MHANGWANE, SHUSHU RIRHANDZU COMFORT

PREDICTING EARLY AND LATE FIRST-LINE ANTIRETROVIRAL THERAPY VIROLOGIC FAILURE, AND SWITCH TO SECOND-LINE THERAPY IN A MILITARY POPULATION IN SOUTH AFRICA

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Predicting early and late first-line antiretroviral therapy virologic failure, and switch to second-line therapy in a military population in South Africa.

by

Shushu Rirhandzu Comfort Mhangwane

A dissertation submitted to the department of pharmacology

at the University of Pretoria in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE IN PHARMACOLOGY.

STUDY LEADERS

Prof Duncan Cromarty, Pharmacology department,

Faculty of Health sciences, University of Pretoria

Dr Judith N Dlamini, Phidisa Project.

Pretoria, 2018

DECLARATION

I, SHUSHU RIRHANDZU COMFORT MHANGWANE, HEREBY DECLARE THAT THIS DISSERTATION IS MY OWN WORK AND HAS NOT BEEN PRESENTED FOR ANY DEGREE OF ANOTHER UNIVERSITY.

THE WORK DESCRIBED IN THIS DISSERTATION WAS CARRIED OUT IN A MILITARY HOSPITAL IN SOUTH AFRICA.

Signature:

DEDICATION

This study is dedicated to my late father Mr MHANGWANE Victor Ghezani, and to my family for your unconditional and unwavering support, belief in me and encouragement.

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ABSTRACT

Context: This study involved retrospective data analysis using statistical methods to reanalyse data collected during a long-term study in a Human Immunodeficiency Virus (HIV) infected population of South African National Defence Force employees and their dependants, where different parameters related to treatment and disease status of HIV infected patients were collected. This study attempted to identify possible predictors of both early and late occurrence of first-line antiretroviral therapy (ART) virologic failure. Potential predictors of first-line ART virologic failure and for switching to second-line therapy were identified.

Objectives: The study had two primary objectives, namely to identify predictors of first-line virologic failure:

In the early period (up to 1 year after ART initiation) and
In the later period (beyond one year of ART initiation)

From data collected during the Phidisa 1a initiated cohort study and to describe the extent of first-line virologic failure in the same cohort;

In addition the time to initiation of second-line antiretroviral therapy among those where first-line antiretroviral therapy failed was assessed.

Research design and methods: This is an observational, retrospective cohort analysis of ART in HIV positive military personnel aged 18 years and older who initiated combined antiretroviral treatment (cART) between 01 April 2008 and 30 April 2011 and had at least a one year follow-up visit by 15 May 2012. First-line ART consisted of a three-drug therapy with two nucleoside reverse-transcriptase inhibitors (NRTIs) and a non-nucleoside reverse-transcriptase inhibitor (NNRTIs), Efavirenz (EFV) or Nevirapine (NVP).

Second-line ART included a boosted protease inhibitor (PI) based treatment regimen.

Results: A total of 1285 patients had complete data for a mean of 4 years. At study entry, the median HIV viral load was 91150 copies per millilitre (cp/mℓ**)** and the median $CD4^+$ cell count was 152 per cubic millimetre. 19.68 % (253/1285) patients were identified as ART failure in this cohort. Of these, 94.1% were on Efavirenz based regimen and 5.9% on NVP based regimen. The percentage of patients with early virologic failure did not show statistical significant difference from the percentage of patients with late virologic failure (12.0% vs. 7.7%).

Missed visits within six months of treatment initiation (HR: 0.232: 95% CI: 0.098-0.546), high viral load before cART initiation (HR: 1.243: 95% CI: 1.086-1.423), NRTI backbone (1) (HR: 0.565: 95% CI: 0.337-0.946) and treatment site displayed strong association with "Early virologic failure'. Predictors of "Late virologic failure" included viral suppression <50 cp/mℓ, high viral load before cART initiation and ART toxicity. Overall, 11.5% of patients were switched to second-line therapy within two years of ART initiation.

TB infection (HR: 0.892: 95% CI: 0.576-1.382) and treatment site (HR: 0.497: 95% CI: 0.288-0.860) were predictive of treatment switching to second-line treatment.

Conclusion: A substantial number of patients with HIV on the first-line cART for less than a year are at greatest risk of early virologic failure. 12.0% (154/1285) patients experienced early virologic failure. Understanding the absolute risk of first-line failure is useful for patient monitoring and for effectively targeting limited resources for second-line ART. Moreover, any missed visit within the first six months of treatment, treatment site, and high viral load before cART initiation and NNTI based treatment regimens all play an important role in early virologic failure of patients. Careful monitoring of patients under HIV/AIDS treatment, particularly in the first three months after cART initiation is necessary.

The major disadvantage of a retrospective observational cohort study design is the unintended limits introduced during data collection, including incomplete or inaccurate data or inconsistent measurements between subjects. This could include variation in disease status, time to initiation of treatment, and other co-morbidities that were not recorded, patient drop-out rate amongst others.

KEY WORDS

HIV-1

Early Virologic Failure

Late Virologic Failure

Resistance

Second-line Protease Inhibitor treatments

Antiretroviral drug resistance

LIST OF ABBREVIATIONS

3TC	Lamivudine
ABC	Abacavir
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
ARV	Antiretroviral
ASSA	Actuarial Society of South Africa
BARC	Bioanalytical Research Corporation
cART	Combined antiretroviral therapy
CD4^{+}	Cluster of differentiation four positive lymphocytes
CDC	Centre for Disease Control and Prevention
CM	Cryptococcal Meningitis
cp/mℓ	Copies per millilitres a measure of viral load
CRO	Conference on Retroviruses and Opportunistic Infections
CVD	Cardiovascular disease
DDI	Didanosine
d4t	Stavudine
EACS	European AIDS Clinical Society
EFV	Efavirenz
EVF	Early virologic failure
GART	Genotypic Antiretroviral Resistance Testing
GCP	Good Clinical Practice
FDA	Food and Drug Administration of the United States of America
FTC	Emtricitabine
Haart	Highly active antiretroviral therapy
Hb	Haemoglobin
HIV	Human immunodeficiency virus
HOPS	HIV outpatient study
IAS-USA	International AIDS Society-United States of America

InSTI	Integrase strand transfer inhibitor
JAMA	Journal of American Medical Association
LDT	Laboratory-developed tests
LVF	Late virologic failure
MDR	Multidrug-resistance
NIAAA	National Institutes on Alcohol and Alcoholism
NIH	National Institutes of Health of the United States of America
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleos(t)ide reverse transcriptase inhibitor
NVP	Nevirapine
OI's	Opportunistic infections
PCP	Pneumocystis Carinii Pneumonia
PCR	Polymerase Chain Reaction
PI/r	Protease inhibitor ritonavir boosted
POD	Progression of diseases
RAMs	Resistance-associated Mutations
RLS	Resource-limited settings
RNA	Ribonucleic acid
SA DoD	South African Department of Defence
SAMHS	South African Military Health Services
SANDF	South African National Defence Force
SD	Standard deviation
SSC	Scientific study committee
TDF/TNV	Tenofovir
TB	Tuberculosis
µl	Micro litre
UNAIDS	United Nations Programme on HIV/AIDS
US DHHS	United States Department of Health and Human Services
US DoD	United States Department of Defence
VL	Viral load

WHO World Health Organization

ZDV Zidovudine

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CHAPTER 1

1.1 INTRODUCTION AND BACKGROUND OF HIV/AIDS

The human immunodeficiency virus (HIV) pandemic is a complex mix of diverse epidemics within and between countries and regions of the world and is undoubtedly the defining public-health crisis of our time. Antiretroviral treatment has transformed Acquired Immune Deficiency Syndrome (AIDS) from an inevitable fatal condition to a chronic manageable disease in some settings. This transformation has yet to be realized in those parts of the world that continue to bear a disproportionately high burden of new HIV-infections (Simon *et al.*, 2006)

The HIV/AIDS is an exceptional epidemic that demands an exceptional response. Although much progress has been made over time in managing (such as treatment and prevention) the HIV/AIDS pandemic, much has also been accompanied by scientific and health care program challenges (Simon *et al.*, 2006). In the absence of a protective vaccine or a cure, prevention and access to antiretroviral treatments are the best options to slow the HIV pandemic. Broad implementation of these options needs improved infrastructure that implies that resource-constrained regions that have been the most affected will continue to be so. Clearly, there is a need for the scale-up of interventions and improvement of current strategies to better manage the HIV/AIDS pandemic.

Heterosexual transmission of the HIV virus remains the dominant mode of transmission and accounts for about 85% of all HIV-1 infections globally (Hayes *et al.*, 2006). Southern Africa remains the epicentre of the pandemic and continues to have high rates of new HIV-1 infections (Cobo 2014). In view of the fact that HIV-1 is predominantly sexually transmitted and disproportionately affects populations that are already socially or economically marginalized, or both, ethical, social, economic and political challenges are posed, calling for constantly finding better strategies to combat the pandemic and provide robust education to the community. The high percentage of the population that are HIV positive indicates that current strategies may not be sufficient to claim the disease is under control. Therefore scientists, society and governments are still faced with a challenge to devise better strategies of managing the spread of HIV-1.

National and International HIV treatment guidelines recommend initiating ART therapy with two nucleoside reverse transcriptase inhibitors (NRTI) and either a non-nucleoside reverse transcriptase inhibitor (NNRTI), a boosted protease inhibitor (bPI), or an integrase inhibitor (INSTI). These are drugs that have different effects on specific virus protein processing enzymes and trans-membrane transporters. The main goal of ART is to achieve and maintain viral suppression while maintaining, if not improving the health of the patient. Although retaining patients in lifelong HIV care is a major challenge in many countries, it is of utmost importance to constantly encourage and support the patients to avoid the risk of mortality and morbidity by encouraging continued use of ART and minimizing further exposures to HIV-1.

1.2 FORMULATING THE RESEARCH PROBLEM

It is now acknowledged that the HIV/AIDS pandemic is currently one of the biggest public health issues that humanity has had to contend with given its global reach (refer to Figure 1 below). By 2010, about 34 million people had been infected by HIV (WHO, 2011). It was only in 1983 that scientists discovered and named the virus that caused AIDS. Once acquired, the virus attacks and weakens the human body's immune system increasing susceptibility to opportunistic infections that are normally not a health risk.

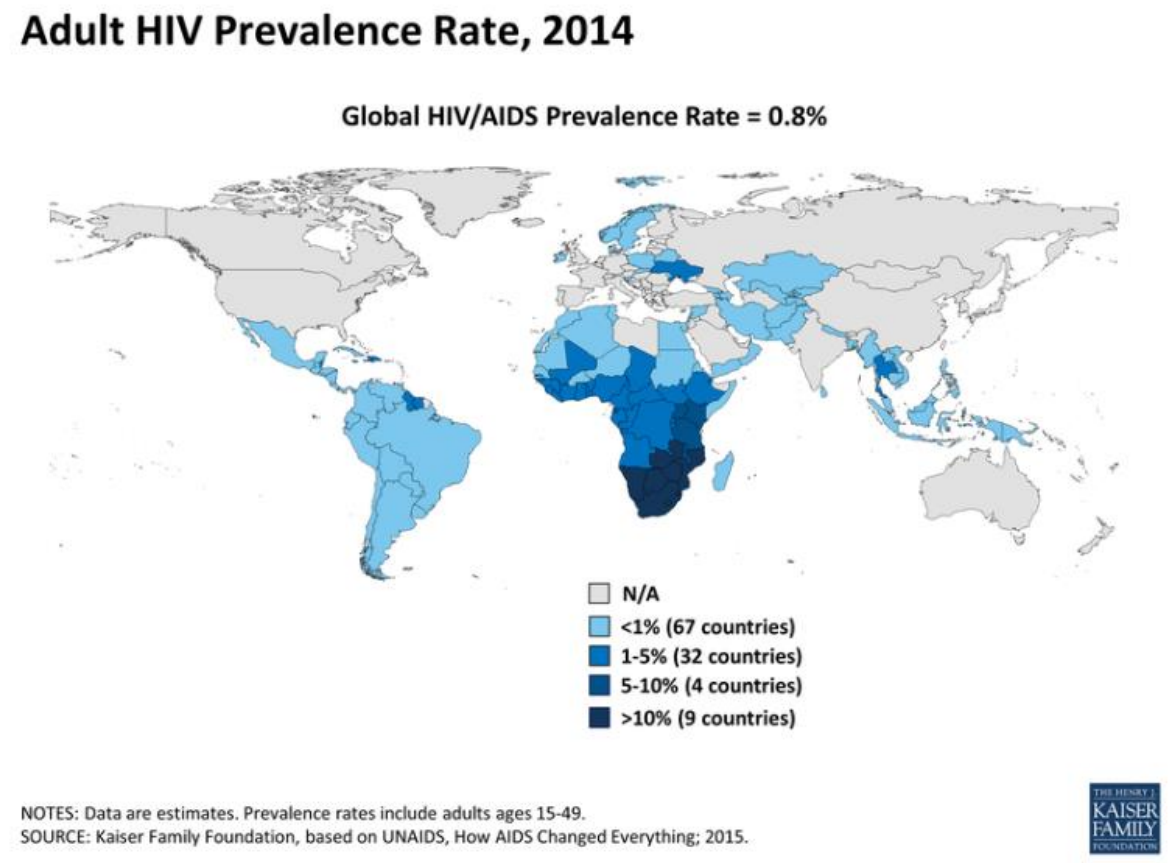


Figure 1: world map of global AIDS epidemic (downloaded from http://www.vox.com/2015/12/1/9814026/world-aids-day-2015-hiv-awareness, on 30 August 2016)

Primarily, for this reason, the focus within the scientific and medical community had been to identify and discover antiretroviral drugs to tackle the HIV virus. While the first antiretroviral drugs were discovered around the late 1980s they were not only expensive, but had severe side effects on patients, the combined effect of which was to limit the number of patients that could receive these drugs.

More than three decades since the discovery of AZT, significant strides have led to the discovery of additional antiretroviral therapy, the discovery of which has led to the significant reduction in their cost as well as fewer adverse side effects, among other factors. As a result, there has been a significant increase in the number of patients enrolled in antiretroviral

therapy. For example the number of patients enrolled in antiretroviral therapy has increased from 400 000 people in 2003 to about 3 million people by 2010 (WHO and UNAIDS 2003). It is presently a practice for HIV infected patients to be treated with a fixed dose combination ART. This fixed dose combination has been found to improve compliance in taking the medication by patients, with fewer side effects, lower costs as well as extending the quality life of the infected patient.

Despite these significant improvements in available drugs and treatment regimens, virologic failure remains a hurdle for HIV-infected patients who need to be committed to lifelong antiviral treatment. Researchers have noted a significant increase in ART resistance mutations in the HIV virus (Wallis *et al.,* 2010). Increasing ART resistance including those due to viral mutations potentially increases the risk of treatment failure. It is therefore prudent to identify and understand the factors that may give rise to virologic failure.

There is currently only a partial understanding of HIV-1 drug resistance patterns that occur in developing countries, where VL testing is uncommon due to cost and lack of infrastructure and where treatment failure is generally defined by clinical and immunological indicators only. HIV-1 drug resistance testing should not be an optional measure in all health settings, but rather compulsory to ensure the drugs are working effectively and efficiently in cases of virologic failure. However, when HIV-1 drug resistance testing is recommended as the standard of care at the time of virologic failure, efforts should be made to ensure that viral load testing becomes affordable, simple and easy to use in resource-limited settings. Moreover, regular VL monitoring could identify the incidence of drug resistance early and this could significantly reduce unnecessary costs.

Accumulation of resistant mutations with the potential to compromise treatment responses and risk of resistant virus infection has also prompted calls for earlier failure detection that can be accomplished with HIV-1 Ribonucleic Acid (RNA) monitoring (Sungkanuparph *et al.,* 2007 and Hosseinipour *et al.,* 2010).

HIV enters the body through the exchange of infected body fluids and initially infect the immune regulating $CD4^+$ lymphocytes in which they replicate and eventually kill the host cell which results in a weakened immune system (Fevrier *et al.,* 2011). Within the first year of HIV infection, the patient $CD4^+$ lymphocyte count decreases by approximately one-quarter then decreases slowly thereafter. Once the $CD4^+$ lymphocytes count is below critical thresholds, HIV-related opportunistic infections become evident due to limited immune response that can be mounted. $CD4^+$ lymphocyte counts were initially studied as markers of the progression of HIV infection, as a measure of the relative risk of developing opportunistic infections (OI's) to estimate the impact of HIV and the use of ARV drugs on the epidemiological progression of TB (Hogg *et al.,* 2001).

HIV can infect a multitude of other cells in an infected person, including brain cells, but the main target is the $CD4^+$ lymphocyte, also called a T-helper cell or $CD4^+$ cell. When a $CD4^+$ cell is infected with HIV, the virus goes through multiple steps to make use of the DNA replication system of the cell to be able reproduce itself and create many more copies of the virus particles. This sequence of intracellular events is summarised in Figure 2.

The process of HIV infection is broken up into the following steps:

Binding and fusion: This is the process by which HIV binds to a specific type of $CD4^+$ receptor and a co-receptor on the surface of the $CD4^+$ cell. This is similar to a key entering the lock. Once properly interacting with the receptors, HIV fuses with the host cell membrane and enters the intact cell ($CD4^+$ cell) where the virus disassembles releasing its genetic material.

Reverse Transcription: A special enzyme called reverse transcriptase changes the genetic material of the virus, so it can be integrated into the host DNA.

Integration: The virus' genetic material enters the nucleus of the $CD4^+$ cell and uses an enzyme called integrase to integrate itself into the host cells own genetic material, where it may remain inactive for several years.

Transcription: When the host cell becomes activated, the virus genetic material is copied using the host cells own enzymes to create more viral genetic material and to code for the required proteins for complete viral particle assembly thereby making multiple viral protein precursors.

Assembly: A viral encoded enzyme called protease cuts the longer HIV proteins into individual proteins that are then combined in the correct manner to form a new viral particle. When these are combined with the virus' genetic material, a viable new virus is assembled.

Budding: This is the stage of the virus' life cycle where the virus pushes itself out of the host cell, thereby compromising the cell membrane and cell deterioration occurs.

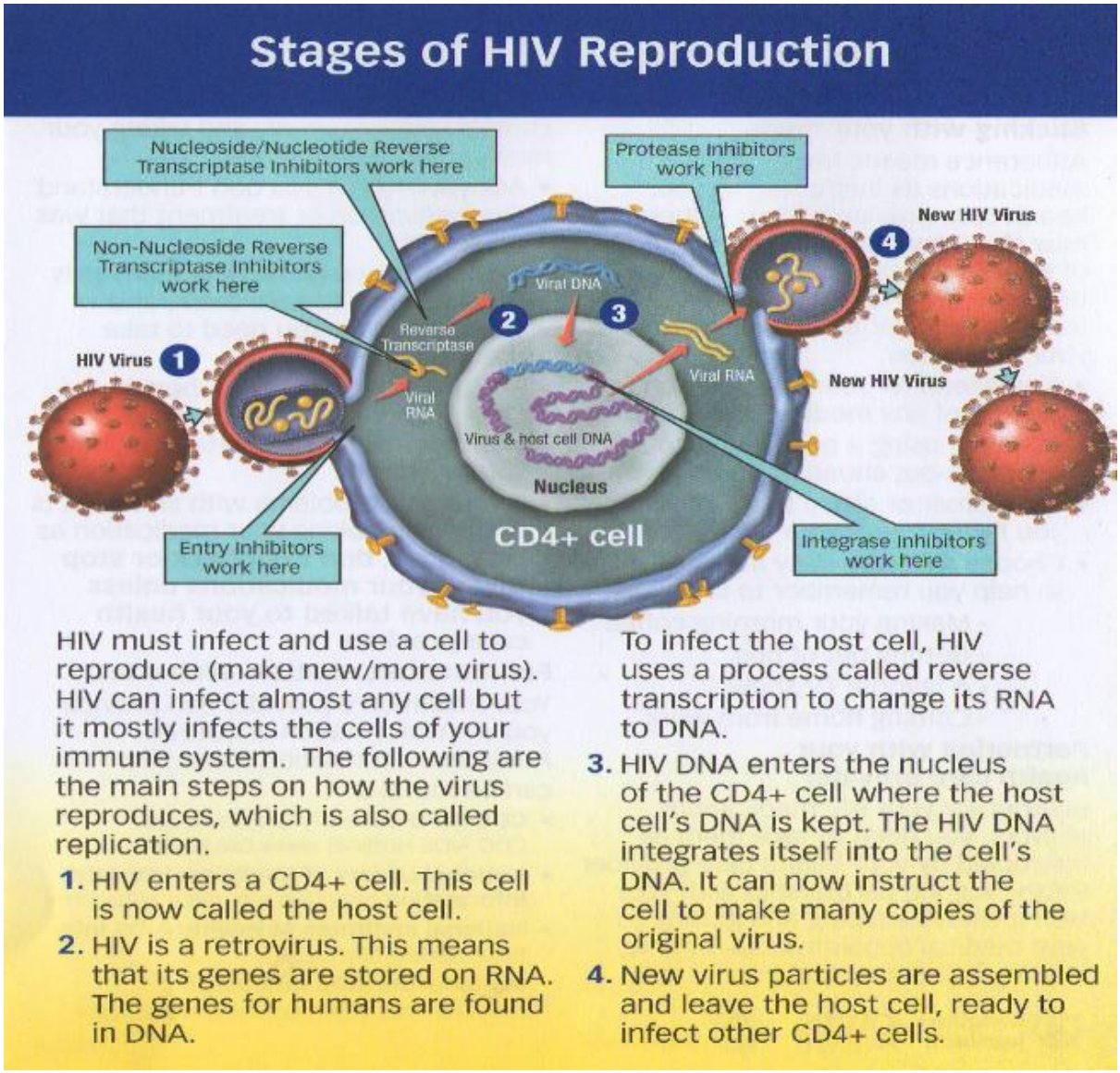


***FIGURE 2**: The reproduction stages of HIV and different treatment targets (downloaded from [http:// www.friendsofaids.org/topics.html](http://www.friendsofaids.org/topics.html) on 11 August 2016)*

During this process many new viral particles are formed and the host cell dies off which results in decreasing numbers of the important $CD4^+$ immune regulating cells. In this way the HIV suppresses the immune system which negatively affects both innate and adaptive immunity which is evident from the opportunistic infections often reported for HIV positive patients. About 30 million AIDS-related deaths have been reported worldwide since the disease first emerged in the early 1980s (Thompson *et al.*, 2012). Over the years since HIV's emergence, researchers have been working on alternative therapies to stem its spread. One of the therapies which has been found to be effective, has been the combination antiretroviral therapy (cART) which inhibits HIV from multiplying and has led to a measurable reduction in HIV associated morbidity and mortality. To be effective however, cART has to be taken continuously on a daily basis for life because it cannot destroy latent viruses hidden in

"reservoir cells". Despite not destroying all the HIV viruses in the body, cART has significantly increased survival rates in affected patients (Uy *et al.,* 2009).

The current goal of cART is suppression of plasma HIV-1 RNA levels, to below 50 copies per millilitre (cp/mℓ) (Li *et al.,* 2012). A proportion of HIV-infected patients, who initiate cART in clinical practice fail to suppress HIV-1 RNA or have HIV-1 RNA level rebound while on therapy. Failure to suppress HIV-1 RNA to below 50 cp/mℓ while patients are on therapy, generally results in further viral replication which ultimately leads to virologic failure as well as the potential emergence of drug resistant mutations. This in turn escalates the costs associated with managing HIV positive patients especially with regard to introducing the second-line Protease Inhibitor (PI) class of drugs. Given the increased costs associated with second-line therapy, affordable strategies to prevent treatment failure on first-line regimens among HIV patients is essential for long-term success of ART in Sub-Saharan Africa. For this reason, identification of patients at risk of virologic failure prior to cART initiation will assist in directing interventions and to provide additional monitoring and support to those patients likely to benefit the most.

Additionally, compliance to long-term therapy has been found to wane with time, potentially resulting in late virologic failure in patients who initially were adherent to cART. Most patients achieve positive response to cART within one year of treatment initiation, demonstrating low viral load and minimal reduction in $CD4^+$ cell counts. It is therefore important to identify patients and factors that potentially explain poor treatment response and waning compliance to long term therapy. This knowledge will benefit future populations initiating cART treatment. Other factors that could explain poor long term compliance to cART may include factors such as provider access, cancelled doctor appointments, drug availability, clinicians experienced in identifying ART failure and waiting times to change treatment (Hosseinipour *et al.,* 2009).

Current ARV treatment guidelines recommend immediate modification of antiretroviral therapy in HIV infected individuals showing incomplete viral suppression, particularly in patients following first-line cART regimens (Petersen *et al.,* 2008). While recent estimates suggest only 2% of those currently on ART are on a second-line regimen, a far greater percentage are showing virological failure but have not been switched from first-line to second-line therapy (Fox *et al.,* 2012). As cART scale-up continues and the average duration already on ART increases, both the absolute number and the relative proportion of patients needing second-line therapy will increase (Boulle *et al.,* 2010). It has previously been estimated that in South Africa, about 14% of patients on ART show virologic failure by year five of ART (Fox *et al.,* 2012).

There are various explanations for difficulties in switching to second-line ART. These difficulties may include, but not be limited to, scarce resources in health facilities, clinicians reluctant to switch patients who are apparently clinically well to second-line cART as they might see second-line therapy as a salvage therapy to be reserved for later when no other options might be available. The difficulties may then result in increased mortality or risk of future virologic suppression. A systematic review and meta-analysis showed that effective switching to second-line ART largely depends on careful clinical assessment and access to

biological measurements (Madec *et al.,* 2013). In the absence of routine viral load assays, detection of treatment failure and subsequent switch to second-line ART usually occur too late to show an effective response to treatment.

Another important explanation is the inability to identify and diagnose patients who have failed first-line therapy. In high-income countries, the diagnosis of ART failure is based on measurements of HIV-1 RNA concentration. For example, in the United Kingdom (UK) and United States of America (USA), a plasma HIV-1 RNA concentration above 50 cp/mℓ on two consecutive occasions is used to define virological failure (Harries *et al.,* 2010). The main reason for not detecting ART failure early in developing countries is lack of resources to determine low levels of HIV-1 RNA in plasma.

Because viral load monitoring is generally not accessible in most resource-limited but high-endemic regions/areas/countries, patients are often continued on first-line ART until the emergence of obvious clinical symptoms of opportunistic infections or until the WHO criteria for immunologic failure are met (El-Khatib *et al.,* 2011). As treatment delay after virologic failure on non-protease inhibitor based regimens has an increased risk of mortality (Levison *et al.,* 2011), the timing of treatment switch after first-line ART failure is particularly important. Furthermore, delay in switching from failed therapy that consisted of thymidine analogues may lead to accumulation of Thymidine Analogue Mutations (TAMs) or K65 resistance mutations, which have been shown to negatively impact to second-line therapy (Hosseinipour *et al.,* 2010).

In this regard, studies have shown that treatment modifications after virologic failure while following a non-protease inhibitor based ART regimen had an increased risk of mortality and increases the odds of poor of virologic suppression which in turn may lead to increased risk of mortality (Levison *et al.,* 2011). Given the reported elevated risk of mortality associated with treatment modification in patients receiving NNRTI-based regimen, efforts should be made to minimize delays in switching to second-line therapy. Also, viral resistance to drugs emerges rapidly during NNRTI-based regimens but is uncommon for most protease-inhibitor based regimens (Peterson *et al.,* 2008). Hence patients who develop resistance to the PI component of their ART regimen may still be able to respond to other PI class drugs. This implies that regular virological monitoring is necessary to detect treatment failure that is evident long before the clinical signs of immunological failure are evident.

This study set out to assess where virologic failure takes place in HIV positive patients being treated with cART. It is a retrospective observational study of the incidence of virologic failure to first-line antiretroviral therapy in HIV positive patients with emphasis on the time from initiation of the treatment to when the virologic failure was noted as well as the time lapse before switching to second–line PI therapy after first-line therapy failure. It should be noted that this study is a statistical analysis of existing data collected during the Phidisa 1a and subsequent studies that were not initially powered to detect these virologic failure predictors. No new data was collected. The investigators attempted to identify risk factors for virologic failure in a population of more than 1200 HIV positive patients who were observed over a cART treatment period of 3 years with regular patient monitoring follow-ups.

1.3 RATIONALE FOR THE STUDY

The aim of this study was to determine which predictors could be associated with early and with late first-line antiretroviral virologic failure that would require that the patient be switched to a second-line ART therapy. It has been established by other studies, (WHO Guidelines, 2003; Chesney, 2006) that effective ART requires strict compliance to long term daily use by patients to ensure sustained HIV suppression, reduced risk of drug resistance, improved overall health, improved quality of life and longer survival.

However, it has also been established that many patients' levels of compliance to ART tends to wane after long term use, rendering ART less effective and increasing the incidence of late virologic failure. This then necessitates the need to switch from first-line to second-line ART therapy. No similar studies have been reported in high endemic areas such as South Africa. As HIV treatment and care enters an era where the use of cART is being increasingly considered as a potential protocol in retarding HIV transmission, it is important that a better understanding of the predictors of Early virologic failure (EVF) and Late virologic failure (LVF) are developed.

1.4 RESEARCH OBJECTIVES

The broad objective of this study was to perform a retrospective statistical analysis on available data in an attempt to identify parameters that can be used as possible predictors of first-line virologic failure during antiretroviral therapy. This aim was met through the specific objectives below:

1.4.1. To identify the predictors of the first-line virologic failure in the early period (up to one year of cART initiation)

1.4.2. To identify the predictors of the first-line virologic failure in the later period (beyond one year after cART initiation)

1.4.3. To ascertain the extent of first-line virologic failure in the early period (up to 1 year of cART initiation) and in the later period (beyond one year of cART initiation).

1.4.4. To establish the average time to initiation of second-line ART among those failing first-line ART in the studied cohort.

1.4.5. To identify predictors of required switching to second-line cART after first-line treatment failure.

The focus of this study was on all uniform wearing SANDF personnel and their registered family members 18 years of age and older who had laboratory confirmed HIV infections and who initiated ART between 01 April 2008 and 30 April 2011 and who had at least one one-year follow-up visit by 15 May 2012. First-line ART provided according to the guidelines then in use generally consisted of a three drug therapy with two NRTIs and one NNRTIs (i.e. EFV or NVP) while second-line ART included a boosted PI (i.e. Lopinavir/ritonavir) based ART.

1.5. LIMITATION OF THE STUDY

It was postulated by De Vos *et al.*, (2011) that potential limitations in a study are often numerous even in the most carefully planned research studies and it is important that they are taken into account. Generally, when identifying limitations, researchers must consider the comparability of the data, completeness of the data, validity and reliability of all data collection instruments, the applicability of the sample measured to the population from which it is drawn, access to comparable data, ethical problems, and the ability to control extraneous factors in the environment and in respondents.

Although problems are never completely eliminated from studies in the clinical setting, researchers must demonstrate the various means by which they attempt to limit problems. Finally, mention of the specific steps proposed to ensure that the sample is as representative as possible of the population from which it is drawn must be made.

A major limitation of this study was that information relating to ART regimen compliance was not captured as an assessment tool to determine treatment compliance and it was not available. All the patients recruited into the study were initially ART-naïve, therefore the findings highlighted in this study may not be applicable to cART treatment-experienced patients.

Although all the patients were from within the military setting, their experiences may differ based on the information provided during counselling, site where the treatment was initiated, the availability of the treatment at all times. Also patients accessing treatment in a community-based setting may experience different limitations or support, making direct comparisons based on measured parameters difficult.

1.6 THE STRUCTURE OF THE THESIS

Chapter one introduces the pathogenesis, epidemiology, and treatment of HIV/AIDS. It further explains how research has deepened our understanding of how the virus replicates, manipulates and evades detection in an infected person. Thereafter, a brief objective is presented leading to the rationale for the work detailed in this dissertation. The last part of this chapter contains limitations of this study as it has been identified that problems are never completely eliminated from any research.

Chapter two contains a literature review covering determination of any association between increased viral load, reducing $CD4^+$ cell count and progression of disease. This chapter also establishes the effect of various variables on early and late virologic failure. The different factors that may lead to a delay in switching to second-line therapy and the risks and benefits of antiretroviral treatment compliance. It further outlines the theoretical model namely; the Cox model that was used in this study. The basics of the model are outlined, including how the model was interpreted.

Chapter three describes the data and methodology of the study. It explains how the study was designed. It also describes explicitly the study settings from six different sites, how patients were recruited and enrolled, eligibility criteria used and the different types of blood samples that were taken from patients to collect the data used in this study. Different definitions used in the study are clarified. Descriptive statistics were employed for patient's baseline demographics, clinical and immunological characteristics. Descriptive statistics such as mean, standard deviation, were calculated for continuous variables. This chapter further describes the data quality and how it was collected. Moreover it describes the comparison between failure, non-failure, early and late virologic failure in a table form.

Chapter four contains the study results. It describes possible predictors identified that elicit a major effect on virologic failure and how they are employed in determining early and late first-line antiretroviral therapy virologic failure. Furthermore it describes the switch from first line treatment failure to second-line treatment. This chapter also provides limitations of this study.

Chapter five analyses the findings of the study and discusses the findings. Finally, this chapter provides the conclusion derived from this study and the recommendations for future work.

CHAPTER 2

2.1 HISTORY OF HIV/AIDS

HIV infection had been found as early as the 1950's but the widespread dissemination did not appear until the late 1970s (Klusacek *et al*., 1992). Prior to the recognition and labelling of AIDS numerous people had died from it during the 1970s but these deaths were attributed to other symptomatic illness. Although AIDS was becoming a medical concern, it was politically ignored until the disease was noted outside the homosexual community. This complex disease was later reported among members of the Haitian community who immigrated to the United States. Nearly all the Haitian patients who reported this complex syndrome were heterosexual, leading to confusion among the scientific community. Further investigations revealed that addicts who shared needles to inject narcotics directly into their bloodstream were also affected. Haemophiliacs had been reported by the Centre for Disease Control (CDC) to contract AIDS through blood transfusion and babies born to mothers who were drug addicts were also falling ill with a similar disease.

By early 1981 several heterosexual men were found to have registered with unusual complaints with complex disease aetiology while it became apparent to the medical community that a distinctive yet nameless disease had erupted within homosexual communities in North America (Klusacek *et al*., 1992). Symptoms varied in different communities.

HIV has more genetic material with which to constantly redesign its surface than any other known virus (O'Malley *et al*., 1988). Therefore, it can alter its surface phenotype somewhat, individuate, in virtually each person infected. Once replicating in infected cells, it can replicate faster than many other viruses destroying the host cell. As it uses the human CD4+ cells as host, the immune system that should protect against infection and diseases becomes compromised. For many years the virus had been spreading invisibly as it was not recognised as the cause of the condition.

2.2 ROUTES OF HIV INFECTION TRANSMISSION

HIV is transmitted in human body fluids (blood, semen and pre-seminal fluid, rectal fluids/anal mucous, vaginal fluids, breast milk) by three major routes: (1) Sexual intercourse through vaginal, rectal or penile tissues; (2) direct injection with HIV-contaminated drugs, needles, syringes, blood or blood products, and (3) from HIV-infected mother to foetus in utero, through intrapartum inoculation from mother to infant or during breast-feeding. Today the risk of contracting HIV from blood transfusions, blood products or organ/tissue transplants that are contaminated with HIV is extremely low because most countries test all related blood products for HIV before being made available to patients. If adequate safety practices are not in place, healthcare workers can also be at risk of HIV from cuts made by sharp objects or a needle (needle stick injury) with infected blood on it. The risk of occupational exposure is very low in most countries.

To get infected, the virus in a body fluid needs to get into the new hosts blood by crossing a mucous membrane, (for example the lining of the vagina, rectum, the opening of the penis, or the mouth), breaks in the skin (like cuts) or be injected directly into your blood stream. According to Centres for Disease Control and Prevention (CDC), HIV is not spread by tears, sweat, coughing or sneezing nor it is transmitted via an infected person's clothing, phone, drinking and eating utensils or other objects that HIV-infected people have used that are free of blood.

HIV is not communicable through contact with inanimate objects or through vectors. Therefore, people cannot become HIV infected in the same way as influenza or the common cold. Transmission via saliva has also been speculated yet clinical research shows that the concentration of HIV in saliva is very low in comparison to blood, breast milk, semen and vaginal secretions. Moreover, saliva contains large sugar protein molecules called glycoproteins, which inhibits HIV infections through formation of giant viral clumps which are not capable of infecting CD4+ cells (Pinsky 1994).

2.2.1 SEXUAL TRANSMISSION

The most frequent mode of transmission of HIV is through sexual contact with an infected person. Sexual transmission of HIV happens when infected semen, blood or vaginal secretions enter the blood stream of a partner. Although HIV can be transmitted during vaginal or oral penetration, unprotected anal sex by males appears to be the most common transmission method. The risk of acquiring HIV depends mainly on three factors: (1) the number of sexual partners; (2) the prevalence of HIV infection in these partners; (3) the probability of virus transmission during sexual contact. Worldwide, the majority of cases of transmission occur through heterosexual contacts (i.e. sexual contacts between people of the opposite sex). However, the pattern of transmission varies significantly between countries. In the United States, as of 2015, (https://www.hiv.gov/hiv-basics/overview/data-and-trends/statistics) most sexual transmission occurred in men who have sex with men, with this population accounting for 67% of all cases.

2.2.2 INJECTION DRUG USERS AND HIV TRANSMISSION

Sharing needles, syringes or other equipment used to prepare and inject drugs with someone who has HIV can increase the risks of contracting the virus. Although HIV does not generally survive well outside the body, it can survive for long periods of time (over 28 days) if sealed in a syringe (Abdala *et al*., 2004). Some injecting drug users may believe, wrongly, that they are not at risk of HIV transmission if they simply avoid intravenous injecting. HIV infection will not automatically occur from a single incident of shared needle/syringe use. In fact, estimates of the infection risk from an injection range from 0.63 to 2.4% (Baggaley *et al*., 2006).

Two factors are likely to determine the chances of HIV infection from any single incident of needle sharing, namely:

The level of HIV copies present in the blood injected. Very low levels of circulating virus in the blood may make HIV infection less likely. But assuming a high viral load, it is thought that amounts of blood invisible to the naked eye may be sufficient to permit HIV infection.

The quantity of blood injected. Evidence from the follow-up of needle stick injuries and other occupational injuries involving blood shows that the likelihood of HIV infection is dose-related – the more blood injected, the more likely it is that seroconversion will take place.

2.2.3 MOTHER-TO-CHILD TRANSMISSION

A mother infected with HIV can pass the virus to her baby via her blood during pregnancy and birth, and through her breast milk when breastfeeding. This is the third most common way in which HIV is transmitted globally. In the absence of treatment, the risk of transmission before or during birth is around 20% and in those who also breastfeed 35% (Teasdale *et al.*, 2011).With appropriate treatment the risk of mother-to-child infection can be reduced to about 1% (AIDS *info* 2017). Preventative treatment involves the mother taking antiretroviral during pregnancy and delivery, an elective caesarean section, avoiding breastfeeding and administering antiretroviral drugs to the new born.

2.2.4 CONTAMINATED BLOOD TRANSFUSIONS AND ORGAN/TISSUE TRANSPLANTS

Receiving blood transfusions, blood products, or organ/tissue that is contaminated with HIV increases the risk of contracting HIV. However, in recent years the risks of transplant-related HIV infection are low, as all organ and tissue donors are screened for risk factors, and tested for HIV and other infectious agents that potentially could be transmitted through transplantation. However, although HIV tests are highly accurate, the tests do not always detect the virus in people with very recent infection. Unexpected transmission of HIV, HBV, and HCV from infected donors has been reported in heart, liver, kidney, and pancreas recipients.

2.3 BACKGROUND OF THE USE OF ART

Antiretroviral treatment is recommended for all HIV-infected individuals to reduce the risk of disease progression. ART is also recommended for HIV-infected individuals for the prevention of transmission of HIV. Without ART, the vast majority of HIV-infected individuals will eventually develop progressive immune-deficiency leading to AIDS-defining illnesses and premature death. The primary goal of ART is to prevent HIV-associated morbidity and mortality. The goal is best accomplished by using combined ARV therapy to totally inhibit HIV replication so that HIV RNA levels are reduced to undetectable levels in plasma.

Furthermore, high plasma HIV RNA has been reported as a major risk factor for HIV transmission and use of effective ART can reduce viraemia and transmission of HIV to

sexual partners (Quinn *et al.*, 2000; Cohen *et al.*, 2011). Thus the secondary goal of ART is to reduce the risk of HIV transmission.

In 2010 WHO revised their guidelines and recommend the initiation of ARV's for all patients with $CD4^+$ count of 350 cells/mm^3 or less in all countries (Thompson *et al.,* 2012). According to the Joint United Nations Programme on HIV/AIDS (UNAIDS) report, these new criteria increased the total number of people medically eligible for ART in South Africa by roughly 50%, from 10 million to 15 million. The policy implemented by the South African National Department of Health was changed to align with this recommendation early in 2010. The current recommendations are based on evidence showing benefit to the health of the HIV-infected individuals and the evidence that earlier initiation of ART reduces AIDS progression and mortality. The threshold previously used by the WHO was $CD4^+$ count of 200 cells/mm^3 or less. This policy was changed in 2010 as patient response to treatment before then was poor due to a severely weakened immune system.

Despite increasing evidence for the benefits associated with early initiation of ART, there are concerns regarding ARV drug toxicities that have adverse effects on the quality of life resulting in poor compliance to treatment regimens. The D:A:D study found an increasing evidence of CVD associated with cumulative exposure to some drugs in the nucleoside reverse transcriptase inhibitor (NRTI) class and the PI drug class (Friis-Moller *et al.,* 2007; Worm *et al.,* 2010). In the SMART study, when compared with interruption or deferral of therapy, continuous exposure to ART was associated with significant loss of bone density (El-Sadr *et al.,* 2006).

There is a strong association between ART treatment and quality of life of people living with HIV/AIDS. ART frequently improves quality of life for patients with symptomatic immune suppression. However, the adverse side effects of ART may impair quality of life for some patients, especially those who are generally asymptomatic at initiation of cART therapy. For example, the drug Efavirenz which belongs to a class of medication called NNRTIs is significantly associated with nervous system and psychiatric side effects, while Lopinavir/ritonavir belonging to a class of drugs called PI, are commonly associated with gastrointestinal side effects.

There is a greater need to provide more support to HIV/AIDS patients and to organize awareness programmes that can address the issue of stigma and discrimination since such awareness programmes could contribute to a better quality of life in people living with HIV/AIDS.

Several assays using different techniques have been used to assess either the HIV status with respect to disease progression verses prognosis of HIV infected patients. The quantitative reverse transcriptase polymerase chain reaction has been used to determine viral load in patient plasma, as this technique allows accurate quantification of plasma viral load in terms of the copies of HIV RNA cp/mℓ of plasma (Piatak *et al.,* 1993; Mulder *et al.,* 1994). Plasma viral load appears to be the best predictor of long-term clinical outcome, whereas $CD4^+$ cell counts predict clinical progression and survival in the shorter term (Galetto-Lacour *et al.,* 1996; Mellors *et al.,* 1997; Yerly *et al.,* 1998). Such data for ART-naïve patients have been used to make decisions about when to initiate ART (CASCADE Collaboration, AIDS 2004)

In HOPS (HIV Outpatient study), a large prospective cohort of HIV-infected patients receiving care in 9 USA based clinics were monitored, many participants achieved virologic suppression on their initial ART regimen but subsequently had virologic failure and underwent genotype resistance testing. These patients started ART at a $CD4^+$ count lower than 200 cells/mm^3. However, patients who started ART at higher $CD4^+$ count experienced the greatest durability of their initial regimen and had lower frequency of resistance mutations at virologic failure despite the fact that their infecting virus had the longest exposure to the selection pressures of a constant ARV regimen that can induce mutations (Uy *et al.*, 2009).

Regardless of $CD4^+$ cell count, the decision to initiate ART should always include consideration of any co-morbid conditions, the willingness and readiness of the patient to initiate therapy and the availability of resources.

As the global scale-up of ART reached nearly five million people in 2012 (Fox *et al.*, 2012), a growing body of evidence from large observational cohorts has demonstrated positive clinical, immunological and virologic outcomes being achieved throughout Sub-Saharan Africa (Ferradini *et al.*, 2006; Stringer *et al.*, 2006; Sanne *et al.*, 2009; Boulle *et al.*, 2010; May *et al.*, 2010; Nglazi *et al.*, 2011). Even though large numbers of new patients are still starting treatment in resource-limited settings, focus is shifting from the short-term stresses of treatment initiation to the long-term problems of managing a lifelong chronic disease.

A critical part of this shift is an emphasis on managing the growing number of public-sector patients who have already failed or will soon fail first-line therapy (Pujades-Rodriguez *et al.*, 2007; Palombi *et al.*, 2009; Keiser *et al.*, 2010; Keiser *et al.*, 2011; Fox *et al.*, 2012). Reports on individual cohorts of patients undergoing ART have reported on durability of first-line regimens, associations with confirmed virologic failure, and delays in switching to second-line, but there is limited understanding of factors predicting the requirement to switch after virologic failure, and how consistent treatment switching to second-line therapy takes place in patients eliciting virologic failure (Keiser *et al.*, 2011).

To limit the impact of virologic failure in any ART programme, the identification, and close monitoring of patients identified to be at risk of virologic failure and early correction of modifiable risk factors, is necessary.

Several clinical and socio-demographic factors have been associated with virologic failure in different settings (routine care vs. clinical trials), but without much consistency between settings. Furthermore, there is particularly little data on the extent of virologic failure in African military healthcare systems.

2.4 BACKGROUND OF PHIDISA PROJECT

Project Phidisa was a HIV/AIDS research collaboration between the South African Department of Defence (SA DoD) through the South African Military Health Service (SAMHS), the US Department of Health and Human Services, the National Institute of Health (NIH), and the US Department of Defence (US DoD). It was established in 2003 with the aim of studying HIV parameters in a sample of SANDF members and their dependents.

Project Phidisa extended over more than ten years and included participants enrolled in 3 clinical protocols. Beginning of January 2004, HIV–infected SANDF members and their dependents could be enrolled in a cohort study (Phidisa 1) or in a randomized trial, Phidisa II (The Phidisa II Writing Team for Project Phidisa; 2010). For Phidisa 1, the inclusion criteria were very broad, any HIV-positive individual in the SANDF or their registered family members could be enrolled. For Phidisa II, HIV-positive individuals could be randomized to one of four ART regimens if their $CD4^+$ count was <200 cells/mm³ or they had a prior AIDS diagnosis and they met set eligibility criteria: a serum liver transaminase level >5 times the upper limit of the normal range, haemoglobin level of 9 g/dℓ or higher in men or 8 g/dℓ or higher in women, a neutrophil count >500 cells/mm³, a platelet count >25,000 platelets/μℓ (Anglaret *et al*., 2012). Participants who were not enrolled in the trial received ART according to South African National Guidelines (National Department of Health South Africa, 2010).

In March 2008, after the clinical trial was completed, all participants in Phidisa 1 and Phidisa II were invited to participate in an amended observational study protocol (Phidisa 1a). Phidisa 1a was 'A prospective, observational cohort study of HIV infection (both treated and untreated) and risk-related co-infections in the South African National Defence Force. Combined antiretroviral therapy management under this observational study adhered to the South African standard of care; therefore the study findings would best reflect those of routine care seen in most community medical support services.

Phidisa 1a included participants who had already participated in either Phidisa 1 or Phidisa II who re-consented for inclusion in the new trial as well as new HIV positive participants that were enrolled. Like Phidisa 1, the inclusion criteria were very broad and any HIV-positive SANDF member or their family members could be enrolled. For ART-naïve participants starting in the Phidisa 1a trial, ART was initiated according to South African National Guidelines of 2010. This study excluded HIV negative individuals.

The study reported here was an observational retrospective analysis of six SAMHS sites that participated in the project Phidisa and included; 1 Military Hospital (Pretoria), 2 Military Hospital (Western Cape), 3 Military Hospital (Bloemfontein), Phalaborwa sickbay (Limpopo), Mtubatuba sickbay (Kwazulu-Natal) and Umthatha sickbay (Eastern Cape). The study was approved by the SANDF and NIH institutional review boards. Written informed consent was obtained from all participants.

TABLE I. LIST OF CURRENTLY AVAILABLE FDA-APPROVED ANTIRETROVIRAL DRUGS

Generic name (US)	Abbreviation	Brand
Nucleoside reverse transcriptase inhibitors (NRTIs)		
Abacavir	ABC	Ziagen
Didanosine	DDI	Videx
Emtricitabine	FTC	Emtriva
Lamivudine	3TC	Epivir
Stavudine	D4T	Zerit
Tenofovir	TDF	Viread
Zidovudine	AZT, ZDV	Retrovir
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)		
Delavirdine	DLV	Rescriptor
Efavirenz	EFV	Sustiva
Etravirine	ETR	Intelence
Nevirapine	NVP	Viramune
Nevirapine extended release	NVP XR	Viramune XR
Rilpivirine	RPV	Edurant
Protease inhibitors (PIs)		
Atazanavir	ATV	Reyataz
Darunavir	DRV	Prezista
Fosamprenavir	FPV	Lexiva
Indinavir	IDV	Crixivan
Lopinavir/ritonavir	LPV/r	Kaletra/ Aluvia
Nelfinavir	NFV	Viracept
Ritonavir	RTV, /r	Norvir
Saquinavir hard gel caps	SQV	Invirase
Tipranavir	TPV	Aptivus
Integrase inhibitors (INIs)		
Raltegravir	RAL	Isentress
CCR5 antagonist		
Maraviroc	MVC	Selzentry
Fusion inhibitor		
Enfuvirtide (T20)	ENF	Fuzeon

ARV= antiretroviral; **CCR5**=CC chemokine receptor 5; /**r** indicates boosted by ritonavir.

Many ARVs may have multiple brand names, depending upon the manufacturing company and location in which they are manufactured, e.g. Efavirenz, is known as Stocrin in Europe; Fosamprenavir is known as Telzir in Europe; Lopinavir/ritonavir is also known as Aluvia in the developing world; and Maraviroc, also known as Elsentri in Europe.

2.5 ASSOCIATION BETWEEN INCREASE VIRAL LOAD, LOW CD4+ COUNT AND PROGRESSION OF DISEASE

HIV is a retrovirus that causes AIDS. The retrovirus primarily attacks the immune system, making the body extremely vulnerable to opportunistic infections due to the immuno-compromised status. HIV can infect different kinds of cells in the human body but the primary target is the CD4 positive T-lymphocytes that are cells central to regulating the immune system. $CD4^+$ T-lymphocytes help coordinate the immune system response to disease and infection and play a central role in targeted antibody production by the B lymphocytes. The HIV binds to the CCR5 and CXCR4 combination of receptors on $CD4^+$ cells and enters these cells, where it starts replicating.

Several studies have shown that people with high viral load are more likely to progress to AIDS faster than people with lower levels of the virus. In adults, progression to AIDS is diagnosed when any condition listed in WHO Stage 4, as shown in Table 2 below is diagnosed and/or patients progress to AIDS when their $CD4^+$ cell counts drop below 200 cells/mm³ blood. Disease progression (the extent to which an HIV positive person shows opportunistic diseases and infections) depends on the viral load as well as the $CD4^+$ cell count in the blood. Patients with lower $CD4^+$ cell count when first infected with HIV are likely to experience faster disease progression, according to an international study by Lodi *et al.,* (2013). The progression to the final phase of AIDS and death will be accelerated when a high viral load is present.

Treatment of patients with advanced HIV disease is becoming increasingly complex with issues of adverse drug effects and requirement for a combination of medications playing a significant role. In a study by Miller *et al.,* (2002), the researcher's reported that in patients with $CD4^+$ cell counts of <50 cells/mm³, disease progression and death were predictable from the latest $CD4^+$ cell count, haemoglobin measurements and BMI while receiving PCP prophylaxis and the number of drugs in the treatment regimen. AIDS status was a predictor of death only, and plasma HIV-1 RNA was a predictor for clinical progression only. A similar analysis of the EuroSIDA cohort indicated that disease progression in patients with low $CD4^+$ cell count was inversely related to intensity of treatment. Similar studies (Cozzi *et al.,* 1998; Lundgren *et al.,* 2002) also found $CD4^+$ cell count to be a better predictor of clinical outcome of AIDS in patients with advanced HIV disease.

Starting ARV's early in asymptomatic HIV infected patients has health benefits. This statement is supported by results in a recent randomized study by INSIGHT START 2015 involving previous untreated HIV-positive adults with a $CD4^+$ cell count of more than 500 cells/mm³. While testing initiation of antiretroviral therapy in early asymptomatic HIV infection the investigators' findings suggest that immediate initiation of antiretroviral therapy

was superior to deferral of therapy until the $CD4^+$ cell count declined to 350 cells/mm³. Moreover, one recently published study found that the efficacy of earlier ART initiation led to lower rates of severe illness than when ART was deferred until $CD4^+$ cell counts dropped below 500 cells/mm³ as proven by the TEMPRAND ANRS 1213 Study Group 2015 'A trial of early antiretroviral and Isoniazid preventative therapy in Africa'.

TABLE 2: WHO CLINICAL STAGE 4 CHARACTERISTICS

HIV wasting syndrome
Pneumocystis pneumonia
Recurrent severe bacterial pneumonia
Chronic herpes simplex infection (orolabial, genital, anorectal or visceral at any site)
Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)
Extra pulmonary tuberculosis
Kaposi sarcoma
Cytomegalovirus infection (retinitis or infection of other organs)
Central nervous system toxoplasmosis
HIV encephalopathy
Extra pulmonary cryptococcosis including meningitis
Disseminated non-tuberculous mycobacteria infection
Progressive multifocal leukoencephalopathy
Chronic cryptosporidiosis
Chronic isosporiasis
Disseminated mycosis (extra pulmonary histoplasmosis, coccidiomycosis)
Recurrent septicaemia (including non-typhoidal Salmonella)
Lymphoma (cerebral or B cell non-Hodgkins)
Invasive cervical carcinoma
Atypical disseminated leishmaniasis
Symptomatic HIV-associated nephropathy or HIV-associated cardiomyopathy

2.5.1 EFFECT OF OPPORTUNISTIC ILLNESS ON HIV RNA VIRAL LOAD AND $CD4^+$ CELL COUNT

Opportunistic infections (OIs) continue to cause morbidity and mortality in patients with human immunodeficiency virus (HIV)-1 infection throughout the world. Combination antiretroviral therapy (cART) has reduced the incidence of OIs for patients with access to care. However, some patients in the developed and developing world do not have access to care and suffer from OIs. Other patients do not have sustained response to antiretroviral agents for several possible reasons, including poor compliance to the treatment regimen, drug toxicities, drug interactions, or initial acquisition of a drug-resistant strain of HIV-1 (Benson *et al.*, 2005) OIs will therefore continue to cause substantial morbidity and mortality in patients with HIV-1 infection. The therapy of OIs has changed substantially during the AIDS epidemic as more information about efficacy, toxicity and drug interactions of the drugs to

treat and prevent OIs and new management strategies have evolved. New drugs have also become available that play important roles in the therapeutic armamentarium.

Opportunistic illness such as malaria, tuberculosis, pneumonia and herpes simplex virus have been associated with elevated HIV RNA viral load and a decrease in $CD4^+$ cell count among HIV infected people not taking ART (Bush *et al.,* 1996; Donovan *et al.,* 1996; Goletti *et al.,* 1996; Mole *et al* 1997; Toossi *et al* 2001). Occurrence of opportunistic infections has also been shown to be predictive of increased risk of mortality (Chaisson *et al* 1998; Osmond *et al.,* 1999).

A number of studies have previously demonstrated increases in HIV RNA viral load following OIs among HIV-positive individuals who were not on ART (Fang *et al*., 1995; Mofenson *et al.,* 1999). In a study done by Ekwau *et al.,* (2013), association was found between having an opportunistic illness and the short-and long-term effects on HIV viral load and $CD4^+$ cell count among people taking ART. Participants who had an OI following an assessment in which their VL was undetectable had four times the odds of having a detectable VL within the three month follow-up assessment compared to when there was no episode of OI. Moreover, the same participants had a significant decline in $CD4^+$ cell count compared to when there was no episode of OI.

It has also been shown, that OI's involving tissue macrophages results in an increase in the HIV VL (Orenstein *et al.,* 1997). In addition to being a predictor of disease progression, HIV VL is also a strong determinant of both sexual and mother-to-child transmission of HIV (Fang 1995; Mofenson *et al.,* 1999). Occurrence of OI may also increase the risk of HIV transmission through their increasing effects on HIV VL highlighting that efforts should be made to prevent OIs, even among patient on ART who have attained suppressed viral loads.

2.6 RISKS AND BENEFITS OF COMPLIANCE TO ANTIRETROVIRAL TREATMENT

Compliance to ART has been correlated strongly with HIV viral suppression, reduced rates of resistance, an increase in survival time and improved quality of life (Quinn *et al.,* 2000; Cohen *et al.,* 2011). Compliance to therapy is essential to achieve continuous viral suppression and prevent the emergence of drug-resistance in the virus due to genetic mutations. In the past few years, ART regimens have been greatly simplified by the introduction of fixed dose combination therapy. Although newer regimens include more fixed-dose combination products and offer once-daily dosing, compliance still remains a challenge. Non-compliance of treatment regimen before the introduction of fixed dose combination is the most common cause of treatment failure and the development of resistance to ARVs (Turner *et al.,* 2002).

When patients exhibit low or non-adherence to ART it may lead to emergence of drug resistance and ultimate treatment failure. Antiretroviral regimen potency and treatment compliance both play a role in the development of antiretroviral medication resistance (Gardner *et al.,* 2009). As the goal of HIV therapy is to achieve sustained virologic suppression, near-perfect dosing compliance is required (Parienti *et al.,* 2008). As more people are given access to ART, there is a greater need to find simple, efficient and replicable

ways of maintaining and assessing near perfect levels of compliance to ensure sustained virologic suppression and to avoid developing virologic failure due to drug combination resistance.

2.6.1 FACTORS ASSOCIATED WITH NON-COMPLIANCE

Compliance remains a challenging and complicated topic. Compliance to ART can be influenced by characteristics of the patient, clinical setting, the provider/patient relationship the drug regimen and the adverse side effects of the ART. To assure compliance, it is significant that the patient be counselled and that they comprehend the implications of HIV related symptoms, disease progression and the goal of treatment therapy and the possible side effects that can be experienced.

The following factors have been associated with poor ART compliance, (Bhat *et al.*, 2010)

- Stigma
- Pill burden
- Medication-related issues e.g. Adverse drug effects
- Missed visits i.e. Irregular clinic attendance for medication refills due to financial constraints, long travelling distances to the clinic, work commitments, discouraged from returning to the clinic because of long delays Psychosocial issues (depression, stressful life events)
- Difficulty in taking medication or the inconvenience
- Religious belief
- Lack of family support
- Active substance abuse e.g. excessive alcohol intake, use of traditional medication
- Low literacy and numeracy
- Younger age

2.6.2 MEASUREMENTS OF COMPLIANCE

Measuring medication compliance in clinical settings has not always been easy because common methods such as pill counts are not objective and relevant enough. There is no gold standard for the assessment of compliance (Quinn *et al.*, 2000), but there are many tools and strategies to choose from. Although self-report of compliance predictably overestimates compliance by as much as 20% (Cain *et al.*, 2011), this measure is associated with viral load responses. More accurate measures of compliance such as drug-level monitoring are expensive, can be inconvenient and difficult to implement in poor resource settings.

One study found that asking patients to rate their compliance on a six-point scale during 1 month was more accurate than asking them about the frequency of missed doses or to estimate the percentage of doses taken during the previous 3 to 7 days (Katahata *et al.*, 2009). Pharmacy records, medication profile, calendar and pill counts can also be used in addition to simply asking the patient about compliance. Other methods of assessing compliance include the use of electronic measurement devices (e.g. bottle caps, dispensing systems). However, these methods are generally not feasible in limited resource settings.

2.6.3 INTERVENTIONS TO IMPROVE COMPLIANCE

Before treatment initiation, a multidisciplinary health care team approach is critical, the clinician, the nurse, the social worker and the pharmacist should all be involved in assessing patient's readiness to start ARVs. The team should endeavour to establish a trusting relationship with the patient, maintain good communication, assess and simplify the regimen, if possible and identify potential barriers to compliance before starting ART. Effective compliance intervention also includes, Directly Observed Therapy (DOT), which has been shown to be effective in provision of ART to active recreational drug users (O'Brien *et al.,* 1996). Other effective methods to encourage compliance are to provide compliance tools such as written calendar of medications, dated pill boxes and encourage the use of alarms, pagers and other mechanical aids as reminders for compliance. An increasing number of interventions has demonstrated efficacy in improving compliance to ART. A meta-analysis of 19 randomized controlled trials of ART compliance interventions found that compliance intervention participants were 1.5 times likely to report 95% compliance and 1.25 times as likely to achieve an undetectable viral load as participants in comparison groups (Geng *et al.,* 2012)

Compliance tool provision does influence the degree of ART compliance. The level of compliance is greatly improved if compliance tools are provided to patients taking ART. The results of the above study provide robust evidence that may improve compliance among patients initiating ART in a resource-limited setting. To prolong patient's life, prevent disease progression, and improve the quality of life, it is critical that patients are provided with as much education and support as possible, given the need for maximal long term compliance to currently available treatment. However, additional studies will be critical for understanding the true benefits and best implementation strategies in ART compliance in developing countries.

2.7 THE EFFECT OF ALCOHOL AND HERBAL REMEDIES ON VIROLOGIC FAILURE

Prohibiting recreational drug and alcohol use alone or concurrently by HIV-infected individuals may have different independent and combination effects on HIV treatment and disease progression outcomes. Importantly, the different effects on HIV treatment outcomes could have implications for HIV treatment and interventions. Of note, several studies examining the role of alcohol on HIV disease progression have used varying definitions of alcohol use.

The National Institutes on Alcohol Abuse and Alcoholism (NIAAA) defines hazardous alcohol use as > 7 drinks per week or > 3 drinks per occasion for woman and > 14 drinks per week or > 4 drinks per occasion in men.

Combining herbal therapies with ART could result in treatment failure. It is recommended that people with HIV tell their providers about all the medications, vitamins, herbs and supplements they are taking concurrently with their ART. This is critical, because when a person starts an HIV regimen it is important to understand the source of any potential side effects and to guard against drug interactions.

2.7.1 ALCOHOL USE AS A RISK FACTOR FOR NON-COMPLIANCE AND LACK OF SUPPRESSION IN HIV INFECTION

Alcohol use is common among HIV-infected patients and is associated with decreased compliance to ART (Berg *et al.,* 2004). Indeed few studies have examined the relationship between alcohol use and viral suppression, and those studies that have evaluated this association have included only small samples, or have combined drugs and alcohol into a single variable, rather than evaluating them independently (Fabris *et al.,* 2000; Miguez *et al.,* 2003; Palepu *et al.,* 2003).

A study conducted by Chander *et al.,* (2006), similar to a published study of compliance among an HIV-infected sample with a history of alcohol dependence, found that both hazardous and moderate levels of alcohol use were associated with decreased compliance with subsequent poor viral suppression (Samet *et al.,* 2004). They also found that hazardous alcohol use was associated with lower odds of compliance than in moderate alcohol users. Hazardous alcohol use was defined as per NIAAA guidelines and moderate alcohol use was defined as any alcohol use at less than hazardous levels.

However, a more recent study (Schneider, 2014) argues that consumption of alcohol appears unrelated to $CD4^+$ levels or treatment failure rates, although heavy drinkers are more likely to interrupt their ART regimen.

2.7.2 IMPACT OF HERBAL REMEDIES AND VIROLOGIC FAILURE

Use of herbal remedies among HIV-infected individuals in Africa increased in the past decade, mainly due to traditional beliefs and at times inconsistent access to antiretroviral drugs. Despite the availability and accessibility of ART, the use of herbal remedies remains high. Concurrent use of herbal medications with ART can either increase or decrease the availability of the active form of the individual drugs used in the ART.

According to a single case report (Wiegman *et al.,* 2009), the researchers reported a virologic failure in a 47 year old HIV–infected patient who had received ART therapy for 10 years. The patient was using EFV, FTC and TDF for 2years. The patient showed excellent treatment compliance. At the end of 2007, a virologic failure developed, and a K103N and M184V mutation in the reverse transcriptase gene was demonstrated. After in-depth questioning about concomitant drugs and supplements he was taking during his time on Efavirenz, the patient appeared to be using *Ginkgo biloba* for some months. No other co-medication was used during that time frame.

Plasma EFV concentrations measurements were conducted on several plasma samples dating back 2 years. Concentration of EFV decreased over time, coinciding with an increase in viral

load (See Table 3 below). The table below indicates that the man had a viral suppression when he first began his ART regimen in 2006, but then significantly changed after he started taking *ginkgo biloba* in 2007. The patient was successfully switched to alternative antiretroviral therapy later on in March 2008.

TABLE 3. HIV-RNA COPIES MEASURED IN PLASMA AND EFAVIRENZ PLASMA CONCENTRATION OF A PATIENT TAKING *GINGKO BILOBA* EXTRACT. (Wiegman *et al.*, 2009)

Sample Date	**HIV-1RNA (cp/mℓ)**	**EFV plasma (concentration mg/mℓ)**
18 December 2006	<50	1.26
14 February 2007	<50	0.92
12 December 2007	618	0.78
8 February 2008	1780	0.48

Ginkgo biloba, a herbal drug, is commonly used because of its assumed beneficial effects on concentration, memory, dementia and depressive disorders. *Ginkgo biloba* extract (GBE) is made of ginkgo leaves and is usually standardized to contain flavonoids and terpenoids (Zhao *et al.*, 2007). Examining the properties of the constituents of GBE, the effects on cytochrome P450 metabolic routes can be anticipated as the flavonoids can inhibit P-glycoprotein (P-gp), and CYP3A4 (Zhou *et al.*, 2004; Zhao *et al.*, 2007). Terpenoids can induce P-gp, pregame X receptor (PXR), multidrug-resistance 1 (MDR-1) and CYP3A4. *Ginkgo biloba* has been confirmed to interact with a number of drugs that are metabolized through the CYP3A4/5 isoenzyme (e.g. trazodone, warfarin, aspirin, ibuprofen and omeprazole) (Hu *et al.*, 2005)

Therefore, based on the above, (Wiegman *et al*., 2009) concluded that an intake of GBE can decrease human plasma EFV levels which may result in virologic failure.

Herbal medications are commonly used by AIDS patients on ART. Some patients use the herbal medication to supplement ARV's, whereas some patients use herbal and traditional medicines due to high cost of ARV's and lack of accessibility. Taking ARV's with herbal remedies may lower the serum levels of specific ARV's leading to decrease effectiveness of the drugs. Efforts are required to determine the safety, efficacy and pharmacological profile of the use of many herbal remedies used in Africa. Moreover, a better understanding of what remedies are in use in HIV and to educate those providing alternative medical services against unsafe practices are highly recommended. Further pharmacological investigations are needed to identify the potential risks, benefits, and interactions or non-interaction associated with concomitant ARV drug and herbal use.

It will also be prudent for future research to explore the effect of herbal use among AIDS patients receiving ART in terms of morbidity and mortality patterns.

However, until future research is available, it is recommended that health-care workers be vigilant about cautioning HIV positive patients on ART who also use herbal remedies that this practice may reduce the treatment effectiveness of ARV drugs.

2.8 EARLY AND LATE VIROLOGIC FAILURE BY VIRAL THRESHOLD

South African and WHO guidelines suggest a pragmatic approach to defining virologic failure which relies on confirmation of viraemia after attempts to optimize compliance. Plasma viral load monitoring, the gold standard used in high-income countries for diagnosing virologic failure, is not available in many resource-limited settings such as the rural clinics in South Africa. Routine VL monitoring in resource-limited settings requires significant infrastructure and expertise and remains prohibitively expensive in most settings. Colebunders and colleagues for example, (Badri *et al*., 2008) proposed an algorithm based on clinical and treatment history and inexpensive laboratory indices such as haemoglobin level and total lymphocyte count. Rajasekaran *et al.*, (2007) came to the same conclusion that monitoring of haemoglobin concentration, absolute lymphocyte count, and body weight during follow-up emerged as inexpensive predictors of treatment failure in a resource-poor setting.

The WHO 2010 guideline revisions recommended that when viral load monitoring is available, "A persistent viral load of >5000 cp/mℓ confirms treatment failure" (Aldous *et al.*, 2009). However this study used a different confirmatory threshold from WHO guidelines. Three subsequent guideline changes in South Africa took effect in April 2010 (Plazy *et al.*, 2010). The recommended NRTI backbone was changed from the stavudine plus lamivudine to tenofovir plus lamivudine combination, while routine viral testing frequently was dropped from six-monthly to annually beyond the first year on the new ART. The threshold for confirming virologic failure was lowered from 5000 to 1000 cp/mℓ with the confirmatory test now required within three months of the initial elevation. This current study used the latter confirmatory viral load threshold as the definition of virologic failure.

2.8.1 DEFINITION OF EARLY AND LATE VIROLOGIC FAILURE

The definitions of early and late virologic failure in this study are:

Early VF is defined as: plasma HIV-RNA ≥1000 cp/mℓ at two consecutive visits after a minimum of six months of ART therapy (first VL at least six months after cART initiation), or failure to suppress VL to <400 cp/mℓ by twelve months (no VL <400 cp/mℓ at any visit between six and twelve months, inclusive), or single VL ≥ 1000 cp/mℓ after at least 6 months of ART therapy followed by death where no second VL is available.

Late VF is defined as: plasma HIV-RNA ≥ 1000 cp/mℓ at two consecutive occasions (first VL at least twelve months after cART initiation) or single VL ≥ 1000 cp/mℓ after at least 12 months ART treatment and death and where no second VL is available and individual did not experienced early VF as defined above.

The approach has been shown to successfully select the patients with high levels of viral load warranting switching to second-line therapy, but low levels of cross-resistance between first and second-line regimens. As evidence by this study however, the exact interpretation of this approach is varied and can profoundly impact the number of patients who meet the definition of virologic failure and require switching to the available second-line therapy.

2.9 ASSOCIATION BETWEEN VIROLOGIC FAILURE AND DRUG RESISTANCE

Viral resistance to antiretroviral treatment remains a limitation to successful HIV therapy. The combination therapy used in ART options have proven remarkably effective in controlling the progression of HIV disease and prolonging survival, but these benefits can be compromised by the rapid development of drug resistance by the virus. Moreover the number of fully active ARVs available diminishes with each successive treatment failure. Therefore, a salvage regimen is likely to be more complicated, in that it may require multiple ARVs with partial residual activity towards compromising genetic resistance, to attain complete virological suppression.

About 50% of patients receiving antiretroviral therapy in the United States are estimated to harbour viruses that express resistance to at least one of the available antiretroviral drugs (Ceccherini-Silberstein *et al*., 2010). Therefore careful measures should be in place to control the development of resistance to minimize the risk of virological failure. The principles of developing drug resistance are the same in all populations, but approaches to drug-resistance testing and regimen switching differs between low-, middle- and high-income countries due to the availability of diagnostic tests for resistance and available drugs to use in the ARTs. As a result, clinicians in developing countries must often treat challenging cases of HIV drug resistance with fewer ARV options than those available to their peers in wealthy parts of the world.

The efficacy of an ARV treatment regimen depends on the compliance to maintain high enough concentrations of the drugs to ensure activity of the individual ARV drugs in the combination therapy and a number of HIV-1 mutations present in the virus for the development of resistance to each ARV, the genetic barrier to resistance. Increased viral replication while on ART therapy leads to evolutionary selection of resistance mutations toward the antiretroviral drugs in the therapy, and potentially cross-resistance to other agents in the same drug class, which decreases the likelihood of response to subsequent ART. On the other hand, determining whether an HIV-positive patient carries an ART resistant virus requires complex genetic testing to determine whether that patient's virus changed or mutated in response to the ART treatment. A multi-step genotyping process allows researchers to determine the emergence of resistant virus.

During the few past years a number of studies designed to evaluate the clinical benefit of drug resistance testing were performed (Reller *et al.*, 2009). The majority of these have evaluated the virologic benefits of ART selected through drug resistance testing compared with treatment chosen without drug resistance testing. In most recent studies, clinical

decision-making guided by genotypic data proved to be more virologically and immunologically beneficial.

HIV drug resistant testing has been considered an emerging asset to modern HIV management. Despite the increased number of antiretroviral agents currently available, as depicted in Table 1 above, virologic failure remains a significant problem. Hence the optimal time to switch ART ensures sustained virologic suppression thereby preventing poor clinical outcomes.

2.9.1 DRUG RESISTANCE TESTING IN CLINICAL PRACTICE

Emergence of drug resistance by the virus is the most common reason for treatment failure. Resistance testing can improve treatment outcomes for HIV infected individuals. Insufficient treatment regimen compliance, drug adverse side-effects, or drug-drug interactions can lead to suboptimum drug concentrations, resulting in viral rebound. Viral resistance has been described for every antiretroviral drug and therefore poses a serious clinical as well as public-health problem (Deeks, 2003; Hirsch *et al.,* 2008; Tang, 2012). The European guidelines recommend that drug-resistance testing should be performed when a patient is first diagnosed with HIV treatment failure. In newly diagnosed patients with HIV, a delay in drug resistance testing would increase the risk that a transmitted drug-resistance mutation would decrease in its proportion relative to the fit wild-type revertants and no longer be detected by standard genotypic resistance testing, which cannot detect variants present at levels below 20% of the total plasma virus population.

Drug resistance testing is design to identify gene mutations or viral growth characteristics that suggest reduced drug susceptibility of the major viral species. Several commercially marketed and/or laboratory-developed tests (LDT) are available to detect known resistance-associated mutations (RAMs) in HIV-1, through genotyping. These genotyping tests mainly comprise polymerase chain reaction (PCR)-amplification and population nucleotide sequencing of a large part of the protease, reverse transcriptase and integrase genes. A PCR step amplifies a specific region of the HIV genome and a specific mutation detection methodology that distinguishes each type of genotyping assays.

The objective of ART is viral suppression with immunologic stability and patient clinical well-being (Yeni et *al.,* 2002). Ideally, this is accomplished through the use of effective combination drug therapy to suppress the viral load to undetectable levels. However later fixed dose combination shows a higher rate of effectiveness (Barlow-Mosha *et al*., 2012). A general concern is that drug-resistant virus may be present even before initiation of ART. Studies of treatment-naïve patients have identified the presence of single-drug and multidrug-resistant HIV strains (Puig *et al.,* 2000; Salomon H *et al*., 2000; Grant *et al*., 2002). It has been demonstrated that drug-resistant virus is transmitted from one adult to another despite ART treatment (Hecht *et al.,* 1998; Yerly *et al.,* 1999) and occasionally transmitted vertically from mother to child.

Although genetic drug resistance testing is available in some settings, it can be very costly and in many poor resource settings, genotype tests are not available at all. As such it is important to consider all other potential causes of virologic failure to avoid hasty and possibly unnecessary drug changes.

HIV-1 drug resistance testing at the time of virologic failure is a standard of care in developed countries but is not widely available in resource-limited settings. There is currently only a partial understanding of patterns of HIV-1 drug resistance that occur during ART failure in developing countries, where VL testing is uncommon and treatment failure is often based on clinical and immunological indicators only. It is therefore recommended that more frequent VL monitoring should be done to reduce RTI mutations and also to reduce the duration of exposure to a failing regimen. A summary of clinical situations in which resistance testing is recommended is depicted in Table 4 below.

TABLE 4: SUMMARY OF CLINICAL SITUATIONS IN WHICH RESISTANCE TESTING IS RECOMMENDED

Clinical setting	**Comments**
Before initiation of therapy Primary (acute and early infection)	Resistance testing is recommended. Initial therapy may be altered based on resistance test results
First evaluation of chronic HIV-1 infection	Resistance testing is recommended, including for patients for whom therapy is delayed, because plasma wild-type isolates may replace drug-resistant virus with time in the absence of treatment.
Treatment initiation for chronic HIV-1 infection	Resistance testing is recommended because of a rising prevalence of baseline HIV-1 drug resistance in untreated patients with chronic infection, unless pre-existing data or stored samples for testing are available.
In antiretroviral-treated patients treatment failure	Resistance testing is recommended. The decision to change therapy should integrate treatment history, new and prior resistance results, and evaluation of compliance and possible drug interactions.
In specific settings Pregnancy	Resistance testing is recommended before initiation of therapy to effectively treat the mother and prevent mother-to child transmission.

2.9.2 TYPES OF DRUG RESISTANCE MUTATIONS

HIV-1 drug resistance can be acquired (developing in a person receiving treatment) or transmitted (occurring because a virus with drug-resistance mutations was transmitted to a virus naïve person). Although both acquired and transmitted HIV-1 drug resistance are public health concerns, transmitted resistance has the potential to more rapidly resist the effectiveness of first-line antiretroviral therapy at the population level. Persons with transmitted drug resistance begin antiretroviral therapy with a lower genetic barrier to resistance, a higher risk of virologic failure, and a higher risk of developing resistance even to those drugs in their regimen that were potentially active (Grant *et al*., 2002; Little *et al*., 2002; Daar *et al*., 2005; Markowitz, 2005).

2.9.2.1 ACQUIRED DRUG RESISTANCE MUTATIONS

Resistance is the consequence of mutations that emerge in the viral proteins targeted by antiretroviral agents (Clavel *et al*., 2004). Patients who acquire or are primarily infected with drug resistant HIV-1 viruses have fewer treatment options and are at increased risk of morbidity and mortality, particularly in developing countries where available drug choices for ART are limited (Hogg *et al*., 2006; Palella *et al.,* 2009). Currently, there are 24 ARV drugs in six classes licensed for the treatment of HIV-1; NRTI's, NNRTI's, PIs, Fusion inhibitors, CC chemokine receptor 5 (CCR5) antagonists and Integrase inhibitors (see Table 1 above). Due to a recent expansion in the number of ARVs and ARV classes, virological suppression has become achievable in most patients in whom numerous prior ARV regimens have failed.

New surveillance data from the U.S. Centre for Disease Control and Prevention (CDC) suggest that HIV resistance to NNRTI was somewhat more common among those with recent infection at 7.6%. The U.S. Variant, Atypical, and Resistant Surveillance (VARHS) system, in which HIV specimens from newly diagnosed individuals are tested for drug-resistance mutations, was established by the CDC to provide the clearest picture of the scope and type of resistance in the United States.

The analysis of the collected data was reported at CROI included a total of 10338 resistance profiles from eight U.S. regions; this consisted of 2339 profiles from individuals with recent infections and 7999 profiles from individuals with confirmed long-standing HIV infection.

2.9.2.2 TRANSMITTED DRUG RESISTANCE MUTATIONS

Drug-resistant HIV-1 is transmissible and can be detected in up to 20% of newly infected individuals in countries with broad access to resistance monitoring and all the antiretrovirals (Hoare *et al*., 2010). The prevalence of viral drug resistance in the untreated population remains low in regions with poor access to treatment (Lurie *et al.,* 2003). Transmitted HIV-1 drug resistance can compromise initial antiretroviral therapy therefore resistance mutation detection is important for patient ART selection. The absence of drug-associated selection pressure in treatment-naïve persons can cause drug-resistant viruses to decline to levels undetectable by conventional bulk sequencing techniques (Johnson *et al.,* 2008).

2.9.2.3 MOTHER-TO-CHILD-TRANSMISSION

Approaches to intervention to reduce the risk of MTCT focus mainly on antiretroviral prophylaxis during pregnancy, labour and in the early neonatal period, but in some settings

also on delivery procedures and avoidance of breastfeeding (Newell 2006). Short-term antiretroviral-based interventions are effective in prevention of mother-to-child transmission. However, these relatively short term interventions could result in drug resistant viral variants in the mother, baby, or both (Toni *et al.,* 2005).

Approximately half the women who received one dose of Nevirapine to prevent mother-to-child transmission were found to harbour viruses resistant to non-nucleoside reverse transcriptase inhibitors (Eshleman *et al.,* 2002; Flys *et al.,* 2006). These resistant viruses replicate efficiently (Lee *et al.,* 2005; Simon *et al.,* 2010)) and minor resistant populations are still present long after the intervention could possibly decrease the effectiveness of subsequent NNRTI-based treatment regimens (Palmer *et al.,* 2006). The combination of short-course zidovudine, lamivudine, and Nevirapine prevents peripartum transmission while reducing the risk of Nevirapine resistant viruses (Chaix *et al.,* 2006).

Prevention of mother-to-child transmission has seen advances in both developed countries and resource-constrained settings (Luzuriaga *et al.,* 2005; McIntyre, 2006). Concerns about drug-resistant viral strains have led to several trials with combination treatments to reduce transmission during the intra-partum period (Cressey *et al.,* 2005; Chaix *et al.,* 2006; McIntyre 2006).

The benefits of breast-feeding have been well-described in the medical literature (Cesar *et al.,* 1999; WHO, 2000; Kramer *et al.,* 2001). These benefits including providing optimal nutrition, preventing common childhood illnesses and improving child spacing are of particular importance in resource poor countries such as sub-Saharan Africa. For this reason, the possibility of HIV transmission through breast milk poses a dilemma, particularly in conditions where breastfeeding is a strong cultural norm, and where large numbers of women are infected with HIV. HIV-1 can be transmitted in utero, peri-partum and post-natally via breastfeeding, (John-Steward, 2007). As such, replacement feeding is recommended in many settings. Access to clean running water in resource-constrained settings may preclude the effective and safe use of formula feeding.

Moreover, the risk of HIV transmission through breast milk must be weighed against the risk of infant malnutrition, infections and mortality posed by formula feeding. The World Health Organization recommends an "either-or" approach based on the available resources. HIV-positive mothers with infants who are not known to be HIV-positive should either:

- Avoid breastfeeding altogether, or
- Breastfeed with accompanying prophylactic antiretroviral therapy to lower the risk of HIV transmission

Exclusive breastfeeding with abrupt weaning is one option for reducing transmission. (Rollins *et al.,* 2004)

A significant factor that can be a burden in a resource-limited setting is affordability in purchasing the feeding formula. Given the wide-spread poverty in this setting, infant formula is not commonly given to infants. It is only in rare cases – when mothers have the economic means to purchase it – that infant formula is used. In a study done by Abiyona *et al.,* (2006),

on infants of HIV-infected mothers, the majority of the study respondents perceived infant formula to be preferable to exclusive breastfeeding because of the risk of contracting HIV through breastfeeding. They explained that the mother should choose infant formula to reduce the chances of her infant contracting the disease. In addition to concerns about transmitting the virus, participants also thought that infected mothers may be too sick to be able to breast feed exclusively.

2.9.2.4 SEXUAL TRANSMISSION

HIV is most easily transmitted through blood and body fluids like semen and vaginal fluids. HIV can enter the body via contact with the blood stream or by passing through delicate mucous membrane, such as inside the vagina, rectum or urethra. As HIV is most common in the blood and body fluids like semen and vaginal fluids, blood or bodily fluids from an infected person introduced into an uninfected person can result in an HIV infection.

Reduction of heterosexual transmission is crucial for control of the epidemic in many parts of the world (Chan, 2005). Prevention is achieved through reduction in the number of discordant sexual acts or reduction of the probability of HIV-1 transmission in discordant sexual acts. The first can be achieved through abstinence and sex between concordantly seronegative individuals. Abstinence and lifelong monogamous relationships might not be feasible solutions for many people and therefore several interventions aimed at lowering the risk of transmission per discordant sexual act are in the process of clinical testing. Male and female condoms provide a proven and affordable prevention option (De Vincenzi 1994; Weller *et al.*, 2002) In combination, these options are also more commonly referred to as the ABC (abstinence, be faithful, condom use) approach.

Other biomedical prevention interventions include male circumcision, antiretroviral for prevention (e.g. pre-exposure or post-exposure), chemo-prophylactic treatment of herpes simplex virus-2 (HSV-2), microbicides, and vaccines. In a study done by Siegfried *et al.*, (2005), circumcised men appear less likely to acquire human immunodeficiency virus (HIV) and this study has contributed to the recent ground swell of support for considering male circumcision as a strategy for preventing sexually acquired infection.

The Orange Farm study was one of three independent Randomized Control Trials conducted in South Africa, Uganda and Kenya to determine whether circumcision reduced the risk of female-to-male transfer of HIV infection during penetrative heterosexual sex. The results showed that the intervention significantly reduced the incidence of HIV infection in the circumcised study group compared with the controls, by 60% in South Africa, 53% in Kenya and 51% in Uganda (Nyayiyana, 2011). The published conclusion of the South African study is more circumspect, declaring simply that ‘Male circumcision provides a degree of protection against acquiring HIV infection, equivalent to what a vaccine of high efficacy would have achieved. Male circumcision may provide an important way of reducing the spread of HIV infection in sub-Saharan Africa.’ The comparison to a vaccine has been contested, but the constrained demeanour is probably closer to reality.

The findings from the South African trial show a 60% protective effect of male circumcision (Auvert *et al.,* 2005). The possible mechanism relates to the fact that the foreskin has apocrine glands that secrete lysozymes but also Langerhans cells expressing CD4 and other receptors (Soto-Ramirez *et al.,* 1996; Svabo *et al.,* 2000). These skin specific dendritic cells can take up virus and are believed to play a part in transport of the virus to susceptible $CD4^+$ T cells. Immunofluorescence studies of foreskin mucosa suggest that these tissues might be more susceptible to HIV-1 infection than cervical mucosa (Halperin *et al.,* 2002). Findings from this proof-of-concept trial in South Africa were at a later stage compared with evidence from the two trials in Kenya and Uganda. Clinical trials conducted in Kenya and Uganda also show similar results compared with findings conducted in South Africa, (Jayathunge *et al*., 2014). The trials demonstrated that male circumcision has an efficacy of around 60% in preventing heterosexual HIV acquisition in men. These findings confirmed earlier observational and ecological studies and prompted the World Health Organization (WHO) recommendations that male circumcision be considered an important component of a comprehensive HIV prevention packages.

Gender disparities lie at the centre of women's vulnerability. Researchers think that most HIV transmission in women happens through the vagina, the cervix and, possibly, the uterus. Moreover, many researchers at first thought that the chance of acquiring HIV sexually through the female genital tract was quite low (Coombs *et al.,* 2003). However, many of the studies did not account for various biological and social risk factors that can make a woman more susceptible to HIV. This means that the probability of sexually transmitting and acquiring HIV in the "real world" may be a lot higher than has been estimated.

Prevention options need to be provided that can be used by women independently of their male sexual partner's knowledge or consent (Duffy, 2005). Notwithstanding that redressing these disparities are a long-term challenge, several preventive interventions can be implemented in the interim on the basis of our incomplete understanding at a biological level of HIV-1 risk for women. For example, there seems to be a correlation between levels of sexual hormones (e.g. progesterone) and transmission risk (Gray *et al.,* 2005) Women with low levels of the hormone oestrogen may be at increased risk for transmission of HIV because low oestrogen levels directly affect the vaginal wall, making it thinner, thereby allowing HIV to easily pass through the vaginal wall (Coombs *et al.,* 2003**)**

Observational studies also highlight the relation between abnormal vaginal flora and increased risk of HIV-1 infection (Schwebke 2005; Van der Straten et al., 2005). The high prevalence of vaginal infections such as bacterial vaginosis (30–50%), vulvo-vaginal candidiasis (10–13%), and *Trichomonas vaginalis* (7–23%) in African women is associated with a substantial risk of HIV-1 acquisition (Schweke, 2005). Some other common sexually transmitted infections (STIs) are Chlamydia, gonorrhoea, syphilis, genital herpes and HPV, a virus that can cause genital warts or cervical and rectal cancer. The risk of transmitting HIV from men to women is much higher than from women to men. This is in part because of the much larger surface area of the vagina and cervix compared with the areas of the penis where transmission can take place (foreskin, urethra and small tears on the head of the penis) (Coombs *et al.,* 2003).

In addition to increasing access to female condoms and treatment of other sexually transmitted infections, trials are underway to assess the use of other barrier methods such as cervical caps, invisible condoms, diaphragms, and diaphragms combined with microbicides (Myer *et al.,* 2005). The control of vaginal infections is a potentially important method for prevention (Simon *et al*.,2010).While most studies targeting genital tract infections as a strategy to reduce HIV incidence have not demonstrated efficacy, it remains plausible that prevention of herpes and bacterial vaginosis (BV) could reduce HIV incidence (Masese *et al.,* 2015). Possible interventions for Herpes Simplex Virus type 2 (HSV-2) controls include vaccination, pre-exposure prophylaxis to prevent HSV-2 acquisition, and preventing BV. Maintaining good vaginal and reproductive health can be an important tool for reducing the risk of HIV transmission.

2.9.3 GENOTYPE AND PHENOTYPE

HIV drug susceptibility tests can evaluate either the genotype or phenotype of the predominant viral quasi-species. The HIV genotype refers to the actual RNA sequence of the virus, the phenotype reflects the physical traits or behaviour expressed by the genotype (Kessler *et al.,* 2000). **HIV genotype assay** detect specific mutations or nucleotide substitutions in the *gag-pol* region of the HIV-1 genome. This region encodes for the reverse transcriptase and protease enzymes, the main targets of current ARV drugs. Specific gene sequences are compared with that of a reference (wild type) virus, and mutations associated with decreased susceptibility to specific antiretroviral drugs are identified.

HIV Phenotypic assays evaluate growth of clinical HIV isolates, relative to wild-type virus, in the presence of a drug. Phenotypic testing directly measures the amount of a drug necessary to inhibit or suppress viral replication *in vitro*. Like genotypic tests, current phenotypic assays also use PCR to amplify the *gag-pol* region of HIV-1.

The Genotypic Antiretroviral Resistance Testing (GART) study, evaluated short-term virologic effects in 153 patients whose therapy was failing (Baxter, 2000). The patients were randomized into GART or no GART arm. Patients in the GART arm received genotypic drug resistance testing along with ‘expert’ advice that included mutation interpretation and specific drug recommendations. Patients in the no-GART arm did not receive genotyping or expert advice. By 12 weeks, the mean drop in viral load in the two groups decrease by 0.94 cp/mℓ and 0.47 cp/mℓ for GART and no-GART arms respectively.

2.9.4 THE MOST COMMON MUTATIONS ASSOCIATED WITH VIROLOGIC FAILURE

Wallis *et al.,* (2010) reported that the highest risk for treatment failure was associated with mutations providing resistance to NNRTIs, which are the most popular first-line drug treatments worldwide. Among patients infected with virus with those mutations, an increased risk was detected even at very low minority variant frequencies (< 0.5% and 10-99 cp/mℓ). A study conducted by (Li *et al*., 2012) confirmed these observations.

Polymerase chain reaction (PCR) was the most common method of detecting the minority variants, and K103N, Y181C, M184V and K65R were the variants most frequently observed. The presence of NNRTIs resistance was associated with an increased risk of virologic failure. Among cohort study participants, the rate of virologic failure for those with NNRTI-resistant minority variants was 37% compared with a failure rate of 15% in patient with virus without detectable variant levels.

Moreover, the same researchers reported that compliance to treatment also made a difference, virologic failure rates were significantly lower among patients who had drug-resistant minority variants but were at least 95% compliant to their medication regimens compared with patients with the same variants in whom compliance was less than 95%. In conclusion the authors wrote "the combined presence of suboptimal medication compliance and drug-resistant minority variants resulted in substantially increased risk of virologic failure".

Minor drug-resistant variants exist in every patient infected with HIV before treatment initiation and this fact underlies the basis of the need for combination therapy for HIV infection. Several studies have demonstrated that drug-resistant HIV variants are present at low frequencies in therapy-experienced patients for prolonged durations not only after virological failure (Hance *et al.,* 2001; Charpentier *et al.,* 2004; Dykes *et al.,* 2004; Kapoor *et al.,* 2004; Lecossier *et al.,* 2005; Palmer *et al.,* 2006) but also after discontinuation of suppressive ART in patients originally infected with wild-type virus (Martinez-Picado *et al.,* 2002;Metzner *et al.,* 2003; Hare *et al.,* 2008). These drug-resistant strains, present as minority populations and often missed by standard genotyping, may be the cause of virological failure (Dykes *et al.,* 2004; Lecossier *et al.,* 2005; Roquebert *et al*., 2006; Svarovskaia *et al.,* 2007; Halvas *et al.,* 2010).

Because these minority variants are usually present at very low-levels, they cannot be detected and quantified using conventional genotypic and phenotypic tests (Gianella *et al.,* 2010). Several assays have been developed to detect minor mutants at levels as low as 0.01% -0.5% (Gianella *et al.,* 2010). Mutations associated with drug resistance in clinical practice are generally detected by direct sequencing of the *pol gene* from the population of HIV RNA extracted from plasma (Hirsch *et al.,* 2008).

2.9.5 RESISTANCE MUTATIONS AFTER FIRST-LINE FAILURE

The 2010 WHO HIV treatment guidelines recommend zidovudine/lamivudine, tenofovir/lamivudine or tenofovir/emtricitabine in combination with Nevirapine or Efavirenz as first-line therapy. Stavudine/lamivudine combination is no longer recommended by WHO due to increased risk of toxicity, though significant numbers of patients in resource-limited regions are still using this combination because of low cost.

In patients developing virologic failure on first-line ART, the extent of drug resistance is roughly proportional to the duration of uncontrolled virus replication in the face of selected drug pressure. In resource limited countries where patients undergo infrequent virological

monitoring, samples from patients with virologic failure generally contain more drug-resistant viruses than virological samples from patients with virological failure in well-resourced regions (Gupta *et al.,* 2008).

The development of drug resistance to NRTIs and NNRTIs has been associated with high baseline HIV VL, low $CD4^+$ cell counts, subtype and treatment failure duration (Wallis *et al.,* 2014). ART can fail as a result of drug toxicity, transmitted drug resistance, inadequate medication compliance or incomplete suppression of viral replication, resulting in the emergence of viral drug resistance (Carr *et al.,* 2000; Haas *et al* 2003;Haas *et al* 2004; Boulle *et al.,* 2007; Johnson *et al.,* 2013). Of the individuals who fail first-line treatment, between 75% and 90% of individuals have one or more drug-resistant mutations associated with NRTI and NNRTIs (Marconi *et al.,* 2008; Hosseinipour *et al.,* 2009; Orrell *et al.,* 2009; Wallis *et al.,* 2010).

Patterns of drug-resistance mutation are associated with specific drugs in the regimen. In a study done by Wallis *et al.,* (2014), drug resistance and levels of susceptibility after first-line virologic failure in individuals from Thailand, South Africa, India, Malawi and Tanzania were reported. Of the 148 individuals screened, HIV-1 drug resistance analysis showed that 93% (n=138) and 96% (n=142) had at least one reverse transcriptase (RT) mutation associated with NRTI and NNRTI resistance, respectively. Moreover 96 % of the individuals were resistant to either EFV or NVP, and 32% were fully susceptible to etravirine (ETR) or rilpivirine (RPV). Only 2 individuals had major PI-associated mutations that were linked to low-level resistance to LPV/r (l90 m and V321I/M46L). The number of NRTI mutations was significantly associated with higher VL *(P<.001*) and lower $CD4^+$ cell count (*P<.001*). Additionally, in the same study, the most prevalent viral subtype was subtype C (66%) followed by subtype AE (18%), subtypes A1 (8%), and lastly sub-types D (7%).

In conclusion (Wallis *et al.*, 2014) it was suggested that widely available RTI (reverse transcriptase inhibitors)-based regimens have resulted in unprecedented access to care in RLS. However, the low genetic barrier and high-level resistance of NNRTI-anchored regimens in the absence of frequent VL monitoring, results in rapid accumulation of drug resistance mutations to NNRTI and NRTI drug, which may limit future treatment options. VL monitoring and earlier virologic failure detection will probably result in lower NRTI resistance.

2.9.6 SALVAGE THERAPY

Important concepts such as ARV potency, genetic barrier to resistance and cross-resistance are to be considered when developing salvage regimens. In resource-limited setting where genotypic resistance testing is not widely available, surveillance of primary resistance and the development of low-cost resistance assays are of utmost importance.

The salvage regimen should be sufficiently potent to suppress virus levels to below the level of detection and should have a sufficiently high genetic barrier to resistance to prevent virological rebound. A boosted protease inhibitor like LPV/r is the drug-class with the highest genetic barrier to resistance, with a minimum of three to four mutations required for Lopinavir/r resistance. This drug can then be considered as a second-line therapy in

combination of NRTI containing backbone. However, proof-of-principle studies have shown that, although Lopinavir/r alone is not as effective as Lopinavir/r plus two NRTIs for initial ARV therapy, it is sufficient to suppress HIV-replication to levels below detection for more than 1 year in the majority of ARV-naïve patients (Delfrassy *et al.,* 2008).

2.10 VIROLOGIC IMPACT OF DELAYED TREATMENT SWITCH TO SECOND-LINE THERAPY

HIV-1 RNA load measurement of plasma from an infected individual defines the success or failure of an antiretroviral treatment. Despite clear guidelines, there is considerable variability by different treatment programs in the time of switching ART treatment in patients showing virologic failure, particularly in response to immunologic and post-failure evolution. WHO recommends switching therapy when a patient has persistent viral load above 5000 viral cp/mℓ (Cässel *et al.,* 2006). Consideration of the timing of treatment switch after first-line ART failure is particularly important in resource-limited settings where salvage regimens are scarce and costly. A study done by Li *et al.,* (2012) concluded that patients who switch treatment within 8 weeks of identifying virologic failure have better clinical outcomes, on average, than patients who delay switching to a new second-line ARV regimen after virologic failure while on the initial regimen

Current guidelines recommend that an individual must be on ART for at least 6 months before it can be determined that a regimen has failed, and biological monitoring ($CD4^+$ count and viral load when available) is recommended every 6 months (Madec *et al.*,2013). Therefore, switching to second-line ART is expected to occur after at least 12 months of first-line ART. The number of virologic failures is also likely to increase as the time spent under-first-line ART increases.

Patients who continue to show virologic failure despite demonstrated compliance (see compliance section) may be changed to second-line therapy. Before changing to second-line therapy, patient should go through the treatment readiness and education program again. These would need to be carefully monitored as some patients might hide their non-compliance. Delays in switching place a patient at greater risk of illness and death through longer durations spent with high viraemia and at lower $CD4^+$ counts (Murray *et al.,* 1999; Murphy *et al.,* 2001). Prospective data from two clinical cohorts suggest that treatment delay after virologic failure on non-protease inhibitor based-regimen had an increased risk of mortality (Peterson *et al.,* 2008).

2.10.1 FACTORS THAT MAY LEAD TO DELAY OF SWITCHING TO SECOND LINE THERAPY

There are clinician-, patient- and clinic- level factors that may lead to delaying the switch to second-line ART after proven treatment failure.

Patient-level factors include: missed or delayed clinic visit or treatment preparedness sessions (Miller *et al.,* 2010).

Clinic-level factors: ART stock outs or inaccessible clinic location

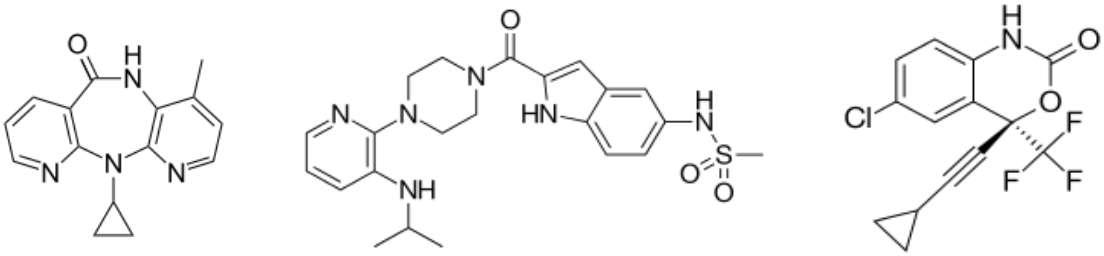
Clinician-level factors: inadequate knowledge

For individuals co-infected with HIV and tuberculosis and in a setting where rifabutin is unavailable, clinicians may delay protease inhibitor-based second-line ART until TB treatment is completed. This is because rifampicin significantly lowers PI levels through induction of metabolic enzyme and cellular drug transporters (Maartens *et al.*, 2009). In this study setting, clinicians prescribed Reyataz or double the dose of lopinavir/ritonavir to counteract the anticipated decrease in protease inhibitor levels by rifampicin rather than delay second-line ART initiation. Since elevated ritonavir levels can increase the risk of GIT side-effects, this course of action may cause long-term tolerability issues (L'Homme *et al.*, 2009). As a result this risk must be balanced against the benefits of integrated treatment of HIV and tuberculosis.

2.10.2 FIRST-LINE AND SECOND-LINE TREATMENT

2.10.2.1 NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS

The name of NNRTIs clearly indicates that they do not have a typical nucleoside analogue structure and show a diversity of chemical structure. Four NNRTIs were developed between 1996 and 2009: Nevirapine (Merluzzi *et al.*, 1990), Efavirenz (Young *et al.*, 1995), Delavirdine (Dueweke *et al.*, 1993), depicted in Figure 3 below and Etravirine (Andries *et al.*, 2004). In the latest guidelines, Nevirapine or Efavirenz is recommended as a part of first-line regimen (WHO guidelines, 2010. What to start, U.S. DHHS 2009 What to start; EACS 2009).



Nevirapine *Delavirdine* *Efavirenz*

Figure 3: Chemical structures of three First generation NNRTIs approved by the FDA

The NNRTIs are antiretroviral drugs that inhibit the reverse transcriptase (RT), an enzyme that controls replication of the genetic material of HIV, depicted in Figure 4 below. The NNRTIs differ from NRTIs in their mechanism of RT inhibition. First, NNRTIs require no intracellular metabolism (Merluzzi *et al.*, 1990; Dueweke *et al.*, 1993; Young *et al.*, 1995; Andries *et al.*, 2004). Second, they bind directly to a hydrophobic pocket near the catalytic site of HIV-1 RT, also referred to as the 'NNRTI-binding pocket' (Kohlstaedt *et al.*, 1992;

Esnouf *et al.*, 1997; Ren *et al.*, 2000; Das *et al.*, 2004). Third, NNRTIs block the chemical reaction step of DNA polymerization (Spence *et al.*, 1995).

First-line ART consisted of three drug therapy with two nucleoside reverse transcriptase (NRTIs) and NNRTIs (i.e. Efavirenz or Nevirapine) in the majority of cases. NNRTIs cannot be used as mono-therapy for the treatment of HIV infected patients but rather as a combination with other antiretroviral agents. A non-nucleoside reverse transcriptase inhibitor or a protease inhibitor boosted with low-dose ritonavir each combined with 2 nucleoside (or nucleotide) reverse transcriptase inhibitors may recommended with choice being based on the individual patient profile (Hammer *et al.*, 2006) .The major rationale for combination ART is to contend with pre-existing drug-resistant variants.

Although medical professionals tend to regard NNRTIs as effective based on potency, less frequent dosing, and low incidence of dangerous side effects and affordability, one of the biggest challenge with NNRTIs is the rapid develop of drug resistance. NNRTIs mutations were discussed above.

Most common possible side effects noted with this class of drugs include organ damage, mild to moderate skin rash and central nervous system disorders (sleep, dizziness etc.) and psychiatric symptoms. In addition, most side effects are the results of the body adapting to the new drug and after few weeks these side-effects usually fade.

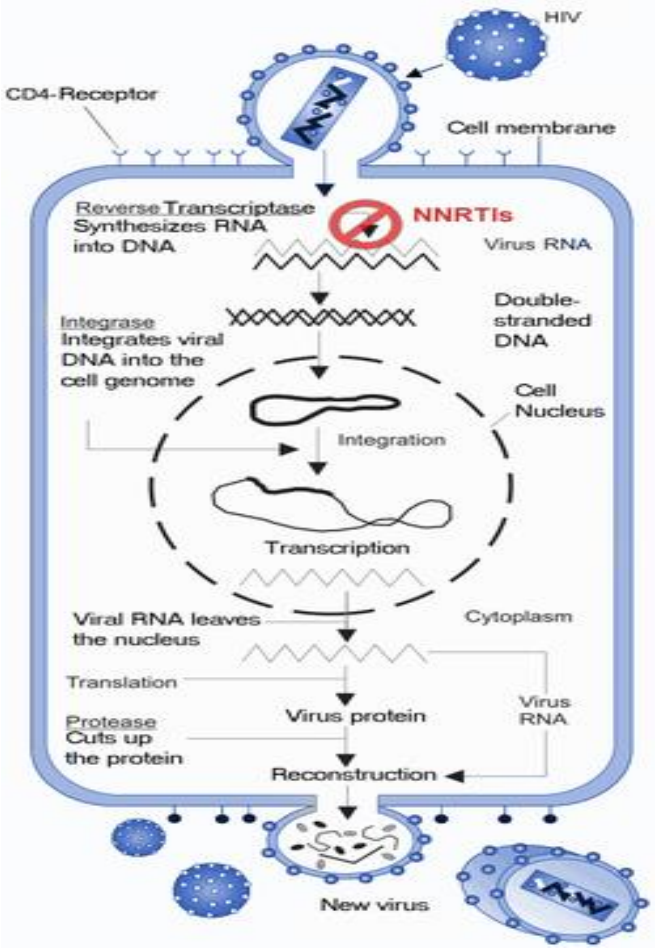


Figure 4: Non-nucleoside reverse-transcriptase inhibitors (NNRTIs) inhibit the reverse-transcriptase enzyme (RT) and therefore the replication of new viruses (Downloaded from https://en.wikipedia.org/wiki/discovery_and_development_of_non-nucleoside_reverse-transcriptase_inhibitors , on 11 August 2016)

2.10.2.2 NUCLEOSIDE/NUCLEOTIDE REVERSE TRANSCRIPTASE INHIBITORS (NRTIs OR 'NUKES')

The NRTIs were the first type of drugs available to treat HIV. They remain effective, powerful and important in the treatment of HIV. However they must be used in combination with other anti-HIV drugs. They are better known as nucleoside analogues or 'nukes'. The NRTIs work by blocking the reverse transcriptase enzyme, thereby blocking HIV from making copies of itself after entering the host cells. Tenofovir was the first nucleotide analogue reverse transcriptase inhibitors approved by the FDA. Like the nucleoside analogue, nucleotide analogues inhibit reverse transcriptase. However, they are active in their native form, unlike nucleoside type inhibitors that only work in cells that have the machinery to activate the drug by the process of phosphorylation. This means that nucleotide analogues may be active against HIV in a wider variety of infected cells. Different types of drugs in NRTIs class and their potential serious side effects are summarised in Table 5 below.

TABLE 5: LIST OF NRTIs AND THEIR POTENTIAL SIDE EFFECTS:

DRUG	ABBREVIATION/ BRAND NAME	POTENTIAL SERIOUS SIDE EFFECT/S
Zidovudine	AZT, ZDV, Retrovir®	Anaemia, neutropenia, hepatotoxicity, myopathy, cardiomyopathy, stomach upset
Lamivudine	3TC, Epivir®	Liver damage, pancreatitis, peripheral neuropathy, lactic acidosis
Abacavir	ABC, Ziagen®	Hypersensitivity, abdominal discomfort, lactic acidosis, liver problems
Didanosine	DDI , Videx EC®	Hepatomegaly with steatosis, lactic acidosis, pancreatitis, fat redistribution
Tenofovir	TDF, Viread®	Decrease bone density, kidney problems, lactic acidosis
Stavudine	D4T, Zerit®	Peripheral neuropathy, lipodystrophy, pancreatitis, lipoatrophy
Emtricitabine	FTC, Emtriva®	Changes in body fat redistribution, headache, diarrhoea, nausea, rash, skin discolouration

2.10.2.3 PROTEASE INHIBITORS (PI'S)

Second-line ART consists of two NRTIs and a protease inhibitor-based ART, lopinavir/ritonavir based regimen. Protease inhibitors are designed to interfere with the activity of the protease enzymes required by many viruses to reproduce themselves. HIV utilizes proteases to truncate the replicated protein encoded by the RNA after synthesis by the host cell. By combining a protease inhibitor with another protease inhibitor the risk of developing resistant viral strains is reduced. Because the protease can change each time a virus replicates, use of multiple inhibitors ensures that random mutations which resist one form of protease inhibitor will be inhibited by another inhibitor. Of note is that the PIs are the drug class with the highest genetic barrier to resistance with a minimum of three to four mutations required for high-level lopinavir/r resistance (Doherty, 2011; Rhee, 2010).

However, several side effects have been noted with this class of drugs, one of the most serious side effects is raising blood sugar and development of diabetes mellitus. These drugs have also been implicated in liver toxicity, a common problem with drugs that are taken chronically and in high dosages because of hepatotoxicity resulting from the metabolites of these drugs. A protease inhibitor also interferes with the way the body process and stores fat, causing an increase in cholesterol levels and formation of unusual fat deposit.

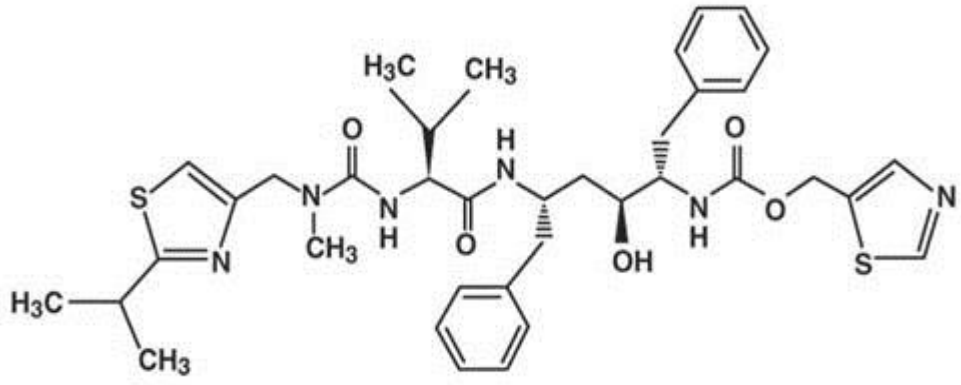


Figure 5: The chemical structure of lopinavir (LPV)

2.11 GENDER DIFFERENCES IN CLINICAL OUTCOME AMONG INDIVIDUALS ON ANTIRETROVIRAL THERAPY

Differences by gender in virologic responses to ART remain inconsistent and have yet to be explored in medical settings. Females tend to be more compliant with ART possibly due to being care givers and nurturers for their children, wanting to live longer to see their children become independent. Furthermore drug and alcohol consumption is less common than in males which in turn can affect treatment compliance, influence survival of HIV-infected

individuals by exacerbating immunosuppression, enhance the toxicity of ARV on liver cells and accelerate liver damage and may depress the immune system allowing increased multiplication of the virus in mononuclear cells (Bagasra, *et al.,* 1996). One study (Cescon *et al.,* 2013) found different results where women were concerned by observing that women on ART are at increased risk of virologic rebound (VL > 400 cp/mℓ following treatment change) after ART initiation although several published studies largely agree that following sero-conversion, women demonstrate higher $CD4^+$ T-cell counts and lower HIV-RNA viral load measures than men (Gandhi, 2002; Napravnik, 2002; Touloumi, 2004; Collazos, 2007; Meditz, 2011). In principle, cohort data examining gender differences in response to therapy and disease progression remain contradictory (Nicastri, 2007) and are likely to have been influenced by context, setting, and other social determinants that affects access to ART and associated support services (Kupyer, 2004). Some studies found that women had higher $CD4^+$ cell count at ART initiation than men (Cornell *et al.,* 2012)

In a study conducted by (Mosha *et al*., 2013), the investigators observed that women were starting treatment at a less advanced disease stage although they had a lower socioeconomic status. Moreover male HIV patients delayed seeking treatment and enter into treatment at a significantly more advanced stage of HIV infection, which predisposes them to increased mortality and worse treatment outcome. However a year later both males and females had similar clinical and immunological conditions.

There are programs aimed at focusing more on educating males and motivating them on ART compliance as well as assisting them with substance abuse and stress-coping techniques can help improve compliance amongst males.

Success of ART in HIV infection may be influenced by numerous factors including: gender, race, cultural beliefs, risk behaviour which may exert an influence on survival rate. It has been reported that a delay of starting ART at WHO clinical stage IV (World Health Organization 2010) or BMI below 16 kg/m² is associated with a significantly higher mortality after starting treatment (Lawn *et al., 2011*). The progression rates to AIDS and clinical manifestations of diseases associated with HIV infection might differ between woman and men because of biological and socioeconomic factors (Nicastri *et al*., 2005). In resource-limited settings, men are more likely to have more advanced disease at HIV diagnosis, which is thought to put them at higher risk of adverse outcomes and less likely to respond well to ART.

Continuous support and follow up are recommended in both males and females despite females appearing to seek treatment at earlier stages than women. Better interventions may improve HIV outcome. Further studies should be done to better understand the underlying causes for these differences.

2.12 ADVANTAGES AND DISADVANTAGES OF SWITCHING FROM FIRST-LINE TO SECOND-LINE ARV THERAPY

There are essentially three parameters that can be used to monitor the success of treatment and determining when to switch ARV regimens: *clinical, immunological* and *virological.* It is

well established that virological failure occurs first, followed by immunological failure and finally clinical failure (Aldous *et al.,* 2009; Barlett *et al.,* 2009).

The current WHO global guidelines define:

Virological failure: when the plasma viral load is >1000 cp/mℓ on consecutive viral load measurements monitored at least 3 months apart, despite proof of ART compliance.

Immunological failure: when $CD4^+$cell counts falls to the pre-therapy baseline (or below) or there is a 50% fall from the on-treatment peak value (if known) or $CD4^+$ cell count levels are persistently < 100 cells/mm^3

Clinical failure: when there is a new or recurrent WHO Stage 4 condition indicating severe immunodeficiency after at least 6 months of ART treatment.

In South Africa, two sequential regimens of ART are available, consistent with WHO guidelines (WHO, 2010). The guidelines recommend that after failure of first-line NNRTI-based regimen, individual be switched to protease inhibitor-based second-line ART. Switching to a second-line ART treatment regimen relies on the observation of persistent virologic failure while still on the initiating ART regimen. Effective and efficient management of first-line ART failure is critical as limited availability of different ART regimen options and the higher costs of second-line ARV drugs.

An early switch can preserve treatment options and the patient's ability to effectively respond to second-line ARVs therapy. A late switch keeps more patients on first-line therapy, keeping medicine and laboratory monitoring costs down. The disadvantages of a late switch are the increased risk of accumulating drug resistance and mutations, resulting in fewer choices of effective ARVs for second-line therapy. A late switch also increases the possibility of a poor response to the new therapy because the patient may be too ill. An increased level of viral NRTI resistance could accumulate when ART failure is not addressed early and as soon after confirming virologic failure and not waiting for immunological and clinical failure. Delayed detection of virologic failure also decreases survival in HIV-infected individuals.

The WHO definitions of first and second-line ART are:

First-line ART: the initial regimen prescribed for patients fulfilling national clinical and laboratory criteria for starting ART. Current WHO treatment guidelines recommend two NRTIs and one NNRTI for initial treatment in a fixed dose combination.

Second-line ART: is the regimen used immediately after first-line therapy failure has been confirmed (virologically, immunologically or clinically). Current WHO treatment guidelines recommend that the PI class of ARV be reserved for second-line ART, preferring ritonavir-boosted protease inhibitors (bPIs) supported by two agents from the NRTI class.

2.12.1 PRINCIPLES GUIDING SWITCH FROM FIRST-LINE TO SECOND-LINE ANTIRETROVIRAL THERAPY

To provide the best possible care for patients infected with HIV, it is significant to correctly decide when to switch from one antiretroviral therapy to another for patients experiencing virologic failure. Chang *et al.,* (2010) conducted a study where they found that in low-

resource settings, monitoring strategies which use immunologic or immunologic and virologic monitoring in addition to clinical monitoring for guiding when to switch therapy resulted in fewer patient deaths, fewer AIDS-defining illnesses and fewer unnecessary ART switching. Little evidence that adding virologic monitoring to immunologic monitoring had added benefits was observed. On-going studies addressing when to switch from a failing regimen to second-line treatment will likely provide information to further clarify optimal monitoring strategies for guidance with respect to when to switch to second-line therapy.

The principles guiding switching form first-line to second-line ART is a complex process that requires consideration of multiple factors including:

- The type of monitoring (e.g. virologic, immunological or clinical) that is available to guide ARV switching,
- Establishing criteria for treatment failure (e.g. viral load > 1000 cp/mℓ)
- Integrating data from different types of monitoring
- Making a decision
- Follow-up and monitoring to determine patient outcomes.

Although viral monitoring (viral load measurement) is the gold-standard for monitoring HIV-infected individuals on ART and determining treatment failure, most resource limited settings only have clinical and/or immunological monitoring ($CD4^+$ cells counts) results available. Efforts to intensify ART compliance should be reinforced prior to switching to second-line as compliance remains the major contributor to first-line virologic failure.

2.12.2 POST IMPLICATIONS OF DELAYED SWITCH TO PROTEASE INHIBITOR-BASED REGIMEN

Current treatment guidelines recommend monitoring of plasma HIV RNA levels during antiretroviral treatment, with a goal of identifying virologic failure as early as possible and modifying therapy once failure is confirmed (Peterson *et al.,* 2008). In practice, however, switching from a failing ART regimen may be delayed as a result of late detection of virologic failure, sporadic follow up and/or lack of access to alternative regimen.

Cost has been a major barrier to the rollout of PI-based second-line ART. Drug-related costs contribute almost three-quarters of the expense of second-line ART in South Africa (Murphy *et al.,* 2010). Recently, the cost of ART has decreased through negotiations with drug manufactures and approval of generic substitutions. A number of strategies may be available for addressing the cost of second-line ART in resource-constrained settings, including further efforts to reduce antiretroviral prices and change to more effective and compliance promoting first-line treatment protocols in an effort to minimize the number of patients requiring the switch to second-line drugs (Long *et al.,* 2010).

In a study by Levison *et al.,* (2013), the researchers used an international model of HIV "cost-effectiveness of preventing AIDS complications" to simulate a South African cohort of HIV-infected adults at first-line ART failure. Two strategies were examined: no viral genotype vs. known viral genotype. The researchers concluded that genotype resistance testing at first-line ART failure would be very cost-effective in South Africa. Furthermore the clinical and economic benefits of genotype testing are particularly critical in patients with

low $CD4^+$ cell counts at ART failure where genotyping results can lead to prompt ART switching. Therefore, program planners should consider operational strategies to implement genotype testing and to reduce delays in processing of the viral genotype test and reporting of the results.

Another consequence of delayed switching to PI-based regimen is significant virologic and immunologic failure. Patients with virologic failure on NNRTI-based regimen who delayed switching treatments were at increased risk for death due to immunological failure (Steegen *et al.*, 2016). However, the same was not true for patients who delayed switching treatments after virologic failure on PI-based regimens. The notion that delaying change from a failing ART regimen would have some untoward consequences (e.g. a fall in $CD4^+$-cell levels, an increase in resistance mutation accumulation, or an increase in clinical events) has always made intuitive sense.

Steegen *et al.*, (2016), suggested that continuing on a failing NNRTI-based regimen has significant clinical consequences. These findings have particular relevance to less-developed countries, where patients are invariably treated with NNRTI-containing regimen and do not have regular viral load monitoring for early detection of virologic failure.

2.13. THE RESEARCH MODEL

2.13.1 BACKGROUND

As already indicated in the foregoing, the objective of this study was to retrospectively investigate data collected from HIV treatment based studies to identify possible predictors of EVF and LVF as well as the time to switch to second-line ART therapy within an HIV positive population in a resource limited setting. To this end, this study attempted to analyse and determine the factors (i.e. predictors) that could explain the rate of the occurrence of an incident (i.e. the number of new EVF or LVF incidents per population at risk per unit of time) as opposed to the cumulative incidence (i.e. the proportion of new cases that develop over time).

As the available data lends itself more to a Cox regression analysis as opposed to a logistic regression analysis, the Cox regression model was used to determine the predictors of EVF, LVF and the requirement to switch to second-line therapy in a sample of HIV positive patients in South Africa. The medical history and treatment regimen data for these patients was available from data collected during participation in HIV treatment trials conducted by Phidisa within the SANDF Medical Corps and this was not powered to find these predictors.

The Cox regression analysis is based on regression like models and could be considered an extension to the common “life tables” popularized by Kaplan and Meier in 1958. Since then, the Cox regression has become a standard statistical tool to analyse incidence rates or survival data in the medical, statistical and related fields (Cox 1972).

2.13.2 CHARACTERISTICS AND ASSUMPTIONS OF THE COX MODEL

The Cox regression (or proportional hazards) model does not require that a specific probability model representing survival times or incidence rates is defined and is more robust than other parametric models (e.g logistic model, etc.). This model can accommodate both discrete and continuous measures and it is easy to incorporate time dependent variables. The Cox regression model does not place undue restrictions in that it attempts to model the effects of explanatory variables on the hazard rate while leaving the baseline hazard rate unspecified and estimating relative as opposed to absolute risks. Finally, Cox regression models assume proportional hazards (i.e. assuming that the hazard of any given individual is a fixed proportion of the hazard of any other individual).

2.13.3 SPECIFICATION OF A COX REGRESSION MODEL

The Cox (or proportional hazards) regression model is a model used to investigate several variables at a time. Essentially, it models (or regresses) the survival times or the hazard function on the explanatory (or predictor) variables where the hazard function is define as the probability that an individual will experience an event (for example, death) within a small time interval, given that the individual has survived from the beginning of the study up to the end of the interval t. In this study, the hazard function is interpreted as the risk that the individual who is HIV positive will experience the hazard (i.e. experiencing either EVF or LVF).

Given the foregoing, the hazard function can be specified as follows:

$$h(t) = h_0(t)e^{(\beta_1 X_1 + \cdots + \beta_k X_k + \varepsilon_i)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where $h(t)$ is the hazard rate (or hazard incidence) at time at time *t*; *e* or *exp* is the exponential to which the explanatory (or predictor) variables are raised as per the equation specification; $h_0(t)$ is the baseline or underlying hazard function and it corresponds to the probability of an event occurring (in this case, EVF or LVF) when all the explanatory variables are zero; the regression coefficients $\beta_1 \; to \; \beta_k$ that are estimated through maximum Likelihood (ML) measures the proportional change in the hazard given changes in the explanatory (or predictor) variables; $X_1 \; to \; X_k$ are explanatory (or predictor) variables; and $\varepsilon_i \; (where \; i = 1,2,3, \ldots, k)$ is the random error term (which measures all the unobservable but equally important factors that explains the probability of the event occurring). For estimation purposes, natural logarithms are taken on both sides of (1) such that:

$$\ln h(t) = \ln h_0(t) + \beta_1 X_1 + \cdots + \beta_k X_k + \varepsilon_i \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

2.13.4 INTERPRETATION OF A COX REGRESSION MODEL

The Cox regression model is included in many appropriate statistical packages such as SPSS; SAS; STATA, etc. A Cox regression output among other things provides two types of coefficients which all tell an interesting story. First is the individual estimated regression coefficients (i.e. the β_i) followed by the hazard (or relative risk) ratio which is obtained by the exponentiation of the respective estimated coefficient (i.e. e^{β}).

The estimated coefficients can be either positive or negative. A positive coefficient suggests an increase or a higher probability in experiencing the hazard, hence poor prognosis or survival time, etc. or as the case may be, while a negative coefficient suggests a decrease or a lower probability in experiencing the hazard, hence an extended survival time. Cox regression models can have either or both categorical (i.e. discrete or dummy) and continuous variables as explanatory (predictor) variables. In this study, a positive estimated coefficient indicates an increase in the probability of experiencing EVF or LVF and vice versa.

The hazard (relative risk) ratio is always interpreted in relation to the control group such that when the relevant explanatory (predictor) variable is a categorical variable, its impact on the hazard function is interpreted as a percentage increase (i.e. if the sign is positive) or a percentage decrease (if the sign is negative) in the risk of experiencing the event (EVF or LVF) after adjusting for all the other explanatory variables. If the explanatory variable is continuous, its impact on the hazard function is interpreted as the number of times the hazard will increase (or decrease) if the sign is positive or negative) given a unit change in the explanatory variable after controlling for the effects of all the other explanatory variables (after assuming *ceteris paribus (i.e. all else remaining the same)*). The hazard (relative risk) ratio must always be interpreted within the associated 95% confidence interval (Walters 2009).

In all instances, attention must be paid to whether the coefficient of the explanatory variable is statistically different (or not different) from zero and for it to be statistically different from zero, then it's associated $\rho\ value \leq 0.05$. When an explanatory variable (if all other explanatory variables remain unchanged) has $\rho \leq 0.05$, we fail to reject the hypothesis that the variable has an impact on the hazard occurring, but if the relevant explanatory variable has $\rho\ value > 0.05$, we reject the hypothesis that the given explanatory variable has an impact on the hazard.

CHAPTER 3: DATA AND METHODS

3.1 STUDY DESIGN

This is a statistical analysis performed retrospectively on an observational cohort analysis of data collected during a linked series of clinical trials related to antiretroviral therapy of HIV positive men and women aged 18 years and older.

3.2 STUDY SETTING

This is a sub-study of the Phidisa HIV research project which was on-going from 2004 to 2014 at six Phidisa investigational sites; 1 Military Hospital (Gauteng), 2 Military Hospital (Western Cape), 3 Military Hospital (Bloemfontein), Phalaborwa sickbay (Limpopo), Mtubatuba sickbay (Kwazulu-Natal) and Mthatha sickbay (Eastern Cape). In the Phidisa project, HIV positive patients were initially enrolled in a cohort study (Phidisa 1) or a randomized trial (Phidisa 2). Following completion of the trial, participants in both studies and a cohort of newly identified HIV positive individuals were invited to enrol in an extended follow-up study (Phidisa 1a). First-line virologic failure and switching to second-line therapy were reported for all participants.

3.3 PATIENT/RESEARCH OBJECT SELECTION

3.3.1 STUDY POPULATION

The study population consisted of all uniform wearing SANDF personnel and their registered family members, of 18 years of age and older and who had laboratory evidence of being HIV positive. The patients had to initiate ART between 01 April 2008 and 30 April 2011 i.e. had a minimum of at least one, one-year follow-up visit by 15 May 2012.

3.3.2 SAMPLING FRAME, PATIENT SELECTION

Patients were recruited into this sub-study if they had initiated ART consisting of a three drug therapy of two NRTIs and one NNRTI (i.e. Efavirenz (EFV) or Nevirapine (NVP)). Second-line ART consisted of boosted PI (Protease inhibitor) based ART (Lopinavir/ritonavir). In project Phidisa, HIV positive participants were initially enrolled in a cohort study (Phidisa 1) or a randomized trial (Phidisa 2). Following completion of these two trials, participants in both studies and newly identified HIV positive individuals were invited to enrol in an extended follow-up study named Phidisa 1a.

3.3.3 SAMPLING STRATEGY AND PATIENT ENROLMENT

A total of 1925 enrolled patients initiated first line ART in the Phidisa 1a trial at one of the six Phidisa investigational sites, were antiretroviral naïve (defined as ART for <7 days before enrolling). Patients were censored at death, loss to follow-up, trial withdrawals or switching to a PI based ART regimen for other reasons than positively confirmed virologic failure, whichever came first.

Patients were referred to Phidisa clinics from ID clinics, Military hospitals or by self-referral. ART-eligible patients attend educational and compliance session reinforced by a pharmacist

and social workers. Patients are assessed by a physician prior to initiating ARV treatment. Medical histories complete physical examination and laboratory studies, including plasma HIV-RNA, $CD4^+$ cell count, haematology and clinical chemistry were obtained prior to ART initiation.

After ART initiation, patients were scheduled for one month post ART initiation visits followed by monthly visits to collect their ART treatment. The frequency of patients visiting the pharmacy was reduced from a visit per month to one visit per quarter if there were no complications or poor response to ART. From 1 April 2008 till 30 April 2012, protocol visits were conducted 3 monthly, and from May 2012 onwards protocol visits were conducted 6 monthly. During the 12 months prior to the end of Phidisa II, and the 12 months following the start of Phidisa 1a, the frequency of protocol visits remained 3 monthly. Patients received all antiretroviral medications free of charge and they were reimbursed for travelling expenses. All changes to ART were recorded. All Stage 4 events that occurred throughout follow-up were reported and coded according to the *Medical Dictionary for Regulatory Activities* (Version 12.0). Patients more than three months late for a scheduled clinic or pharmacy visit were actively traced by a phone call or a home visit if needed to ascertain the reason for loss to follow-up.

As per previous South African guidelines, the majority of patients received a first-line ART regimen of Stavudine, Lamivudine and either Efavirenz or Nevirapine. The WHO guidelines was changed in 2010 to recommend Tenofovir, Lamivudine and Efavirenz as first line regimen.

All data were stored in the Phidisa database from where it was extracted and transferred to MS Excel worksheets in CSV format, recoded to suit the possible variables for each parameter and finally analysed using Statistical Analysis Software (SAS) version 9.2.

3.3.4 INCLUSION CRITERIA

Inclusion criteria were selected to include all volunteers who tested HIV positive with the finger prick rapid test and from blood drawn for confirmatory testing using a validated ELISA and Western blot assay:

All included participants were initiated on NNRTI based ART regimens for antiretroviral treatment while on the Phidisa 1a trial.

All included participants were ART naïve except for those who were included in the PMTCT (Prevention of mother to child transmission) with single dose NVP or dual therapy i.e. AZT (Zidovudine) and single dose NVP.

3.3.5 EXCLUSION CRITERIA

Participants were excluded from the study if one or more of the following were present:

All the participants who were found to be pregnant at initiation of ART.

All the participants who had less than six months of follow-up after initiating ART.

3.4 MEASUREMENTS

3.4.1 RECORDS

The following information was obtained directly from the patient's records: Age, Enrolment year, BMI, Haemoglobin concentration, $CD4^+$ cell count, HIV RNA viral load, Gender, Education level, Marital status, BMI, Haemoglobin (Hb) categories, $CD4^+$ cell count categories, WHO Stage 4, Current TB treatment, use of Traditional Medicine, Concomitant chronic medication, History of depression, NRTI backbone, NNRTI drugs prescribed and NVP/ZDV exposure.

3.4.2 BLOOD TESTS

Clinical and laboratory (including $CD4^+$ cell count, viral load, haemoglobin) data were collected at all schedule visits. Follow-up measurements of $CD4^+$ cell count and HIV viral load were obtained every 3 months for those prescribed ART and every 6 months for those not prescribed ART until May 2012 at which time all participants were seen at 6 months intervals.

3.5 ETHICAL CONSIDERATION

An approval was obtained from the Research Ethics Committee of the University of Pretoria before commencing the study. Other organizations that approved the study were the Scientific Study Committee Project Phidisa, SANDF Defence Intelligence Department, the Phidisa project/SANDF Institutional Review board, the General Officer Commanding 1 Military Hospital and Office of Clinical Research Project Phidisa. The study adhered to the principles of ICH Good Clinical Practice and WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. The study was financially supported by SANDF, NIAID, and the US Department of Defence. None of these entities nor their employees or contractors had any financial interest in the drugs used.

3.6 SAMPLE SIZE

Data capture from the Phidisa database provided complete required information for 1925 subjects who initiated ART within the cut-off dates of the study and who had follow-up data available from participation in one of the Phidisa trials. This data was filtered, cleaned and coded to ensure that all required information could be analysed statistically. This data filtering resulted in many participants being disqualified from the study as they did not have complete data or were found to not meet the inclusion criteria.

3.7 STATISTICAL METHODS

Initial data was extracted from the Phidisa databases and transferred as CSV values which were imported into Microsoft Excel 2010 software in the form of multiple columns per participant. Digital variables were defined for each criteria within the data to set numerical coded values for each of the measured variables to enable statistical analysis and these codes were substituted into the database manually. Frequency and descriptive analysis was initially

performed on the digitalised data. Data was filtered to remove any participants where discrepancies existed and where data sets were incomplete or missing.

The data was imported into STATA version 11, and again assessed for completeness and any invalid inputs to insure integrity of the collected and converted data. Statistical (regression) analysis of the data was then performed using the following models that were tested in this study; the predictors (determinants) of (a) Virologic failure versus non-failure, (b) Early Virologic Failure; (c) Late Virologic Failure; and (d) Switch time to second-line ART treatment. The dependent (response) variables for all three models were categorical (binary/dummy dependent variables) which are hypothesised to be determined by a set of explanatory variables that are either common or unique across the three models. The dependent (response) variables as well as the explanatory (predictor) variables are fully defined and described below. In all three models, the hypothesis that the set of explanatory variables explains variations in the dependent variables when the respective level of significance, $\rho > 0.05$ rejected. By the same token, the hypothesis that the respective explanatory variables explains the variations in the dependent variable was not rejected when the level of significance; $\rho \leq 0.05$. Means (± standard deviations), actual numbers or ratios were used to describe the data.

Kaplan-Meier estimates of cumulative failure probabilities were derived using each of the three different failure definitions (as described in the definitions section below) and stratified by predictors of failure. Hazard ratios (HR) for associations of patient characteristics with either EVF or LVF and switch to second-line antiretroviral treatment were then estimated using the Cox regression for determining the proportional hazard ratio.

To test for evidence of EVF, the following predictors or variables were considered; age, gender, marital status, highest level of education, body mass index, haemoglobin levels, $CD4^+$ cell count at initiation of ART, single dose NVP in women, self-reported use of traditional medicine, first HIV-RNA load after cART initiation in terms of cp/mℓ, WHO clinical staging status, initiating ART regimen, TB treatment, treatment for other chronic conditions, past/current history of depression, month/year of ART initiation, any missed visit within the first 6 months of initiating ART and the initiating treatment site.

To test for evidence of LVF, the same predictors or variables as for the EVF model were used and over and above that included, the following potential additional predictors; namely; month twelve updated clinical factors such as $CD4^+$ cell count; viral suppression <50 cp/mℓ; diagnosis of disease progression; new concomitant chronic medication; ART adverse effects requiring ART change; inter-site transfer within the first year of initiating ART and missed clinic visit in first year of initiating ART and initiating site.

Finally, for assessing the predictors of requiring switching to second-line ART treatment, the following predictor variables were used; cohort; calendar year of failure; months on ART at failure; viral load at failure; $CD4^+$ cell count at failure; ART regimen at failure; current TB status; and site of treatment when virologic failure was noted.

3.8 VARIABLE AND PREDICTOR DEFINITIONS OF THE MODELS

The empirical analysis of this study is based on a subset sample from a total sample of 1 925 subjects who participated in the Phidisa trials. From the total sample of 1 925 subjects, the empirical models were performed for only 1285 after filtering out patients with missing values or observations.

The definitions of the dependent variables as well as all the explanatory variables (predictors) used across all three models are provided here:

To re-iterate, the study empirically estimates the determinants (or predictors) of three models; namely the **Early Virology Failure, Late Virology Failure and the Switch to Second-Line Therapy**. The dependent variables (namely; Early Virology Failure, Late Virology Failure and the Switch to Second-Line Therapy) are dummy (categorical) variables which were defined as follows:

Early virology failure was defined in four different ways;

> **Early virologic failure condition 1 (EVFC1),** plasma HIV-RNA ≥ 1000 cp/mℓ at two consecutive occasions after a minimum of six months on ART therapy.
> **Early virologic failure condition 2 (EVFC2),** failure to suppress VL to less than 400 cp/mℓ within twelve months of initiating ART.
> **Early virologic failure condition 3 (EVFC3),** single VL ≥1000 cp/mℓ after at least 6 months of ART, with follow-up visits and death, but where no second VL data was available.
> **Early virologic failure condition 4 (EVFC4),** when EVFC1, EVFC2 and EVFC3 are not indicated but treatment failure was confirmed within 12 months of ART initiation.

Late virology failure is defined in three different ways:

> **Late virologic failure condition 1 (LVFC1**), plasma HIV-RNA ≥1000 cp/mℓ at two consecutive occasions after at least 12 months of compliant ART treatment.
> **Late virologic failure condition 2 (LVFC2**), single VL ≥ 1000 cp/mℓ after at least 12 months of ART treatment and death where no follow-up VL is available.
> **Late virologic failure condition 3 (LVFC3**), when LVFC1 and LVFC2 are not indicated and there treatment failure was confirmed after at least 12 months of compliant ART.

Switch to second-line ARV due to virologic failure is defined as switch to PI based ART treatment and at least one new NRTI. These dependent variables are hypothesised to be determined by the explanatory variables that are defined as follows across all three models:

Body Mass Index (BMI) is defined as a ratio of the subject's weight and the squared value of their height. A subject is categorized as underweight when $BMI \leq 18.5 kg/m^2$; is normal weight when $19 kg/m^2 \leq BMI \leq 24.9 kg/m^2$ and a subject is categorized as overweight when $BMI \geq 25\ kg/m^2$.

Haemoglobin before is defined as the levels of haemoglobin (found in red blood cells to carry oxygen from the lungs to the body's tissues and return carbon dioxide from the tissue back to the lungs) that were recorded before ART initiation. Haemoglobin is categorised as low when the reading of Haemoglobin ≤ 11.35g/dℓ in females and when the reading of Haemoglobin≤ 12.35g/dℓ for males. Haemoglobin is considered normal when the Haemoglobin reading > 11.35g/dℓ for females and when the Haemoglobin reading > 12.35g/dℓ for males.

Age is defined as the age of the subject in years and months.

Gender is the gender if the respondent where gender = 1 if subject is female and 0 otherwise.

Education is the highest level of education attained by the subject where primary education level or no formal education = 1, high school level education = 2 while tertiary education level = 3.

Marital status is the status of the subject's marriage where Married = 1, Single = 2, divorced = 3 and widowed = 4.

CD4 before is the CD4+ cell count of the subjects prior to ART initiation.

Traditional medicine refers to whether the subject was taking traditional medicine concurrently with ART (yes =1; or 0 otherwise).

Viral Load refers to the viral load reading of the subjects at the visit reported.

WHO4 refers to the progression from HIV to AIDS; immune system is compromised and involves severe symptoms such as wasting, pneumonia and other life threatening conditions.

NRTI's refers to Nucleoside Re-uptake Inhibitor combinations used for initial ART. In this study the use of NRTI drug combinations: 3TC+D4T/ABC = 1; the use of the drugs TNV+3TC/FTC = 2; while the use of the drugs ZDV+3TC = 3

Tuberculosis (TB) refers to whether the subject was suffering from TB (yes =1 or 0 otherwise) before ART initiation.

Concomitant Chronic Medication refers to whether the subject was taking any chronic medication simultaneously with ART (yes =1; or 0 otherwise).

Depression refers to whether the subject was diagnosed with depression (yes=1 or 0 otherwise).

Enrolment Year refers to the year in which the subject enrolled for the Phidisa ART.

Missed Visits refers to whether the subject missed any scheduled visits before six months (yes= 1 or 0 otherwise); and

Current Site refers to the site where the subjects were treated such that 1 Military Hospital = 1; 2 Military Hospital = 2, 3 Military Hospital = 3, Mtubatuba = 4, Mthatha = 5 and Phalaborwa = 6.

CD4+ count (twelve months updated) refers to the CD4+ level of the subjects recorded after twelve months of ART initiation.

Viral suppression not < 50cp/mℓ refers the values of viral suppression not below 50 **cp/mℓ**

New concomitant chronic medication refers to whether the subject were simultaneously taking new chronic medication after twelve months (yes=1; 0 otherwise).

ART toxicity refers to whether the ARVs that the subjects were taking after ART initiation caused any adverse side effect (yes = 1; 0 otherwise)

Inter site transfers refers to whether the patients were transferred from one site to another (yes=1; 0 otherwise)

Any missed visits in the first year refers to whether the subjects missed any scheduled visits to the treatment site(s) after a year of ART initiation (yes = 1; 0 otherwise)

3.9 PREDICTORS OF EARLY AND LATE VIROLOGIC FAILURE, AND COMPARISONS OF MEANS AND PROPORTIONS.

Tables 6, 7 and 8 below respectively presents descriptive statistics (means and standard deviations (for continuous variables) and proportions (for categorical variables) in respect of subjects in the overall sample (n = 1285) versus those who experienced virologic failure (i.e. early and late) (n = 253) as well as descriptive statistics for subjects who experienced early virology failure (n = 154) versus those who experienced late virology failure (n = 99). Furthermore, the two tables test for independence within the two samples (i.e. all vs. failures and early virologic failure vs. late virologic failure). When the $\rho\ value < 0.05$, the hypothesis is not rejected that the two samples are independent.

TABLE 6: DESCRIPTIVE STATISTICS: OVERALL

Variable (n = 1285)	**Means, (proportions) & Standard deviation (±)**
Age (years) (min 19, max 69)	38.8 (±5.6)
Gender (M) (F)	855 (66.5%) 430 (33.5%)
Enrolment year (2008) (2009) (2010) (2011)	 376 (29.3%) 419 (32.6%) 401 (31.2%) 89 (6.9%)
Viral load (cp/mℓ)	186683(±292204)
CD4+ count (cells/mm³)	146,34 (± 92.56)
BMI (kg/m²)	24.6 (±10.9)
Haemoglobin (g/dl)	12.56 (±2.13)
Educational level (PS &NFE) (HS) (TE) (MD)	 13 (1.0%) 1170 (91.1%) 99 (7.8%) 3 (0.1%)
Marital status (S) (M) (D) (W) (MD)	 351 (27.3%) 851 (66.2%) 36 (2,8%) 44 (3.4%) 3 (0.3%)
WHO stage 4 (Y) (N)	 150 (11.7%) 1135 (88.3%)
Current TB (Y) (N)	 214 (16.7%) 1071 (83.3%)
History of depression (Y) (N) (MD)	 11 (0.9%) 1264 (98.3%) 10 (0.8%)
Traditional medicine use (Y) (N) (MD)	 152 (11.9%) 1132 (88%) 1(0.1%)
Any missed visit before 6 months (Y) (N)	 15 (1.2%) 1270 (98.8%)

ART Toxicity	
(Y)	536 (41.7%)
(N)	749 (58.3%)
NRTI backbone	
(3TC+D4T/ABC)	772 (60%)
(TNV+3TC/FTC)	389 (30%)
(ZDV+3TC)	124 (10%)
Initiating site	
(1Military Hospital)	428 (34%)
(Mtubatuba)	206 (16%)
(2 Military Hospital)	128 (10%)
(Mthatha)	151 (11%)
(3 Military Hospital)	218 (17%)
(Phalaborwa)	154 (12%)

Abbreviations;
M/F: male/female, **PS**: primary school, **NFE**: No formal education, **HS:** High school, **TE**: Tertiary Education, **MD**: Missing data,
Y: Yes, **N**: No,
S: Single, **M:** Married, **D**: Divorce, **W:** Widowed.
Statistical differences have been determined as appropriate. Rounding off decimals and missing data may result in numbers not adding up.

TABLE 7: DESCRIPTIVE STATISTICS: EVF

VARIABLE (n = 154)	**Means, (proportions) & Standard deviation (±)**
Age (years) (min 19, max 57)	38.3 (±5.4)
Gender (M) (F)	109 (70.8%) 45 (29.2%)
Enrolment year (2008) (2009) (2010) (2011)	 50 (32.5%) 54 (35.1%) 42 (27.2%) 8 (5.2%)
Viral load (cp/ mℓ)	283296.77 (±391091.67)
CD4 count (cells/mm³)	126.32 (±90.54)
BMI (kg/m²)	23.23 (±6.34)
Haemoglobin (g/dl)	12.18 (±2.37)
Educational level (PS/NFE) (HS) (TE) (MD)	 4 (2.6%) 140 (90.9%) 8 (5.2%) 2 (1.3%)
Marital status (S) (M) (D) (W) (MD)	 33 (21.4%) 114 (74.0%) 2 (1.3%) 4 (2.6%) 1 (0.7%)
WHO stage 4 (Y) (N)	 23 (14.9%) 131 (85.1%)
Current TB (Y) (N)	 31 (20.1%) 123 (79.9%)
Traditional medicine use (Y) (N)	 16 (10.4%) 138 (89.6%)
Concomitant chronic medicine use (Y) (N) (MD)	 18 (11.7%) 55 (35.7%) 81 (52.6%)
Missed visit before 6 months (Y) (N)	 3 (2.0%) 151 (98.1%)
NRTI backbone (3TC+D4T/ABC) (TNV +3TC/FTC) (ZDV+3TC)	 110 (71%) 34 (22%) 10 (7%)

ART Toxicity	
(Y)	71 (46.1%)
(N)	83 (53.9%)
Initiating site	
(1Military Hospital)	38 (24.7%)
(Mtubatuba)	17 (11.0%)
(2 Military Hospital)	22 (14.3%)
(Mthatha)	34 (22.1%)
(3 Military Hospital)	24 (15.6%)
(Phalaborwa)	19 (12.3%)

TABLE 8: DESCRIPTIVE STATISTICS: LVF

VARIABLE (n = 99)	**Means, (proportions) & Standard deviation (±)**
Age (years) (min 24, max 50)	38.16 (±5.09)
Gender	
(M)	71 (71.72)
(F)	28 (28.3%)
Enrolment year	
(2008)	52 (52.5%)
(2009)	32 (32.3%)
(2010)	14 (14.1%)
(2011)	1 (1.0%)
Viral load (cp/ mℓ)	229471 (±229110)
CD4 count (cells/mm^3)	115.1 (±67.7)
BMI (kg/m^2)	22.9 (±6.1)
Haemoglobin	12.25 (±2.34)
Educational level	
(PS/NFE)	1 (1.0%)
(HS)	92 (92.9%)
(TE)	5 (5.1%)
(MD)	1 (1.0%)
Marital status	
(S)	32 (32.3%)
(M)	62 (62.6%)
(D)	4 (4.0%)
(W)	1 (1.0%)
WHO stage 4	
(Y)	13 (13.1%)
(N)	86 (86.9%)
Current TB	
(Y)	16 (16.2%)
(N)	83 (83.8%)
Traditional medicine use	
(Y)	10 (10.1%)
(N)	89 (89.9%)
Concomitant chronic medicine use	
(Y)	7 (7.1%)
(N)	24 (42.2%)
(MD)	66 (66.7%)
Missed visit before 6 months	
(Y)	3 (3.0%)
(N)	96 (97.0%)
NRTI backbone	
(3TC+D4T/ABC)	81 (81.8%)
(TNV +3TC/FTC)	10 (10.1%)
(ZDV+3TC)	8 (8.1%)

ART Toxicity	
(Y)	41 (41.4%)
(N)	58 (58.6%)
Initiating site	
(1Military Hospital)	34 (34.3%)
(Mtubatuba)	15 (15.2%)
(2 Military Hospital)	10 (10.1%)
(Mthatha)	11 (11.1%)
(3 Military Hospital)	19 (19.2%)
(Phalaborwa)	10 (10.1%)

3.10 DATA DESCRIPTION AND QUALITATIVE HYPOTHESIS

The testable hypotheses with respect to the predictors of the two models (i.e. EVF, LVF) are indicated in full in Tables 9 and 10 below respectively. The time taken to switch to second-line therapy after virologic failure was identified and it was not statistically analysed owing to the number of subjects found to have been switched to a new treatment regimen being less than 30. The percentage relative to the total that showed late and early virologic failure is 12% and 8% respectively.

3.10.1 TESTABLE HYPOTHESES OF EVF MODEL

The investigators hypothesise that the following explanatory variables will increase the risk (hazard) of a subject experiencing early virology failure, namely being female, being single, having minimal schooling, a previous low CD4+ count, a high Viral Load, WHO4, NRTI treatment, concomitant use of chronic medication, being depressed, missing visits before six months and receiving treatment from facilities other than military hospitals.

The investigators further hypothesised that being older would reduce the risk of a subject experiencing EVF on account of being less risky in behaviour and presumably more street wise and that a higher level of education would also reduce the risk of a subject experiencing EVF. It was expected that an inverse relationship between age and EVF and between education level and EFV would be observed.

Even though the standard of care and treatment in the military is expected to be the same for all clinical facilities, the average subject may perceived the level of care received from a rural treatment facility other than a urban hospitals to be inferior (Visser *et al*., 2015; Ndou *et al.,* 2016). For this reason, it was hypothesised that patients who received treatment from the "rural" treatment sites or facilities such as Mthatha, Phalaborwa and Mtubatuba would experience a higher risk of experiencing EVF than those who receive their treatment from 1 Military Hospital (Pretoria), 2 Military Hospital (Cape Town) or 3 Military Hospital (Bloemfontein).

Receiving treatment from any other treatment facility but a military hospital where added support was available from social workers and pharmacists trained to counsel HIV positive patients could be interpreted as a proxy for whether the subjects would adhere to the drug administration regime and take their treatment regularly or not. This behaviour has been observed in a number of different settings in South Africa and elsewhere on the continent where for example, the average patient perceives the level of care received from a primary health care facility such as a clinic to be inferior to that received from a hospital and may be less prone to adhere to instructions and be less compliant to the drug administration regimen than if personally counselled (Mukora *et al*., 2011; Nabbuya-Sekandi *et al.,* 2011; Becker *et al.,* 2012; Visser *et al*., 2015 and Ndou *et al.,* 2016).

The investigators hypothesised that single (or people who were never married), divorced or widowed people are likely to lack the support they would otherwise receive from their partners, and this is likely to increase their risk of experiencing EVF. Finally, simultaneous administration of chronic medication (such as for hypertension, diabetes, etc.) would increase the risk of a subject experiencing EVF on account of drug-drug interaction and poly-pharmacy.

The detailed hypothesised relationships and the testable hypotheses with respect to the predictors of EVF are indicated in full in Table 9 below.

TABLE 9: HYPOTHESES TESTED (DETERMINANTS OF EVF)

Variable	**Hypothesized sign**
EFV (dependent/indicator variable)	
Age	−
Gender (Female = 1, 0 otherwise)	+
Marital Status (where being married=reference variable)	
Single	+
Widowed	+
Divorced	+
Education	
Primary or no Education (reference variable)	
High school	−
Tertiary	−
BMI	+
Haemoglobin	+
$CD4^+$ cell count	+
WHO Stage IV	+
Current TB treatment	+
Use of Traditional Medicine	+
Concomitant chronic medication	+
History of depression	+
NRTI backbone	+
Current site (1 Military= reference site)	
2 Military Hospital	−
3 Military Hospital	−
Mthatha	+
Mtubatuba	+
Phalaborwa	+
Missed visit before 6 months	+
Enrolment year (2008= reference year)	
2009	−
2010	−
2011	−

3.10.2 TESTABLE HYPOTHESES OF LVF MODEL

Since the investigators assume that the same predictors of EVF are relevant for LVF with similar hypotheses, only the additional hypothesised parameters for the additional six predictors in respect of the LVF model that are not relevant for the EVF model will be explained in this section. These variables are suppressed $CD4^+$ count after twelve months, insufficient viral suppression (not <50 cp/mℓ), new concomitant chronic medication, ART toxicity, inter-site transfer; and any missed visits in the first year of ART.

An increase in the $CD4^+$ count after twelve months of ART and subjects transferring within treatment sites resulted in a lower risk of LVF while the concomitant use of new chronic medication, subjects experiencing ART toxicity, subjects missing visits in the first year after initiation of ART increased the risk of LVF.

TABLE 10: HYPOTHESES TESTED (DETERMINANTS OF LVF)

Variable	**Hypothesized sign**
Age	−
Gender (Female)	+
Marital Status (where being married = reference variable)	
Marital Status (1) (Single)	+
Marital Status (2) (Widowed)	+
Marital Status (3) (Divorced)	+
Education Level (Primary or no education = reference variable)	
Education Level (1) (High School)	−
Education Level (2) (Tertiary education level)	−
BMI	+
Haemoglobin	+
CD4+ count	+
Use of traditional medicine (Yes = 1, 0 otherwise)	+
Prior levels of Viral load	+
WHO 4 (Yes = 1, 0 otherwise)	+
NRTI backbone (the drug 3TC+D4T = reference first line NRTI drug)	
NRTI backbone (1) (3TC+TDF)	+
NRTI backbone (2) (ZDV+3TC)	−
TB treatment (Yes)	+
Concomitant use of chronic medication	
Chronic medication(1)	+

Chronic medication(2)	+	
History of depression	+	
Enrolment year (2008 = reference year)		
2009	−	
2010	−	
2011	−	
Missed visits before 6 months	+	
Current site (1 Military = reference treatment site)		
Mtubatuba	+	
2MilitaryHospital	−	
3MilitaryHospital	−	
Mthatha	+	
Phalaborwa	+	
Low $CD4^+$ Count after 12 months		+
Viral suppression not < 50 cp/mℓ	+	
New concomitant chronic medication	+	
ART toxicity	+	
Inter-site transfer	+	
Any missed visit in first year	+	

CHAPTER 4: RESULTS AND STUDY LIMITATIONS

4.1 OVERALL BASELINE CHARACTERISTICS RESULTS.

This study specifically attempted to assess variables that are significantly associated with an increased risk of virologic failure. Identifying factors that are associated with an increased risk of virologic failure is advantageous as these can be used to put measures in place to try delay or prevent the development of progression of disease.

Of the 1925 identified participants for whom data was captured, only 1285 participants were eligible to be included in this study due to 640 patients being excluded due to not meeting the inclusion criteria or missing information; patients with less than six months follow-up, pregnancy at ART initiation, initiation of ART before 2008 and due to obvious data capture errors. The site that initiated highest number of patients on ART was 1 Military Hospital in Pretoria with 33.3% and the lowest initiation site was 2 Military Hospital in Cape Town with 10.0%. Complete data from 1285 patients was available for analysis.

At enrolment, mean age was 38.8 years (range 19 – 69 years); 33.5% were female; 66.5% were males. The majority of participants, 94.1% initiated ART with an Efavirenz based regimen. The majority (60 %) initiated ART using the NRTI backbone of D4T+3TC with 30 % initiating with 3TC+TNV and 10 % initiated with 3TC+ZDV. At the time of ART initiation, more than half (66.2%) of patients were married. Most patients who initiated the treatment had high school education, 91.1%.

Patients presented with advanced HIV disease, with median baseline $CD4^+$ cell count of 146.34 cells/mm³ and Viral load of 186683 cp/ml.

At initiation of ART 16.7% of patients were already on treatment for tuberculosis while 11.7% had a history of AIDS-related symptoms, but no history of extra-pulmonary tuberculosis. Use of traditional medication and history of depression was significantly low at 11.9% and 0.9% respectively. Participants who initiated ART with concomitant medication were 12.4%.

4.2 PREDICTORS OF EARLY VIROLOGIC FAILURE

Out of 1285 subjects for whom complete data is available a total of 154 (12.0%) participants were classified as experiencing EVF in terms of the definition of EVF (Section 3.8). A population of 154 subjects is statistically large enough to permit reliable statistical analysis.

The results for the determinants or predictors of EVF are summarised in Table 10 below. Overall, the empirical results are as per the hypothesized relationship albeit there are a few instances where some of the predictors did not have hypothesised outcome (mostly having incorrect hypothesised sign). However in those instances where the predictors have the wrong hypothesised signs, such predictors were statistically not different from zero. There are however instances where the trend did not confirm the hypothesised effects but showed the opposite effect. In those instances, the associated values of $\rho > 0.05$, which means that the tested predictor or explanatory variable is not statistically significant.

This applies to predictors like being in possession of tertiary education, gender, use of traditional medicine, TB, initiating treatment at Mtubatuba and concomitant use of chronic medication. On the other hand, most predictors were hypothesized as having an influence on the outcome, however the associated values of $\rho > 0.05$ means that these parameters are statistically not different from zero (insignificant). The variables that have the hypothesized signs but are statistically not different from zero include: Age, being divorced or widowed, and having high school education.

Given the foregoing, the results indicates that being single; having higher prior levels of viral load; the initiating NRTI backbone treatment; having missed follow-up visits within six months of ART initiation and receiving treatment at rural sites of Mthatha and Phalaborwa were found to be significant determinants of EVF since the associated $\rho\ values \leq 0.05$. Specifically, not missing follow up visits within six months of ART initiation; and being administered NRTI backbone of 3TC+TDF or ZDV+3TC significantly reduces the hazard of the subject experiencing EVF compared to initiating on D4T+3TC, while being single; having higher initial viral loads; and initiating treatment at the rural sites of Mthatha and Phalaborwa showed significantly increased hazard odds for the subject to experience EVF.

A brief interpretation of the results may also be in order. In this regard, it is noted that holding all other variables constant, subjects who are single will experience a 46 % increase in the probability of experiencing EVF compared with married subjects. And based on the hazard risk ratio, the risk of a responded who is single experiencing EVF increases by 58% compared with that of a married people (95% CI 1.083-2300). Subjects who receive their treatment in the rural clinic sites of Mthatha and Phalaborwa will respectively experience a 71% and 70% increase in the probability of experiencing EVF compared with those receiving treatment at the 1 Military Hospital in Pretoria.

When the effects of all the other predictors are held constant, patients who are on NRTI drug combination treatment of TNV+3TC/FTC will experience a 57% reduction in the probability of experiencing EVF compared with those on an NRTI drug combination of 3TC+D4T/ABC. By the same token patients on NRTI drug combination of ZDV+3TC will experience a 100.3 % decrease in the probability of experiencing EVF compared with those on the combined therapy of 3TC+D4T/ABC. Subsequently, the risk of experiencing EVF as measured by the hazard ratio decreases respectively by 56.5% (95% CI 0.337 - 0.946) and 36.7% (95% CI 0.195 - 0.691) when subjects were administered the NRTI combinations of 3TC+TNV/FTC and ZDV+3TC compared with those administered the NRTI drug combination of 3TC+D4T/ABC.

Finally, subjects who have higher viral loads before ART initiation will experience a 22% increase in the risk of EVF, and the hazard ratio of 1.243 at the 95% CI of 1.086 to 1.423 suggest that the risk of experiencing EVF for these high viral load patients will increase by 1.243 fold (or 1.243 times) for every unit increase in the cp/mℓ of viral load after adjustment for the effects of the other predictors in the model or while holding the other predictors constant.

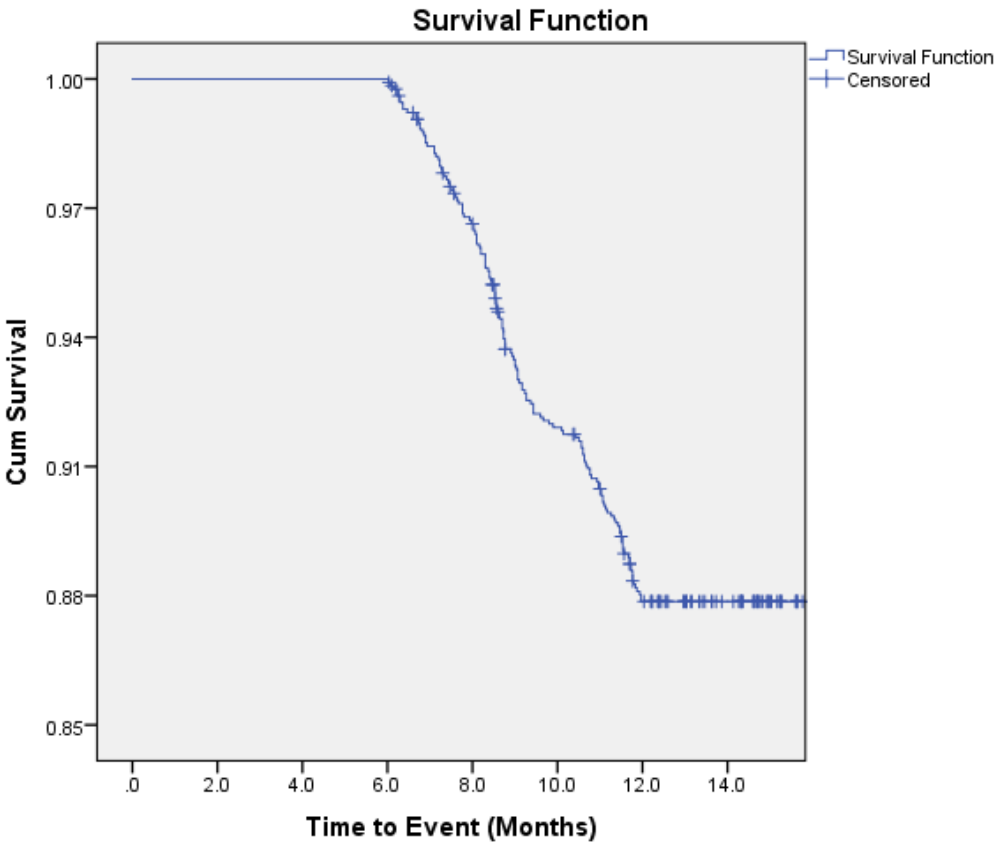


Figure 6: Kaplan Meier curve-indicating the time course of measured early virologic failure for patients on ART treatment.

TABLE 11: COX PROPORTIONAL HAZARD MODEL: EARLY VIROLOGIC FAILURE

Variables in the Equation

	B	SE(β)	Wald	Df	Sig.	Exp(β)	95.0% CI for Exp(β)	
							Lower	Upper
Age (-)	-0.026	0.018	1.975	1	0.160	0.975	0.941	1.010
Gender	-0.381	0.236	2.618	1	0.106	0.683	0.430	1.084
Marital status			5.717	3	0.126			
Marital status(1)	0.457	0.192	5.652	1	**0.017**	1.579	1.083	2.300
Marital status(2)	0.247	0.474	0.273	1	0.601	1.281	0.506	3.240
Marital status(3)	0.225	0.468	0.232	1	0.630	1.253	0.501	3.135
Education level			1.139	2	0.566			
Education level(1)	-10.605	211.008	0.003	1	0.960	0.000	0.000	1.010
Education level(2)	0.427	0.401	1.137	1	0.286	1.533	0.699	3.363
BMI	-0.002	0.011	0.042	1	0.838	0.998	0.976	1.020
HB before	-0.028	0.046	0.383	1	0.536	0.972	0.888	1.063
CD4+_before	0.000	0.001	0.132	1	0.716	1.000	0.997	1.002
Traditional Medicine	-0.030	0.285	0.011	1	0.915	0.970	0.555	1.697
Log (VL before)	0.218	0.069	9.987	1	**0.002**	1.243	1.086	1.423
WHO4	-0.142	0.252	0.316	1	0.574	0.868	0.530	1.422
NRTI backbone			9.936	2	**0.007**			
NRTI backbone(1)	-0.572	0.263	4.721	1	**0.030**	0.565	0.337	0.946
NRTI backbone(2)	-1.003	0.323	9.631	1	**0.002**	0.367	0.195	0.691
TB	-0.114	0.223	0.261	1	0.610	0.892	0.576	1.382
Concomitant chronic medication			0.908	2	0.635			
Concomitant chronic medication(1)	-0.208	0.291	0.511	1	0.475	0.812	0.459	1.437
Concomitant chronic medication(0)	-0.170	0.213	0.635	1	0.425	0.844	0.556	1.281
Depression	10.902	208.860	0.003	1	0.958	0.823	0.000	3.284
Any missed visit before 6 months	1.463	0.437	11.193	1	**0.001**	0.232	0.098	0.546
Current site			15.633	5	**0.008**			
Mtubatuba (2)	-0.192	0.306	0.395	1	0.530	0.825	0.453	1.502
Cape Town (3)	0.287	0.312	0.844	1	0.358	1.332	0.722	2.456
Bloemfontein (4)	0.366	0.308	1.416	1	0.234	1.442	0.789	2.635
Mthatha (5)	0.705	0.235	9.021	1	**0.003**	2.024	1.278	3.208
Phalaborwa (6)	0.698	0.280	6.241	1	**0.012**	2.010	1.162	3.477

4.3 PREDICTORS OF LATE VIROLOGIC FAILURE

Of the 1285 patients who were placed on ART, 99 (7.7%) patients met the definition of late virologic failure at N=99, this group was large enough to enable reliable statistical analysis. The full results of the LVF model are presented in Table 12 below. As already indicated in the previous section, the LVF model not only includes all the predictors of the EVF model (including the hypothesised relationships), but is supplemented by an additional six predictors, namely the $CD4^+$ count after twelve months, viral suppression < 50 cp/mℓ after twelve months, the use of new concomitant chronic medication within a year of ART initiation, ART toxicity, inter-site transfer; and whether the subjects missed any scheduled visits after a year of ART initiation.

A number of predictors (such as age, gender, marital status, haemoglobin levels at ART initiation, being administered the combination of NRTI backbone treatment of the drugs, TNV + 3TC/FTC and ZDV + 3TC as opposed to the combination NRTI backbone treatment of the drug D4T +3TC, use of concomitant chronic medication, prior history of depression, inter-site transfer and missing scheduled visits after a year of being in ART) have the correct sign but have p values > 0.05 which means they are not statistically different from zero or we reject the hypothesis that they are predictors of LVF.

On the other hand, a number of predictors (such as being single, all measures of education level, BMI, prior levels of $CD4^+$ count, use of traditional medicine, being in the WHO stage 4, being administered the combination NRTI backbone treatment of the D4T+3TC (1), any missed visits before 6 months, receiving treatment at Phalaborwa, Mtubatuba and Mthatha; and the $CD4^+$ count after 12 months) have the wrong sign but are also statistically not different from zero.

As a result, four predictors, namely; (a) prior levels of viral load, (b) receiving treatment at the rural site of Phalaborwa sickbay, (c) viral suppression not < 50 cp/mℓ and (d) ART toxicity were found to significantly predict LVF since the associated $\rho\ values \leq 0.05$. Specifically, all four predictors are associated with an increase in the risk of a subject experiencing LVF. Briefly, if all the other predictors were held constant, an increase in viral load will increase in the risk of experiencing LVF by 33%. By the same token, there will be a 73% increase in the probability that a subject who experiences ART toxicity will experience LVF, while subjects whose viral load cannot be less than 50 cp/mℓ will experience a 255% increase in the risk of experiencing LVF. Finally, and assuming *ceteris paribus*, there will be a 94.8% probability that a subject who receive treatment at the Phalaborwa sickbay will experience LVF.

Taking into account the relative risk (hazard) ratio, the investigators find that holding all other predictors constant, (a) an increase in the levels of viral load before ART initiation will increase the hazard of such subjects experiencing LVF by 1.39 times at the 95% CI 1.12-1.72 ($\rho = 0.002$). (b) the risk of experiencing LVF increases by 39% for subjects who receive treatment from the Phalaborwa sickbay compared with those who received treatment from 1 Military Hospital in Pretoria at the 95% CI 0.164-0.916 (ρ = 0.031). (c) assuming *ceteris*

paribus, failure by a subjects to supress viral loads to less than 50 cp/mℓ will increase the hazard of experiencing LVF by more than twelvefold (12.8) at the 95% CI 7.4-22.2 (ρ = 0.000).

Finally, subjects who experienced ART toxicity experienced a 207% increase in the risk of experiencing LVF compared with those who did not experience ART toxicity 95% CI 1.218-3.506 ($\rho = 0.007$) when holding the effects of all the other predictors constant.

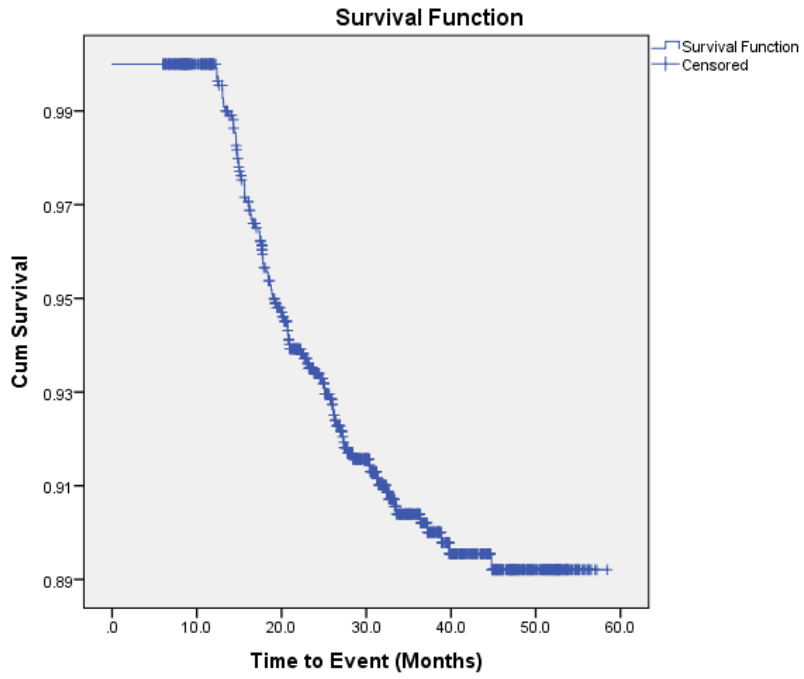


Figure 7: Kaplan Meier curve- indicating the time course of measured late virologic failure for patients on ART treatment.

TABLE 12: COX PROPORTIONAL HAZARD MODEL: LATE VIROLOGIC FAILURE

Variables in the Equation

	B	SE(β)	Wald	Df	Sig	Exp(β)	95.0% CI for Lower	95.0% CI for Upper
Age	-0.028	0.028	0.939	1	0.333	0.973	0.920	1.029
Gender	0.060	0.359	0.028	1	0.866	1.062	0.525	2.148
Marital status			1.553	3	0.670			
Marital status(1)	0.103	0.297	0.119	1	0.730	1.108	0.619	1.982
Marital status(2)	0.428	0.577	0.550	1	0.458	1.534	0.495	4.750
Marital status(3)	-0.972	1.035	0.883	1	0.347	0.378	0.050	2.874
Education level			3.856	2	0.145			
Education level(1)	1.982	1.244	2.540	1	0.111	7.259	0.634	83.090
Education level(2)	1.258	0.694	3.286	1	**0.070**	3.518	0.903	13.705
BMI	-0.002	0.030	0.003	1	0.958	0.998	0.941	1.059
HB_before	-0.028	0.070	0.162	1	0.687	0.972	0.848	1.115
CD4_before	-0.003	0.002	3.176	1	0.075	0.997	0.993	1.000
Traditional Medicine	-0.309	0.402	0.589	1	0.443	0.734	0.334	1.616
Log (VL before)	0.330	0.108	9.230	1	**0.002**	1.390	1.124	1.720
WHO4	-0.489	0.377	1.686	1	0.194	0.613	0.293	1.283
NRTI backbone			5.868	2	**0.053**			
NRTI backbone(1)	0.314	0.399	0.622	1	0.430	1.370	0.627	2.993
NRTI backbone(0)	-0.820	0.557	2.166	1	0.141	0.440	0.148	1.313
TB	0.442	0.366	1.453	1	0.228	1.555	0.759	3.188
concomitant chronic medication			0.767	2	0.681			
concomitant chronic medication(1)	0.446	0.535	0.696	1	0.404	1.563	0.548	4.457
concomitant chronic medication(2)	0.139	0.300	0.215	1	0.643	1.149	0.639	2.068
Depression	1.024	1.079	0.900	1	0.343	2.785	0.336	23.097
Any missed visit before 6 months	-1.953	1.181	2.733	1	0.098	0.142	0.014	1.437
Enrolment Year	-0.200	0.204	0.961	1	0.327	0.819	0.550	1.221
Current site			6.866	5	0.231			
Mtubatuba (2)	-0.934	0.564	2.742	1	0.098	0.393	0.130	1.187
Cape Town (3)	-0.109	0.393	0.077	1	0.781	0.897	0.415	1.935
Bloemfontein (4)	-0.200	0.497	0.161	1	0.688	0.819	0.309	2.171
Mthatha (5)	-0.448	0.351	1.637	1	0.201	0.639	0.321	1.269
Phalaborwa (6)	0.948	0.439	4.670	1	0.031	0.388	0.164	0.916
$CD4^+$ count (twelve month updated)	0.000	0.000	1.983	1	0.159	1.000	1.000	1.001
Viral Suppression not <50 cp/mℓ	2.551	0.279	83.762	1	**0.000**	12.823	7.425	22.145
New concomitant chronic medication	0.686	0.397	2.991	1	0.084	1.986	0.913	4.324
ART toxicity	0.726	0.270	7.249	1	**0.007**	2.067	1.218	3.506
Inter-site transfer	0.369	0.447	0.682	1	0.409	0.691	0.288	1.661
Any missed visit in the first year	2.197	1.469	2.238	1	0.135	9.000	0.506	160.18

Table 11 and 12 above: the 95 % Confidence Interval[1] for the upper and lower limit for the mean gives an estimate of how much uncertainty there is in the precision of the estimate of the mean. The narrower the interval, the more precise the estimate and vice versa. The beta value is supposed to lie between the upper and lower limit. Confidence interval consists of a range of values that act as a good estimate of the unknown population parameter. To obtain a more precise estimate for the mean, the sample size would need to be increased.

4.4 SWITCHING TO SECOND-LINE TREATMENT

Among the total of 253 patients meeting either the early or late virologic failure definition, only 11.5% (29/253) were switched from first-line treatment to second-line PI based ART. However, owing to the small number of cases (i.e. n<30), the model suffers from consistency[2] and as such we cannot make meaningful inferences on the resulting regression results owing to a number of potential problems including biased estimates[3]. For this reason as well as the advice of my supervisor, we will not present an analysis of the predictors of the switch to second-line treatment.

Table 13 displays association of variables in a Cox proportional hazards model with switching to second-line therapy.

[1] A Confidence interval consists of a range of values that act as a good estimate of the unknown population parameter. A more precise estimate for the mean can be obtained the bigger the sample size.
[2] Consistency means that, as the sample size increases, the sampling distribution of the estimator becomes increasingly concentrated at the true parameter value which implies that the estimator will be unbiased.
[3] An estimator is biased if, on average, it fails to hit the true parameter value or if the estimated parameter is not equal to the true population parameter.

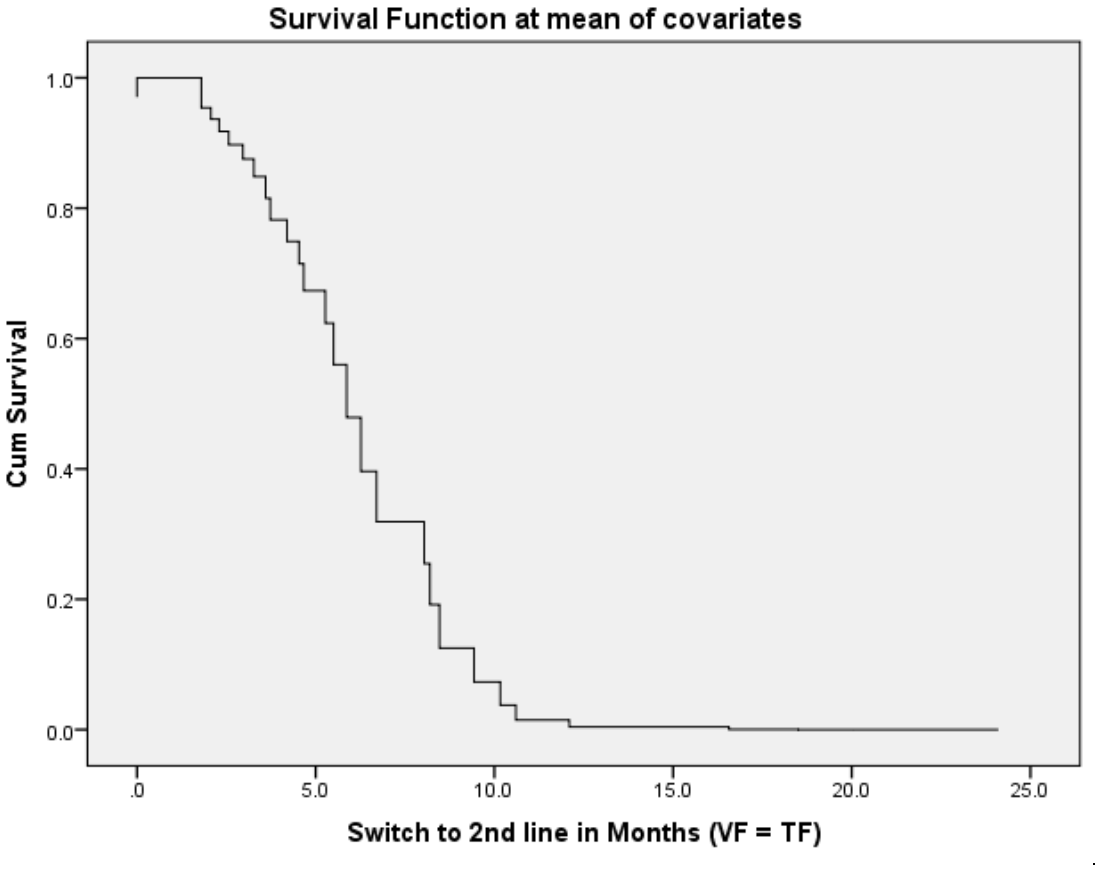


Figure 8: Kaplan Meier curve indicating the time course for patients on ART treatment switching to a second-line PI based ART after first-line treatment failure.

TABLE 13: COX PROPORTIONAL HAZARD MODELS: SWITCH TO SECOND-LINE PROTEASE INHIBITOR BASED ART

Variables in the Equation

		B	SE(β)	Wald	Df		Exp(β)	95.0% CI for	
								Lower	Upper
Step 1	log (VL at Failure)	0.318	0.130	6.030	1	0.014	1.375	1.066	1.772
Step 2	log (VL at Failure)	0.755	0.200	14.261	1	0.000	2.128	1.438	3.149
	Current site			14.542	5	0.013			
	Mtubatuba(2)	-1.058	0.892	1.407	1	0.236	0.347	0.060	1.994
	Cape Town(3)	1.570	0.783	4.025	1	0.045	4.806	1.037	22.279
	Bloemfontein(4)	1.252	0.741	2.852	1	0.091	3.497	0.818	14.952
	Mthatha(5)	2.176	0.649	11.245	1	0.001	8.807	2.469	31.409
	Phalaborwa(6)	-0.799	0.822	0.944	1	0.331	0.450	0.090	2.253
Step 3	Calendar year art failure	0.825	0.379	4.746	1	0.029	2.281	1.086	4.790
	log (VL at Failure)	0.767	0.217	12.463	1	0.000	2.154	1.407	3.298
	Current site			15.493	5	0.008			
	Mtubatuba(2)	-1.472	0.932	2.495	1	0.114	0.230	0.037	1.425
	Cape Town(3)	1.976	0.836	5.585	1	0.018	7.210	1.401	37.109
	Bloemfontein(4)	1.357	0.754	3.236	1	0.072	3.886	0.886	17.050
	Mthatha(5)	1.769	0.670	6.968	1	0.008	5.864	1.577	21.804
	Mthatha(6)	-1.930	0.983	3.855	1	0.050	0.145	0.021	0.997
Step 4	Calendar year art failure	0.767	0.390	3.872	1	0.049	2.153	1.003	4.620
	log (VL at Failure)	0.652	0.219	8.896	1	0.003	1.919	1.250	2.945
	TB	1.221	0.723	2.853	1	0.091	3.391	0.822	13.989
	Current site			16.508	5	0.006			
	Mtubatuba(2)	-1.592	0.943	2.853	1	0.091	0.203	0.032	1.291
	Cape Town(3)	2.296	0.881	6.796	1	0.009	9.934	1.768	55.817
	Bloemfontein(4)	1.349	0.760	3.151	1	0.076	3.853	0.869	17.083
	Mthatha(5)	1.543	0.667	5.346	1	0.021	4.677	1.265	17.294
	Phalaborwa(6)	-2.236	0.989	5.115	1	0.024	0.107	0.015	0.742

In Table 13 above, a stepwise approach was done.
Variable entered at Step 1 was log (VL at failure)
Variables entered at Step 2: Current site
Variables entered at Step 3: Calendar year ART failure
Variable entered at Step 4: Active TB

4.5 LIMITATIONS

The information contained in the medical records is only as accurate as the person/s that entered it into the spread sheet. Similarly, this study was based on the availability of laboratory results at periodic intervals; hence unavailability of these results and information on the variables of interest in the sites' record would ultimately impact the results. Missing data for some patients would have had an impact on the results.

Reliable PMTCT data to be able to examine the role of single-dose NVP exposure was lacking.

The overall virologic failure rate was 253, yet the number of patients who were switched to second-line PI based therapy was only 29.

Assumptions of virologic failure were made in patients with single VL>1000 cp/mℓ after at least 6 months of ART followed by death where no second VL was available, resulting in uncertainty whether those patients represent true virologic failures or not.

No data with respect to an important potential predictor of Early Virologic Failure, estimated Glomerula Filtration Rate (eGFR) which is used to screen for and detect early kidney damage and to help diagnose chronic kidney disease, was collected during the initial Phidisa 1a trials.

CHAPTER 5

5.1 DISCUSSION

To the best of the investigators knowledge, this is the first study to investigate the predictors of early and late virologic failure among HIV positive individuals who are on ART regimens in a military setting in South Africa. The country is considered resource poor and this impact on the drugs that are available for ART and the routine testing that is carried out.

The study was conducted in a military setting under the auspices of Phidisa which was a joint collaboration between South Africa Military and the USA's Department of Defence and National Institute of Health. The study included data initially collected from 1925 participants but was reduced to 1285 participants prior to any analysis as the balance of participants were found to have incomplete data or did not match the inclusion criteria. Only 1285 participants met all inclusion, exclusion and data integrity criteria and reliable data had been captured. Due to the sample size and time over which the study was conducted this study has contributed a number of unique findings on the impact of ART for treatment of HIV positive individuals in South Africa.

For example, the current government recommendations (April 2015) are based on evidence supporting earlier switch from first line ART to second-line ART following confirmed virologic failure than was advocated in previous guidelines.

The distribution of patients per site were relatively skewed but this was due to the Phidisa trials being co-ordinated from Pretoria and that the 1 Military Hospital is the hospital most commonly used for referred medical cases in the military setting of South Africa. Amongst the six Phidisa ART initiating sites, 1 Military Hospital had almost double the number of participants than any of the other sites.

There were more males subjects in the study which is not unexpected as the study was conducted in a military setting which is male dominant. The majority of patients had achieved a high school education level and a higher proportion of patients were married. Both males and females showed late initiation of ART treatment even though the guidelines at the time recommended initiation of ART when $CD4^+$cell counts were below 200 cells/mm^3. The late initiation of ART resulted in a baseline median $CD4^+$ cell count of 149 cells/mm^3, where in an ideal situation the majority of patients would start ART with higher $CD4^+$ cell counts and present with a less advanced disease status. Starting ART with low $CD4^+$ cell counts has been shown to be associated with early mortality mainly caused by Immune Reconstitution Inflammatory Syndrome (IRIS), which appears after starting ART when the patient is showing advanced HIV disease status (WHO stage 4), with $CD4^+$ cell counts below 50 cells/mm^3. $CD4^+$ cell counts of these low levels were evident in some of the patients who were included in the Phidisa studies and included in this study as part of the cohort.

Among the different ART regimens that were used in the Phidisa studies and then assessed in this study, the highest number of patients were initially on the combination of EFV+D4T+3TC, mainly due to the previous ART guidelines used by the Department of Health prior to 2010. In 2010 the ART guidelines were revised and updated and the use of

D4T on new patients was discouraged due to growing evidence of D4T toxicities. As noted in the descriptive analysis Table in the appendix, EFV+TDF+3TC is the ART regimen with the highest number of participants' recruited post-2010 in accordance with the updated Department of Health guidelines for ART. This regimen was then considered regimen 1a by the South African Department of Health and became more readily available, with once daily dosing, convenience and better patient compliance and tolerance.

The HIV/AIDS treatment approach has significantly changed since this study was conducted. In 2015, the WHO published new guidelines recommending that anyone infected with HIV should begin antiretroviral treatment as soon after diagnosis as possible. This 'treat all' recommendation responded to findings from clinical trial (The INSIGHT START Study group 2015) confirming that early use of antiretroviral keeps people living with HIV healthier and reduces the risk of transmitting the virus to others.

While a cure for HIV would be the best possible outcome, a vaccine to protect against the virus was entered in a clinical trial in South Africa in July 2016. The trial, called HVTN 702, will run over the course of three years in South Africa, across four sites receiving the experimental drug. The researchers are confident that the vaccine will be effective. Noting that the South African HIV/AIDS government guidelines have recently (September 2016) adopted the WHO guidelines of initiating patient treatment as soon as they test positive for the HIV virus.

It would be interesting to investigate the determinants of EVF, LVF and the switch to second-line therapy when patients initiate treatment as soon as they test positive for the HIV virus in the future, even when their $CD4^+$ cell count shows a high value. However, this study is still relevant in terms of initiating ARV treatment regimen, prevention, viral load and $CD4^+$ cell count monitoring. One factor that stood out in this study is the fact that patients initiated treatment when their $CD4^+$ cell count was 350 cells/mm^3 and below.

At the moment, research has shown that creating drugs with a lower dosage could potentially save the world billions of rands. Researchers have started to push Dolutegravir (an integrase inhibitor) to be part of the first-line ART regimen. The introduction of Dolutegravir drug will allow people to stay on first-line regimens for longer, allowing them to skip the complications that come with medication available for second and third-line regimen. Moreover, this would allow patients a more cost-effective treatment plan, as costs of the second and third-line regimen are very highly priced. When patients are taking Dolutegravir, they will benefit greatly in that they will be experiencing less side-effects, the size of the tablet will be smaller, as would be the dosage. While Efavirenz is generally a good drug, it causes disturbing psychological side effects in small percentage of people. There would also be a greater reduction level of resistance cases as compared with efavirenz.

5.2 STUDY FINDINGS AND COMPARISONS WITH OTHER STUDIES

At the onset, it is important to point out that this study intended to investigate possible predictors of early and late virologic failure as well as the predictors of the switch to second-line therapy. While there were enough cases to enable a statistical analysis of EVF and LVF, the same could not be said about the switch to second-line therapy model. As such, detailed analyses of the predictors of the switch to second-line therapy were not undertaken.

The following predictors were found to statistically explain EVF, namely being single; having higher prior levels of viral load; taking specific drug combinations of nucleoside reverse transcriptase inhibitors; having missed follow-up visits within six months of ART initiation and receiving treatment at rural sites of Mthatha and Phalaborwa.

Specifically, not missing follow up visits within six months of ART initiation; and taking 3TC+TDF and ZDV+3TC compared the drug combination of 3TC+D4T will significantly reduce the hazard of the subject experiencing EVF while being single; having higher prior levels of viral loads; and receiving treatment in the rural sites of Mthatha and Phalaborwa significantly increased the hazard of the subjects experiencing EVF.

On the other hand, the following variables were found to be significant predictors of LVF, namely; prior levels of viral load, receiving treatment at the rural site of Phalaborwa, subjects where viral load was not suppressed to below 50 cp/mℓ. All these predictors were statistically different from zero suggesting that the hypothesis that they are not predictors of LVF be rejected. Four other predictors; subjects having tertiary levels of education, low levels of $CD4^+$ count before ART initiation, missed visits before six months and receiving treatment at Mtubatuba sick bay were also found to have the hypothesized signs but were not statistically different from zero.

The present study may not necessarily be generalizable to all ART settings in South Africa, as the data was collected from a multi-site military setting with routine access to viral load measurements and better access to both clinical and financial resources compared with public health care settings in the same regions where the Phidisa study groups were living.

Some of the results may warrant further exposition. Ordinarily and given that the military provides the same standard of care to its personnel throughout, the location of where a patient was treated should not really make a difference particularly within a military setting.

It is important to note that of the five sites, 1 Military, 2 Military and 3 Military are referral hospitals offering high standard of care while the three other sites (Mtubatuba, Mthatha and Phalaborwa) are sickbays that nonetheless offer a superior level of care but would refer complicated cases to any of the three military hospitals. The fact that receiving treatment at some of the sickbays such as Mtubatuba and Phalaborwa increases the risk of subjects experiencing both EVF and LVF may be ascribed more to perceptions by patients about the perceived level of care received at sickbays. Phalaborwa sick bay for example, is located in the remote area and the personnel are often out in the bush due to being an active base.

The results, indicate that subjects who received their treatment at Phalaborwa experience both early and late virologic failure and the investigators speculate that the level of adherence may be comprised in certain instances due to delay in delivery of medication, transport issues (patients travelling long distances from home to the site). Patients may also perceive the level of care received from sickbays to be inferior as compared with that received from military hospitals, and we could speculate that this may have an impact on their levels of adherence to treatment. In that regard, current site may actually assume a status of or act as a proxy for patient adherence to treatment, a variable that was not measured explicitly in this study. To this end, the investigators could speculate that current site is a significant predictor not so much of the fact that there is a difference in the standard or level of care received by patients across the six different sites (in fact the level of care should have been exactly the same), but it is significant because patients perceive sickbays to be inferior to hospitals. This perception has been noted both in South Africa and the African continent where patients tend to associate primary health care facilities such as clinics and sickbays with inferior level of care and this tend to affect their level of adherence (see for example Mukora *et al*., 2011; Nabbuya-Sekandi *et al*., 2011; Becker *et al.*, 2012; Visser *et al*., 2015; and Ndou *et al.*, 2016).

5.3 CONCLUSIONS, RECOMMENDATIONS AND DIRECTIONS FOR FUTURE RESEARCH

To the best of the authors' knowledge, this study is the first of its kind in South Africa to investigate EVF, LVF and the switch to second-line ARV treatment using data from South Africa's military personnel from both urban and rural settings.

Treatment intervention may alleviate short-term problems but prevention interventions must have long lasting impacts. Education is imperative, values and beliefs are passed on from one person to another and generation to generation. Education enables individual empowerment, which is prerequisite of HIV prevention. Consequently, preventative intervention should be focussed on the very young, as they are more likely to learn and change than adults who may have already adopted certain ideas and values. Additionally, overcoming issues surrounding stigma is critical, as well as providing psychological support for patients and families.

HIV is no longer a new infection, and it cannot be ignored as it continues to take lives. It must continue to be a priority for every community, nation and region. Ignoring it can only cause serious damage. Awareness must be linked with adequate support to protect the affected. One of the greatest challenges is to maintain public interest in HIV/AIDS. Without public interest, public support will be lacking. Awareness must be linked with adequate support to protect the affected. Priority must be given to those who can amplify and transmit key messages, such as governmental leaders, representatives of media, religious and community leaders. They must continue to speak out and influence the public's attitude toward the situation and call for active involvement. Education and communication is vital, as it is through communication that people initiate, develop, maintain and change their attitude.

As with all empirical investigations, there will always be limitations in studies and this study is no exception. This is a retrospective observational study; there may be unmeasured underlying determinants that might have affected the final results. There were missing data, mainly due to patients being lost to follow-up. As such, in order to have a better understanding of the predictors of both EVF and LVF, it would be interesting to establish study outcomes if there could be better ways of managing the loss of patients during follow up as well as finding better ways of measuring important underlying predictors that the current study is unable to explicitly assess such as patient adherence which are otherwise important predictors.

Future guideline revisions should make explicit mention of the rational for the thresholds chosen to define and confirm virologic failure. Patients on the first-line cART for less than a year have high risk of virologic failure. A careful monitoring of patients particularly in the first three months of cART initiation is necessary and should include viral load at initiation and at regular intervals without requiring clinical symptoms to be evident before these tests are done. With enough commitment, political will and funding, we have the tools to avert the HIV epidemic since we live in a technologically advanced era.

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Appendices

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 20 Oct 2016.
- IRB 0000 2235 IORG0001762 Approved dd 13/04/2011 and Expires 13/04/2014.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

27/03/2014

Approval Certificate
New Application

Ethics Reference No 69/2014

Title PREDICTING EARLY AND LATE FIRST-LINE ANTIRETROVIRAL THERAPY VIROLOGIC FAILURE, AND SWITCH TO SECOND-LINE THERAPY IN A MILITARY POPULATION IN SOUTH AFRICA

Dear Ms SHUSHU MHANGWANE

The **New Application** as supported by documents specified in your cover letter for your research received on the 28/02/2014, was Provisionally approved by the Faculty of Health Sciences Research Ethics Committee on the 26/03/2014.

Please note the following about your ethics approval:
- Ethics Approval is valid for 4 years.
- Please remember to use your protocol number (**69/2014**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:
- The ethics approval is conditional on the receipt of 6 monthly written Progress Reports, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

Additional Conditions:
- Provisionally approved, pending approval from the MSc committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers; MBChB; MMed (Int); MPharMed.
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

♦ Tel:012-3541330 ♦ Fax:012-3541367 Fax2Email: 0866515924 ♦ E-Mail: fhsethics@up.ac.za
♦ Web: //www.healthethics-up.co.za ♦ H W Snyman Bld (South) Level 2-34 ♦ Private Bag x 323, Arcadia, Pta, S.A., 0007

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DEPARTMENT OFSTATISTICS

LETTER OF STATISTICAL SUPPORT

Date: 17 February 2014

This letter is to confirm that **SRC Mhangwane** of the University of Pretoria discussed the project with the title **PREDICTING EARLY AND LATE FIRST-LINE ANTIRETROVIRAL THERAPY VIROLOGIC FAILURE, AND SWITCH TO SECOND-LINE THERAPY IN A MILITARY POPULATION IN SOUTH AFRICA** with me.

I hereby confirm that I am aware of the project and also undertake to assist with the statistical analysis of the data generated from the project.

The estimated sample size of 1800 patient will allow the statistical analysis of the data.

The data analysis will consist of basic descriptive statistics (means, standard deviations, percentiles and proportions), as well as Kaplan-Meier curves to estimate the extent of virologic failure. Cox proportional hazard models will be utilized to evaluate the predictors of first-line virologic failure and of switching to second-line therapy.

L. Fletcher

Dr Lizelle Fletcher
Department of Statistics
Internal Consultation Service
Tel 012 420 3967

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health
National Institute of Allergy
and Infectious Diseases

DATE: 2/19/2008

TO: Michael Polis, M.D., M.P.H.
PRINCIPAL INVESTIGATOR

FROM: Chair, NIAID-IRB

APPROVAL LETTER

PROTOCOL NUMBER: 04-I-N096
PROTOCOL TITLE: Phidisa I: A Prospective Observational Cohort Study of HIV Infection (Both Treated and Untreated) and Risk-Related Co-Infections in the South African National Defence Force (SANDF)
MEETING DATES: 10 22/2007 and 11/19/2007
EXPIRATION DATE: 8/5/2008
ADULT RISK/BENEFIT CATEGORY: The research involves no more than minimal risk to subjects (45 CFR 46.102(h)(i)). / The research involves the prospect of direct benefit (45 CFR 46.102(h)(i)).
CHILD RISK CATEGORY: Research not involving greater than minimal risk (45 CFR 46.404).
REQUEST: Amendment and Response to Amendment

To address the current issues on the Phidisa project, the following changes are proposed: To amend Phidisa I to allow for the monitoring of both treated and untreated HIV-infected persons, especially to allow for the continued follow-up and laboratory support for persons currently enrolled on Phidisa II. After approval of this amendment by the NIAID and Phidisa IRBs, Phidisa II participants will have a close-out visit and have their monitoring continued under the Phidisa I protocol. All current Phidisa II participants will remain under the care of the Phidisa clinic. HIV infection and treatment will be monitored by the clinic staff. The project will continue to arrange for antiretroviral therapy to be provided for these participants as before, but the antiretroviral therapy will be guided by South African National Guidelines and not driven by protocol. This process should be relatively transparent to the participants on Phidisa II. The Phidisa II data set will be unblinded and analyzed. The amended Phidisa I protocol will serve as a platform from which to conduct new treatment substudies.

To initiate the above process, the following changes are proposed to the Phidisa I protocol:

Changes to the protocol:
- Title: To change the title to read, Phidisa I: A prospective observational cohort study of HIV infection (both treated and untreated) and risk-related co-infections in the South African National Defence Force;
- To remove the words epidemiologic and incidence throughout the protocol. The study will continue to enroll a selected population; we will be unable to determine incidence data from the population at large;
- Cover page: To update cover page address information for Major Marumo and Dr. Polis and removal of Drs. Andrew Ratsela and Nommso Stubbs;
- This an observational study with no treatment intervention. Administration of combined antiretroviral therapy (cART) consistent with the applicable South African treatment guidelines for HIV infection, will be made available to adult and pediatric participants in this protocol as specified. Language has been modified throughout the protocol to outline this change in the protocol both in the objectives and several sections through the protocol. Specific changes include: