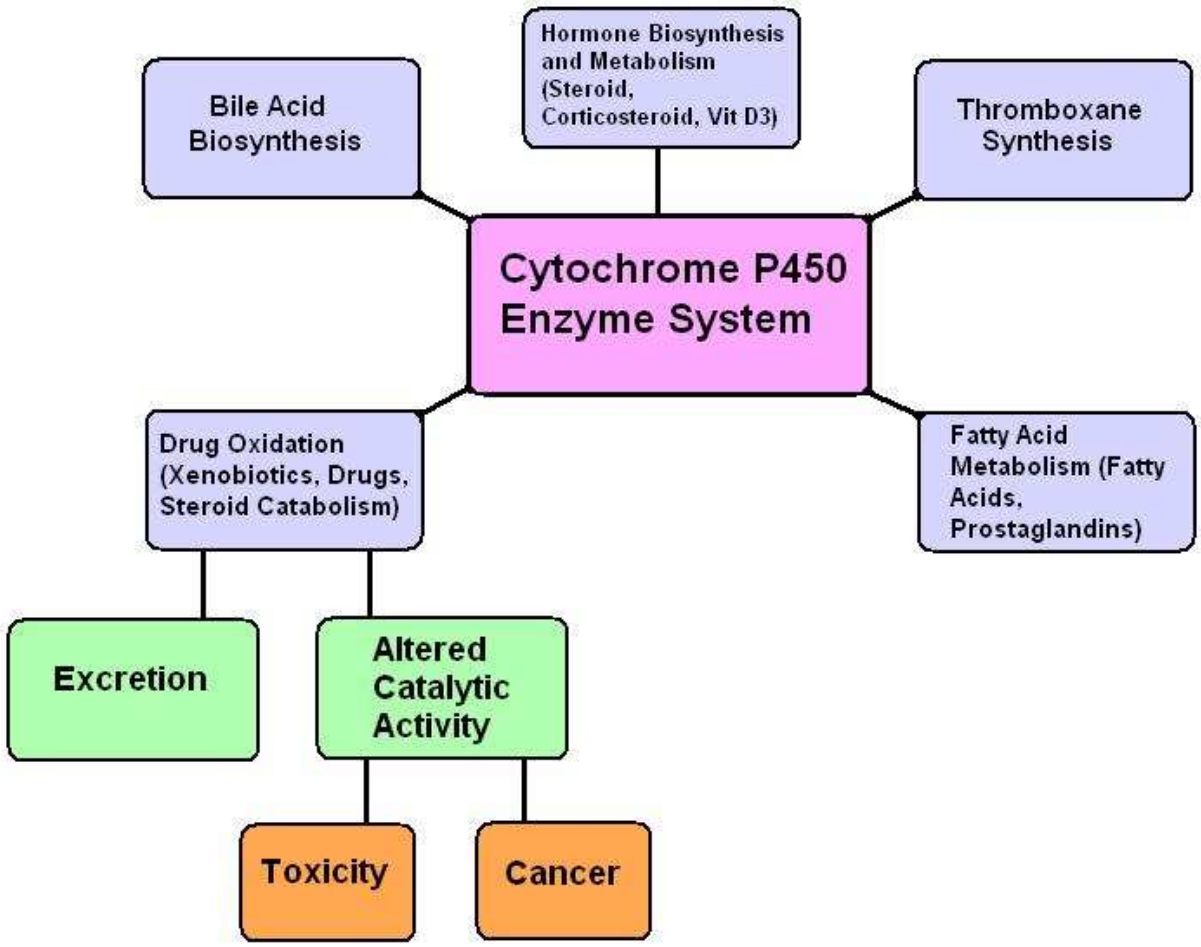
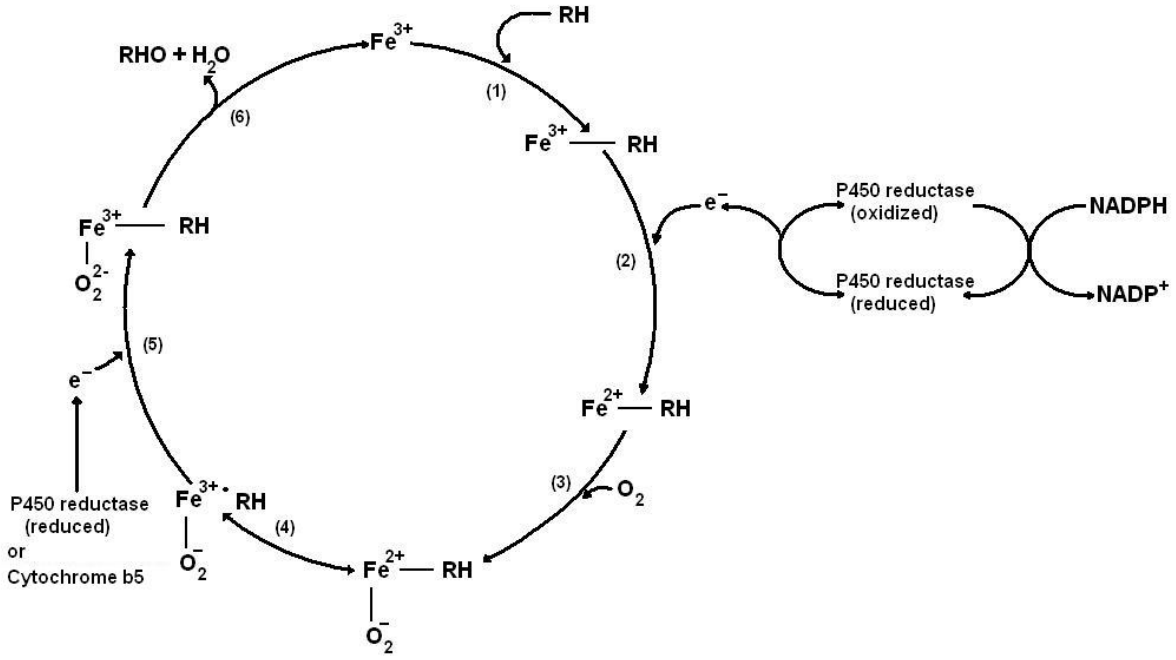


Figure 4: Different metabolism roles of Cytochrome P450 enzymes (copied from Simpson (1997).

Estabrook *et al*. (1971) proposed a cyclic reaction to explain the catalytic action of CYP450 enzymes (Figure 5) (Omura 1999; Estabrook 2003). The substrate binds to the active site of the CYP450 enzyme and forms a ferric-haem substrate complex. Following this, two electrons are donated to the CYP450 enzyme by the reducing co-factor NADPH (Shet *et al.* 1993; Omura 1999; Estabrook 2003). During the first electron donation from the reductase co-enzyme, the iron (Fe^{3+}) is reduced to the ferrous state (Fe^{2+}). Following this, a second electron is donated by the co-enzyme, P450 reductase or cytochrome b5. Finally, the substrate is oxidized with a water molecule produced (Guengerich 1991; Shet *et al.* 1993; Estabrook 2003). This process completes the biotransformation of the substrate to a hydrophilic form that facilitates its excretion from the body.



Summary of cyclic reaction:

$NADPH + RH + O_2 + H^+ \longrightarrow NADP^+ + H_20 + ROH$

Figure 5: Catalytic cycle for cytochrome P450 reactions (copied from Estabrook (2003), Omura (1999) and Shet *et al.* (1993). (Fe^{3+} = Oxidised CYP450 enzyme, Fe^{2+} = Reduced CYP450 enzyme, Cytochrome b5 = Co-enzyme for CYP450, RH = Substrate, ROH = Product of biosynthesis and e^- = electron). (1) Binding of substrate to active site of CYP450 enzyme. (2) Iron (Fe^{3+}) is reduced to ferrous state (Fe^{2+}). (3) Oxygen is incorporated into substrate-enzyme complex. (4) and (5) second electron is donated by co-enzyme P450 reductase or Cytochrome b5. (6) substrate is oxidised with water molecule produced.

1.5.4 Human drug metabolising CYP450 enzymes

Even though most of the functions of the enzymes within these families have been determined, there are still a few enzymes whose function is unknown (Nelson 1999). Only specific enzymes from the CYP1, CYP2 and CYP3 families are responsible for the active metabolism of therapeutic drugs (Table 3) (Shimada *et al.* 1994; Capdevila *et al.* 2000).

Table 3: Cytochrome P450 enzymes involved with drug metabolism. This table was adapted from the following sources: Lewis *et al.* (2002), Frye (2004) and van Schaik (2005).

Family	Subfamily	Enzyme
CYP1	CYP1A	CYP1A2
CYP2	CYP2A	CYP2A6
	CYP2B	CYP2B6
	CYP2C	CYP2C8, CYP2C9, CYP2C19
	CYP2D	CYP2D6
	CYP2E	CYP2E1
CYP3	CYP3A	CYP3A4, CYP3A5, CYP3A7

1.5.4.1 CYP1A2

The CYP1 family consists of three genes namely, *CYP1A1, CYP1A2* and *CYP1B1* (Sim *et al.* 2008). All three CYP1 enzymes are involved with the metabolism of polycyclic aromatic hydrocarbons (PAH), petroleum derivatives and insecticide derivatives (Shimada *et al.* 1996). Of these, only CYP1A2 has been associated with drug metabolism (Raunio *et al.* 1995; Shimada *et al.* 1996;). *CYP1A2* expression is thought to be restricted to the liver with a total content of approximately 13% (Figure 6) and is estimated to be responsible for the metabolism of approximately 11% of all drugs (Figure 7) (Murray *et al.* 1993; Shimada *et al.* 1994; Koskela *et al.* 1999).

1.5.4.2 CYP2 family

The CYP2 family contains the subfamilies CYP2A, CYP2B, CYP2C, CYP2D, CYP2E, CYP2F and CYP2J (Sim *et al.* 2008). Of these, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP2E1 have been reported to be involved in drug metabolism (Lewis *et al.* 2002; Frye 2004; van Schaik 2005).

1.5.4.2.1 CYP2A6

CYP2A6 is generally associated with the metabolism of nicotine and cotinine as well as the metabolism of nitrosamines, carcinogens (aflatoxin B1), coumarin-type

alkaloids and certain pharmaceuticals (Kitagawa *et al.* 2001; Rautio 2003). Although this enzyme is estimated to constitute a total of 10% of total hepatic CYP450 content (Figure 6) it is only responsible for the metabolism of approximately 0.5% of drugs (Figure 7) (Shimada *et al.* 1994). SNPs in the *CYP2A6* gene have been associated with the difference in the metabolism of nicotine between individuals (Oscarson 2001; Rautio 2003).

1.5.4.2.2 CYP2B6

CYP2B6 is regarded as a minor form of the CYP450 enzymes, as it only has a supporting role in the metabolism of most compounds, with a total CYP450 hepatic content of 3 to 5% (Figure 6A) (Imaoka *et al.* 1996; Guan *et al.* 2006; Korhonen *et al.* 2007). Guan *et al*. (2006) confirmed that CYP2B6 participates in the metabolism of approximately 3% of therapeutic drugs (Figure 7). This enzyme is mainly expressed in the liver, but has also been detected in extrahepatic tissue, including the kidneys, intestine, brain, skin and lungs (Guan *et al.* 2006; Korhonen *et al.* 2007). SNPs in *CYP2B6* have been associated with altered catalytic activity of certain substrates between individuals (Heyn *et al.* 1996; Lang *et al.* 2001; Guan *et al.* 2006).

1.5.4.2.3 CYP2C8, CYP2C9, and CYP2C19

The CYP2C subfamily consists of four highly homologous enzymes, CYP2C8, CYP2C9, CYP2C18 and CYP2C19. Of these, CYP2C8, CYP2C9 and CYP2C19 have been associated with drug metabolism (Jiang *et al.* 2002; Allabi *et al.* 2004; Grasmäder *et al.* 2004; Chang *et al.* 2008). Certain SNPs in the different *CYP2C* genes, have been associated with the decreased enzyme activity *in vivo*, and has led to preliminary screening for these allelic variants before administering doses of certain drug types (Allabi *et al.* 2004). It is estimated that CYP2C is responsible for the metabolism of 24% of all therapeutic drugs (Figure 7).

1.5.4.2.4 CYP2D6

The *CYP2D* subfamily consists of four pseudogenes and one functional gene, *CYP2D6* (Shimada *et al.* 1996). CYP2D6 was the first CYP enzyme identified to have polymorphisms linked to decreased catalytic activity (Kapitany *et al.* 1998; Ishida *et al.* 2002; Markowitz *et al.* 2003a; Markowitz *et al.* 2003b; Grasmäder *et al.* 2004). CYP2D6 is estimated to be responsible for the metabolism of approximately

25% of all therapeutic drugs (Figure 7) even though it only constitutes 2 to 5% of the total CYP450 hepatic content (Figure 6) (Bernard *et al.* 2006).

1.5.4.2.5 CYP2E1

CYP2E1 is the only member of the CYP2E subfamily (Shimada *et al.* 1996). This enzyme has been identified as an important enzyme for ethanol metabolism (Kunitoh *et al.* 1997; Nolin *et al.* 2003). CYP2E1 has been estimated to metabolise approximately 4% of therapeutic drugs (Figure 7) but is mainly implicated in the metabolism of toxins and other exogenous compounds such as protoxicant and procarcinogenics (Nolin *et al.* 2003).

1.5.4.3 CYP3 family

The CYP3 family consists of only one subfamily, CYP3A (Shimada *et al.* 1996). CYP3A contains the CYP3A4, CYP3A5, CYP3A7 and CYP3A43 enzymes (Finta and Zaphiropoulos 2000; Boulton *et al.* 2001). The CYP3A subfamily is considered to be one of the most multifunctional CYP450 biotransformation enzyme systems as it facilitates the elimination of drugs, other xenobiotics compounds and endogenous molecules from the human body (Lamba *et al.* 2002). It is thought that the CYP3A group of enzymes is responsible for 50 to 60% of the metabolism of therapeutic drugs (Lamba *et al.* 2002; Yamaori *et al.* 2002; Hirota *et al.* 2004).

1.5.4.3.1 CYP3A4

CYP3A4 is considered to be the most important drug metabolising enzyme in the human body and represents approximately 30 to 40% of total CYP450 liver content (Figure 6) and is involved in over 50% of therapeutic drug metabolism (Figure 7) (Westlind *et al.* 1999; Boulton *et al.* 2001; Hsieh *et al.* 2001; O'Brien *et al.* 2003b; Eap *et al.* 2004; Lopes *et al.* 2004; Peng *et al.* 2005). Initially the CYP3A4 enzyme was thought to be non-polymorphic, however 68 polymorphisms have been identified, of which a few have altered catalytic activity (Table 4, Table 5 and Appendix A) (Dai *et al.* 2001; van Schaik *et al.* 2001; Sim *et al.* 2008). There are currently 28 known allelic variants of CYP3A4, of which CYP3A4*8, CYP3A4*11, CYP3A4*12, CYP3A4*13, CYP3A4*16, CYP3A4*17 and CYP3A4*18 have been shown to have altered catalytic activity *in vitro* (Table 4). However, recently Kang *et al.* (2009) determined that CYP3A4*18 has altered catalytic activity *in vivo* as well (Table 4)

(Sim *et al.* 2008; Kang *et al.* 2009). Even though certain polymorphisms of CYP3A4 have been associated with altered catalytic activity, a great deal of uncertainty still exists to what extent these polymorphisms impact drug efficacy in general.

1.5.4.3.2 CYP3A5

CYP3A5 is estimated to account for approximately 10 to 25% of the entire CYP450 content in the human liver (Figure 6) (Tateishi *et al.* 1999; Liu *et al.* 2002; Eap *et al.* 2004). However, despite its high prevalence, studies have concluded that approximately only 20% of adults express CYP3A5 (Dai *et al.* 2001; Daly 2006). CYP3A5 generally metabolises the same substrates as CYP3A4, but is thought only to play a supporting role in the metabolism of these compounds (Daly 2006). For example, the metabolism of CYP3A4 substrates midazolam, nifedine and docetaxel were no different in the presence or absence of CYP3A5 (Dai *et al.* 2001; Daly 2006).

1.5.4.3.3 CYP3A7

CYP3A7 is a foetal specific isoform that is also expressed in adults (Yamaori *et al.* 2002; Daly 2006). Altough this enzyme is involved in the metabolism of substrates similar to that of CYP3A4 and CYP3A5, it is only thought to play a supporting role in the metabolism (Tateishi *et al.* 1999).

1.5.5 CYP450 enzymes and pharmacogenetics

Even though there are a great number of different CYP450 enzymes, it is interesting to note that the majority of all drugs are metabolised by approximately only 11 of these enzymes, all belonging to the CYP1, CYP2 and CYP3 families. These families contribute to the majority of all CYP450 enzymes in the liver (Figure 6, Table 3). Of these, CYP3A4 and CYP2D6 metabolise approximately 55% of all therapeutic drugs (Figure 7). However, the remaining CYP450 enzymes are thought to play an important part in CYP3A4 and CYP2D6 mediated metabolism (Peng *et al.* 2005). In addition to this, CYP3A4 and CYP2D6 can play a supporting role in the metabolism of each other, for example, imatinib is mainly metabolised by CYP3A4, but CYP2D6, like CYP3A5, CYP1A2, CYP2C9 and CYP2C19 is thought to play a minor supporting role (Schmidli *et al.* 2004; de Kogel and Schellens 2007). Thus, any change in metabolic activity of these enzymes, especially CYP3A4 with regard to CML patients being treated with imatinib, may have a range of therapeutic corollaries. These

include, limited to no therapeutic result, due to an increased catalytic activity, ADRs, due to a decreased metabolic rate of a drug, or compromised treatment, due to the limited activation of a drug (Wolf and Smith 1999).

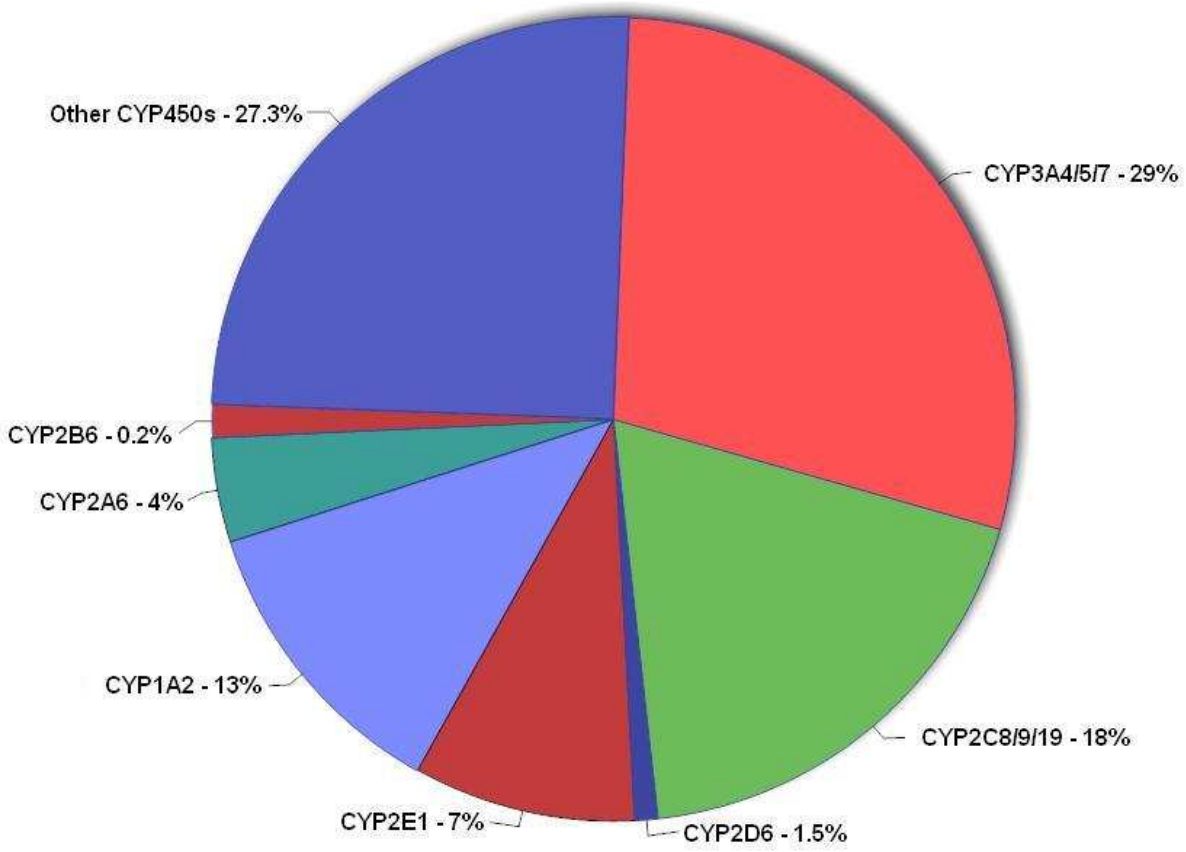


Figure 6: Relative liver abundance of CYP450 enzymes in the human liver (adapted from Shimada *et al.* (1994), Wolf and Smith (1999), Lamba *et al.* (2002), Nolin *et al.* (2003), Hirota *et al.* (2004), Bernard *et al.* (2006), Guan *et al.* (2006), Korhonen *et al.* (2007)).

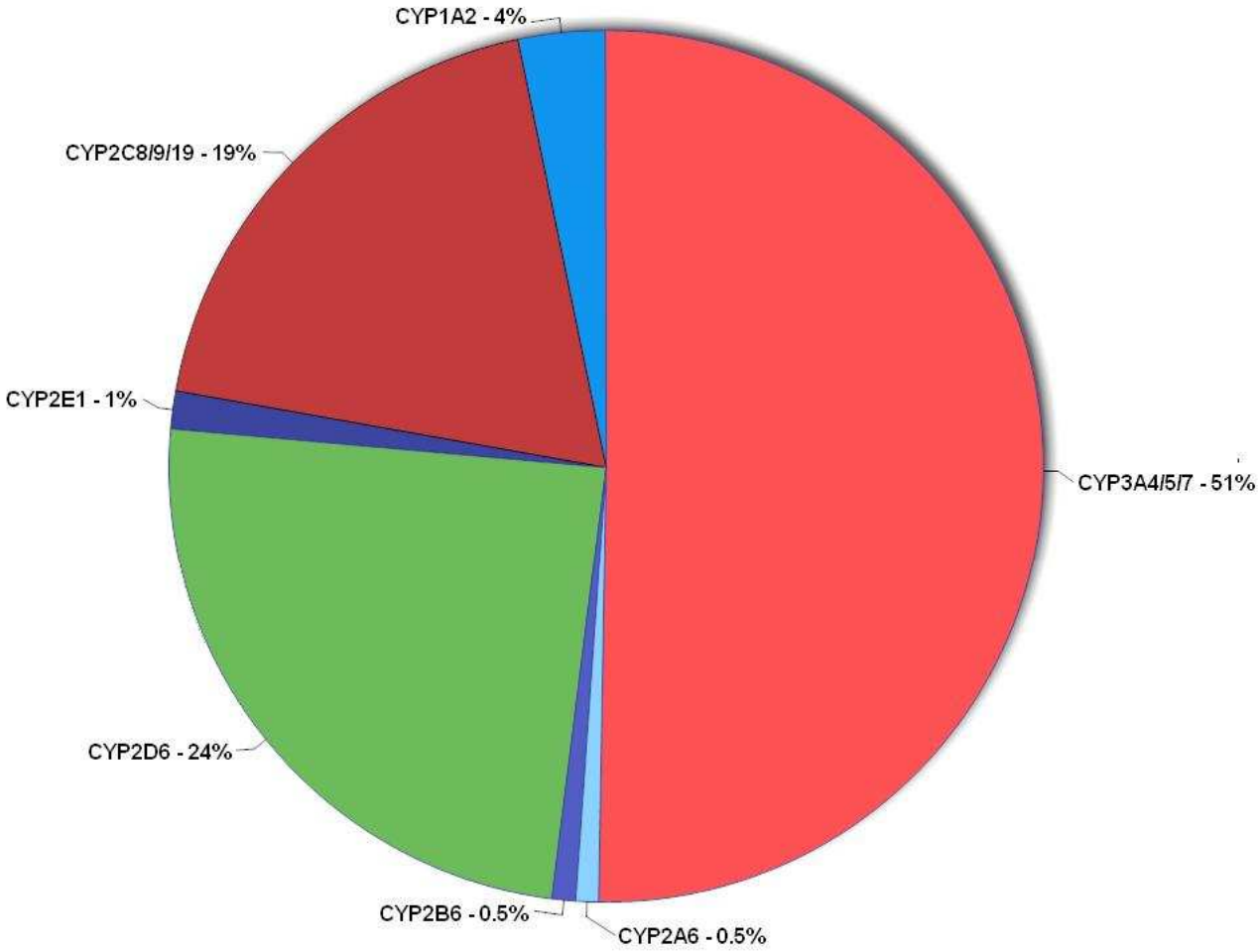


Figure 7: Relative proportion of drugs metabolised by CYP450 enzymes (adapted from Shimada *et al.* (1994), Wolf and Smith (1999) Lamba *et al.* (2002), Nolin *et al.* (2003), Hirota *et al.* (2004), Bernard *et al.* (2006), Guan *et al.* (2006), Korhonen *et al.* (2007)).

1.5.5.1 Impact of SNPs on the catalytic activity of CYP3A4

Various SNPs, resulting in amino acid changes have been identified in the *CYP3A4* gene (Table 5). SNPs are usually detected through sequencing and evaluated using probe drugs to determine whether these have an impact on catalytic rate (Dai *et al.* 2001; Eiselt *et al.* 2001; Murayama *et al.* 2002). For example, a study done by Dai *et al.* (2001), showed that CYP3A4*17 has a decreased clearance rate of testosterone and chlorpyrifos *in vitro* and recently Kang *et al.* (2009) showed that the allelic variant CYP3A4*18 had a decreased rate of the same probes *in vivo.* However, these findings have not been investigated for other substrates such as imatinib.

1.5.5.2 Population differences of CYP3A4

The allelic variants of *CYP450*, including *CYP3A4,* have a low frequency. However, allelic differences in CYP450 enzymes do not only occur between individuals, but can also be population specific (van Schaik *et al.* 2001; Lamba *et al.* 2002; Yamaori *et al.* 2002). Differences in CYP450 enzymes between populations can prove problematic when a standard treatment regimen is employed for therapeutic drugs (Wolf and Smith 1999; van Schaik *et al.* 2001; Garcia-Martin *et al.* 2002). For example, a study by Lamba *et al*. (2002), determined that the most common variant of *CYP3A4, CYP3A4*1B*, thought to influence the expression of *CYP3A4*, is present in Caucasians at a frequency of 2.0 to 9.6%, in African Americans at 40% but absent in Japanese and Chinese. In South Africa, the same variant was detected in 81.3% of Indians, 42.9% of Caucasians and 16.4% of Africans (Chelule *et al.* 2003). These studies have demonstrated the difference in *CYP3A4* allelic distribution between different populations. If such a variant was shown to impact therapeutic drug metabolism, it would be crucial to screen for such an allelic variant. Thus, in the case where allelic variants have been shown to alter catalytic activity for specific drugs, routine pharmacogenetic screening should be required prior to treatment to ensure adequate dosage for drug efficacy.

1.6 Conclusion

CML can be effectively treated with the tyrosine kinase inhibitor, imatinib. Imatinib is metabolised by the specific CYP450 enzyme, CYP3A4. Allelic differences of CYP3A4 exist due to SNPs, which could alter the catalytic activity of *CYP3A4* and lead to ADRs or inefficient treatment. Despite the extensive research that has been done involving CYP3A4 and various substrates, no studies involving the impact of *CYP3A4* polymorphisms on imatinib treatment have been conclusive. Studies involving other substrates have been used to elucidate the impact of the different *CYP3A4* polymorphisms on catalytic rate, but do not address whether the same impact will arise with imatinib (Dai *et al.* 2001; Murayama *et al.* 2002). Currently the variability in drug treatment makes the effects from treatment such as ADRs and under-dosing, highly unpredictable. By understanding the impact of polymorphisms in the CYP450 enzymes responsible for drug metabolism, a more accurate treatment regimen can be established for each patient, depending on which allelic variants are present.

Table 4: *CYP3A4* allelic variants with altered catalytic activity. Data was obtained and adapted from Hamzeiy *et al.* (2002), Keshava *et al.* (2004), Sim *et al*. (2008).

Allele	Position in GenBank AF280107	SNP Change and Sequence Context	Location Exon	Enzyme Activity	
				In vivo	*In vitro*
*CYP3A4*8*	75944	agattac**G/A**atcattg	5	Unknown	Decreased
*CYP3A4*11*	83903	gaatgaaa**C/T**gctcaga	11	Unknown	Decreased
*CYP3A4*12*	83932	gctatgaga**C/T**ttgaga	11	Unknown	Decreased
*CYP3A4*13*	84062	agttcctcc**C/T**tgaaagg	11	Unknown	Decreased
*CYP3A4*16A*	77639	tgtgatca**C/G**tagcac	7	Unknown	Decreased
*CYP3A4*17*	77651	cacatcat**T/C**tggagt	7	Unknown	Decreased
*CYP3A4*18A*	82106	ctgtccgatc**T/C**ggag	10	Decreased	Increased/ Decreased

Table 5: DNA variations identified within the *CYP3A4* promoter region, 3'-UTR region, intron regions and exon regions. Each table entry is denoted with a number, which is used in, and referred by Appendix A. Data was obtained and adapted from Keshava *et al.* (2004) and Hirota T *et al.* (2004).

CYP450 Nomenclature	Distance from ATG start signal	Position in Genbank (AF280107)	Nucleotide Change and sequence context
*(1) - CYP3A4*1B*	- 392	61645	agggca**A/G**gagagag
*(2) - CYP3A4*1C*	- 444	61593	ggcttgt**T/G**gggatgaa
*(3) - CYP3A4*1D*	- 62	61975	gcccag**C/A**aaagagca
*(4) - CYP3A4*1x*	- 66	61971	gcacatagc**C/G**cagcaaaga
*(5) - CYP3A4*1M*	- 156	61881	gctgcagct**C/A**cagccctgc
*(6) - CYP3A4*1E*	- 369	61668	aatagatt**T/A**tatgccaa
*(7) - CYP3A4*1w*	- 666	61371	gaaacaggc**G/A**tggaaacac
*(8) - CYP3A4*1K*	- 655	61382	tggaaacaca**A/G**tggtggtaa
*(9) - CYP3A4*1L*	- 630	61407	aagaggacaa**A/G**taggattgc
*(10) - CYP3A4*1F*	- 747	61290	acagcac**C/G**ctggtagg
*(11) - CYP3A4*15B*	- 844^845	61191^2	aagaa**ATGGAGTGA**gtca
(12) - 3′UTR	25958	87994	taaggacttc**T/G**gctttgct
*(13) - CYP3A4*1T*	26011	88049	aaattactt**T/C**gtgaataga
*CYP3A4*1T*	26082	88120	ttctgtaca**T/G**gcattgagc
*(14) - CYP3A4*1H*	26204	88242	tccaccacc**C/A**ccagttagc
*CYP3A4*1H*	26269	88305	tcaataattt**C/T**ctccacaa
*CYP3A4*1H*	26418	88454	ctttcctgca**C/T**attaagga

CYP450 Nomenclature	Distance from ATG start signal	Position in Genbank (AF280107)	Nucleotide Change and sequence context	Intron No.
*(15) - CYP3A4*1y*	3856	65993	tatctataa**A/-**gtcacaatc	1
(16) - T5922C	5917	67953	tctgatttca**T/C**tggcttcg	2
*(17) - CYP3A4*1J*	6076	68113	gaaacttcc**A/G**ttggataga	3
(18) - T6165A	6159	68195	gggatgaagctc**T/A**tgtca	3
(19) - SNP4	13804	75840	ccacaactg**A/T**tgtaggaca	4
(20) - G13875A	13757	75793	tgaataagt**G/A**ttcctgttta	4
(21) - G13947C	13829	75865	tgtttctgctttg**G/C**aactctag	4
(22) - SNP5	14200	76236	tatgggtggtg**T/G**tgtgtttt	5
(23) - M10	14323	76359	aagcgcagc**C/T**atggggtt	6
(24) - M11	14329	76365	gccatggg**G/T**tctgagctgtc	6
(25) - T14475G	14357	76393	cccctccagc**T/G**gcctgcca	6
*(26) - CYP3A4*1Z*	15552	77589	taattttcc**A/C**catctttct	6
*(27) - CYP3A4*1P*	15726	77763	aagtatgtg**G/A**actactatt	7
(28) - T15871G	15753	77789	ttttatttatctt**T/G**ctctcttaaa	7
(29) - T15901C	15783	77819	tttattgaga**T/C**ataaatcacca	7
(30) - SNP10	15804	77840	tgtaattca**T/G**ccacttaaaa	7
*(31) - CYP3A4*1Q*	15808	77845	ttcatccac**T/C**taaaatata	7
(32) - T15955A	15837	77873	gtgatttgtag**T/A**acatttgaag	7
(33) – C78013T	15977	78013	tcaactttctgc**C/T**tctatggattt	7
(34) – C78649T	16613	78649	actgctgtag**C/T**ggtgctcctta	7
(35) - CYP3A41R	16774	78811	acattcaca**A/G**tgaatttct	7
(36) - C17141T	17024	79060	gtgcagttac**C/T**tgtatgttttta	8
*(37) - CYP3A4*11b*	17717	79754	tttctgagg**G/A**ctacttgtg	9
(38) - SNP14	17815/6	79851/2	agaacgacac**AT/-**gtttgaat	9
*(39) – CYP3A4*1G*	20230	82266	tgagtggatg**G/A**tacatggag	10
(40) - SNP16	20265	82301	gaaaccttag**C/T**aaaaatgcc	10
(41) - G20417C	20309	82345	tttttataaaaa**G/C**cataatcact	10
(42) - A21891C	21785	83821	caatttatcca**A/C**atctgtttcgt	10
(43) - SNP18	21795	83831	caaatctgtttc**G/A**ttctttccagg	10
(44) - M16	22041	84077	aggtacaagg**C/T**ccctgggaa	11
(45) - SNP21	23024	85060	aagtaagaa**A/G**ccctaacatg	11
(46) - C23187T	23081	85117	aaaaatctaccaa**C/T**gtggaac	11

CYP450 Nomenclature	Distance from ATG start signal	Position in Genbank (AF280107)	Nucleotide Change and sequence context	Exon No.
*(47) - CYP3A4*2*	77749	15713	attctttctc**T/C**caataa	7
*(48) - CYP3A4*3*	85208	23171	gcattggca**T/C**gaggttt	12
*(49) - CYP3A4*4*	75907	13871	aagtgcc**A/G**tctctat	5
*(50) - CYP3A4*5*	77738	15702	ttttggatc**C/G**attcttt	7
*(51) - CYP3A4*6*	79698^9	17661^176622	gatgattgac_**A**_tctcag	9
*(52) - CYP3A4*7*	68040	6004	tcccagg**G/A**cttttgt	3
*(53) - CYP3A4*8*	75944	13908	agattac**G/A**atcattg	5
*(54) - CYP3A4*9*	76328	14292	gcaagcct**G/A**tcacct	6
*(55) - CYP3A4*10*	76340	14304	ccttgaaa**G/C**agtaag	6
*(56) - CYP3A4*11*	83903	21867	gaatgaaa**C/T**gctcaga	11
*(57) - CYP3A4*12*	83932	21896	gctatgaga**C/T**ttgaga	11
*(58) - CYP3A4*13*	84062	22026	agttcctcc**C/T**tgaaagg	11
*(59) - CYP3A4*14*	62080	44	gcttctcc**T/C**ggctgt	1
*(60) - CYP3A4*15A*	76305	14269	tctgaggc**G/A**ggaag	6
*(61) - CYP3A4*16A*	77639	15603	tgtgatca**C/G**tagcac	7
*(62) - CYP3A4*17*	77651	15615	cacatcat**T/C**tggagt	7
*(63) - CYP3A4*18A*	82106	20070	ctgtccgatc**T/C**ggag	10
*(64) - CYP3A4*19*	85273	23237	tccttcaaa**C/T**cttgtaa	12
*(65) - CYP3A4*20*	87925	25889^25890	agaaaaa_**A**_cccgt tg	13
(66) – I193I #	15628	77664	gagtgaacat**C/T**gactct	7
(67) - A297A #	20083	82119	ctcgtggc**C/T**caatcgaa	10
(68) - T346T #	21817	83853	ccacccac**C/A**tatgata	11
(69) – T363T #	21868	83904	tgaatgaaac**G/A**ctcaga	11
(70) – T499T #	25925	87961	gggatggcac**C/T**gtaagt	13

^ - DNA Nucleotide Insertion

/ - DNA Nucleotide change [e.g. **G/C** - A (G)-Nucleotide changed to a (C)- Nucleotide]

- Synonymous SNP

Chapter 2: Research Aim and Methodology

2.1 Rationale for this study

The CYP3A4 gene consists of 13 exons and 12 introns, which comprise approximately 27,000 nucleotides (Hashimoto *et al.* 1993). Only 10 of the 13 *CYP3A4* exons harbour SNPs known to alter the amino acid sequence of CYP3A4 and only four of these have been shown to alter the catalytic activity *in vitro* or *in vivo* (Table 4). There are currently 68 polymorphisms identified within the *CYP3A4* gene of which 14 are located in the promoter and 3'-UTR regions, 30 in the intron regions and 24 in the exon regions (Keshava *et al.* 2004; Sim *et al.* 2008).

2.2 Aim of study

The aim of this preliminary study was to screen for SNPs in the *CYP3A4* gene of CML patients being treated with imatinib and determine whether these SNPs result in a functional change in the CYP3A4 enzyme as well as their association with ADRs.

2.3 Study Design

The study group consisted of an experimental group and control group. The 13 exons of *CYP3A4* were sequenced in both groups and SNPs identified. SNPs were investigated to determine whether they result in a change in amino acid sequence of CYP3A4 and whether they are associated with the presence of ADRs.

2.4 Study group

The study group consisted of CML patients, being treated with imatinib (Table 6). The patients routinely visit the Haematology clinic at the Universitas Hospital (Bloemfontein, South Africa) for diagnosis and treatment. Since CML is an uncommon condition, it was difficult to acquire sufficient patients for statistical purposes. The experimental group consisted of nine CML patients reported to experience ADRs from imatinib. The control group consisted of 16 CML patients who did not experience ADRs at the standard or higher dose of imatinib.

Table 6: CML patients and imatinib dosage.

Patient Number	Ethnic Group	Imatinib Dose[1]	Study Group[3]
1	Sesotho	400 mg/day	Control group
2	Sesotho	400 mg/day	Control group
3	Sesotho	600 mg/day	Control group
4	Sesotho	400 mg/day	Control group
5[2]	Sesotho	400 mg/day	Experimental Group
6	Sesotho	800 mg/day	Control group
7	Sesotho	600 mg/day	Control group
8	Caucasian	400 mg/day	Control group
9	Sesotho	200 mg/day	Experimental Group
10	Sesotho	200 mg/day	Experimental Group
11	Sesotho	200 mg/day	Experimental Group
12	Sesotho	200 mg/day	Experimental Group
13	Sesotho	400 mg/day	Control group
14	Sesotho	800 mg/day	Control group
15	Sesotho	400 mg/day	Control group
16	Sesotho	800 mg/day	Control group
17	Sesotho	200 mg/day	Experimental Group
18	Sesotho	400 mg/day	Control group
19	Sesotho	300 mg/day	Experimental Group
20	Coloured	200 mg/day	Experimental Group
21	Sesotho	400 mg/day	Control group
22	Sesotho	400 mg/day	Control group
23[2]	Coloured	400 mg/day	Experimental Group
24	Caucasian	400 mg/day	Control group
25	Sesotho	800 mg/day	Control group

[1] – Dose at the time of sampling.

[2] – Not able to tolerate a dose higher than 400 mg/day

[3] – Experimental group consisted of patients not able to tolerate the drug at recommended levels

2.4.1 Inclusion criteria

Cohort selection was based on CML patients receiving a lower than the recommended dose of 400 mg/day of imatinib due to the development of ADRs as

well as patients experiencing ADRs at 600 or 800 mg/day (Table 6). Patients who received a dose of 400 mg/day or higher of imatinib, who did not experience ADRs were selected for the control group. Patient sex or age was not considered a significant parameter as the SNPs that may affect the functioning of the CYP3A4 enzyme are not *de novo*. The study group consisted of a mixed population and race was not considered to be a significant parameter in this preliminary investigation.

2.4.2 Exclusion criteria

Patients, who were on concomitant therapy where the functioning of the CYP3A4 enzyme was suspected to be affected, were excluded from this study. The loss of response to treatment was not considered as exclusion, since patient response is affected by other factors including mutation development in the kinase domain of BCR-ABL.

2.5 Methods

2.5.1. Blood sampling

Informed consent was obtained from each patient before participating in this study. Patient data was documented according to the ethics approval protocol (ETOVS 32/07). A unique identifier code was assigned to each consenting patient to identify their blood or DNA sample. Approximately 20 ml of blood was collected from each patient.

2.5.2 Sample stabilization

Within six hours of sampling, lysis buffer (consisting of equal parts of 0.144 M NH_4CL and 0.01 M NH_4HCO_3) was added to each blood sample to a volume of 50 ml and left to stand for 10 min at room temperature with periodic mixing. The lysis buffer sample was centrifuged for 10 min at 3000 rpm and the supernatant discarded. Thereafter, each sample was made up to 20 ml with lysis buffer and incubated for 5 min at room temperature. Following this, each sample was centrifuged at 3000 rpm and the supernatant discarded, leaving only white blood cells. Each white blood cell pellet was homogenized in 3.2 ml of Trizol® (Invitrogen) reagent. Where the cells did not dissolve properly, additional Trizol reagent was added to ensure complete homogenization. The homogenate was stored at -70℃ until used for DNA extraction.

2.5.3 DNA extraction

The DNA extraction from the Trizol®/Blood samples was performed using the DNA/RNA isolation protocol from Trizol (http://humanvaccine.duke.edu). The Trizol®/Blood samples were thawed at room temperature before use. For each DNA extraction, 1 ml of sample was used. To each 1 ml of sample, 200 µl of chloroform was added and mixed thoroughly for 15 sec. The samples were incubated at room temperature for 15 min and then centrifuged for 15 min at 12,000 rpm. The supernatant was discarded and 300 µl of absolute ethanol added to precipitate the nucleic acids for 3 min at room temperature. This was followed by centrifugation for 5 min at 12,000 rpm. The supernatant was removed and the DNA pellet washed twice with 1 ml of 0.1 M sodium citrate containing 10% absolute ethanol. Following the washing steps, the DNA pellet was washed in 1 ml of 75% ethanol for 20 min with periodic mixing. This was followed by centrifugation for 5 min at 12,000 rpm. The supernatant was discarded and the DNA pellet was air dried for 5 min and re-suspended in 100 µl of sterile water. Where necessary, the sample was heated to 55℃ for 10 to 20 min to aid solubility. The DNA concentrations were determined for each sample using the Qubit® Fluorometer (Invitrogen).

2.5.4 PCR amplification

The PCR reactions were performed using specific DNA primers, for each of the 13 exons (Table 7). DNA primer sequences were obtained from Hamzeiy *et al.* (2002). Where PCR amplification proved problematic with the primers from Hamzeiy *et al.* (2002), additional primers were designed using the Lightcycler Probe Design Software v0.99.34 (Table 7).

PCR reactions were performed in a total volume of 50 µl. Each reaction contained 5 µl of 10x PCR Gold buffer (500 mM potassium chloride and 150 mM Tris-HCl pH 8.05), 3 µl of 25mM $MgCl_2$, 0.5 µl of 10 mM dNTP mixture, 0.16 µl Amplitaq Gold® (5 U/µl), 15 pmol of forward and reverse primer (Table 7) and between 50 to 150 ng of genomic DNA. The cycling conditions were: 10 min at 95℃ for one 1 cycle; 30 sec at 95℃, 1 min at 58℃, and 1 min at 72℃ for 40 cycles, followed by 7 min at 72℃ for 1 cycle.

Table 7: DNA primers used for the amplification of *CYP3A4* exons. Primer sequences were obtained from Hamzeiy *et al.* (2002) and In House primer design.

Primer Pairs	Primer Sequence	Annealing Temp	Fragment Size (bases)
Exon 1 F[2] Exon 1 R[2]	5' – AGTGGTGTGTGTGTGA - 3' 5' – TTAGCACCCCAAGTCC - 3'	58℃	375
Exon 2 F[2] Exon 2 R[2]	5' – GTGTCTCATGGTGGAGG - 3' 5' – TGTTCTGGGTGATGCC - 3'	58℃	426
Exon 3 F[2] Exon 3 R[2]	5' – GCCAGCAAGTCTGATTT - 3' 5' – TGCAGATTCCCATTGCA - 3'	58℃	348
Exon 4 F[2] Exon 4 R[2]	5' – TTTGGCCCACATTATCCT - 3' 5' – GGACGTGGAACCTTCC - 3'	58℃	428
Exon 5 F[1] Exon 5 R[1]	5' – CATCACCCAGTAGACAGTCAC - 3' 5' – GGCAGCTCAAATTCAGTGGAC - 3'	58℃	351
Exon 6 F[2] Exon 6 R[2]	5' – GGGAAAGCCATGTCCT - 3' 5' – GGATATGTAAACCCTGGCC - 3'	58℃	390
Exon 7 F[1] Exon 7 R[1]	5' – CCTGTTGCATGCATAGAGG - 3' 5' – GATGATGGTCACACATATC - 3'	58℃	366
Exon 8 F[2] Exon 8 R[2]	5' – TAGACCTGCCCTTGCT - 3' 5' – TGTGCTGTCTCTGACTCA - 3'	58℃	523
Exon 9 F[2] Exon 9 R[2]	5' – CACTGGTGATTCAGGC - 3' 5' – GCCTCCAACTACCACTT - 3'	58℃	362
Exon 10 F[1] Exon 10 R[1]	5' – CCAGTGTACCTCTGAATTGC - 3' 5' – CAGAGCCTTCCTACATAGAG – 3'	58℃	430
Exon 11 F[1] Exon 11 R[1]	5' – CCAGTATGAGTTAGTCTCTGGA - 3' 5' – TGTCCTGTAGATTAAGAGAGGC - 3'	58℃	416
Exon 12 F[1] Exon 12 R[1]	5' – AGGGGTGGCCCTAAGTAAG – 3' 5' – GATCACAGATGGGCCTAATTG - 3'	58℃	396
Exon 13 F[2] Exon 13 R[2]	5' – TGAAGGAGTGTCTCACTCAC - 3' 5' – CTGACAAAGGCCCCAC - 3'	58℃	750

1 - Primers sequences obtained from Hamzeiy *et al.* 2002.

2 - Primers sequences designed in-house.

F – Forward Primer.

R – Reverse Primer.

2.5.5 PCR product purification

PCR amplicons were purified prior to DNA sequencing using the illustra™ GFX™ PCR DNA and gel band purification kit, according to the kit protocol PCR product was mixed with 500 µl of Capture Buffer and mixed gently. Thereafter, the samples were loaded onto a filter column and centrifuged for 30 sec at 16,000 rpm. The flow through was discarded and the column washed twice with 500 µl Wash Buffer and centrifuged for 30 sec at 16,000 rpm. The DNA was eluted by the addition of 50 µl Elution Buffer and centrifugation for 1 min at 16,000 rpm.

2.5.6 Gel electrophoresis

PCR amplicons were resolved with 2% agarose (molecular biology grade) gel electrophoresis, in 1x TAE buffer (0.438 g/L Tris, 0.022 g/L EDTA pH 8 and 0.09 ml/L Acetic Acid) for approximately 25 min at 130 V and 200 mA. Thereafter, the gel was stained in ethidium bromide (0.5 µg/ml) for approximately 25 min and visualised using a Kodak Gel Logic 200 Imaging system with the Kodak1D v3.6.5 software, under UV light.

2.5.7 DNA sequencing reactions

All sequencing reactions were performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The reactions were set up according to the manufacturer's protocol. Sequencing reactions were performed in a total volume of 10 µl. Each reaction contained 2 µl of Terminator mix, 1 µl of Sequencing buffer, 5 µl of purified PCR product and 0.8 pmol of primer (Table 7). Sequencing reactions were performed with forward and reverse primers, respectively. The cycling conditions were: 1 min at 96℃ for one cycle; 10 sec at 96℃, 5 sec at 58℃, and 4 min at 60℃ for 25 cycles.

2.5.8 DNA sequencing product precipitation

The sequencing products were precipitated prior to capillary electrophoresis using ethanol/sodium acetate precipitation according to the ABI PRISM BigDye Terminator v3.0 Ready Reaction Cycle Sequencing kit protocol. Sequencing reaction products were added to a pre-mixed solution containing 3 µl of 3M sodium acetate (pH 5) kept at 4℃, 62.5 µl of absolute ethanol and 14.5 µl of sterile water, mixed thoroughly and stored in the dark for 30 min followed by centrifugation for 30 min at 13,200 rpm. The

supernatant was discarded, and the pellet washed with 250 µl 70% ethanol by mixing, followed by centrifugation for 10 min at 13,200 rpm. The supernatant was discarded and the DNA pellet dried at 90℃ for 1 min. Following this, 25 µl Hi-Di™ Formamide (Applied Biosystems) was added mixed to homogenate. Finally, the sequencing sample was denatured at 95℃ for 2 min, and quick cooled on ice for 5 min.

2.5.9 DNA sequencing

DNA sequencing was performed using the ABI Prism 310 Genetic Analyzer (Applied Biosystems). Separation of DNA fragments was performed using polymer POP-6 (Performance Optimized Polymer – 6). The samples were electro-kinetically injected into the analyzer for 30 sec at 12.2 kV and seperated for approximately 70 min at 50 volts/cm, 5 µA and 50℃. The data was analysed by the Sequence Analysis Software v5.31 (Applied Biosystems), and interpreted with Chromas v2.31 (http:www.technelysium.com.au) against the GenBank (http://www.ncbi.nlm.nih.gov) reference sequence AF280107, using the online software, LALIGN (http://www.ch.embnet.org/software/LALIGN_form.html).

2.5.10 Three dimensional (3D) protein structure imaging

The 3D structure of CYP3A4 was generated with the software package RasMol (http://www.openrasmol.org) together with a PDB file specific to CYP3A4, obtained from the Protein Data Base (PDB) (http://www.rcsb.org/pdb/home/home.do). The CYP3A4 PDB file contains the information regarding folding patterns, amino acids involved with the beta sheets and alpha helices of the protein. The RasMol software renders the PDB file into a visible 3D structure. The location of amino acids forming the active site of CYP3A4 was obtained from Williams *et al.* (2004), and illustrated using the RasMol software. Any amino acid substitutions can also be shown in the 3D structure.

2.5.11 Statistical analysis

SNP data was analysed using SNPator, a web based statistical analyses package for SNPs (Morcillo-Suarez *et al.* 2008) (http://bioinformatica.cegen.upf.es/public/principal/dataMngA.php). Allele and genotype frequencies were calculated and deviation from Hardy-Weinberg equilibrium assessed by Chi-square and Fisher's exact probability test. Confidence

Intervals (95% CI) were calculated using the Odds Ratio with Haldane correction. Associations were determined between SNPs and adverse drug reactions or imatinib dosage, respectively. A p-value of less than 0.05 was considered statistically significant.

Chapter 3: Results and Discussion

3.1 Results and discussion

A total of 25 samples were analysed from which the 13 exons of *CYP3A4* were PCR amplified and sequenced, respectively. From this, six SNPs were detected, of which four were located in introns and two in exons (Table 8). Two of the SNPs detected were novel, I369V and G73239A, as they have not previously been described in the literature and were identified in an exon and intron, respectively.

The novel SNP, I369V, was identified in exon 11 at base 83920 (Table 8). The SNP is a base change from A to G (Figure 8). The SNP results in a codon change from ATT to GTT that encodes a valine instead of an isoleucine. This SNP is denoted as I369V, based on the position of the amino acid substitution. The altered amino acid is situated in close proximity to the active site of the enzyme (Figure 9). However, although the SNP alters the peptide sequence of CYP3A4, it was not statistically associated with ADRs in patients treated with imatinib (Table 9 and Table 12).

SNP I369V has not previously been reported in literature, and was only identified in two Sesotho patients (Table 10) associated with the experimental group, with an allele frequency of 0.04 (Table 11). Further research involving a population study will be required to determine whether this is a rare SNP, as in the case with *CYP3A4*3*, where it was only detected in one of 91 Chinese individuals investigated. Although thought to be rare, this SNP was later shown to be present in the Dutch Caucasian population as well at a frequency of 0.011 (Sata *et al.* 2000, van Schaik *et al.* 2001).

The novel intron SNP is located in intron three at base 73239 (Table 8). This SNP involves a base substitution of a G with an A, and is subsequently referred to as G73239A (Figure 10). This SNP was heterozygous in one Sesotho patient with an allele frequency of 0.02 (Table 11) This SNP was not statistically associated with the experimental group (Table 9 and Table 12) even though it was detected in a patient experiencing ADRs (Table 10).

The remaining four SNPs detected, included a known synonymous SNP, and three known intron SNPs. The synonymous SNP, I193I, is located at base 77664, in exon 7 (Table 8). This SNP was heterozygous in two patients (Sesotho) associated with the experimental group (Table 10) with an allele frequency of 0.08 (Table 11). This SNP involves a base change of a C with a T, that results in a codon change from ATC to ATT, coding for isoleucine (Figure 11). Synonymous SNPs do not produce altered coding sequences and are therefore not expected to change the function of the protein in which they occur (Purvis *et al.* 1987). However, synonymous SNPs are thought to have an impact on protein folding as a result of translational pause (Parmley and Hurst *et al.* 2007; Sauna *et al.* 2007).

Protein folding has been shown to occur immediately after peptide formation, while the remainder of the protein is still being translated (Horton *et al.* 2002). A synonymous SNP is thought to slow the translational process by forcing the ribosome to pause during translation while producing a suitable tRNA molecule corresponding to the new codon (Sauna *et al.* 2007). During this translational pause, the translated protein undergoes folding, despite the fact that the entire peptide chain is not fully translated. This premature folding may result in an altered protein structure and altered activity (Horton *et al.* 2002; Sauna *et al.* 2007). A study by Kimchi-Sarfaty *et al.* (2007) found that a synonymous SNP affects the timing of translational folding of the P-glycoprotein, thereby altering the protein structure with regard to substrate interaction.

The three known intron SNPs detected in this study included *CYP3A4*1G*, T15871G and C23187T (Table 8). From all the SNPs detected, *CYP3A4*1G* was calculated to have a significant association with the experimental group ($P = 3.217 \times 10^{-8}$) (Table 9). The *CYP3A4*1G* SNP is located in intron 10 at base 82266. This SNP involves a base change of a G with an A (Figure 12). Five patients were homozygous (Sesotho: $f^4 = 0.2$) for this polymorphism, and five were heterozygous (Sesotho: $f = 0.06$ and Coloured: $f = 0.04$), with a combined allele frequency of 0.30 (Table 10). This frequency is considerably higher than all the other SNPs detected in this study. In previous research, this SNP was found to be present at a frequency of 0.146 in Caucasians, 0.73 in African Americans and 0.375 in Asians (Dai *et al.* 2001).

[4] - Frequency

However, different results were obtained from a study by Eiselt *et al.* (2001) and Lamba *et al.* (2002) for Caucasians and African Americans, with a frequency of 0.095 and 0.5 respectively.

The intron SNP T15871G was also statistically associated with ADRs (P = 0.0172) (Table 9). However, this SNP was only detected in two patients (Sesotho), and had a higher allelic frequency (0.06) due to its homozygous status in one patient. In previous research, this SNP is reported to have a frequency of 0.065 in Caucasians and 0.48 in African Americans (Lamba *et al.* 2002). T15871G is located in intron 7 and involves a base change of a T with a G (Figure 13). The intron SNP C23871T was not significantly associated with ADRs, even though it is grouped with the experimental group (Table 9 and Table 12). This SNP is located in intron 11 and involves a base change of a C with a T (Figure 14). This SNP was homozygous and only detected in one patient (Sesotho), with an allele frequency of 0.04. Previous research indicates this polymorphism to be present at a frequency of 0.21 in African Americans (Dai *et al.* 2001).

Since the cohort comprised a mixed South African population (Black (Sesotho), Caucasian and Coloured), it is possible that SNPs, especially those occurring at low frequency and found only in patients experiencing ADRs may prove significant in a larger population study. For example, the majority of the patients that participated in this study were Sesotho, with a few Caucasians and Coloured patients. The SNPs detected were all associated with the Sesotho ethnic (Black) group, and only two coloured patients presented the *CYP3A4*1G* SNP. No SNP was detected within Caucasian patients, which consisted of 3 patients. Due to the cohort only consisting of a small number of patients, a significant population study could not be carried out.

It is not certain how an intron SNP could impact on the metabolic rate of an enzyme. However, Hirota *et al.* (2004) reported on two SNPs in intron seven of *CYP3A4*, C78013T and C78649T are significantly associated with lower levels of CYP3A4 mRNA. The lower levels of CYP3A4 expression result in reduced metabolic capability. Although this was confirmed by Schrimer *et al.* (2007), the possible effect of intronic SNPs on gene expression in still unknown.

It has been hypothesised that an SNP can alter intron splice sites (Swida *et al.* 1986; Jap *et al.* 2002; Mathijssen *et al.* 2006). Swida *et al.* (1986) found that an SNP in an intron of *CYH2* (ribosomal protein) in the yeast Saccharomyces cerevisiae, inhibits splicing efficacy during mRNA processing. Other studies have found similar results. For example, an intron SNP in the cholesterol ester transfer protein (*CETP*) gene, has been associated with hyperalphalipoproteinemia disease (Jap *et al.* 2002). In contrast to these studies, it has also been shown that intron SNPs can induce protein expression. Sachse *et al.* (1999) and Basile *et al.* (2000) found that an SNP in intron one of *CYP1A2* was associated with the ADR, tardive dyskinesia (TD). This potentially irreversible ADR is reported to develop during long term treatment with typical antipsychotic drugs and involves involuntary muscle movements. This study demonstrated that the severity of TD was 3.4 fold greater in the presence of the intron SNP than in patients without this allele (Basile *et al.* 2000).

3.2 DNA primers

DNA primer sequences for the amplification of the 13 exons of *CYP3A4* were obtained from Hamzeiy *et al.* (2002) (Table 7). However, only exons 5, 7, 10, 11 and 12 were successfully amplified using these primers (Figure 15). For exons 1, 2, 3, 4, 6, 8, 9, and 13 the PCR reactions did not result in amplification or produced more than one amplicon. Sequencing of these PCR products was unsuccessful and new primers for these exons were designed. The new primers for exons 1, 2, 3, 4, 6, 8, 9, and 13 produced single amplicons (Figure 16) of the expected size that were possible to sequence (Table 7).

3.3 Conclusion

ADRs can adversely affect treatment, in CML patients treated with imatinib. It is recommended that the treatment is stopped, should ADRs become a problem. Thus ADRs can adversely affect the outcome of treatment. There is currently little understanding of the link between ADRs in patients being treated with imatinib and CYP3A4 metabolism. An understanding of the effect of SNPs on the activity of CYP3A4 and subsequently also possibly ADRs, will allow for more effective management of CML patients experiencing ADRs from imatinib.

In this study six SNPs were detected of which two, I369V and G73239A, were not previously described in literature. These two SNPs were not statistically associated with the presence of ADRs in this study. Two known intron SNPs, *CYP3A4*1G* and T15871G, were significantly associated with the presence of ADRs in CML patients being treated with imatinib. It is currently hypothesised that intron SNPs can delay intron splicing in mRNA resulting in a decreased expression level of the enzyme. Two other SNPs, previously described, were detected in patients experiencing ADRs. However they were not statistically associated with the occurrence of ADRs. It is possible that these SNPs were not significantly associated as a result of a combination of low frequency and small population size.

This study was not intended to analyse different populations in SA. Furthermore, the effect of SNPs on gene expression was not investigated and the normal frequency of these SNPs in different SA populations not determined. Thus future research should focus on these aspects. It is hoped that this research will act as a basis for future studies and assist in understanding the association between ADRs and SNPs, since this could affect the treatment of CML patients with imatinib as well as other tyrosine kinase inhibitors.

Table 8: SNPs identified in the *CYP3A4* gene in CML patients treated with Imatinib.

SNP	Position in Genbank (AF280107)	Nucleotide Change	Amino Acid Change	Homo/ Heterozygous	Intron/ Exon
I 369 V (*Novel*)	83920	A to G	Ile to Val	Heterozygous	Exon 11
I193I	77664	C to T	None	Heterozygous	Exon 7
G73239A (*Novel*)	73239	G to A	None	Heterozygous	Intron 3
T15871G	77789	T to G	None	Homozygous and Heterozygous	Intron 7
*CYP3A4*1G*	82266	G to A	None	Homozygous and Heterozygous	Intron 10
C23187T	85117	C to T	None	Homozygous	Intron 11

Table 9: Allelic association with experimental and control group.

SNP	P	Odds Ratio	CI 95	P-Fisher
I 369 V (*Novel*)	0.0543	9.8485	0.45-217.24	0.1249
I193I	0.0543	9.8485	0.45-217.24	0.1249
G73239A (*Novel*)	0.1780	5.5714	0.22-144.12	0.3600
T15871G	0.0172 *	14.6774	0.71-302.08	-
*CYP3A4*1G*	3.217×10^{-8} **	108.5000	11.09-1061.22	-
C23187T	0.0543	9.8485	0.45-217.24	0.1249

* - Significant at $P < 0.05$

** - Significant at $P < 0.01$

Table 10: SNPs identified within the study group illustrating homozygote and heterozygote individuals relative to the experimental and control groups.

SNP / Patient	I 369 V (*Novel*)	I193I	G73239A (*Novel*)	T15871G	*CYP3A4* 1G*	C23187T	STATUS[1]	Dosage
1	A/A	C/C	G/G	T/T	G/G	C/C	C	400
2	A/A	C/C	G/G	T/T	G/G	C/C	C	400
3	A/A	C/C	G/G	T/T	G/G	C/C	C	600
4	A/A	C/C	G/G	T/T	G/G	C/C	C	400
5	**A/G**	C/C	**G/A**	T/T	**G/A**	C/C	E	400
6	A/A	C/C	G/G	T/T	**G/A**	C/C	C	800
7	A/A	C/C	G/G	T/T	G/G	C/C	C	600
8	A/A	C/C	G/G	T/T	G/G	C/C	C	400
9	A/A	**C/T**	G/G	T/T	**A/A**	C/C	E	200
10	A/A	**C/T**	G/G	**G/G**	**A/A**	C/C	E	200
11	A/A	C/C	G/G	T/T	**A/A**	C/C	E	200
12	**A/G**	C/C	G/G	**T/G**	**A/A**	C/C	E	200
13	A/A	C/C	G/G	T/T	G/G	C/C	C	400
14	A/A	C/C	G/G	T/T	G/G	C/C	C	800
15	A/A	C/C	G/G	T/T	G/G	C/C	C	400
16	A/A	C/C	G/G	T/T	G/G	C/C	C	800
17	A/A	C/C	G/G	T/T	**G/A**	C/C	E	200
18	A/A	C/C	G/G	T/T	G/G	C/C	C	400
19	A/A	C/C	G/G	T/T	**A/A**	**T/T**	E	300
20	A/A	C/C	G/G	T/T	**G/A**	C/C	E	200
21	A/A	C/C	G/G	T/T	G/G	C/C	C	400
22	A/A	C/C	G/G	T/T	G/G	C/C	C	400
23	A/A	C/C	G/G	T/T	**G/A**	C/C	E	400
24	A/A	C/C	G/G	T/T	G/G	C/C	C	400
25	A/A	C/C	G/G	T/T	G/G	C/C	C	800

[1] - **C** is for control group indicating those patients that did not experience ADRs to Imatinib.

E refer to the experimental group, including the patients who experience ADRs to Imatinib.

Table 11: Allele and Genotype frequencies within the study group.

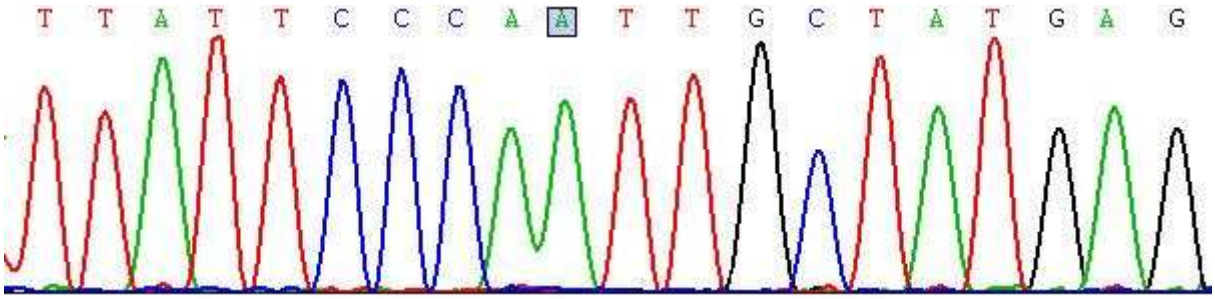
SNP	Base Change	Allele Frequency		Genotype Frequency		
I 369 V (*Novel***)**	A to G	A: 0.9600	G: 0.0400	AA: 0.9200	AG: 0.0800	GG: 0.0000
I193I	C to T	C: 0.9600	T: 0.0400	CC: 0.9200	CT: 0.0800	TT: 0.0000
G73239A (*Novel*)	G to A	G: 0.9800	A: 0.0200	GG: 0.9600	GA: 0.0400	AA: 0.0000
T15871G	T to G	T: 0.9400	G: 0.0600	TT: 0.9200	TG: 0.0400	GG: 0.0400
*CYP3A4*1G*	G to A	G: 0.7000	A: 0.3000	GG: 0.6000	GA: 0.2000	AA: 0.2000
C23187T	C to T	C: 0.9600	T: 0.0400	CC: 0.9600	CT: 0.0000	TT: 0.0400

Table 12: Hardy-Weinberg Equilibrium of SNPs detected.

SNP	Observed	Expected	X^2	P
I 369 V (*Novel***)**	0.0800	0.0768	0.0036	0.9521
I193I	0.0800	0.0768	0.0036	0.9521
G73239A (*Novel*)	0.0400	0.0392	0.0004	0.9836
T15871G	0.0400	0.1128	1.3240	0.2499
*CYP3A4*1G*	0.2000	0.4200	4.9672	0.0258 *
C23187T	0.0000	0.0768	2.0797	0.1493

* - Significant at $P < 0.05$

(i) Reference Sequence (Exon 11)



(ii) Sequencing Results - Exon 11 (Heterozygous)

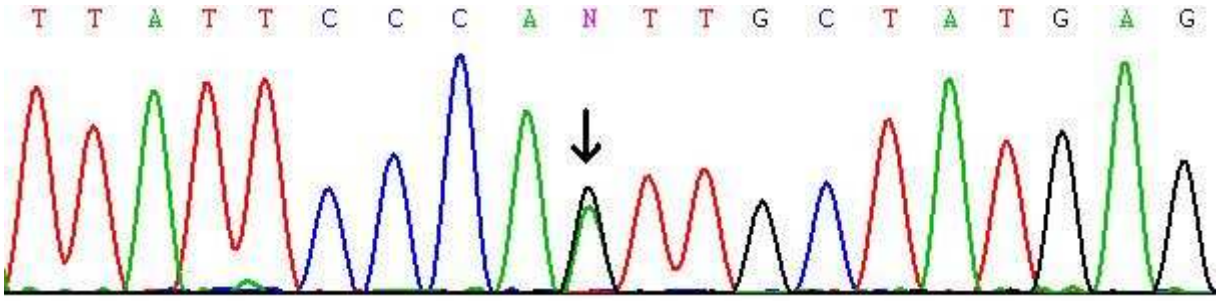


Figure 8: Sequence results of I369V SNP located in exon 11. In this sequence a **A**-Nucleotide is substituted for a **G**-Nucleotide. The SNP is located at the arrow, where a black peak (**G**-Nucleotide) is at the same location as a green peak (**A**-Nucleotide). The **N** denotes two bases called at the same location, signifying a heterozygous SNP. **(i)** – Reference DNA sequence for exon-11; **(ii)** – Exon 11 sequence results.

(i)

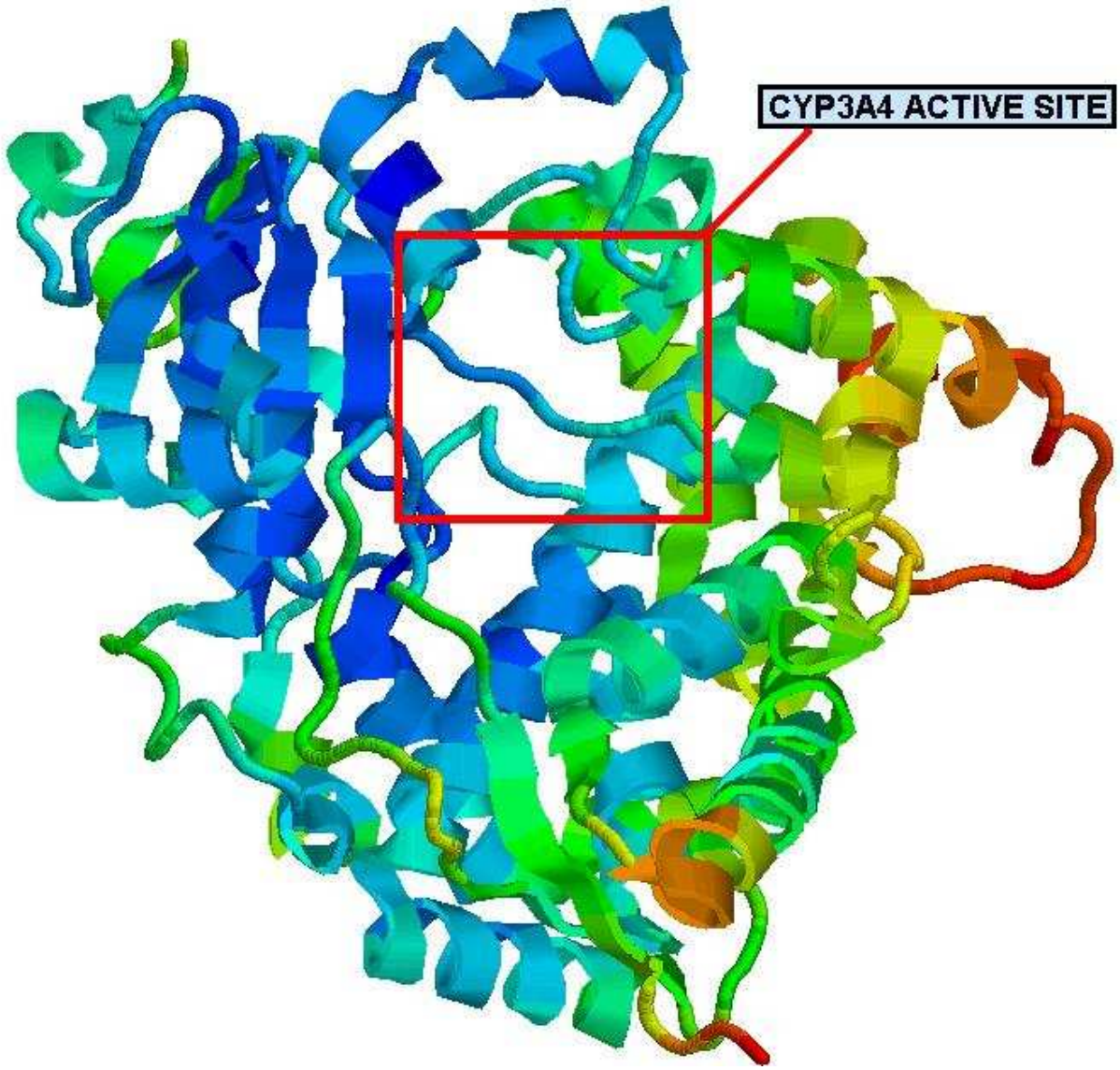


Figure 9 (i): CYP3A4 three dimensional structure. Area within red box illustrates the active site of CYP3A4 where a substrate would bind to be metabolised. Visualized using RasMol (Available from: http://www.openrasmol.org).

(ii)

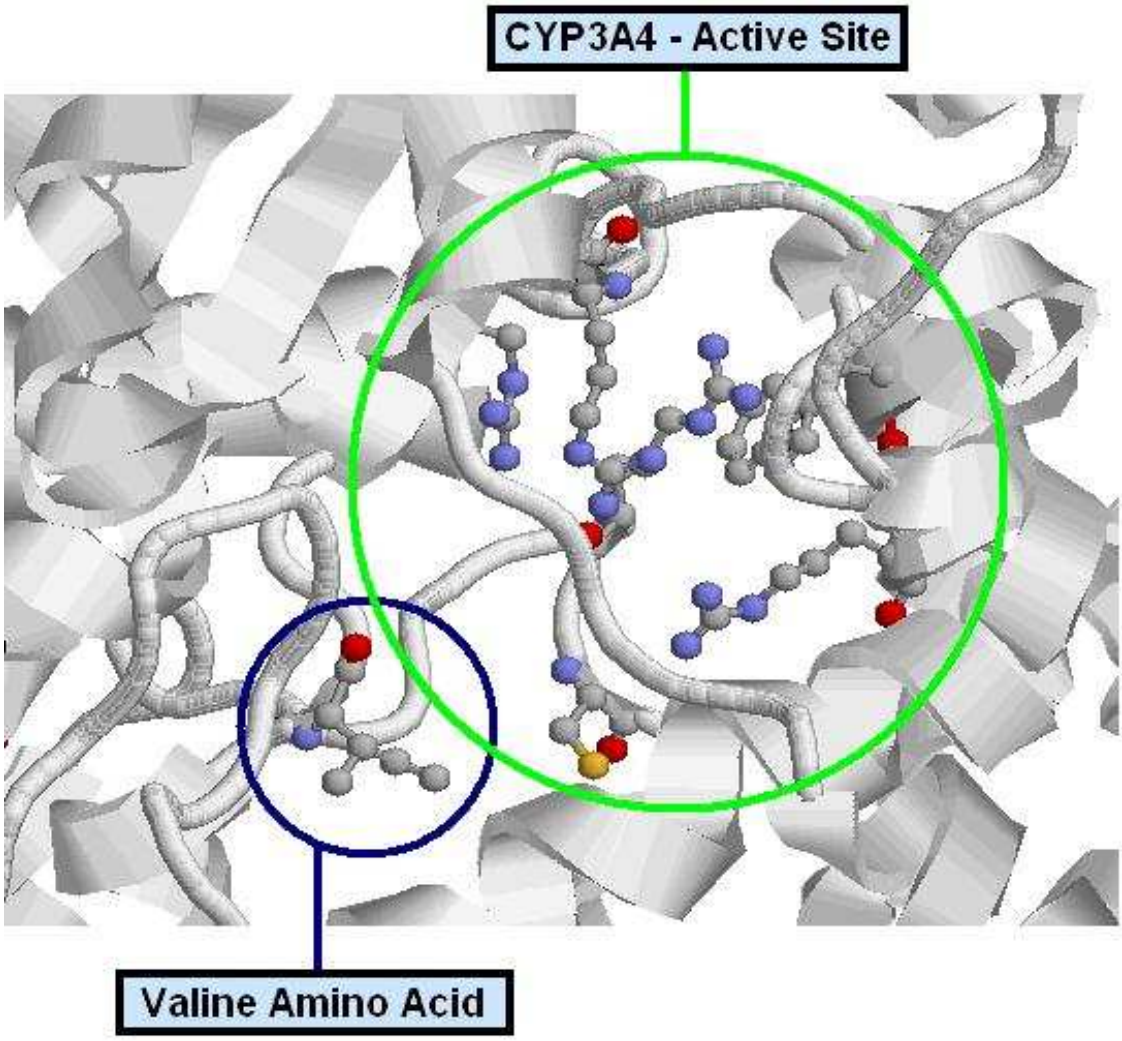
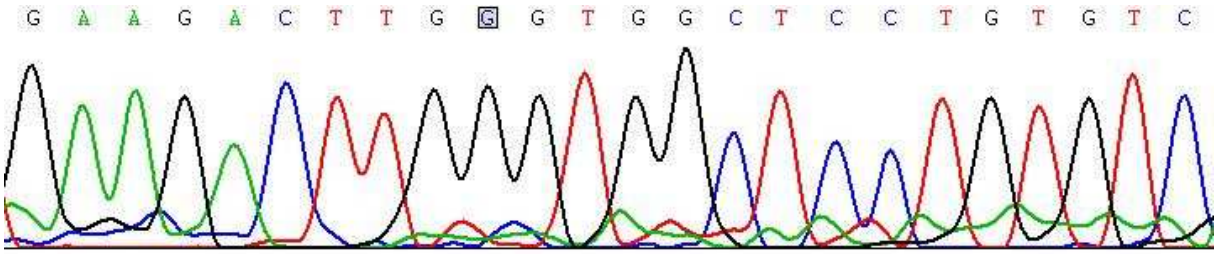


Figure 9 (ii): CYP3A4 Three Dimensional structure. A close-up view of the CYP3A4 active site. The area within the green circle illustrates the active site of CYP3A4, and the amino acids (ball and stick structures) that make up the active site of CYP3A4. The amino acid circled in blue (ball and stick structure) is the Valine amino acid which is the result of the I 329 V SNP. The blue coloured ball in the amino acid structure represent the Nitrogen, the red ball an oxygen atom, and the grey balls are carbon atoms.

(i). Reference Sequence



(ii). Sequencing Results (Heterozygous)

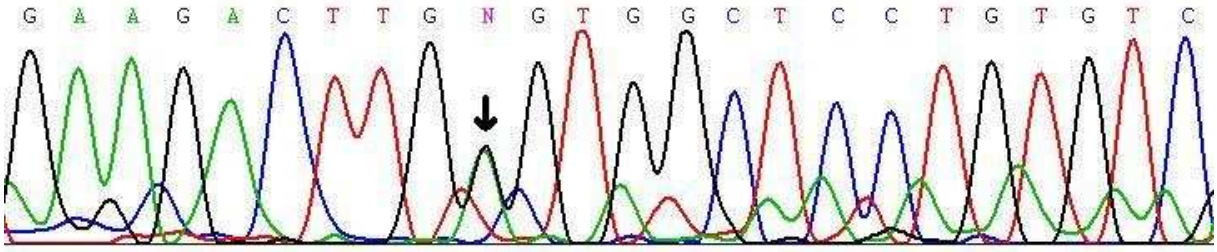
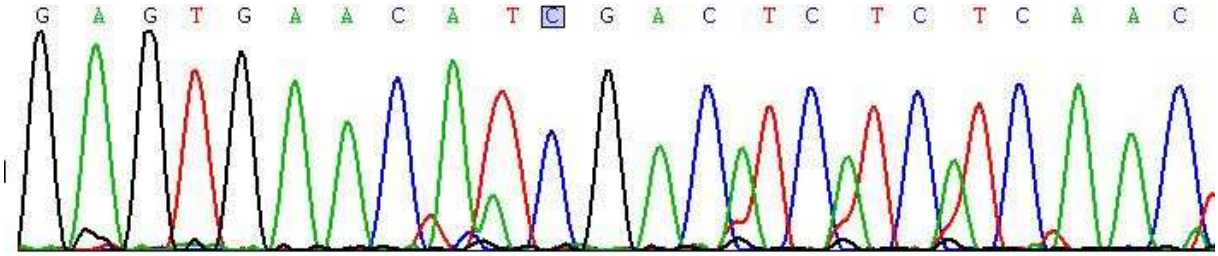


Figure 10: Sequence results of G73239A SNP located in intron 3. In this sequence a **G**-Nucleotide (reference sequence) is substituted for an **A**-Nucleotide. The SNP is located at the arrow, where a black peak (**G**-Nucleotide) is at the same location as a green peak (**A**-Nucleotide). In figure **(ii)**, the **N** denotes two bases called at the same location, signifying a heterozygous SNP. **(i)** – Reference DNA sequence; **(ii)** – Intron 3 sequence results.

(i) Reference Sequence – Exon 7



(ii) Sequencing Results –Exon 7 (Heterozygous)

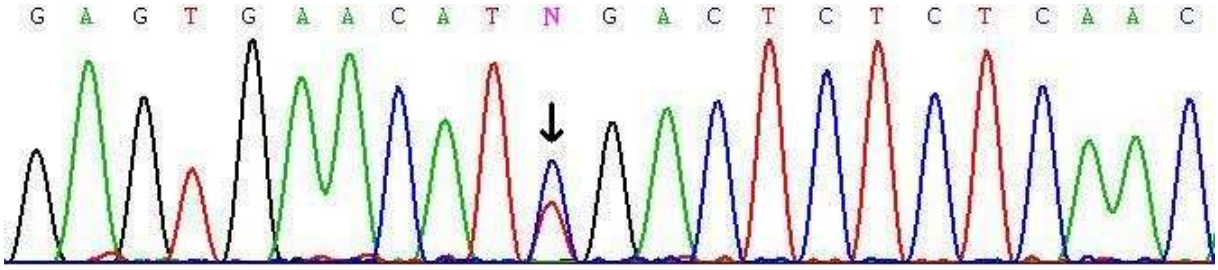
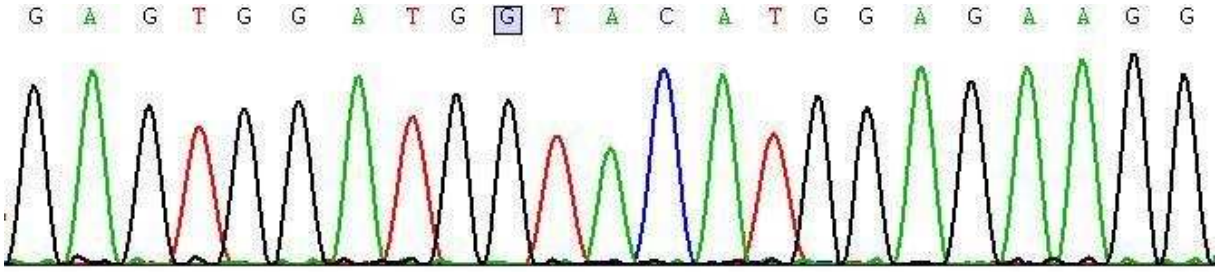
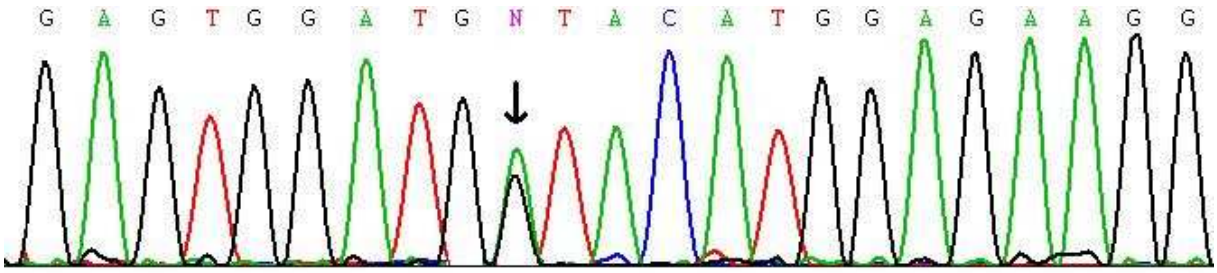


Figure 11: Sequence results of I193I located in exon 7. In this sequence the **C**-Nucleotide is substituted for a **T**-Nucleotide. The SNP is located at the arrow, where a blue peak (**C**-Nucleotide) is at the same location as a red peak (**T**-Nucleotide). The **N** denotes two bases called at the same location, signifying a heterozygous SNP. **(i)** – Reference DNA sequence. **(ii)** – Exon 7 sequencing results.

(i) Reference Sequence (Intron 10)



(ii) Sequencing Results - Intron 10 (Heterozygous)



(iii) Sequencing Results - Intron 10 (Homozygous)

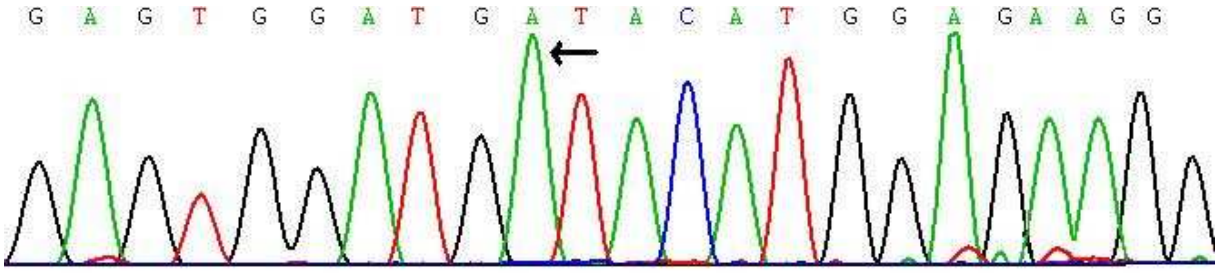
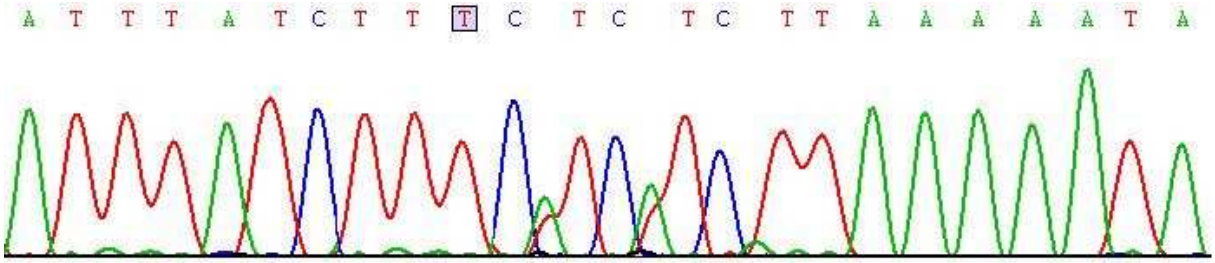
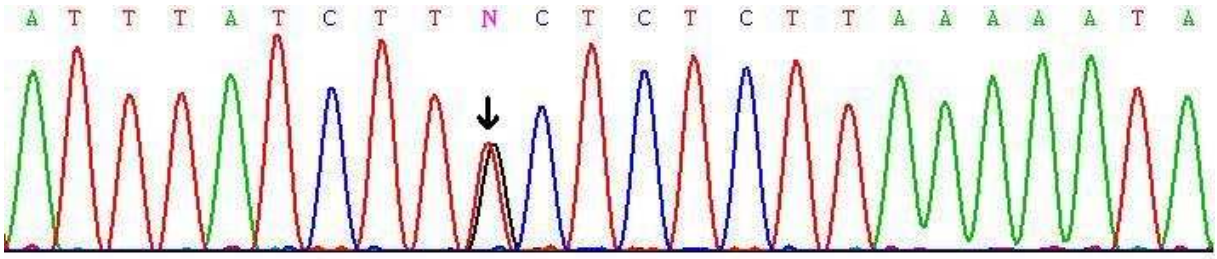


Figure 12: Sequence results of *CYP3A4*1G* located in intron 10. In this sequence the **G**-Nucleotide is substituted for a **A**-Nucleotide. The SNP is located at the arrow in both **(ii)** and **(iii).** In Figure **(ii)**, the black peak (**G**-Nucleotide) is at the same location as a green peak (**A**-Nucleotide). The **N** denotes two bases called at the same location, signifying a heterozygous SNP. In Figure **(iii)**, there is only one green peak (**A**-Nucleotide), in the position of the original black peak (**G**-Nucleotide), signifying a homozygous SNP. **(i)** – Reference DNA (Intron-10) sequence. **(ii)** – Intron-10 sequencing results with heterozygous SNP. **(iii)** - Intron-10 sequencing results with homozygous SNP.

(i) Reference Sequence (Intron 7)



(ii) Sequencing Results - Intron 7 (Heterozygous)



(iii) Sequencing Results - Intron 7 (Homozygous)

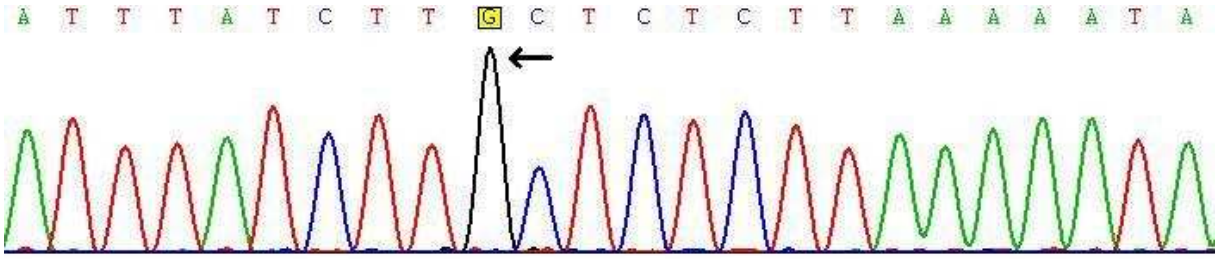
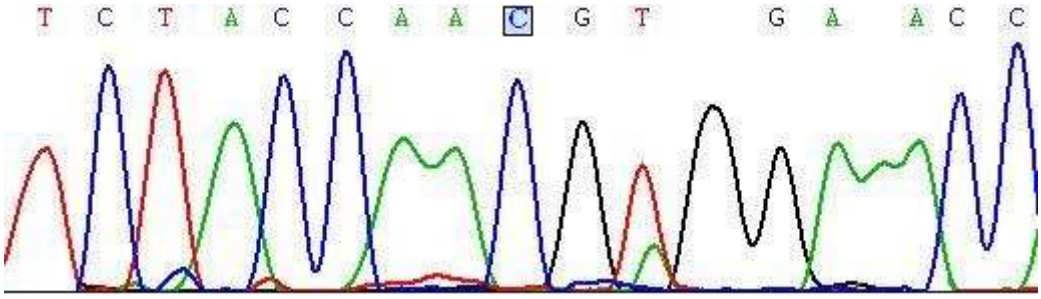


Figure 13: Sequence results of T15871G located in intron 7. In this sequence the **T**-Nucleotide is substituted for a **G**-Nucleotide. The SNP is located at the arrow in both **(ii)** and **(iii).** In Figure **(ii)**, the black peak (**G**-Nucleotide) is at the same location as a red peak (**T**-Nucleotide). The **N** denotes two bases called at the same location, signifying a heterozygous SNP. In Figure **(iii)**, there is only one black peak (**G**-Nucleotide), in the place of the original red peak (**T**-Nucleotide), signifying a homozygous SNP. **(i)** – Reference DNA (Intron-7) sequence. **(ii)** – Intron-7 sequencing results with heterozygous SNP. **(iii)** - Intron-7 sequencing results with homozygous SNP.

(i) Reference Sequence (Intron 11)



(ii) Sequencing Results - Intron 11 (Homozygous)

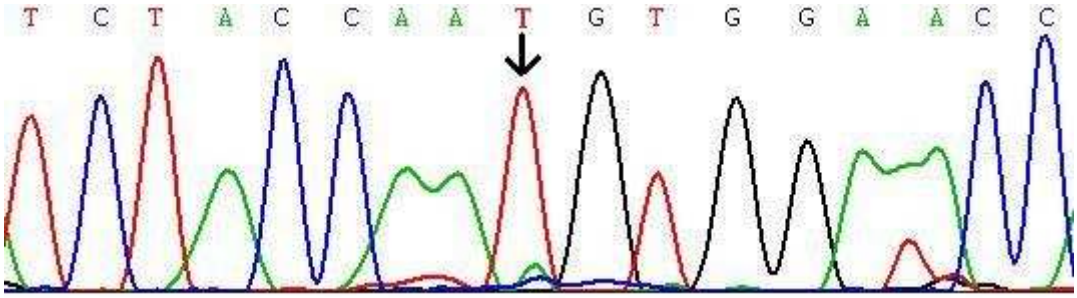


Figure 14: Sequence results of C23187T located in intron 11. In this sequence the **C**-Nucleotide is substituted for a **T**-Nucleotide. The SNP is located at the arrow in **(ii)**. In Figure **(ii)**, there is only one red peak (**T**-Nucleotide), in the place of the original blue peak (**C**-Nucleotide), signifying a homozygous SNP. **(i)** – Reference DNA (Intron-11) sequence. **(ii)** - Intron-11 sequencing results with homozygous SNP.

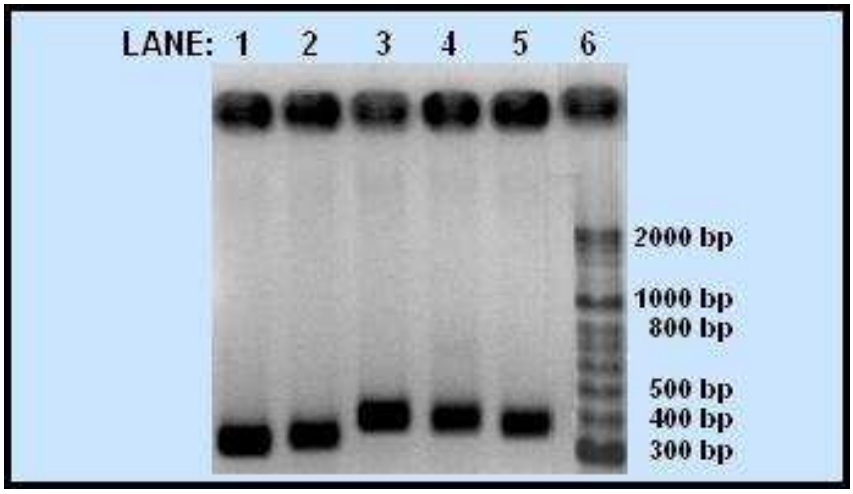


Figure 15: Gel electrophoresis image for Exon 5, 7, 10, 11, 12.

Lane 1: Exon 5 (351 bp); **Lane 2:** Exon 7 (366 bp); **Lane 3:** Exon 10 (430 bp);

Lane 4: Exon 11 (416 bp); **Lane 5:** Exon 12 (396 bp); **Lane 6:** HyperLadder II

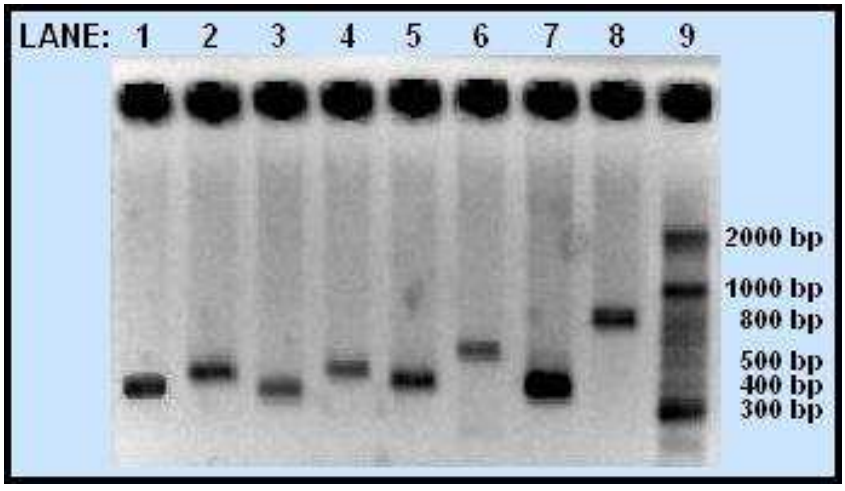


Figure 16: Gel electrophoresis image for Exon 1, 2, 4, 6, 8, 9, 13.

Lane 1: Exon 1 (375 bp); **Lane 2:** Exon 2 (426 bp); **Lane 3:** Exon 3 (348 bp);

Lane 4: Exon 4 (428 bp); **Lane 5:** Exon 6 (390 bp); **Lane 6:** Exon 8 (523 bp);

Lane 7: Exon 9 (362 bp); **Lane 8:** Exon 13 (750 bp), **Lane 9:** HyperLadder II

Chapter 4: References

Ali R, Özakalemkas F, Özkocaman V, Özcelik T, Ozan U, Kimya Y and Tunali. (2004). Successful pregnancy and delivery in a patient with Chronic Myelogenous Leukemia (CML), and management of CML with Leukapheresis during pregnancy: a case report and review of the literature. *Japanese Journal of Clinical Oncology* 34(4): 215–217.

Allabi AC, Gala JL, Horsmans Y, Babaoglu MO, Bozkurt A, Heusterspreute M and Yasar U. (2004). Functional impact of CYP2C9*5, CYP2C9*6, CYP2C9*8 and CYP2C9*11 in vivo among black Africans. *Clinical Pharmacology and Therapeutics* (76)2: 113–18.

Basile VS, Özdemir V, Masellis M, Walker ML, Meltzer HY, Lieberman JA, Potkin SG, Alva G, Kalow W, Macciardi FM and Kennedy JL. (2000). A functional polymorphism of the cytochrome P450 1A2 (*CYP1A2*) gene: association with tradive dyskinesia in schizophrenia. *Molecular Psychiatry* 5: 410–417.

Bernard S, Neville KA, Nguyen AT and Flockhartb DA. (2006). Interethnic differences in genetic polymorphisms of CYP2D6 in the U.S. population: Clinical implications. *The Oncologist* 11: 126–135.

Blume-Jensen P and Hunter T. (2001). Oncogenic kinase signalling. *Nature* 411(6835): 355–365.

Boulton DW, Arnaud P and DeVane L. (2001). A single dose of methadone inhibits cytochrome P-4503A activity in healty volunteers as assessed by the urinary cortisol ration. *Clinical Pharmacology* 51: 350–354.

Capdevila JH, Falck JR and Harris RC. (2000). Cytochrome P450 and arachidonic acid bioactivation: molecular and functional properties of the arachidonate monooxygenase. *Journal of Lipid Research* 41: 163–181.

Chang S, Li W, Traeger SC, Wang B, Cui B, Zhang H, Wen B and Rodrigues AD. (2008). Confirmation that cytochrome P450 2C8 (CYP2C8) plays a minor role in (*S*)-(+)- and (*R*)-(-)-Ibuprofen hydroxylation *in vivo. Drug Metabolism and Disposition*: 1–42.

Cellule PK, Gordon M, Palanee T, Page T, Mosam A, Coovadia HM and Cassol S. (2003). *MDR1* and *CYP3A4* polymorphisms among African, Indian, and white populations in KwaZulu-Natal, South Africa. *Clinical Pharmacology and Therapeutics* 74(2): 195–196.

Choudhary DR, Mishra P, Kumar R, Mahapatra M and Choudhry VP. (2006). Pregnancy on Imatinib: fatal outcome with meningocele. *Annals of Oncology* 17(1): 178–179.

Cohen MH and Tang S. (2006). NDA 21-588-S016-2006-03-27 Executive Summary Gleevec for Pediatric Ph+ CML. *Novartis*: 1–6.

Cohen MH, Williams G, Johnson JR, Duan J, Gobburu J, Rahman A, Benson K, Leighton J, Kim SK, Wood R, Rothmann M, Chen G, U KM, Staten AM and Pazdur R. (2002). Approval summary for Imatinib Mesylate capsules in the treatment of Chronic Myelogenous Leukemia. *Clinical Cancer Research* 8: 935–942.

Coon MJ, Ding X, Pernecky SJ and Vaz AND. (1992). Cytochrome P450: progress and predictions. *The FASEB Journal* 6: 669–673.

Dai D, Tang J, Rose R, Hodgson E, Bienstock RJ, Mohrenweiser HW and Goldstein JA. (2001). Identification of variants of CYP3A4 and characterization of their abilities to metabolise testosterone and chlorpyrifos. *The Journal of Pharmacology and Experimental Therapeutics* 299(3): 825–831.

Daly AK. (2006). Significance of the minor cytochrome P450 3A isoforms. *Clinical Pharmacokinetics* 45(1): 13–31.

de Jong FA, de Jonge MJ, Verweij J, Mathijssen RH. (2006). Role of pharmacogenetics in irinotecan therapy. *Cancer Letters* 234(1): 90-106.

de Kogel CE and Schellens JHM. (2007). Imatinib. *The Oncologist* 12: 1390–1394.

Deininger MWN, O'Brien SG, Ford JM and Druker BJ. (2003). Practical management of patients with Chronic Myeloid Leukemia receiving Imatinib. *Journal of Clinical Oncology* 21(8): 1637–1647.

Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lyndon NB, Kantarjian H, Capdeville R, Ohno-Jones S and Sawyers CL. (2001). Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in Chronic Myeloid Leukemia. *The New England Journal of Medicine* 344(4): 1031–1037.

Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian H, Gattermann N, Deininger MWN, Silver RT, Goldman JM, Stone RM, Cervantes F, Hochhaus A, Powell BL, Gabrilove JL, Rousselot P, Reiffers J, Cornelissen JJ, Hughes T, Agis H, Fischer T, Verhoef G, Shepherd J, Saglio G, Gratwohl A, Nielsen JL, Radich JP, Simonsson B, Taylor K, Baccarani M, So C, Letvak L and Larson RA. (2006). Five year follow-up of patients receiving Imatinib for Chronic Myeloid Leukemia. *The New England Journal of Medicine* 255(23): 2408–2417.

Eap CB, Buclin T, Hustert E, Bleiber G, Golay KP, Aubert AC, Bauman P, Telenti A and Kerb R. (2004). Pharmacokinetics of midazolam in CYP3A4- and CYP3A5-genotyped subjects. *European Journal of Clinical Pharmacology* 60: 231–236.

Ebnöether M, Stentoft J, Ford J, Buhl L and Gratwohl A. (2002). Cerebral oedema as a possible complication of treatment with imatinib. *Lancet* 359(9319): 1751–1752.

Eiselt R, Domanski TL, Zibat A, Mueller R, Presecan-Siedel E, Hustert E, Zanger UM, Brockmoller J, Klenk HP, Meyer UA, Khan KK, He YA, Halpert JR, Wojnowski L. (2001). Identification and functional characterization of eight CYP3A4 protein variants. Pharmacogentics 11(5): 447–458.

Ekins S, Nikolsky Y and Nikolskaya T. (2005). Techniques: Applications: Application of systems biology to absorption, distribution, metabolism, excretion and toxicity. *Trends in Pharmacological Science* 26(4): 202–209.

Estabrook RW. (2003). A passion for P450's (Remembrances of the early history of research on cytochrome P450). *Drug Metabolism and Disposition* 31(12): 1461–1473.

Evans WE and Relling MV. (1999). Pharmacogenomics: Translating functional genomics into rational therapeutics. *Science* 286: 487–491.

Finta C and Zaphiropoulos PG. (2000). The human P450 3A locus. Gene evolution by capture of downstream exons. *Gene* 260: 13–23.

Frye RF. (2004). Probing the world of cytochrome P450 enzymes. *Molecular Interventions* 4(3): 157–162.

Gambacorti-Passerini CB, Gunby RH, Piazza R, Galietta A, Rostagno R and Scapozza L. (2003). Molecular mechanisms of resistance to imatinib in Philadelphia-chromosome-positive leukaemias. *The Lancet Oncology* 4:75–85.

Garcia-Martin E, Martinez C, Pizarro RM, Garcia-Gamito FJ, Gullsten H, Raunio H and Agundez JAG. (2002). CYP3A4 variant alleles in white individuals with low CYP3A4 enzyme activity. *Clinical Pharmacology and Therapeutics* 71: 196–204.

Garfinkel D. (2003). Studies on pig liver microsomes I. Enzymatic and pigment composition of different microsomal fractions. *Archives of Biochemistry and Biophysics.* 409: 7–15.

Golas JM, Arndt K, Etienne C, Lucas J, Nardin D, Gibbons J, Frost P, Ye F, Boschelli DH and Boschelli F. (2003). SKI-606, a 4-Anilino-3-quinolinecarbonitrile dual inhibitor of Src and Abl kinases, is a potent antiproliferative agent against Chronic Myelogenous Leukemia cells in culture and causes regression of K562 xenografts in nude mice. *Cancer Research* 63: 375–381.

Grasmäder K, Verwohlt PL, Rietschel M, Dragicevic A, Müller M, Hiemke C, Freymann N, Zobel A, Maier W and Roa ML. (2004). Impact of polymorphisms of cytochrome-P450 isoenzymes 2C9, 2C19 and 2D6 on plasma concentrations and clinical effects of antidepressants in a naturalistic clinical setting. *European Journal of Pharmacology* 60: 329–336.

Gschwind H, Pfaar U, Waldmeier F, Zollinger M, Sayer C, Zbinden P, Hayes M, Pokorny R, Seiberling M, Ben-Am M, Peng B and Cross G. 2005. Metabolism and disposition of Imatinib Mesylate in healthy volunteers. *Drug Metabolism and Disposition* 33(10): 1503–1512.

Guan S, Huang M, Li X, Chen X, Chan E and Zhou S. (2006). Intra- and Inter-ethnic differences in the allele frequencies of cytochrome P450 2B6 gene in Chinese. *Pharmaceutical Research* 23(9): 1983–1990.

Guengerich FP. (1991). Reactions and significance of cytochrome P-450 enzymes. *The Journal of Biological Chemistry* 266(16): 10019–10022.

Gupta A and Prasad K. (2007). Hematological and molecular response evaluation of CML patients on Imatinib. *Journal of Association of Physicians of India* 55: 109–113.

Hamada A, Miyano H, Watanabe H and Saito H. (2003). Interaction of Imatinib with human P-glycoprotein. *The Journal of Pharmacology and Experimental Therapeutics* 307(2): 824–828.

Hamzeiy H, Vahdati-Mashhadian N, Edwards HJ and Goldfarb PS. (2002). Mutation analysis of the human *CYP3A4* gene 5' regulatory region: Population screening using non-radioactive SSCP. *Mutation Research* 500: 103–110.

Hashimoto H, Toide K, Kitamura R, Fujita M, Tagawa S, Itoh S and Kamataki T. (1993). Gene Structure of *CYP3A4*, an adult-specific form of cytochrome P450 in human livers, and its transcriptional control. *European Journal of Biochemistry*, 218(2): 585-595.

Hellmold H, Rylander T, Magnusson M, Reihnér, Warner M and Gustafsson. (1998). Characterization of cytochrome P450 enzymes in human breast tissue from reduction mammaplasties. *Journal of Clinical Endocrinology and Metabolism* 83(3): 886–895.

Henkes M, van der Kuip H and Aulitzky WE. (2008). Therapeutic options for chronic myeloid leukemia: focus on Imatinib (Glivec®, Gleevec™). *Therapeutics and Clinical Risk Management* 4(1): 163–187.

Heyn H, White RB and Stevens JC. (1996). Catalytic role of cytochrome P4502B6 in the N-demethylation of S-mephenytoin. *Drug Metabolism and Disposition* 24: 948–954.

Hirota T, Ieiri I, Takane H, Maegawa S, Hosokawa M, Kobayashi K, Chiba K, Nanba E, Oshimura M, Sato T, Higuchi S, and Otsubo K. (2004). Allelic expression imbalance of the human *CYP3A4* gene and individual phenotypic status. *Human Molecular Genetics* 13(23): 2959–2969.

Hitchen L. (2006). Adverse drug reactions result in 250 000 UK admissions a year. *British Medical Journal* 332: 1109.

Horton HR, Moran LA, Ochs RS, Rawn JD and Scrimgeour KG. (2002). Principles of biochemistry. Third Edition. *Prentics Hall Inc.* 701–733.

Hsieh K, Lin Y, Cheng C, Lai M, Lin M, Siest J and Huang J. (2001). Novel mutations of CYP3A4 in Chinese. *Drug Metabolism and Disposition* 29(3): 268–273.

Huang S, Strong JM, Zhang L, Reynolds KS, Nallani S, Temple R, Abraham S, Habet SA, Baweja RK, Burckart GJ, Chung S, Colangelo P, Frucht D, Green MD, Hepp P, Karnaukhova E, Ko H, Lee J, Marroum P, Norden JM, Qiu W, Rahman A, Sobel S, Stifano T, Thummel K, Wei X, Yasuda S, Zheng JH, Zhao H and Lesko LJ. (2008). New era in drug interaction evaluation: US Food and Drug Administration update on CYP enzymes, transporters and the guidance process. *The Journal of Clinical Pharmacology* 48: 662–670.

Hughes TP, Kaeda J, Branford S, Rudzki Z, Hochhaus A, Hensley ML, Gathmann I, Bolton, van Hoomissen IC, Goldman JM and Radich JP. (2003). Frequency of major molecular responses to Imatinib or Interferon Alfa plus Cytarabine in newly diagnosed Chronic Myeloid Leukemia. *The New England Journal of Medicine* 349: 1423–1432.

Imaoka S, Yamada T, Hiroi T, Hayashi K, Sakaki T, Yabusaki Y and Funae Y. (1996). Multiple forma of human P450 expressed in *Saccharomyces cerevisiae*. *Biochemical Pharmacology* 51: 1041–1050.

Ingelman-Sundberg M, Oscarson M and McLellan RA. (1999). Polymorphic human cytochrome P450 enzymes: an opportunity for individualised drug treatment. *Trends in pharmacological sciences* 20: 342–349.

Innocenti F, Iyer L and Ratain MJ. (2000). Pharmacogenetics: A tool for individualising antineoplastic therapy. *Clinical Pharmacokinetics* 39(5): 315–325.

Iqbal Z, Siddiqui RT and Qureshi JA. (2004). Two different point mutations in ABL gene ATP-binding domain conferring primary Imatinib resistance in a Chronic Myeloid Leukemia (CML) patient: A case report. *Biological Procedures Online (www.biologicalprocedures.com)* 6(1): 144–148.

Ishida S, Soyama A, Saito Y, Murayama N, Saeki M, Sai K, Ozawa S and Sawada J. (2002). Determination of CYP2D6 gene alleles by the CYP450 probe array using the Afymetrix GeneChip System: Comparison with sequencing results. *Drug Metabolism and Pharmacokinetics* 17(2): 157–160.

Jap T, Wu Y, Tso Y and Chiu C. (2002). A novel mutation in the intron 1 splice donor site of the Cholesterol Ester Transfer Protein (*CETP*) gene as a cause of Hyperalphalipoproteinemia. *Metabolism* 51(3): 392–397.

Jiang Z, Shu Y, Chen X, Huang S, Zhu R, Wang W, He N and Zhou H. (2002). The role of CYP 2C19 in amitriptyline *N*-demethylation in Chinese subjects. *European Journal of Clinical Pharmacology* 58: 109–113.

Johnson JR, Bross P, Cohen M, Rothman M, Chen G, Zajicek A, Gobburu J, Rahman A, Staten A and Pazdur R. (2003). Approval summary: Imatinib Mesylate capsules for treatment of adult patients with newly diagnosed Philadelphia chromosome-positive Chronic Myelogenous Leukemia in chronic phase. *Clinical Cancer Research* 9: 1972–1979.

Kang Y, Park S, Yim C, Kwak H, Gajendrarao P, Krishnamoorthy N, Yun SC, Lee K and Han K. (2009). The CYP3A4*18 genotype in the cytochrome P450 3A4 gene, a rapid metabolizer of sex steroids, is associated with low bone mineral density. Clinical Pharmacology and Therapeutics 85(3): 312–318.

Kapitany T, Meszaros K, Lenzinger E, Schindler SD, Barnas C, Fuchs K, Sieghart W, Aschauer HN and Kasper S. (1998). Genetic polymorphisms for drug metabolism (CYP2D6) and tardive dyskinesia in schizophrenia. *Schizophrenia Research* 32: 101–106.

Keshava C, McCanlies EC and Weston A. (2004). CYP3A4 polymorphisms—Potential risk factors for breast and prostate cancer: A Huge Review. *American Journal of Epidemiology* 160(9): 825–841.

Kimchi-Sarfaty C, Oh JM, Kim I, Sauna ZE, Calcagno AM, Ambudkar SV and Gottesman MM. (2007). A “silent” polymorphism in the *MDR*1 gene changes substrate specificity. *Science* 315: 525–528.

Kitagawa K, Kunugita N, Kitagawa M and Kawamoto T. (2001). *CYP2A6*6*, a novel polymorphism in cytochrome P450 2A6, has a single amino acid substitution (R128Q) that inactivates enzymatic activity. *The Journal of Biological Chemistry* 276(21): 17830–17835.

Korhonen LE, Turpeinen M, Rahnasto M, Wittekindt C, Poso A, Pelkonen O, Raunio H and Juvonen RO. (2007). New potent and selective cytochrome P450 2B6 (CYP2B6) inhibitors based on three-dimensional quantitative structure-activity relationship (3D-QSAR) analysis. *British Journal of Pharmacology* 150: 932–942.

Koskela S, Hakkola J, Hukkanen J, Pelkonen O, Sorri M, Saranen A, Anttila S, Fernandez-Salguero P, Gonzalez F and Raunio H. (1999). Exression of CYP2A genes in human liver and extrahepatic tissues. *Biochemical Pharmacology* 57: 1407–1413.

Kühr T, Burgstaller S, Apfelbeck U, Linkesch W, Seewann H, Fridrik M, Michlmayr G, Krieger O, Lutz D, Lin W, Pont J, Köck L, Abbrederis K, Baldinger C, Buder R, Geissler D, Hausmaninger H, Lang A, Zabernigg A, Duba C, Hilbe W, Eisterer W, Fiegl M, Greil R, Gastl G and Thaler J. (2003). A randomized study comparing Interferon (IFN alpha) plus low-dose Cytarabine and Interferon plus Hydroxyurea (HU) in early chronic-phase Chronic Myeloid Leukemia (CML). *Leukemia Research* 27(5): 405–411.

Kunitoh S, Imaoka S, Hiroi T, Yabusaki Y, Monna T and Funae Y. (1997). Acetaldehyde as well as ethanol is metabolised by human CYP2E1. *The Journal of Pharmacology and Experimental Therapeutics* 280(2): 527–532.

Lalli E and Sassone-Corsi P. (1994). Signal transduction and gene regulation: The nuclear response to cAMP. *The Journal of Biological Chemistry* 269(26): 17359–17362.

Lamba JK, Lin YS, Schuetz EG and Thummel KE. (2002). Genetic contribution to variable human CYP3A4-mediated metabolism*. Advanced Drug Delivery* 54: 1271–1294.

Lang T, Klein K, Fischer J, Nussler AK, Neuhaus P, Hofmann U Eichelbaum M, Schwab M and Zanger UM. (2001). Extensive genetic polymorphism in the human CYP2B6 gene with impact on expression and function in human liver. *Pharmacogenetics* 11: 399–415.

le Coutre P, Kreuzer K, Pursche S, Bonin MV, Leopold T, Baskaynak G, Dörken B, Ehninger G, Ottmann O, Jenke A, Bornhäuser M and Schleyer E. (2004). Pharmacokinetics and cellular uptake of Imatinib and its main metabolite CGP74588. *Cancer Chemotherapy and Pharmacology* 53: 313–323.

Lee Y, Im S, Nam S, Lee S, Kim Y, Seong C, Han WS, Lee SW, Park S and Lee SM. (2003). Challenging problems in advanced malignancy. *Journal of Clinical Oncology* 21(16): 2168–3176.

Leeder JS. (2001). Pharmacogenetics and pharmacogenomics. Pediatric Clinics of North America 48(3): 765–781.

Lewis DFV, Jacobs MN, Dickins and Lake BG. (2002). Quantitative structure-activity relationships for inducers of cytochromes P450 and nuclear receptor ligands involved in P450 regulation within the CYP1, CYP2, CYP3 and CYP4 families. *Toxicology* 176: 51–57.

Li E and Hristova K. (2006). Role of receptor tyrosine kinase transmembrane domains in cell signalling and human pathologies. *The American Chemical Society* 45(20): 6241–6251.

Lin J, Sahakian DC, de Morais SM, Xu JJ, Polzer RJ and Winter SM. (2003). The role of absorption, distribution, metabolism, excretion and toxicity in drug discovery. *Current Topics in Medical Chemistry* 3(10): 1125–1154.

Liu T, Lin S, Chen T and Chang J. (2002). Polymorphism analysis of CYP3A5 in myeloid leukemia. *Oncology Reports* 9: 327–329.

Lopes LF, Piccoli FDS, Paixão VA, Latorre MdRDO, de Camargo B, Simpson AJG and Caballero OL. (2004). Association of CYP3A4 genotype with detection of Vγ/Jβ trans-rearrangements in the peripheral blood leukocytes of paediatric cancer patients undergoing chemotherapy for ALL. *Leukemia Research* 28: 1281–1286.

Lund EG, Guileyardo JM, Russell DW. (1999). cDNA cloning of cholesterol 24-hydroxylase, a mediator of cholesterol homeostasis in the brain. *Biochemistry* 96: 7238–7243.

Markowitz JS, DeVane CL, Chavin KD, Taylor RM, Ruan Y and Donovan JL. (2003a) Effects of garlic (*Allium sativum* L.) supplementation on cytochrome P450 2D6 and 3A4 activity in healthy volunteers. *Clinical Pharmacology and Therapeutics* 74(2): 170–177.

Markowitz JS, Donovan JL, DeVane CL, Taylor RM, Ruan Y and Wang Y. (2003b) Multiple doses of saw palmetto (*Serenoa repens*) did not alter cytochrome P450 2D6 and 3A4 activity in normal volunteers. *Clinical Pharmacology and Therapeutics* 74(6): 536–542.

Mathijssen RHJ and van Schaik RHN. (2006). Genotyping and phenotyping cytochrome P450: Perspectives for cancer treatment. *European Journal of Cancer* 42: 141–148.

Mauro MJ and Druker BJ. (2001). STI571: Targeting BCR-ABL as therapy for CML. *The Oncologist* 6: 233–238.

McKinnon RA and Evans AM. (2000). Cytochrome P450. *Australian Journal of Hospital Pharmacy* 30: 102–105.

Monroe KR, Murphy SP, Kolonel LN and Pike MC. (2007). Prospective study of grapefruit intake and risk of breast cancer in postmenopausal women: the Multiethnic cohort study. *Bristish Journal of Cancer* 97: 440–445.

Monroy RH, Viveros PV and Franco JC. (2007). Chronic Myeloid Leukemia current concepts in physiopathology and treatment. *Cancerologia* 2: 137–147.

Morcillo-Suarez C, Alegre J, Sangros R, Gazave E, de Cid R, Milne R, Amigo J, Ferrer-Admetlla A, Moreno-Estrada A, Gardner M, Casals F, Pérez-Lezaun A, Comas D, Bosch E, Calafell F, Bertranpetit J, Navarro A. (2008). SNP analysis to results (SNPator): a web-based environment oriented to statistical genomics analyses upon SNP data. Bioinformatics 24(14): 1643–1644.

Mukherjee S, Tessema M and Wandinger-Ness A. (2006). Vesicular Trafficking of Tyrosine Kinase Receptors and Associated Proteins in the Regulation of Signaling and Vascular Function. *Circulation Research* 98: 743–756.

Murayama N, Nakamura T, Saeki M, Soyama A, Saito Y, Sai K, Ishida S, Nakjima O, Itoda M, Ohno Y, Ozawa S and Sawada J. (2002). CYP3A4 gene polymorphims influence testosterone 6*β*-hydroxylation. *Drug Metabolism and Pharmacokinetics* 17(2): 150–156.

Murray BP, Edwards RJ, Murray S, Singleton AM, Davies DS and Boobis AR. (1993). Human hepatic CYP1A1 and CYP1A2 content, determined with specific anti-peptide antibodies, correlates with mutagenic activation of PhIP. *Carcinogenesis* 14(4): 585–592.

Murray M. (2006). Role of CYP pharmacogenetics and drug-drug interactions in the efficacy and safety of atypical and other antipsychotic agents. *Journal of Pharmacy and Pharmacology* 58: 871–885.

Nakamura Y. (2008). Pharmacogenomics and drug toxicity. *The New England Journal of Medicine* 359(8): 856–858.

Nebert DW, Adesnik M, Coon MJ, Estabrook RW, Gonzalez FJ, Geungerich FP, Gunsaius IC, Johnson EF, Kemper B, Levin W, Phillips IR, Sato R and Waterman MR. (1987). The P450 gene superfamily: recommended nomenclature. *DNA* 6: 1–11.

Nelson DR. 1999. Cytochrome P450 and the individuality of species. *Archives of Biochemistry and Biophysics* 369(1): 1–10.

Nolin TD, Gastonguay MR, Bies RR, Matzke GR and Frye RF. (2003). Impaired 6-hydroxychlozoxazone elimination in patients with kidney disease: Implication for cytochrome P450 2E1 pharmacogenetic studies. *Clinical Pharmacology and Therapeutics* 74(6): 555–568.

Nowell PC and Hungerford DA. (1960). A minute chromosome in human granulocytic leukemia. *Science* 142: 1497.

O'Brien S, Kantarjian H and Talpaz M. (1996). Practical guidelines for the management of chronic myelogenous leukemia with interferon alpha. *Leukemia and Lymphoma* 23(3-4):247–252.

O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, Cornelissen JJ, Fischer T, Hochhaus A, Hughes T, Lechner K, Nielsen JL, Rousselot P, Reiffers J, Saglio G, Shepherd J, Simonsson B, Gratwohl A, Goldman JM, Kantarjian H, Taylor K, Verhoef G, Bolton AE, Capdeville R and Druker BJ. (2003a). Imatinib compared with Interferon and low-dose Cytarabine for newly diagnosed chronic-phase Chronic Myeloid Leukemia. *The New England Journal of Medicine* 384: 994–1004.

O'Brien SG, Meinhardt P, Bond E, Beck J, Peng B, Dutreix C, Mehring G, Milosavljev S, Huber C, Capdeville R and Fischer T. (2003b). Effects of Imatinib Mesylate (STI571, Glivec) on the pharmacokinetics of Simvastatin, a cytochrome P450 3A4 substrate, in patients with chronic myeloid leukaemia. *British Journal of Cancer* 89: 1855–1859.

Omura T and Sato R. (1964). The carbon monoxide-binding pigment of liver microsomes: Evidence for its hemoprotein nature. *Journal of Biological Chemistry* 239: 2370–2378.

Omura T. (1999). Forty years of Cytochrome P450. *Biochemical and Biophysical Research Communications* 266(3): 690–698.

Oscarson M. (2001). Genetic polymorphisms in the cytochrome P450 2A6 (CYP2A6) gene: implications for interindividual differences in nicotine metabolism. *Drug Metabolism and Disposition* 29(2): 91–95.

Ottmann OG and Wassmann B. (2003). Imatinib in the treatment of Philadelphia chromosome-positive acute lymphoblastic leukaemia: Current status and evolving concepts. *Best Practice and Research Clinical Heamatology* 15(4): 757–769.

Parmley JL and Hurst LD. (2007). How do synonymous mutations affect fitness? *BioEssays* 29(6): 515–519.

Peng B, Dutreix C, Mehring G, Hayes MJ, Ben-Am M, Seiberling M, Pokorny R, Capdeville R and Lloyd P. (2004). Absolute bioavailability of Imatinib (Glivec®) orally versus intravenous infusion. *Pharmacokinetics and Pharmacodynamics* 98: 2039–2042.

Peng B, Lloyd P and Schran H. (2005). Clinical pharmacokinetics of Imatinib. *Clinical Pharmacokinetics* 44(9): 879–894.

Pestina TI, Cleveland JL, Yang C, Zambetti GP and Jackson CW. (2001). Mpl ligand prevents lethal myelosuppression by inhibiting p53-dependent apoptosis. *Blood* 98: 2084–2090.

Purvis IJ, Bettany AJE, Santiago TC. (1987). The efficiency of folding of some proteins is increased by controlled rates of translations *in vivo*: a hypothesis. *The Journal of Molecular Biology* 193: 413–417.

Raunio H, Husgafvel-Pursiainen K, Anttila S, Hietanen E, Hirvonen A and Pelkonen O. (1995). Diagnosis of polymorphisms in carcinogen-activating and inactivating enzymes and cancer susceptibility – a review. *Gene* 159: 113–121.

Rautio A. (2003). Polymorphic CYP2A6 and it's clinical and toxicological significance. *The Pharmacogenomics Journal* 3: 6–7.

Ridruejo E, Cacchione R, Villamil AG, Marciano S, Gadano AC and Mandó OG. (2007). Imatinib-induced fatal acute liver failure. *World Journal of Gastroenterol* 13(48): 6608–6611.

Robertson SC, Tynan J and Donoghue DJ. (2000). RTK mutations and human syndromes: when good receptors turn bad. *Trends in Genetics* 16(8): 368.

Rogers JF, Nafziger AN and Bertino JS. (2002). Pharmacogenetics affects dosing, efficacy, and toxicity of cytochrome P450 – metabolized drugs. *The American Journal of Medince* 113: 746–750.

Rule SAJ, O'Brien SG and Crossman LC. (2002). Managing cutaneous reactions to Imatinib therapy. *Blood* 100(9): 3434–3435.

Sacchi S, Kantarjian H, O'Brien S, Cohen PR, Pierce S and Talpaz M. (1995). Immune-mediated and unusual complications during Interferon alfa therapy in Chronic Myelogenous Leukemia. *Journal of Clinical Oncology* 13(9): 2401–2407.

Sachse C, Brockmöller J, Bauer S and Boots I. (1999). Functional significance of a C-A polymorphism in intron I of the cytochrome P450 *CYP1A2* gene tested with caffeine. *British Journal of Clinical Pharmacology* 47: 445–449.

Sata F, Sapone A, Elizondo G, Stocker P, Miller VP, Zheng W, Raunio H, Crespi CL and Gonzalez FJ. (2000). CYP3A4 allelic variants with amino acid substitutions in exon 7 and 12: Evidence for an allelic variant with altered catalytic activity. *Clinical Pharmacology and Therapeutics* 67(1): 48–56.

Sauna ZE, Kimchi-Sarfaty C, Ambudkar SV and Gottesman MM. (2007). Silent polymorphisms speak: How they affect pharmacogenomics and the treatment of cancer. *Cancer Research* 67(20): 9609–9612.

Sawyers CL. (1999). Chronic Myeloid Leukemia. *The New England Journal of Medicine* 340(17): 1330–1340.

Sawyers CL, Hochhaus A, Feldman E, Goldman JM, Miller CB, Ottmann OG, Schiffer CA, Talpaz M, Guilhot F, Deininger MWN, Fischer T, O'Brien SG, Stone RM, Gambacorti-Passerini CB, Russell NH, Reiffers JJ, Shea TC, Chapuis B, Coutre S, Tura S, Morra E, Larson RA, Saven A, Peschel C, Gratwohl A, Mandelli F, Ben-Am M, Gathmann I, Capdeville R, Paquette RL and Druker BJ. (2002). Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crises: results of a phase II study. *Blood* 99: 3530–3539.

Schindler T, Bornmann W, Pellicena P, Miller WT, Clarkson B and Kuriyan J. (2000). Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science* 289(5486): 1938–1942.

Schirmer M, Rosenberger A, Klein K, Kulle B, Toiliat MR, Nürnberg P, Zanger UM and Wojnowski L. (2007). Sex-dependent genetic markers of CYP3A4 expression and activity in human liver microsomes. *Pharmacogenetics* 8(5):443–453.

Schmidli H, Peng B, Riviere GJ, Capdeville R, Hensley M, Gathmann I, Bolt AE and Racine-Poon A. (2004). Population pharmacokinetics of imatinib mesylate in patients with chronic-phase chronic myeloid leukaemia: Results of a phase III study. *British Journal of Clinical Pharmacology* 60(1): 35–44.

Shenoy SK. (2007). Seven-transmembrane receptors and ubiquitination. *Circulation Research 100*: 1142–1154.

Shet MS, Fisher CW, Holmans PL and Estabrook RW. (1993). Human cytochrome P450 3A4: Enzymatic properties of a purified recombinant fusion protein containing NADPH–P450 reductase. *Pharmacology* 90:11748–11752.

Shimada T, Hayes CL, Yamazaki H, Amin S, Hecht SS, Guengerich P and Sutter TR. (1996). Activation of chemically diverse procarcinogens by human cytochrome P450 1B1. *Cancer Research* 56: 2979–2984.

Shimada T, Yamazaki H, Mimura M, Inu Y and Geungerich P. (1994). Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *The Journal of Pharmacology and Experimental Therapeutics* 270(1): 414–423.

Silver RT, Woolf SH, Hehlmann R, Appelbaum FR, Anderson J, Bennett C, Goldman JM, Guilhot F, Kantarjian HM, Lichtin AE, Talpaz M and Tura S. (1999). 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: Developed for the American Society of Hematology. *Blood* 94: 1517 – 1536.

Silver RT, Peterson BL, Szatrowski TP, Powell BL, Stock W, Carroll AJ, Bloomfield CD, Schiffer CA and Larson RA. (2003). Treatment of the chronic phase of Chronic Myeloid Leukemia with an intermittent schedule of recombinant Interferon alfa-2b and Cytarabine: results from CALGB study 9013. *Leukemia and Lymphoma* 44(1): 39–48.

Sim S.C, Sundberg M.I, Nebert D.W. (2008). Home Page of the Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee. Available at: http://www.cypalleles.ki.se. Accessed May 21, 2008.

Simmons P, Kaushansky K and Torok-Storb B. (1990). Mechanisms of cytomegalovirus-mediated myelosuppression: Perturbation of stromal cell function versus direct infection of myeloid cells. *Medical Sciences* 87: 1386–1390.

Simpson AECM. (1997). The cytochrome P450 4 (CYP4) family. *General Pharmacology* 28(3): 351–359.

Sneed TB, Kantarjian HM, Talpaz M, O"Brien S, Rios MB, Bekele BN, Zhou X, Resta D, Wierda W, Faderl S, Giles F and Cortes JE. (2003). The significance of myelosuppression during therapy with Imatinib Mesylate in patients with Chronic Myelogenous Leukemia in chronic phase. *Cancer* 100(1): 116–121.

Swida U, Thüroff E and Käufer NF. (1986). Intron mutations that affect the splicing efficiency of the *CYH2* gene of *Saccharomyces cerevisiae. Molecular Genetics and Genomics* 203: 300–304.

Talpaz M, Kantarjian H, Kurzrock R, Trujillo JM and Gutterman JU. (1991). Interferon-alpha produces sustained cytogenetic responses in Chronic Myelogenous Leukemia. Philadelphia chromosome-positive patients. *Annals of Internal Medicine* 114(7): 532–538.

Talpaz M, Silver RT, Druker BJ, Goldman JM, Gambacorti-Passerini C, Guilhot F, Schiffer CA, Fischer T, Deininger MWN, Lennard AL, Hochhaus A, Ottman OG, Gratwohl A, Baccarani M, Stone R, Tura S, Mahon F, Fernandes-Reese S, Gathmann I, Capdeville R, Kantarjian HM and Sawyers CL. (2002). Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study. *Blood* 99: 1928–1937.

Tang X, Feng Y and Ye K. (2007). Src-family tyrosine kinase fyn phosphorylates phophatidylinositol 3-kinase enhancer-activating Akt, preventing its apoptotic cleavage and promoting cell survival. *Cell Death and Differentiation* 14: 368–377.

Tateishi T, Watanabe M, Moriya H, Yamaguchi S, Sato T and Kobayashi S. (1999). No ethnic difference between Caucasian and Japanese hepatic samples in the expression frequency of CYP3A5 and CYP3A7 proteins. *Biochemical Pharmacology* 57: 935–939.

Ushma M, Durrheim DN, Blockman M, Kredo T, Gounden R, Barnes KI. (2007). Adverse drug reactions in adult medical inpatients in a South African hospital serving a community with high HIV/AIDS prevalence: Prospective observational study. *British Journal of Clinical Pharmacology* 65(3): 396–406.

van Schaik RHN, de Wildt SN, Brosens R, van Fessem M, van den Anker JN and Lindemans J. (2001). The CYP3A4*3 allele: is it really rare? *Clinical Chemistry* 47(6): 1104–1106.

van Schaik RHN. (2004). Implications of cytochrome P450 genetic polymorphisms on the toxicity of antitumor agents. *Therapeutic Drug Monitoring* 26(2): 236–240.

van Schaik RKN. (2005). Cancer treatment and pharmacogenetics of cytochrome P450 enzymes. *Investigational New Drugs* 23: 513–522.

Veltmann J. (2005). Pharmacogenomics. The Online Journal for the American Association of Integrative Medicine. Available at: http://www.aaimedicine.com/jaaim/aug05/pharmacogenomics.php. Accessed on 6 May 2008

Veronese ML, Gillen LP, Burke JP, Dorval EP, Hauck WW, Pequignot E, Waldman SA and Greenberg HE. (2003). Exposure-dependent inhibition of intestinal and hepatic CYP3A4 *in vivo* by grapefruit juice. *The Journal of Clinical Pharmacology* 43: 831–839.

Wang X and Seed B. (2003). A PCR primer bank for quantitative gene expression analysis. *Nucleic Acid Research* 31(24): 1–8.

Wauthier V, Verbeeck RK and Calderon PB. (2007). The effect of ageing on Cytochrome P450 enzymes: Consequences for drug biotransformation in the elderly. *Current Medicinal Chemistry* 14: 745–757.

Westlind A, Löfberg L, Tindberg N, Andersson TB and Ingelman-Sundberg M. (1999). Interindividual differences in hepatic expression of CYP3A4: Relationship to genetic polymorphism in the 5'–upstream regulatory region. *Biochemical and Biophysical Research Communications* 259(1): 201–205.

Wetzler M, Kantarjian H, Kurzrock R and Talpaz M. (1995). Interferon-alpha therapy for chronic myelogenous leukemia. *The American Journal of Medicine* 99(4):402–411.

White JA, Beckett-Jones B, Guo Y, Dilworth FJ, Bonasoro J, Jones G and Petkovich M. (1997). cDNA cloning of human retinoic acid-metabolizing enzyme (hP450RAI) identifies a novel family of cytochromes P450 (CYP26). *The Journal of Biological Chemistry* 272(30): 18538–18541.

Williams PA, Cosme J, Vinkovic DM, Ward A, Angove HC, Day PJ, Vonrhein C, Tickle IJ and Jhoti H. (2004). Crystal structures of human cytochrome P450 3A4 bound to metyrapone and progesterone. *Science* 305: 683–686.

Wolf CR and Smith G. (1999). Pharmacogenetics. *British Medical Bulletin* 55(2): 366–386.

Wolf CR, Smith G and Smith RL. (2000). Pharmacogenetics: Science, medicine, and the future. *British Medical Journal* 320:987–990.

Xu P, Huang S, Zhu R, Han X and Zhou H. (2002). Phenotypic polymorphism of CYP2A6 activity in a Chinese population. *European Journal of Clinical Pharmacology* 58: 333–337.

Yamaori S, Yamazaki H, Iwano S, Kiyotani K, Matsumura K, Saito T, Parkinson A, Nakagawa K and Kamataki T. (2005). Ethnic differences between Japanese and Caucasians in the expression levels of mRNA for CYP3A4, CYP3A5 and CYP3A7: lack of co-regulation of the expression of CYP3A4 in Japanese livers. *Xenobiotica* 35(1): 69–83.

Chapter 5: Summary

Chronic myelogenous leukaemia (CML) is a malignant clonal disorder that results in the uncontrolled production of white blood cells. This disease is a result of a reciprocal translocation of the long arms of chromosome 9 and 22 resulting in a shortened chromosome 22, harbouring the *BCR-ABL* fusion gene, known as the Philadelphia chromosome. The *BCR-ABL* oncogene encodes for a constitutively activated tyrosine kinase that interferes with normal cell differentiation and apoptosis. CML can be effectively treated with tyrosine kinase inhibitors, such as imatinib mesylate (Gleevec®). However, some CML patients experience adverse drugs reactions (ADRs) to imatinib and cannot be treated at the recommended dose. There is a concern that lowering the dose of imatinib to reduce the side effects can result in the development of resistant cancer cells, and thus a cessation in treatment is rather recommended. Imatinib is metabolized by the cytochrome P450 enzyme, CYP3A4. However, if the ADRs were a result of decreased metabolic effect of CYP3A4, it would be possible to reduce the dose of imatinib without effecting efficacy.

It is hypothesised that single nucleotide polymorphisms (SNPs) can alter the catalytic activity of the CYP3A4 enzyme. Thus a decrease in metabolic rate can result in ADRs due an increased exposure to the drug. Therefore, the aim of this study was to determine whether SNPs in the *CYP3A4* gene are associated with ADRs from imatinib treatment. In this study, the DNA sequence of the *CYP3A4* gene from 25 CML patients treated with imatinib were compared to a reference DNA sequence obtained from Genbank. The SNPs identified during this study was statistically analysed, and their association with the presence of ADRs was determined using the online statistics package, SNPator.

A total of six SNPs were detected, I193I, T15871G, *CYP3A4*1G*, C23187T, I369V and G73239A. Of these, I369V and G73239A are novel and not described previously in literature. It was found that I369V resulted in an amino acid change, involving a substitution of isoleucine with valine. The remaining SNPs identified in this study were located in intron regions, with the exception of I193I which is a synonymous SNP.

There is little information available on the frequency of SNPs located in introns, since these SNPs are generally regarded to have no impact on the expression or activity of a protein. However, in this study an SNP located in intron 10 was significantly associated with the presence ADRs. Current hypothesises suggest that intron SNPs could affect the expression levels of a protein by influencing the splicing efficiency of mRNA and subsequently translation efficacy. Future research needs to elaborate on the role of *CYP3A4*1G* on *CYP3A4* expression as well as on the prevalence of other alleles identified in this study in South African populations.

Keywords: ADRs, BCR-ABL, Chronic myeloid leukemia (CML), CYP3A4, imatinib, intron, tyrosine kinase, single nucleotide polymorphism (SNP), synonymous

Chapter 6: Opsomming

Chroniese meloïede leukemie (CML) is ’n kwaadaardige klonale toestand wat lei tot onbeheersde produksie van witbloedselle. Die toestand ontstaan as gevolg van ’n resiproke translokasie tussen die lang arms van chromosoom 9 en 22. Dit lei tot ’n verkorte chromosoom 22, die sogenaamde Philadelphia chromosoom, wat die *BCR-ABL* fusiegeen bevat. Die *BCR-ABL* onkogeen kodeer vir ’n tirosienkinase wat permanent aangeskakel is en inmeng met normale selfdifferensiasie en apoptose. CML kan effektief behandel word met tirosienkinase inhibitore soos imatinib mesylate (Gleevec®). Sommige CML pasiënte ervaar egter newe-effekte teen imatinib en die aanbevole dosis moet aangepas word. Die gevaar bestaan ook dat indien die dosis verlaag word om newe-effekte te verminder, dit kan lei tot die ontwikkeling van imatinib bestande kankerselle. Om weerstand te voorkom word opskorting van behandeling voorgestel. Imatinib word deur die sitochroom P450 ensiem, CYP3A4, gemetaboliseer. Indien die newe-effekte onstaan as gevolg van ’n verlaagde metaboliese effek van die CYP3A4-ensiem, sou dit moontlik wees om die dosis van imatinib te verlaag sonder om die effektiwiteit daarvan te beïnvloed.

Daar word gespekuleer dat enkelnukleotiedpolimorfismes (SNPs) die katalitiese aktiwiteit van die CYP3A4-ensiem kan beïnvloed. ’n Verlaging van die metaboliese tempo kan dus lei tot newe-effekte as gevolg van ’n verlengde blootstelling aan die middel. Die doel van die studie was dus om te bepaal of SNPs in die *CYP3A4* geen gekoppel kon word aan newe-effekte as gevolg van imatinib behandeling. In hierdie studie is die DNA opeenvolging van die *CYP3A4*-geen vanaf 25 CML pasiënte vergelyk met die standaard DNA opeenvolging vanaf Genbank. Statistiese analises is gedoen om die assosiasie van die SNPs wat voorgekom het met die verskyning van newe-effekte te bepaal. Die SNPator program is hiervoor aangewend.

In totaal is ses SNP’s waargeneem nl. I1931I, T15871G, *CYP3A4*1G*, C23187T, I369V en G73239A. Twee van hierdie SNPs, I369V en G73239A, is nog nie voorheen beskryf nie. Daar is vasgestel dat I369V lei tot ’n aminosuur verandering nl. die vervanging van isoleusien met valien. Die ander SNPs wat voorgekom het was in introne geleë, met die uitsondering van I193I wat ’n sinverwante SNP is.

Daar is min inligting bekend aangaande die frekwensie van SNPs wat in introne voorkom, aangesien daar gereken word dat hierdie SNPs geen invloed het op die uitdrukking of aktiwiteit van proteïene nie. In hierdie studie is daar egter gevind dat twee intron SNPs, *CYP3A4*1G* en T15871G, statisties beduidend met die teenwoordigheid van newe-effekte geassosieer kon word. Huidige voorstelle dui daarop dat intron SNPs die uitdrukkingsvlakke van 'n proteïen kan beïnvloed deur middel van 'n effek op die snydingseffektiwiteit van mRNA te hê en dus ook die vertalingseffektiwiteit. Toekomstige navorsing moet uitbrei op die rol van *CYP3A4*1G* en T15871G op *CYP3A4* uitdrukking asook die voorkoms van ander allele wat in hierdie studie gevind is, in Suid Afrikaanse bevolkings.

Sleutelwoorde: BCR-ABL, Chroniese meloïede leukemie (CML), CYP3A4, enkelnukleotiedpolimorfismes (SNPs), imatinib, intron, newe-effekte, tirosienkinase, sinverwante SNP

Appendix A

The precise location of each *CYP3A4* SNP listed in Table 5. These tables served as a database of all known *CYP3A4* gene variants in the respective exons and introns, in sequence context.

- Nucleotides underlined, highlighted and in bold represent the nucleotide which can vary
- Sections of DNA sequence within box and shaded in grey, represent the Exon DNA.
- Regions not within a box, represent the Intron DNA
- SNPs number (33) and (34) are not presented in these tables, as they are located further within in the intron 7 sequence, and not amplified by the DNA primers designed for the CYP3A4 exons.

Promoter region DNA sequence of *CYP3A4* with specific locations of DNA variants as described by Table 5. Data was obtained and adapted from Sim *et al.* (2008), Keshava *et al.* (2004), GenBank Accession No. AF280107 and NM_017460.

CYP3A4 Promoter Region	Distance From ATG start codon	Position in GenBank AF280107	DNA variant
	– 896.	61141	
acagcagctgaggcacagccaagagctctggctgtattaat			
gacctaagaa_gtcaccagaaagtcagaagggatgacatgca			***CYP3A4*15B*** **(11)**
gaggcccagcaatctcagctaagtcaactccaccagcctttt			
ctagttgcccactgtgtgtacagcacCctggtagggaccag			***CYP3A4*1F*** **(10)**
agccatgacagggaataagactagactatgcccttgaggag			
ctcacctctgttcagggaaacaggcGtggaaacacAatggt			***CYP3A4*1w*** **(7)**
ggtaaagaggaaagaggacaAtaggattgcatgaaggggat			***CYP3A4*1K*** **(8)**
ggaaagtgcccaggggaggaaatggttacatctgtgtgagg			***CYP3A4*1L*** **(9)**
agtttggtgaggaaagactctaagagaaggctctgtctgtc			
tgggtttggaaggatgtgtaggagtcttctaggggcacag			
gcacactccaggcataggtaaagatctgtaggtgtggcttg			
tTgggatgaatttcaagtattttggaatgaggacagccata			***CYP3A4*1C*** **(2)**
gagacaagggcaAgagagaggcgatttaatagattTtatgc			***CYP3A4*1B*** **(1)**
caatggctccacttgagtttctgataagaacccagaaccct			***CYP3A4*1E*** **(6)**
tggactccccagtaacattgattgagttgtttatgatacct			
catagaatatgaactcaaaggaggtcagtgagtggtgtgtg			
tgtgattctttgccaacttccaaggtggagaagcctcttcc			
aactgcaggcagagcacaggtggccctgctactggctgcag			
ctCcagccctgcctccttctctagcatataaacaatccaac			***CYP3A4*1M*** **(5)**
agcctcactgaatcactgctgtgcagggcaggaaagctcca			
tgcacatagcCcagCaaagagcaacacagagctgaaaggaa			***CYP3A4*1x*** **(4)**
gactcagaggagagagataagtaaggaaagtagtg			***CYP3A4*1D*** **(3)**

The 3'UTR region DNA sequence of *CYP3A4* with specific locations of DNA variants as described by Table 5. Data was obtained and adapted from Sim *et al.* (2008), Keshava *et al.* (2004), GenBank Accession No. AF280107 and NM_017460.

CYP3A4 3'UTR	Distance From ATG start codon	Position in GenBank AF280107	DNA variant
	25940	87977	
attttcctaaggacttcTgctttgctcttcaagaaatctgtgc			3'UTR (12)
ctgagaacaccagagacctcaaattacttTgtgaatagaactc			CYP3A4*1T (13)
tgaaatgaagatgggcttcatccaatggactgcataaataacc			
ggggattctgtacaTgcattgagctctctcattgtctgtgtag			CYP3A4*1T (13)
agtgttatacttgggaatataaaggaggtgaccaaatcagtgt			
gaggaggtagatttggctcctctgcttctcacgggactatttc			
caccaccCccagttagcaccattaactcctcctgagctctgat			CYP3A4*1H (14)
aagagaatcaacatttctcaataatttCctccacaaattatta			CYP3A4*1H (14)
atgaaaataagaattattttgatggctctaacaatgacattta			
tatcacatgttttctctggagtattctataagttttatgttaa			
atcaataaagaccactttacaaaagta			

Exon 1-13 DNA sequence of CYP3A4 including adjacent intron DNA sequence. Specific locations of DNA variants as described by Table 5 are illustrated where applicable. Data was obtained and adapted from Sim *et al.* (2008), Keshava *et al.* (2004), GenBank Accession No. AF280107 and NM_017460.

CYP3A4 Exon 1	Distance From ATG start codon	Position in GenBank AF280107	DNA variant
	1	62037	
ATGgctctcatcccagacttggccatggaaacctggcttctc cTggctgtcagcctggtgctcctctatct			***CYP3A4*14*** **(59)**

CYP3A4 Exon 2	Distance From ATG start codon	Position in GenBank AF280107	DNA variant (Refer to Table 8B +8C)
	3997	66034	
ccctatatctataaAgtcacaatcgctgtgacttgatttct gtttcactttgtagatatggaacccattcacatggacttttt aagaagcttggaattccagggcccacacctctgccttttttg ggaaatattttgtcctaccataag			***CYP3A4*1y*** **(15)**

CYP3A4 Exon 3	Distance From ATG start codon	Position in GenBank AF280107	DNA variant
	6002	68039	
gccagcaagtctgatttcaTtggcttcgactgttttcatcc caattagaggcagggttaagtacattaaaaataataatcaa atattattttgtttctcctcccaggGctttgtatgtttga catggaatgtcataaaaagtatggaaaagtgtggggtgtgag tattctggaaacttccAttggatagacttgtttctatgatg agtttaccccactgcacagaggacagtctcagcccaaagcc tcttgggatgaagctcTtgtcaacctaactacaaacagaga gaagttctctgaaagaagaagatatttatttgggtgtagag			***T5922C*** **(16)** ***CYP3A4*7*** **(52)** ***CYP3A4*1J*** **(17)** ***T6165A*** **(18)**

CYP3A4 Exon 4	Distance From ATG start codon	Position in GenBank AF280107	DNA variant
	11383	73420	
tgtagaataaggctgttgatgtttaatcaactctgtttttt tcacacagcttttatgatggtcaacagcctgtgctggctat cacagatcctgacatgatcaaaacagtgctagtgaaagaat gttattctgtcttcacaaaccggagg			No mutations identified within this exon

CYP3A4 Exon 5	Distance From ATG start codon	Position in GenBank AF280107	DNA variant
	13837	75874	
catcacccagtagacagtcactaaatagttgttgaataagt Gttcctgtttaacacattttctacaaccatggagacctcca caactgAtgtaggacaaaatgtttctgctttGaactctagc cttttggtccagtgggatttatgaaaagtgccAtctctata gctgaggatgaagaatggaagagattacGatcattgctgtc tccaaccttcaccagtggaaaactcaaggag			G13875A (20) SNP4 (19) G13947C (21) *CYP3A4*4* (49) *CYP3A4*8* (53)

CYP3A4 Exon 6	Distance From ATG start codon	Position in GenBank AF280107	DNA variant
	14206	72543	
ggaaagccatgtccttctgggactagagtctgcacatttaa ctatgggtggtgTtgtgttttgtgcttagatggtccctatc attgcccagtatggagatgtgttggtgagaaatctgaggcG ggaagcagagacaggcaagcctGtcaccttgaaaGagtaag tagaagcgcagcCatgggGttctgagctgtcatgaacccct ccagcTgcctgccatggagctgatattcctgctgttgggtt attccagtgaccagacaaaaggagggctgtggtaatgcaac			SNP5 (22) *CYP3A4*15A* (60) *CYP3A4*9* (54) *CYP3A4*10* (55) M10 (23) M11 (24) T14475G (25)

CYP3A4 Exon 7	Distance From ATG start codon	Position in GenBank AF280107	DNA variant
	15570	77607	
cctgttgcatgcatagaggaaggatggtaaaaaggtgctgat tttaattttccAcatctttctccactcagcgtctttggggcc tacagcatggatgtgatcaCtagcacatcatTtggagtgaac atCgactctctcaacaatccacaagacccctttgtggaaaac accaagaagcttttaagatttgatttttggatcCattcttt ctcTcaataagtatgtgGactactatttccttttatttatct tTctctcttaaaaataactgctttattgagaTataaatcacc atgtaattcaTccacTtaaaatatacagttcagtgatttgta gTacatttgaagatatgtgtgaccatcatc			CYP3A4*1Z (26) CYP3A4*16A (61) CYP3A4*17 (62) I193I(66) CYP3A4*5 (50) CYP3A4*2 (47) CYP3A4*1P (27) T15871G (28) T15901C (29) SNP10 (30) CYP3A4*1Q (31) T15955A (32)
CYP3A4 Exon 8	Distance From ATG start codon	Position in GenBank AF280107	DNA variant
	16814	78851	
aggtaaattttgcacattcacaAtgaatttcttttctgttt ttgttttgttttcctacagcagtctttccattcctcatccc aattcttgaagtattaaatatctgtgtgtttccaagagaagt tacaaatttttaagaaaatctgtaaaaaggatgaaagaaag tcgcctcgaagatacacaaaaggtaaaatgtggtggtagtta taggaggatgtttagttttcataatttttagataatatac atatgatcagtgcagttacCtgtatgttttaaagaatgctt ttaa			CYP3A4*1R (35) C17141T (36)

CYP3A4 Exon 9	Distance From ATG start codon	Position in GenBank AF280107	DNA variant
	17629	79666	
caccgagtggatttccttcagctgatgattgac tctcagaat tcaaaagaaactgagtcccacaaaggtaaccagagtgtttctg aggGctacttgtggggcactcagagggaaggccttgttctgaa aatgtgcaggaagtattccaggatgatgagaatttctgccaca tagcagaacgacacATgtttgaatgttataagtggtagttgga ggc			CYP3A4*6 (51) CYP3A4*11b (37) SNP14 (38)

CYP3A4 Exon 10	Distance From ATG start codon	Position in GenBank AF280107	DNA variant
	20057	82094	
cagtgtacctctgaattgcttttctattctttcccttaggg atttgagggcttcacttagatttctcttcatctaaactgtga tgccctacattgatctgatttacctaaaatgtctttcctctc ctttcagctctgtccgatcTggagctcgtggcCcaatcaatt atctttatttttgctggctatgaaaccacgagcagtgttctc tccttcattatgtatgaactggccactcaccctgatgtccag cagaaactgcaggaggaaattgatgcagttttacccaataag gtgagtggatgGtacatggagaaggagggaggaggtgaaacc ttagCaaaaatgcctcctcaccacttcccaggagaattttta taaaaaGcataatcactgattctttcactgactctatgtagg aaggctctg			CYP3A4*18A (63) A297A (67) CYP3A4*1G (39) SNP 16 (40) G20417C (41)

CYP3A4 Exon 11	Distance From ATG start codon	Position in GenBank AF280107	DNA variant
	21805	83842	

ccagtatgagttagtctctggagctcctaatacttcattagt
actgcatggactgagttaaaagttaattcaaaatctcaattt
atccaAatctgtttcGttctttccaggcaccacccacCtatg
atactgtgctacagatggagtatcttgacatggtggtgaatg
aaaCGctcagattattcccaattgctatgagaCttgagaggg
tctgcaaaaaagatgttgagatcaatgggatgttcattccca
aaggggtggtggtgatgattccaagctatgctcttcaccgtg
acccaaagtactggacagagcctgagaagttcctccCtgaaa
ggtacaaggCccctgggaagggagccctccctgaaccagcct
ggttcaagcatattctgcctctcttaatctacaggaca

A21891C (42)
SNP18 (43)
T346T (68)
*CYP3A4*11* (56)
T363T (69)
*CYP3A4*12* (57)

*CYP3A4*13* (58)
M16 (44)

CYP3A4 Exon 12	Distance From ATG start codon	Position in GenBank AF280107	DNA variant
	23091	85128	

tagttgaggggtggcccctaagtaagaaAccctaacatgtaac
tcttaggggtattatgtcattacttttaaaaatctaccaaC
gtggaaccagattcagcaagaagaacaaggacaacatagatcc
ttacatatacacacccttggaagtggacccagaaactgcatt
ggcaTgaggtttgctctcatgaacatgaaacttgctctaatca
gagtccttcagaacttctccttcaaaCcttgtaaagaaacaca
g

SNP21 (45)
C23187T (46)

CYP3A4*3 (48)
CYP3A4*19 (64)

CYP3A4 Exon 13	Distance From ATG start codon	Position in GenBank AF280107	DNA variant
	25844	87881	
atccccctgaaattaagcttaggaggacttcttcaaccagaaa aaAcccgttgttctaaaggttgagtcaagggatggcacCgtaa gtggagcctga			CYP3A4*20 (65) T499T (70)