

**Virological failure among adult HIV positive patients
three years after starting antiretroviral treatment at
Mankweng Hospital, Limpopo Province, RSA**

By

Matome Abel Lekoloana

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Supervisor:

Prof Nyazema NZ

Co-supervisor:

Mr Kekana MP

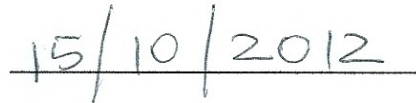
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DECLARATION

"I declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Public Health, has not previously been submitted by me for a degree at this university or any other university, that is my work in design and in execution, and that all the material contained herein has been duly acknowledged"

A handwritten signature in black ink, appearing to read 'Lekoloana MA', written over a horizontal line.

Lekoloana MA

A handwritten date '15/10/2012' written in black ink over a horizontal line.

Date

DEDICATION

This paper is dedicated to all HIV positive patients in the hope they all receive high quality care to optimize their health while patiently waiting for a cure.

ACKNOWLEDGEMENTS

To everyone who offered their support for the research, from Mankweng Hospital management and the Department of Family Medicine, all the research and ethics committees (University of Limpopo and Polokwane-Mankweng Hospital Complex), the Limpopo Department of Health, to the statisticians (Mr TS Ntuli and Mr MV Netshidzivhani) who patiently assisted whenever their expertise was sought. And last but not least, a special thank you to the team of Mr MP Kekana and Prof N Nyazema for their guidance from the beginning, through the challenges right till the end. Thank you all for indulging my interests.

ABSTRACT

Background: The main goal of HAART is to achieve maximal viral suppression. However, with poor adherence to therapy the chances of achieving and maintaining successful viral suppression are decreased, leading to virological failure. And virological failure has been recognized by WHO as one of the early warning signs of drug resistance. This operational research sought to explore virological failure as a treatment outcome to evaluate program performance at a facility level.

Methods: Purposive sampling as per inclusion and exclusion criteria was used to retrospectively review clinical records of the first 700 adult HIV positive patients (350 males and 350 females) who initiated antiretroviral treatment between April 2004 and December 2007 at this adult HIV clinic, were followed up for at least 3 years and treated according to the South African government's National Department of Health 2004 HIV treatment guidelines for adults and adolescents.

Major Results: 268 clinical records, 97 (27.71%) male and 171 (58.86%) female records were eligible for inclusion in the study. The proportion of females was higher (63.8%) than males (32.8%) with an average age of 38.95 years. 24 (8.9%) patients in the study sample experienced virological failure during the study period; 11 (11.3%) males and 13 (7.6%) females. Two-thirds (66.6%) of patients who failed to suppress at their first viral load measurement proceeded to develop virological failure. Overall, there was no association of statistical significance between age, sex, baseline CD4 cell count and baseline regimen, and virological failure at various intervals, $p > 0.05$.

Conclusion: It was a challenge to keep patients in care but those that remained in care had good treatment outcomes with only 8.9% developing virological failure. Failure to suppress at first viral load preceded virological failure in the majority of patients.

DEFINITION OF CONCEPTS

1. **Adherence** means adhering to a treatment regimen; for the patient it means, taking all the pills and doses in accordance with the manner prescribed by the doctor, and also means maintaining certain lifestyle patterns (e.g. stop smoking and alcohol intake), attending follow-up appointments, collecting all prescriptions, maintaining a healthy diet and other therapeutic behaviours like exercise (NDoH ART Guidelines 2004).

2. **Antiretroviral drugs** (ARVs) are drugs that interfere with the replication of retroviruses like HIV and are used to stop the progression of HIV disease by reducing the viral load and thereby allowing some recovery of the immune system. Commonly used ARVs are divided into fusion inhibitors, nucleoside reverse transcriptase inhibitors (NRTIs), non- nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs). They are also used in post-exposure prophylaxis after sexual assault and occupational exposure to potentially infected blood and bodily fluids, and for the prevention of mother to child transmission of HIV (Wilson 2004, p 329 – 334, Spencer 2005, p 14).

3. **CD4 T-lymphocyte cells** are immune cells central to and coordinating immune function. They are the primary target following HIV infection; used to assess prognosis for progression to AIDS or death (Bartlett et al 2008, p 16).

4. **HAART**- Highly Active Antiretroviral Treatment also called combination antiretroviral treatment (c-ART) involves the use of at least three different antiretroviral drugs with a backbone of two NRTIs and either a NNRTI or a PI, to fully suppress viral replication during HIV infection. A combination of three NRTIs has been demonstrated to possess inferior antiretroviral activity (Spencer 2005, p 14).

5. **HIV RNA** (ribonucleic acid) also called the viral load measures the concentration of free virus particles in the blood. This concentration predicts the rate of immune depletion and subsequent disease progression (Wilson 2004, p 44).

6. **HIV drug resistance** is the selection of viral mutations, due to inadequate drug levels, that decrease the susceptibility of HIV to known antiretroviral drugs (Abdool Karim & Abdool Karim, 2005 p 484; Bartlett et al 2008, p 46).
7. **Immunological response** is an increase in CD4 cell count by at least 50 – 120 cells/ μ l during the first 3 months on HAART and correlates with the duration of viral suppression (Bartlett et al 2008, p 47).
8. **Re-suppression** is a lower than detectable viral load while on the same HAART regimen following a virological rebound of greater than 1000 copies/ml (Hoffman et al 2009).
9. **Treatment failure** can be classified as clinical failure (new or recurrent AIDS-defining illnesses after at least 6 months of ART), immunological failure (fall to baseline CD4 level or 50% fall of CD4 cell count from treatment peak or levels of CD4 cells persistently less than 100 cells/ μ l) and virological failure (a persistent increase in viral load more than 5000 copies/ml on ART (NDoH 2004 ART guidelines).
10. **Virological response (Optimal viral suppression)** is described as the decrease in viral load less than 50copies/ml by 24 weeks or a sustained suppression of viral load of less than 50copies/ml (Spencer 2004). In the 2004 South African ART guidelines viral loads are performed every 6 months. Some authorities regard a viral load of less than 400copies/ml as optimal response, but it is good practice to strive for lower than detectable of lower than 50copies/ml viral loads.
11. **Virological failure** is a sustained increase in viral load of more than 5000copies/ml at least 3 months apart in a patient on HAART for at least 6 months (NDoH ART guidelines 2004).
12. **Viral or virological rebound/ breakthrough** is a detectable viral load of greater than 1000copies/ml after an optimally suppressed viral load.
13. **Viral blip** is a transient increase in viral load less than 1000copies/ml preceded and followed by viral loads less than 50 copies/ml or lower than detectable viral load.

14. **Viraemia** refers to a detectable plasma viral load irrespective of whether or not one is on HAART .

LIST OF ABBREVIATIONS

AIDS – Acquired immunodeficiency syndrome

ART – Antiretroviral treatment

HAART – Highly active antiretroviral treatment

HIV – Human immunodeficiency virus

NRTI – Nucleoside reverse transcriptase inhibitor

NNRTI – Non-nucleoside reverse transcriptase inhibitor

RNA – Ribonucleic acid

UNAIDS – United Nations organization on AIDS

WHO – World Health organization

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CHAPTER 1

This chapter gives a background to the study, a summary of what is generally known about the topic, why the study was done and what it seeks to achieve.

1.1 INTRODUCTION

Highly active antiretroviral treatment (HAART) involves the use of at least three different antiretroviral drugs, in combination, with the goal of achieving maximal viral suppression (Spencer, 2005). However, if HIV positive patients do not adhere to therapy, they decrease their chances of maintaining successful viral suppression leading to increase in viral load in the presence of HAART, a phenomenon called virological failure (Wilson et al, 2004).

Virological failure has been recognised by WHO as one of the early warning signs of drug resistance; and in this setting, more feasible to measure as a surrogate for resistance, especially since there is currently no provision for individual patient monitoring for resistance testing in the South African HIV treatment guidelines (WHO, 2003; Bartlett et al, 2008).

The prospect of resistance not only makes individual patients vulnerable to HIV strains that are unresponsive to available treatment; it also raises a spectre of a public health threat that could potentially neutralise the collective benefit of these therapies, especially if new infections occur with HIV viral strains which already acquired genetic resistance characteristics against actual therapies (Hecht et al, 1998). And the paucity of resources and the massive numbers of patients eligible for antiretroviral therapy in South Africa makes the development of wide spread resistance a valid concern (Abdool Karim & Abdool Karim, 2005).

Where HAART has been made available to HIV-infected populations there have been dramatic decreases in rates of mortality and morbidity, improved quality of life and changed perceptions of HIV infection from that of a death sentence to a manageable chronic illness (Abdool Karim & Abdool Karim, 2005). High levels of viral replication lead to the destruction of most facets of the immune system with subsequent acquisition or

development of opportunistic infections and/or malignancies; while HAART suppresses viral replication halts disease progression and allows host immunity to return to a more normal state (Wilson et al, 2004).

However, some patients experience increases in viral load in the presence of HAART, often resulting in the selection of resistant viral mutations with subsequent disease progression (Deeks, 2003). This failure to achieve or maintain viral suppression is called virological failure and the initial approach to virological failure is to assess adherence (Joly & Yeni, 2000). It is reported that the incidence of virological failure is only 2,4% with 95% adherence and rises to 17,4% with 75% adherence because inconsistent adherence leads to reduced drug levels which allows HIV to acquire resistance to all known classes of antiretroviral drugs(in use at the time) with notorious ease; and the sexual transmission of resistant virus is well established (Bhattacharya & Oman, 2009; Abdool Karim & Abdool Karim, 2005).

Along with non-adherence, several other factors also contribute to sub-optimal drug exposure, namely: altered drug metabolism, the presence of tissue sanctuaries with poor drug penetration, poor drug absorption, drug-drug interactions or drug toxicities, while complicated dosing schedules and a high pill-burden of antiretroviral drugs and concurrent medications may also have a negative impact on adherence (Deeks, 2003; Bhattacharya & Oman, 2009). Each of the factors that contribute to failure of an initial regimen need to be addressed before a successful response to any subsequent regimen can be certain; especially since the first antiretroviral treatment regimen offers the best chance of optimal viral suppression (Deeks, 2003).

For this reason, the process of initiating antiretroviral therapy involves assessing the patient's readiness to commence therapy and an understanding of its implications [life-long therapy, high-level adherence, toxicities]; and the South African government's antiretroviral treatment roll-out plan incorporates a compulsory drug readiness programme into decisions for starting treatment which requires education on HIV/AIDS, antiretroviral treatment and its side-effects and the importance of adherence (WHO, 2006; Gebrekristos et al, 2005).

In order to monitor response to antiretroviral treatment and identify patients with virological failure, the 2004 South African National Department of Health antiretroviral treatment guidelines for the management of HIV and AIDS require that viral load measurements be conducted at six monthly intervals from the start of therapy. It is through operational research assessing various treatment outcomes and their time of occurrence that may assist with the evaluation of program performance at a facility level and probably even propose ways of improving them.

1.2 RESEARCH PROBLEM

The South African ART program is still in its fairly early stages and the problem of virological failure and viral resistance should be monitored closely, recognised early and corrective measures be instituted promptly. Phillips et al (2007) observed that extensive virological failure of the three main classes of drugs occurs slowly in routine clinical practice; while antiretroviral drug resistant virus was detected in more than 80% of South African patients who experienced failure of a first antiretroviral treatment regimen (Marconi et al, 2008).

Local studies were conducted at various facilities to assess treatment outcomes in different parts of the country: Western Cape, Free State, Kwazulu-Natal and Gauteng Provinces in both the public and private sector with enviable suppression rates sometimes above 90%, but there were limited studies assessing treatment outcomes found in the literature search for any ART facility in the Limpopo Province. In a guidance set by WHO, one of the targets for antiretroviral therapy programmes in resource-limited countries is a virological suppression rate of greater than 70% at one year (Barth et al, 2010).

While embarking on antiretroviral therapy scale-up, wider surveillance is warranted to monitor program quality and limit the development of drug resistance, which remains a major potential challenge for the future of antiretroviral treatment in Africa (Maldonado et al, 2009). But this can only be done within available resources.

In Mankweng Hospital, information about patient management is regularly recorded in the patients' clinical records but collation of such information to synthesise data for

analysis to monitor program performance is lacking; and it is the characterisation of treatment response that will inform future actions. The aim of the present study was therefore to assess virological response over a 3 year period, and then explore the problem of virological failure among adult HIV positive patients 3 years after starting antiretroviral treatment in Mankweng Hospital.

1.3 PURPOSE OF THE STUDY

To explore virological failure among adult HIV positive patients 3 years after starting antiretroviral treatment in Mankweng Hospital

1.4 OBJECTIVES OF THE STUDY

- To obtain the baseline characteristics of the HIV positive patients from their records
- To identify patients with severe drug toxicities and co-morbidities
- To determine the level of virological and immunological response of the patients
- To determine the prevalence of virological failure
- To identify the reasons for virological failure

1.5 RESEARCH QUESTIONS

- Who is experiencing virological failure among adult HIV positive patients 3 years after starting antiretroviral treatment in Mankweng Hospital?
- What is the prevalence of virological failure among adult HIV positive patients 3 years after starting antiretroviral treatment in Mankweng Hospital?
- What are some of the reasons for virological failure among adult HIV positive patients 3 years after starting antiretroviral treatment in Mankweng Hospital?

CHAPTER 2

This chapter presents a summary of the research work that has already been done in other parts of South Africa and the world around virological failure and other treatment outcomes of patients on HAART.

LITERATURE REVIEW

The efficacy of HAART in the suppression of plasma HIV-1 RNA to undetectable levels has been documented in clinical trials, however, the virological and immunological success rates reported from such trials may not be generalisable to the whole population of HIV-1 infected patients (Fätkeuheuer et al, 1997). Literature review for this study will focus mainly on virological response to HAART since achieving and maintaining viral suppression is the goal of therapy.

Several studies were conducted in townships across South Africa in which suppression rates at 12 months ranged from 84% to 100%, even systematic reviews reported suppression rates above 75% at 6 and 12 months but most of these studies had a follow-up duration of 3 years or less, highlighting the scarcity of long-term data (Keiser et al, 2008; Barth et al, 2010; Boulle et al, 2008; Coetzee et al, 2004; Bekker et al, 2006).

Most low-income and middle-income countries with high HIV burden have adapted the WHO treatment guidelines: a public health approach, to frame their national recommendations which focuses on maximising survival at the population level through standardised sequencing of the available antiretroviral drugs; as opposed to the highly individualised approach by resource rich countries (Keiser et al, 2008; Wallis et al, 2010). However, results from studies conducted in Europe (individualized approach) were comparable to those conducted locally and in neighbouring countries (standardized approach) in terms of treatment outcomes (ART-LINC and ART-CC 2006; Keiser et al, 2008; Lederberg et al, 2009; Ferradini et al, 2006; Maldonado et al, 2009). This effectively means that the provision of HAART in both settings yielded comparable results with no approach superior to the other.

An observational cohort study conducted on 7916 patients who started antiretroviral therapy with three or more drugs and followed up from the time therapy started until the last viral load measure showed that 167 patients developed extensive triple class failure (failure of the three sub-classes of NRTIs, NNRTIs and ritonavir-boosted PIs) during 27441 person-years follow-up. The study concluded that extensive virological failure of the three main classes of antiretroviral drugs occurred slowly in routine clinical practice (Phillips et al, 2007). The similar observation was made by both Barth et al (2010) and Coetzee et al (2004) who demonstrated declining viral suppression rates over time, supporting the above conclusion.

In contrast, Bekker et al (2006) observed maintenance of very high suppression rates over a three year period, even Boulle et al (2008) also reported that over 85% of adults tested had viral loads below 400 copies/ ml at 6 monthly durations until 4 years on ART.

In a Cape Town community-based ART service, it was observed during 760 person-years that of 929 ART-naive adults, a total of 893 (96%) remained on first-line therapy and 16 (1,7%) switched to second-line due to hypersensitivity reactions (n=9) or lactic acidosis (n=7). Virological breakthrough (viral load of >1000 copies/ml) occurred in 67 (7,2%) patients, but, following the use of targeted adherence intervention, virological failure was confirmed in just 20 (2,2%). Kaplan-Meier estimate at 32 months were 20% for virological breakthrough but only 5,6% for confirmed virological failure. The study concluded that although the follow-up time was limited, over 95% of individuals remained on first-line regimen using a combination of viral monitoring and a targeted adherence intervention (Orrell et al, 2007).

The most important long-term benefit of achieving a viral load < 50 copies/ml is that clonal sequence analyses show no viral evolution with resistance mutations at that level, and the implication is that there is no viral replication and little likelihood of developing resistance (Bartlett et al, 2008). However, incompletely suppressive therapy result in viremia (detectable virus particles in the blood) in the presence of HAART which if it persists can lead to virological failure and subsequent selection for resistance mutations, unless corrective measures are instituted (Bartlett et al, 2008).

A retrospective analysis was conducted in a workplace HIV Program in SA; of a cohort of 3727 patients, of the 1007 patients who developed viremia, 815 had subsequent RNA assays, and 331 (41%) of these re-suppressed without regimen switch. At identification of viremia, 45 (66%) of 68 patients had HIV-I drug resistance. Resistance was associated with a reduced probability of re-suppression; however, 50% of patients with NNRTI resistance re-suppressed while receiving an NNRTI. Findings support maximising first-line use while minimising risk of significant cross-resistance by implementing intensive adherence support and repeat HIV RNA testing 3 – 6 months after detecting viremia, with regimen switch only if viremia persists (Hoffmann et al, 2009).

In a case-control study conducted in the Western Cape to investigate factors associated with virologic treatment failure in the government antiretroviral therapy facilities, nevirapine in the initial ART regimen or for PMTCT, low CD4 at ART initiation (of less than 50 cells/ μ l) and treatment interruptions were all independently associated with virologic treatment failure (Dantay, 2010).

Further, the prevalence of resistance among patients from Kwazulu-Natal, South Africa was assessed following failure of their first HAART regimen. From January 2005 through August 2006, a total of 124 antiretroviral treated adults who experienced virological failure were enrolled. Virus samples from 83,5% of participants carried one or more resistance mutations. Dual class resistant virus was present in 64,3% of participants, and 2,6% had virus with triple class drug resistance. The study concluded that antiretroviral drug resistant virus was detected in more than 80% of South African patients who experienced virological failure of the first HAART regimen and suggested that continued surveillance of resistance patterns is warranted to guide selection of second-line regimens (Marconi et al, 2008).

The CD4 count typically increases by 50 cells/ μ l and greater at 4 to 8 weeks after viral suppression with HAART and then increases an additional 50 – 100 cells/ μ l per year thereafter; while the maximum antiviral effect is expected by 4 to 6 months. Since the CD4 response generally correlates with viral load suppression, sometimes, despite

good virological response, there may be an initial delay in CD 4 response that cannot be explained; and discordant results are common in both directions (Bartlett et al, 2008).

A cross-lagged regression analysis was conducted on a sample of 268 patients enrolled in a public sector ART program in the Free State Province, South Africa. It showed that after 24 months of ART, 76,4% were classified as treatment successes (viral load < 400 copies/ ml, CD4 200 cells/ μ l or greater), compared with 64,1 % at 12 months and 46,1% at 6 months. The proportion of patients not responding to treatment was 7,5% at 2 years (Wouters et al, 2009).

It is difficult in most facilities in resource-limited settings to make an accurate diagnosis of clinical (or aetiological) treatment failure because the possibilities for laboratory investigation are limited, notably; viral load measurements are not widely available and will remain restricted because of cost and accessibility (WHO, 2006 revision). The WHO recommends, however, that other factors be taken into consideration before switching antiretroviral therapy if treatment failure is suspected: timing (i.e. after a reasonable trial of the first-line therapy of 6 – 12 months), addressing and resolving adherence issues, waiting until the successful treatment of concurrent opportunistic infections, and excluding the possibility of immune reconstitution inflammatory syndrome (WHO, 2006 revision).

Evidence of national databases suggests that a substantial proportion of patients in low and middle income countries do not switch therapy early enough despite experiencing virological failure. In an assessment of data from 62 Médecins Sans Frontières programmes, 48338 adult patients followed on first line antiretroviral therapy, only 370 switched to a second line regimen after 20 months (Pujades-Rodriguez et al, 2008). If patients are left on a failing regimen for too long they increase their chance of developing viral resistance, sometimes even to an entire class of antiretroviral drugs due to accumulating mutations.

Recent data from the TREAT Asia Observational Database showed that among 2446 patients who initiated antiretroviral therapy in rural Kenya, 447 developed treatment failure over 5697 person-years (7,8/100 person-years). Of these, 253 patients modified

at least one drug after failure, meaning that nearly half of the cohort remained on a failing antiretroviral therapy (Adero et al, 2008).

Recent data from the national ART programme in Malawi in over 100000 patients started on standard first-line therapy (lamivudine, stavudine and nevirapine) showed that, from 2004 to 2007, 64,9% of patients were kept alive on antiretroviral therapy; 96,4% on first-line regimen, 2,9% on first-line substitutions, with only 0,3% switching to second-line therapy (Jahn et al, 2008) .

Intermittent viral blips with transient HIV-RNA levels above detection limits do occur in patients with apparently suppressive treatment, and several factors contribute to this, namely: residual viral replication in the presence of HAART, virus released from the reservoir of latently infected CD4 T cells, and other possible fluid, anatomical and cellular compartments that have reduced drug penetrance. And, latently infected CD4 cells can rekindle productive viral infection when treatment is withdrawn . . . , the latent reservoir, in resting CD4 cells as pro-viral DNA, has been considered as the major obstacle to viral eradication (Rong & Perelson, 2009; Bhattacharya & Oman, 2009).

The characteristics of viral blips were examined by intensive sampling of 10 patients over a period of 90 days. In the sample, blips were common (9 out of 10 patients), brief in duration (median less than 3 days), low in magnitude (median 79 copies/ml) and poorly reproducible on independent testing. Moreover, blip frequency was not related to illness, vaccination or drug concentration. These observations suggest that random biological or statistical variation around a mean viral load of less than 50copies/ml might be responsible for the aberrant viral load measurements observed (Nettles et al, 2005).

Macias et al (2005) found that viral blips (intermittent relapses of viremia of less than 1000 copies/ml) were not associated with virological failure after a median follow-up of two years; however, transient rebounds of HIV plasma viremia (viral load equal or greater than 1000 copies/ml) were associated with the emergence of drug resistance mutations in patients on HAART. Possible explanations were suggested for the suppression of genotypically drug resistant HIV by HAART. First, HIV strains with multiple mutations that confer resistance to individual antiretroviral drugs may still be

phenotypically sensitive to a combination of those drugs. Second, multidrug resistant variants could have a compromised capacity to replicate. And thirdly, some patients harbouring drug resistant mutants could have hyper-susceptibility to certain NRTIs.

Research to assess treatment outcomes in HIV management is performed differently, depending on the setting, methodology, endpoints, resources and the level of expertise among other things. But since the goal of HAART is to achieve maximal viral suppression, virological failure will remain one of the most important treatment outcomes to monitor especially in resource limited settings where viral resistance testing remain largely unavailable. It is also pertinent that events surrounding this important outcome are well understood.

CHAPTER 3

This chapter reports on how the study was actually conducted, from study design, site, sampling right through to data collection and analysis.

RESEARCH METHODOLOGY

3.1 RESEARCH DESIGN

A retrospective review of clinical records of a cohort of patients who were followed up at the adult HIV clinic in Mankweng Hospital was conducted.

3.2 STUDY SITE

The study was conducted at Mankweng Hospital which is situated in the Capricorn district of Limpopo Province and about 32 kilometers east of the city of Polokwane, roughly 2 kilometers from the University of Limpopo, Turfloop Campus. The hospital has a catchment area with an estimated population of over 210000, in sparsely distributed, poverty stricken largely rural 18 wards, serviced by 19 primary health care clinics which make up the Mankweng area (Stats SA 2001). Mankweng hospital was the only government accredited antiretroviral treatment roll-out site where all treatment eligible patients were initiated on highly active antiretroviral therapy in this region during the study period.

3.3 RESEARCH POPULATION

The study population comprised of clinical records of the first 700 adult HIV positive patients (350 males and 350 females) who enrolled at the clinic for initiation of antiretroviral treatment between April 2004 and December 2007 at Mankweng Hospital's adult HIV clinic and were followed up at the clinic for at least 3 years; they also had to be treated according to the South African government's National Department of Health 2004 HIV treatment guidelines for adults and adolescents.

The standardized clinical records were completed upon enrolment and updated at each visit with manual filing of the results. See Appendix 3 for treatment regimens and follow-up schedules.

Patients' clinical records were filed separately for both males and females, and according to their time of enrolment at the clinic, for instance, F1 represented the first female patient to be enrolled at the clinic and similarly, M1 would represent the first male patient to be enrolled at the clinic. Patients would then be worked up, counseled and followed up until they are eligible or are initiated on ART. Once initiated, patients are followed up monthly and monitored for adherence to treatment, treatment of side-effects, the occurrence and treatment of opportunistic infections and response to treatment as per ART guidelines.

The clinic utilized a booking system whereby patients are given a review date for their next appointment which helped control the number of patients seen per day and assisted with monitoring compliance to follow-ups. Patients who missed their appointments were regarded as defaulters and their clinical records filed separately until they returned to care and started honoring their appointments.

3.4 SAMPLING

A homogenous purposive sampling technique was utilized to identify records that satisfied the inclusion criteria. The clinical records of the first 350 male and the first 350 female patients were sequentially reviewed for inclusion in the study. The clinical records of patients in care at the time of the study period were reviewed.

3.5 INCLUSION CRITERIA

Eligibility criteria for inclusion in the analysis

Patients who were at least 18 years old and treatment-naive at the time of enrolment at the clinic, honored all their follow-up appointments and initiated antiretroviral treatment at Mankweng Hospital's adult HIV clinic between April 2004 and December 2007 according to the South African government 2004 HIV treatment guidelines, and followed up for at least 3 years at the HIV clinic were included in the study.

3.6 EXCLUSION CRITERIA

Eligibility criteria for exclusion from analysis

Patients who were lost to follow up, inconsistent clinic attendance (not honoring appointments) and transferred out before 3 years of follow up or initiated ART elsewhere were not included in the final analysis in the study. Patients who did not use the clinic services for 6 consecutive months without being down-referred or transferred-out before at least three years of follow up were considered as lost to follow-up or defaulters and therefore not included in the study. Reasons for records being excluded from the final analysis were recorded. However, the excluded records were not reviewed.

3.7 DATA COLLECTION

The clinical records of the first 350 male and 350 female patients were sequentially reviewed and assessed for inclusion and exclusion as per set criteria above. Clinical records that met the inclusion criteria had their information recorded in the data collection tool (see appendix 1).

Baseline characteristics: unique identification number, age, sex, baseline CD4 cell count, baseline body weight, baseline haemoglobin measurement, WHO classification, initial ART regimen and date of initiation of ART.

Follow up characteristics: CD4 cell count, viral load, body weight and current regimen, were recorded at 6 monthly intervals or consecutively as measured to detect viral suppression, viral rebound, virological failure, a viral blip or re-suppression and immunological response.

Endpoints were recorded along with time of occurrence in relation to time of ART start.

Endpoints (as applicable in this study)

Major outcome was **virological failure** with subsequent regimen change. Secondary outcomes include:

- **Virological suppression** (a viral load of lower than detectable or at least a viral load of less than 400 copies/ml six months after a initiating a regimen)

- **Failure to suppress** (a viral load of 1000 or more copies/ml at six months after ART initiation)
- **Suboptimal viral suppression** (a viral load of ≥ 400 copies/ml but less than 1000 copies/ml at six months after ART initiation)
- **Viral rebound** (a detectable viral load of more than 1000 copies/ml in a patient who has been on ART for more than 6 months and was previously suppressed, it was classified as more or less than 5000 copies/ml in this study)
- **Virological failure** (a viral load of more than 5000 copies/ml on two or more consecutive occasions, usually followed by regimen change)
- **Viral blip** (a viral load of less than 1000 copies/ml in a patient who is on ART for more than 6 months)
- **Re-suppression** (a lower than detectable viral load, or viral load less than 400 copies/ml, following a detectable viral load greater than 1000 copies/ml)

3.8 DATA ANALYSIS

Data was captured using Microsoft excel and later coded with the assistance of a statistician. Data analysis was performed by Statistical Package for Social Sciences (IBM SPSS version 20). ANOVA and Fisher's exact test were used as appropriate to assess associations. Analysis was adjusted for baseline CD4 count, stage of disease, gender and age. Descriptive statistics like the mean, median, proportion, rates, frequencies, tables, graphs etc. were used to display and summarize the results.

3.9 ETHICAL CONSIDERATIONS

An ethical clearance certificate was received from the University of Limpopo (MEDUNSA)'s Research Ethics Committee (MREC) and the permission to collect data was granted by the Limpopo Provincial Department of Health. The proposal was further evaluated by the Polokwane-Mankweng Hospital Complex Ethics Committee which also issued an ethics clearance certificate and granted permission to use the files.

CHAPTER 4

This chapter presents a summary of the results and data analysis.

RESULTS

4.1 Baseline characteristics

From a population of 700 (350 male and 350 female) clinical records, purposive sampling as per inclusion and exclusion criteria identified 268 records to be included in the study with 97 (27.71%) and 171 (58.86%) female records selected for the study. Table 1 below summarizes the main reasons from the exclusion of most records. Note, the records of loss to follow up were filed separately in the filing room and were thus not reviewed.

Table1. The main reasons for records being ineligible for analysis in the study

Reason	Male.	Females
	N (%)	N (%)
1. Loss to follow up	230 (90.91)	137 (76.54)
2. History of default*	4 (1.58)	11 (6.14)
3. Transfer in	5 (1.98)	7 (3.91)
4. Transfer out	0 (0)	1 (0.56)
5. Other reason [#]	14 (5.53)	23 (12.85)
Total	253 (100)	179 (100)

*History of default but still in care; [#] Inadequate information in the file

Loss to follow up accounted for majority of exclusions, 90% and 76% of all exclusions in the males and females respectively. A total of 253 (72.3%) of male records were excluded from the analysis while 179 (51.1%) female records were excluded as shown in Table 1 above.

Table2. The proportion of patient records according to sex

Sex	Number (N)	Percentage (%)
Male	97	36.2
Female	171	63.8
Total	268	100

The proportion of females was higher (63.8%) than males (32.8%) in the study sample as shown in Table 2 above, with an overall average age of 38.95 years. Females in this study sample were younger with a mean age of 36.11±8.5; while males were older with a mean age of 41.79±9.5 as shown in Table 3 below. Also note that there was one missing value not recorded for age in one of the female records. The youngest patient was a female aged 19 years while the oldest patient was a male aged 65 years.

Table3. An analysis of the age of the study population according to sex

	N	Mean age	Standard deviation	Standard error	95% Confidence interval for mean		Minimum	Maximum
					Lower bound	Upper bound		
Male	97	41.79	9.584	.973	39.86	43.73	21	65
Female	170	36.11	8.596	.659	34.81	37.41	19	64
Total	267	38.18	9.359	.573	37.05	39.30	19	65

The females dominated the young adult (18 – 35 years) age group at 51.5% while the majority of males (59.8%) were in the older adult (36 – 59 years) age group, see Table4 below.

Table4. A breakdown of sex according to different age groups

		Age			Missing values	Total
		18 -35 years	36 -59 years	60 years and above		
Sex	Count	33	58	6		97
	Male % within sex	38.0%	59.8%	6.2%		100%
	Count	88	79	3	1	171
	Female % within sex	51.5%	46.2%	1.7%	0.6%	100%
Total	Count	121	137	9	1	268
	% within sex	45.1%	51.1%	3.4%	0.4%	100%

The average baseline CD4 count was comparable for both sexes with an overall average of 114.38cells/ μ l, as shown in Table 5 below. So was the minimum and maximum CD4 count for both sexes which was 3cells/ μ l and 281cells/ μ l respectively for females and 4cells/ μ l and 270cells/ μ l respectively for males. There were more males than females (6.2% versus 1.7%) in the elderly group of 60 years and above.

Table 5. The average baseline CD4 count and body weight for both sexes

	N	Mean	Standard Deviation	Standard Error	95% Confidence Interval for Mean	95% Confidence Interval for Mean
					Lower bound	Upper bound
Male CD4 count	96	114.05	61.720	6.299	101.55	126.56
Female	167	114.57	60.977	4.719	105.26	123.89
Average	263 (Total)	114.38	61.132	3.770	106.96	121.81
Male Body weight	96	58.289	10.8278	1.1051	56.095	60.482
Female	167	54.967	12.7348	0.9855	53.021	56.913
Average	263 (Total)	56.179	12.1586	0.7497	54.703	57.656

The mean baseline body weight was also comparable for both sexes at overall average of 56.179 ± 0.7497 kg.

Table 6. The frequencies for CD4 count per category at baseline for both sexes

Sex	CD4 cell count category						Total
	0-50	51-100	101-150	151-200	>200	Missing	
Male	16	25	22	30	3	1	97
Female	32	38	46	41	10	4	171
Total (%)	48(18.0)	63(23.5)	68(25.4)	71(26.5)	13(4.8)	5(1.8)	268(100)

Very few patients presented with a preserved immune system with only 4.8% of the patients presenting with CD4 counts above 200 cells/ μ l; while on the other hand, 18 % of patients presented very late with CD4 counts of 50 cells/ μ l and below, as shown in Table 6 above, with a cumulative total of 41.5% of patients presenting with a CD4 cell count of less than 100 cells/ μ l.

Table 7. The frequency table for WHO clinical staging at enrolment

WHO Stage	Frequency (N)	Percent (%)	Cumulative Percent
Not recorded	160	59.7	59.7
Asymptomatic (WHO I)	1	0.4	60.1
Mild disease (WHO II)	12	4.5	64.6
Moderate Disease (WHO III)	67	25.0	89.6
Severe Disease (WHO IV)	28	10.4	100.0
Total	268	100.0	

Likewise, only 4.9% of patients presented with asymptomatic and mild disease, whereas 35.4% of patients presented with moderate and severe disease as

demonstrated in Table 7 above and Figure1 below. Note that in the majority of patients(59.7%) the WHO staging was not recorded.

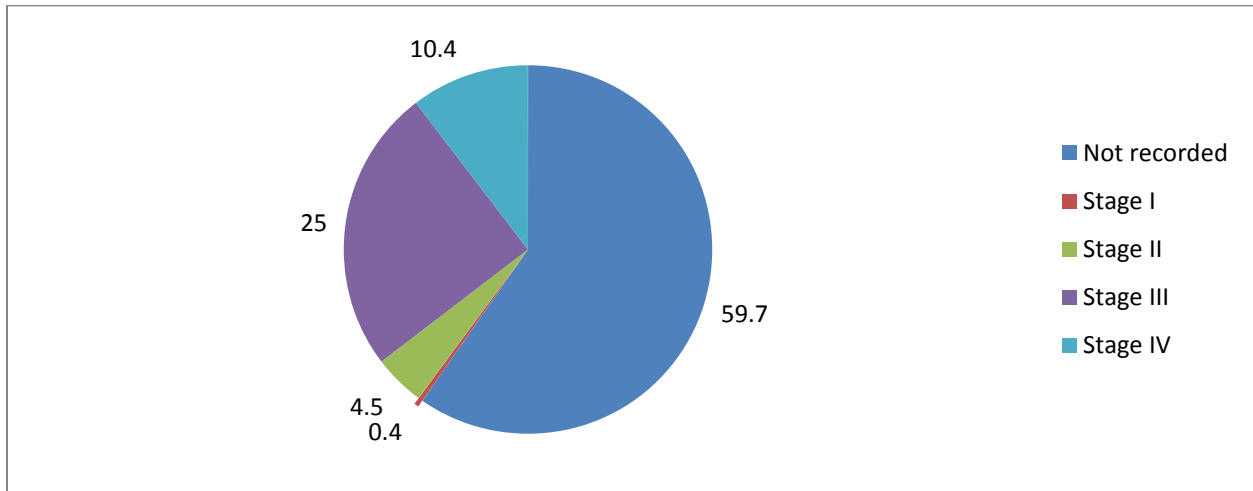


Figure1. The WHO clinical staging at baseline in percentages

The pie-chart above illustrates that in 59.7% of the patients the clinical staging was not recorded with only 0.4% presenting in WHO stage 1.

4.2 Regimens

Table 8.The frequency table for the choice of initial regimen

Regimen	Frequency (N)	Percent (%)	Cumulative Percent
d4T/3TC/EFV (1a)	246	91.8	91.8
d4T/3TC/NVP (1b)	22	8.2	100.0
Total	268	100.0	

Majority of patients were initiated on regimen 1a, as high as 91.8%, as shown in Table 8 above.

Table 9.The choice of initial regimen according to sex

Sex	Initial regimen		Total
	d4T/3TC/EFV (1a)	d4T/3TC/NVP (1b)	
Male	95	2	97
Female	151	20	171
Total	246	22	268

The majority of patients, 98% of males and 88% of females were initiated on regimen 1a. And, of the 8.2% of patients who were initiated on regimen 1b, 90.9% were females.

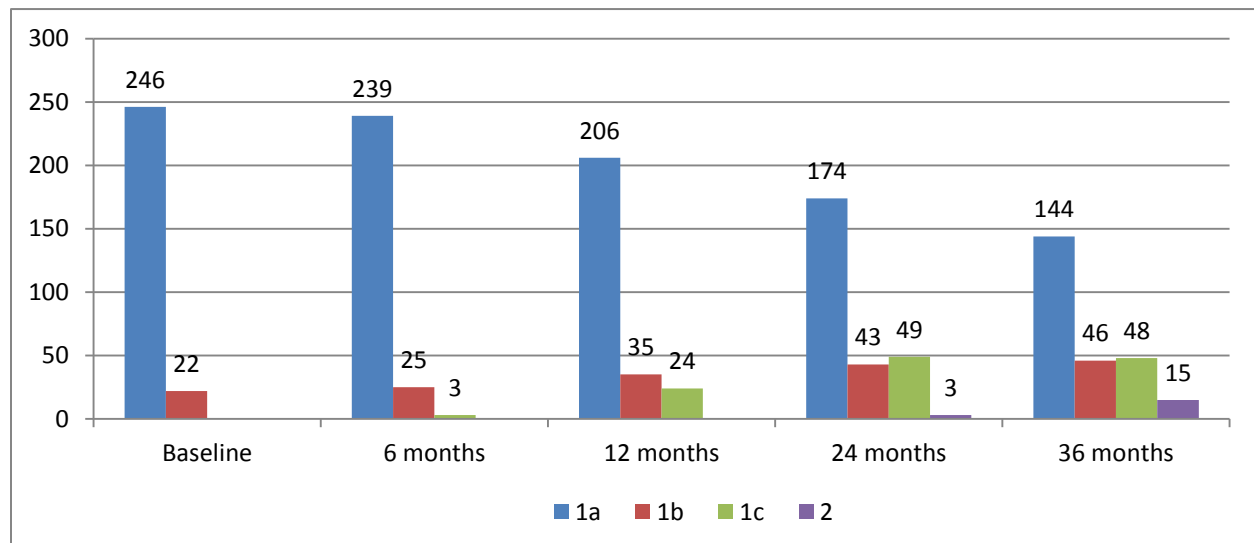


Figure2. The bar graph of regimen utilization over time

{The Regimens: 1a (d4T/3TC/EFV); 1b (d4T/3TC/NVP); 1c (AZT/3TC/EFV) and 2 (AZT/ddI/L/r)}

The utilization of regimen 1a declined gradually over time with stavudine (d4T) being the most commonly substituted drug as evidenced by the increased utilization of the AZT containing regimen from 3 (1.1%) at 6 months to 48 (17.9%) at 36 months, see Figure 2 above. There was also a gradual increase in the number of patients on regimen 1b to more than double the initial value. At 36 months there were 15 (5.6%) patients on second-line regimen. And below, Table 10 illustrates a breakdown of the reasons as documented in the clinical records for regimen change or drug switch.

The events in Table 10 below represent only those that were documented in the clinical records; there were regimen changes and drug switches that occurred without clear documentation or reason. For instance, 15 (5,6%) of patients were on regimen 2 at 36 months but the diagnosis of virological failure was documented in the clinical records of only 2 (0,7%) patients, see Figure 2 above. The reduced utilization of regimen 1a corresponds with the documented side effects of stavudine (d4T).

Table 10. Documented frequencies for drug toxicities, comorbidities and other significant events

Drug side effects (Frequency)	Other conditions (Frequency)
Peripheral neuropathy (34)	Pregnancy (21)
Unspecified Efavirenz side effects (6)	Psychiatric condition (4)
Lactic acidosis (7)	Child-wish (2)
Lipodystrophy (7)	Virological failure (2)
	Tuberculosis (9)

None of the female patients who fell pregnant during the study period experienced virological failure, in fact, they continued to suppress optimally even after the pregnancy.

4.3 Immunological response

The average CD4 count gain was calculated with the baseline CD4 cell count of 114.38 cells/ μ l as a reference point, i.e. CD4 count at 6, 12, 24 and 36 months minus the baseline CD4 count. Table 11 below shows that there was an average CD4 count gain from baseline of 51.93cells/ μ l at 6 months to 225.99cells/ μ l at 36 months. There was a steady CD4 cell count recovery throughout the study period with the most significant rise occurring between 24 and 36 months as shown in Table 11 below. There was on average about 10% of missing values.

Table 11 and Figure 3 below demonstrate progressive increases in CD4 cell counts throughout the study period. Overall, there was on average about 10% of missing values.

Table11. Immunological response over time

	CD4 cell count (cells/ μ l)			
	6 months	12 months	24 months	36 months
Count (N)	235	241	233	246
CD4 gain (cells/ μ l)	51.93	94.48	143.47	225.99
Missing values	33	27	35	22
Mean CD4 count (cells/ μ l)	166.31	208.86	257.85	340.37

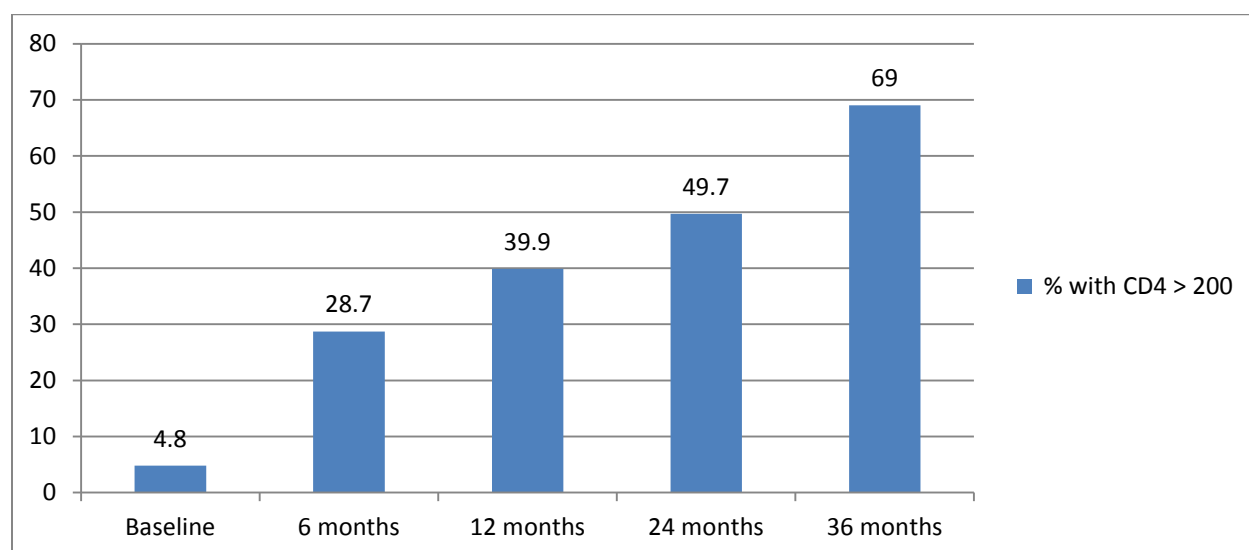


Figure3. Patients with CD4 cell counts above 200cells/ μ l

The proportion of patients with CD4 cell counts above 200cells/ μ l rose steadily from 4.8% at baseline to 69% at 36 months. Significant percentage increases were noted between baseline and the 6th month (23.9%) and later between the 24th and 36th months (19.3%). There were only \pm 10% increases at other intervals, see Figure 3 above.

4.4 Bodyweight response

The body weight increased from baseline until it peaked at 24 months and then seem to decline at 36 months as summarized in Table 12 below and illustrated by Figure 4.

Table12. The average weight gain over time

	Body weight			
	6months	12 months	24 months	36 months
Valid	248	244	244	238
Missing values	20	24	24	30
Average	5.20	6.15	6.28	5.81

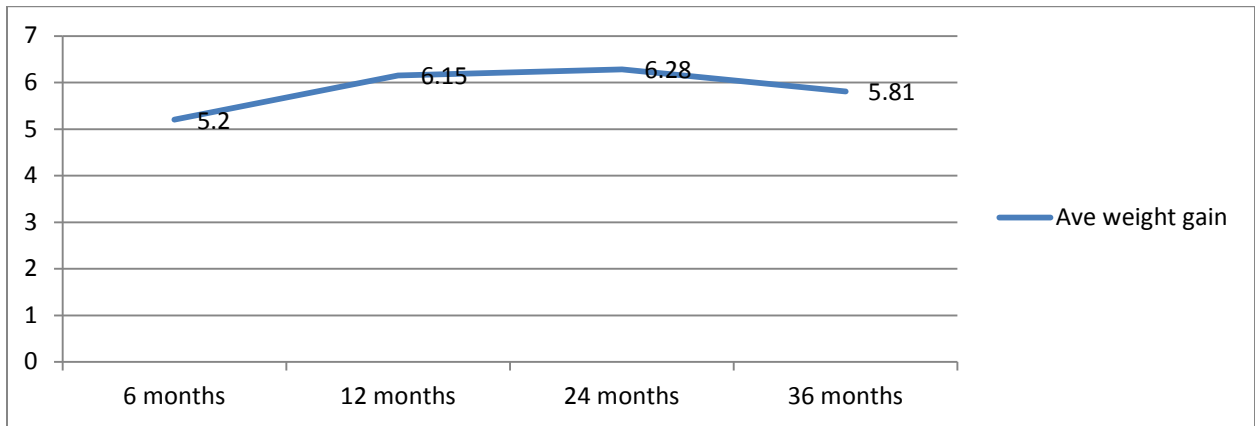


Figure4. The average weight gain in kilograms plotted over time

4.5 Virological response

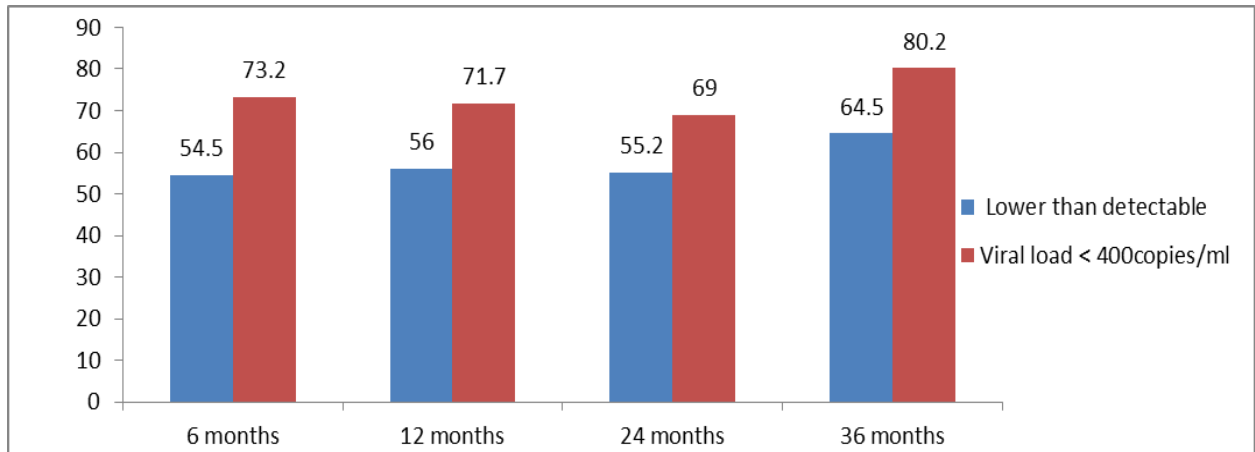


Figure5. Proportion of patients with optimal viral

suppression The occurrence of various endpoints was comparable for both sexes, except for **failure to suppress** which was higher for males at 14.4%

compared to 6.4% for females at 6 months. Also, overall 11.7% of patients in the older adult age group **failed to suppress** at 6 months compared to 7.4% of patients in the younger adult age group. However, majority of patients, between 69 and 80 %, managed to maintain optimal viral suppression with viral loads below 400copies/ml as shown in Figure5 above.

Patients achieving lower than detectable viral loads rose from 54.4% at 6 months to 64.5% at 36 months, these percentages improved markedly to 80% at 36months when viral load less than 400copies/ml was utilized as a measure of good adherence.

4.5.1 Viral blip

Table 13.The frequency table for the number of viral blips per patient

Number of blips per patient	Frequency (N)	Percentage (%)
1	92	34.3
2	54	20.1
3	11	4.1
4	5	1.9
>4	2	0.7
Total	164	61.1

164 (61.1%) patients experienced one or more episode of viral blip as shown in Table 13. About 1 in 5 patients developed two viral blips during the study period; while 1 in 3 patients developed one viral blip. Only 2 patients had virological failure preceded directly and only by viral blip.

4.5.2 Virological failure

24 (8.9%) patients of the study sample experienced virological failure during the 3 year period; 11 (11.3%) males and 13 (7.6%) females. Of these, 15 (62.5%) patients failed within 18 months. 14 (58.3%) patients who failed their first antiretroviral regimen failed to suppress (viral load > 1000 copies/ml) at 6 months; a further 2 (8.3%) patients who only had their first viral load at 12 months had viral loads above 1000 copies/ml

subsequently experienced virological failure. This means that two-thirds (66.6%) of patients of patients who failed to suppress at their first viral load measurement proceeded to develop virological failure.

And, of the 14 (58.3%) patients who failed to suppress at 6 months, 5 (20.8%) subsequently re-suppressed on the same regimen; while 4 (17%) patients appeared to fail even a second line regimen within 36 months. 15 (5.6%) of the study sample were switched to a second line regimen which constituted 62.5% of all patients who experienced virological failure. 13 (54.2%) of patients experiencing virological failure stayed on a failing regimen longer than it was necessary, 3 (12.5%) of which had a single drug switch.

Using Fisher's exact test, association was assessed between age, sex and baseline regimen, and virological failure at various intervals. Overall, there was no association of statistical significance between these parameters and virological failure at different intervals, $p > 0.05$. However, there was an association noted between baseline CD4 cell count and virological failure of statistical significance but only at 24 months, $p = 0.011$. The CD4 cell count categories were stratified into 1 – 50, 51 – 200 and >200 cells/ μ l; and 12.5%, 1.5% and 7.6% of patients respectively experienced virological failure at 24 months. Such an association could not be assessed for baseline WHO clinical staging and virological failure as in majority of patients (59.7%) clinical staging was not done or recorded.

None of the 21 patients who fell pregnant during the study period developed virological failure. But of the 9 patients who were treated for tuberculosis during the study period, 4 developed virological failure. Thus, 44% of patients treated for both tuberculosis and HIV at the same time developed virological failure.

No reason for virological failure was documented for any of the patients who experienced virological failure. In the two files where the diagnosis of virological failure was documented, the plan was to use condoms, re-adherence counseling three times (by an HIV/AIDS counselor) and a referral to the social worker with immediate change of a regimen.

CHAPTER 5

This chapter tries to explain the implications of the results presented in this study.

5.1 DISCUSSION

As ART continues to be scaled up in South Africa, with more Primary Health Care (PHC) facilities providing ART services, increasingly more efforts and resources need to be directed at ensuring that patients who continue to enroll at these facilities receive quality care to optimize their health. This operational research sought to identify treatment outcomes, mainly virological response, as a way to assess programme performance at this ART facility and propose recommendations that will form part of quality assurance.

In the first instance, there was a challenge of keeping HIV positive patients in care as a majority, 90.91% of male patients and 76.54% of female patients, were excluded from the final analysis in the study on the basis of loss to follow up. This left only 27.71% and 58.86% of male and female clinical records respectively, eligible for inclusion in the final analysis of the study. Research suggests that unascertained deaths and transfers may contribute substantially to loss to follow up (Lamb et al, 2012). This is a serious point of concern considering that the current National Strategic Plan (NSP) has as part of its goals that at least 70% of people receiving ART should be alive and still on treatment after five years (NSP 2012 – 2016).

Boulle et al remarked that locating care in primary care sites was associated with a good retention in care (Boulle et al, 2008). So with the unacceptably high rate of attrition observed in this study, the decentralization of ART services may provide some solutions to that problem. But the PHC facilities will need to be well supported on a continuous basis, both administratively and clinically in the form of mentoring if quality care is envisaged at that level. Also, updated patient held records (summaries or appointment cards) will also go a long way in ensuring continuity and good quality care as HIV positive patients are mobile and sometimes consult in various hospitals and health

facilities other than their usual point of care with no access to the patients' clinical records.

Since the start of the ART roll-out by the government of South Africa, strategies for recruiting more and more patients for ART care have been on the rise to this day, including the HIV Counseling and Testing (HCT) campaign, the decentralization of ART services to PHC facilities, fast tracking of selected groups of patients, increasing the CD4 cell count threshold for ART eligibility to 350cells/ μ l and so on. However, quality assurance strategies to ensure that ART-providing facilities are effective and efficient are sometimes not given as much attention at all these facilities except by some non-governmental organizations (NGOs) that form partnerships with the Department of Health (DoH). In fact, HIV clinical support service is supposed to be a competency of the DoH which should endeavor to empower and encourage hospitals to mentor and support feeder PHC facilities both directly and telephonically.

It has also been reported that the provision of free ART, food supplementation, high quality adherence support services and active (outreach) patient tracing improve retention in care following ART initiation (Lamb et al, 2012).

The public health significance of this high attrition raises concerns about outcomes of the patients lost to follow up. Questions of whether such patients are still alive or not, how many have actually died and of the patients still alive, what proportion is harboring drug resistant virus, remain unanswered. For instance, if the majority patients lost to follow up are alive, are harboring drug resistant virus and are sexually active, then new HIV infections in the community may start occurring with an already drug resistant virus. This will create a public health problem given the limited treatment options currently on offer. On the other hand, if the majority of these patients lost to follow up have died, then it raises concerns about program effectiveness in keeping people alive and in an optimum state of health.

So, perhaps some of the lessons to be learned from this operational study would have been found in the large numbers of patients lost to follow up. But this study sought to answer the question of what happens if patients stayed in care as expected in chronic

care offered by ART programs. After all, the goal of ART programs is not only to initiate patients on treatment but to also keep such patients in care for the longer term.

The proportion of the study sample was predominately females at 63.8% perhaps owing to a lower rate of attrition compared to their male counterparts mentioned earlier. Apart from the males being at a higher risk of being lost to follow up as shown in this study, there may be issues of health-seeking behavior and lifestyle among others, which are usually different for both sexes. There may be a question of how 'man-friendly' these ART providing facilities are, especially if in some clinics the majority of personnel may be female. These issues need closer attention in the form of research to better understand and address them appropriately.

The average age was also different with females being younger than males. In agreement with the widely publicized observation that the majority of females tend to be affected at a younger age than their male counterparts, perhaps owing to gender inequality and increased vulnerability among other issues. This is despite the similar age range for both sexes being between 19 and 65 years.

In fact, the UNAIDS reports that in the 15 to 24 years age group, HIV infection rates among females are twice as high as among males of the same age group (UNAIDS 2012). And the current NSP reports that young women between the ages 15 and 24 years are four times more likely to have HIV than males of the same age, and that on average, young females become HIV positive about five years earlier than males (NSP 2012 – 2016).

The mean baseline CD4 cell count was comparable for both sexes at 114.38cells/ μ l. It was however noted with concern that 18% of patients presented very late with CD4 cell count level of 50cells/ μ l and below, with a cumulative 41.5% presenting with a CD4 cell count level below 100cells/ μ l. Initiating ART at these very low CD4 count levels have been associated with poor outcomes, including immunological failure, that is partly why the eligibility criteria for ART initiation have been increased to a CD4 count level of 350cells/ μ l (Jevtovic et al, 2005; NSP 2012 – 2016).

The mean baseline body weight was also comparable for both sexes at 56.179 kg perhaps underscoring the late presentation with both sexes presenting at an already advanced stage of disease with some degree of wasting. Both the clinical and immunological criteria need to be adhered to in order to assess ART eligibility to ensure timely ART initiation of eligible HIV positive patients.

Also in almost 60% of patients the WHO clinical staging was not done. This raises concerns about the reliance on CD4 cell count (immunological criteria) as the main determinant for ART eligibility with limited attention being given to the clinical (WHO staging) indication. And this could lead to patients with AIDS defining illnesses being initiated late on ART with detrimental consequences. Less than 5% of patients presented with asymptomatic and mild disease, while as high as 34.5% presented with moderate and severe disease which also emphasizes the problem of late presentation.

It is important to strive for ART initiation in patients with a preserved immune system as chances of full immune recovery are enhanced with reduced risk of opportunistic infections and immune reconstitution inflammatory illness.

Regimen 1a was preferred as the initial regimen in both males and females, but its utilization tended to decline with time with 246 patients at baseline compared to 144 patients at 36 months representing about a 40 % drop over 3 years. The most common drug substituted was predictably stavudine (d4T), as shown in Table 10. And peripheral neuropathy was the commonest reported reason, in 34 patients, for switching d4T to the then only option of Zidovudine (AZT) followed by lipodystrophy and lactic acidosis at 7 apiece. The possibility exists of under-reporting and a delayed switch while treating symptomatically, which can disadvantage the patient as a delayed switch may lead to severe and even irreversible adverse effects.

Clinicians need to be pro-active and enquire about known side-effects of d4T as some patients may not readily report non-life threatening adverse events but respond positively when asked.

The introduction of the Tenofovir (TDF) in the first line regimen to substitute d4T was most welcome and will definitely improve the quality of the life of patients, at least until

the long-term side-effects of TDF are apparent. Lipodystrophy requires an observant clinician and a vigilant patient as it needs to be identified early because it is irreversible; while lactic acidosis calls for a high index of suspicion due to non-specific symptoms and its potentially life-threatening nature. Stavudine still warrants special mention because in some patients, both TDF and AZT maybe contradicted due to renal failure and anemia respectively, and temporarily d4T may be used until one of the parameters recovers and then a switch to a safer drug can be made.

With regard to immune response, there was a progressive increase in CD4 cell count over time showing good immune recovery with an average increase of 51.93cells/ μ l at 6 months rising to 225.99cells/ μ l at 36 month, increasing the number of patients with CD4 cell count levels above 200cells/ μ l from 4.8% at baseline to 69% at 36 months. It is widely documented that an improved CD4 cell count level is associated with a lower incidence of opportunistic infections, offering some protection from some of the most serious conditions seen in the severely immunosuppressed patients.

Although immune recovery generally correlates with good viral load suppression, it is by no means an accurate marker of treatment response as discordant responses to ART may occur in both directions (Barlett et al, 2008). This means that patients may experience poor increments in CD4 cell count despite good viral suppression or significant increments of CD4 cell count with poor viral suppression (Molina-Pinelo, 2005). In this study, however, there was generally a good correlation between viral suppression rates and CD4 cell count recovery, as a larger proportion (up to 80%) of patients suppressed to viral loads less than 400 copies/ml, a proportionately high percentage (up to 69%) attained CD4 cell counts above 200cells/ μ l.

And mechanisms proposed to clarify immune responses include lack of adherence to therapy, unfavorable pharmacokinetics, diminished fitness of the mutant viruses, inability of certain viruses to replicate in the human thymus, decreased cytopathic effect of the virus, increased half-life of the CD4 cells and a decreased T-cell apoptosis modulated by protease inhibitors (Molina-Pinelo, 2005). Jetvonic et al identified CD4 cell count below 100cells/ μ l and a combination ART regimen comprised of three nucleoside reverse transcriptase inhibitors (NRTIs) as risk factors for immunological

failure (Jetvonic et al, 2005). However, the good immunological response (with almost 70% of patients having CD4 cell counts above 200cells/ μ l at 36 months) was observed in this study despite 41.5% of patients presenting with CD4 cell count below 100cells/ μ l.

The viral load measurement is regarded as an accurate measure of treatment response as ART halts viral replication and allows CD4 cell count recovery, the latter of which depends mostly on the body's regenerative capacity. Suppression rates, or viral loads below 400copies/ml, were maintained around 70% in 70% of patients and peaked to 80% at 36 months, proving durability of ART regimens used in South Africa and a high standard of care provided at this clinic. This may mean that if most patients had stayed in care, adhered to a prescribed treatment regimen and honored all appointments, even higher viral suppression rates would have been achieved.

The significance of high suppression rates lies in the fact that patients who manage to suppress their viral loads to undetectable levels are less likely to develop drug resistant mutants and also have a reduced probability of transmitting the virus to others.

It is ideal to strive for an undetectable viral load, or viral load less than 50copies/ml, as at that level there is virtually no viral replication, making it almost impossible for the virus to select for resistant mutations and later resistance. But viral loads less than 400copies/ml are a good compromise as laboratory variability and the type of viral load assay and its limit of detection may influence the results. Also, a viral load below 400copies/ml may be perceived as just a variation about a mean of 50copies/ml but may require close monitoring and continued adherence counseling. More than 50% of patients maintained undetectable viral loads over time, peaking at 36 months with 64.5% of patients achieving undetectable viral loads which may indicate fairly high levels of adherence in the population under study.

Viral blips (viral load less than 1000copies/ml) were fairly common in this study with a total of 164 (61.1%) patients developing at least one viral blip. 92 (56%) patients developed one blip while 7 (4.3%) patients developed at least four viral blips (Table 13). Consistent with other studies, viral blips were observed to be common, brief in duration, low in magnitude and not reproducible on independent testing; moreover, viral blip

frequency was not related to illness, vaccination or drug concentration. And as mentioned above with viral loads less than 400copies/ml, these aberrant viral load measurements are considered to represent a random biological or statistical variation around a mean viral load less than 50copies/ml (Nettles, 2005). Viral blips were not observed to be associated with progression to virological failure as in only two patients was virological failure preceded directly and only by a viral blip.

Consistent with this study, in the assessment of emergence of new drug resistance mutations, Marcias et al observed that intermittent relapses of viraemia, which in this case was an equivalent of a viral blip, were not associated with virological failure after a median follow up of two years (Marcias et al, 2005). However, viral blips of high magnitude (> 400copies/ml) or progressively increasing magnitude warrant close monitoring as they may represent an early sign of poor adherence, the commonest cause of virological failure. Unlike with viral blip, transient rebounds of HIV plasma viraemia (viral rebound with viral load > 1000copies/ml) were found to be associated with the emergence of drug resistant mutations in patients on triple ART (Marcias et al, 2005).

And, at 6 months, 11.7% of older adults (36 – 59 years) failed to suppress to viral loads less than 400copies/ml, compared to 7.4% of younger adults; also, 14.4% of males failed to suppress at 6 months which was significantly (more than two times) higher than 6.4% of females. This is important since two-thirds (66.6%) of patients who failed to suppress at their first viral load measurement proceeded to develop virological failure. In Table1 male patients were shown to be more likely to be lost to follow up; and in Table4, males dominated the older adult age group. This may imply that males in this study population were more likely to develop virological failure.

If two thirds of patients who failed to suppress at their first viral load developed virological failure, and 62.5% of patients experience virological failure within 18 months of ART initiation, then there may be a worrying inability by the clinic to deal effectively with increased viral loads. And Hoffmann et al supported the strategy of maximizing first line regimen and avoiding cross-class drug resistance and premature switching to second line regimen by implementing intensive adherence support with any episode of

viremia identified while the patient is on ART (Hoffmann et al, 2009). This intensified adherence support should then be followed by a repeat viral load testing 3 – 6 months later to assess its effectiveness and the persistence of viremia, with regimen switch only if viremia persists. This strategy was incorporated in the SA 2004 National ART Guidelines although it seems poorly implemented at this clinic.

Targeted adherence intervention (also called intensive adherence support) often involves intensification of adherence counseling, probing to identify modifiable behaviors that may hamper adherence, increasing the frequency of clinic visits and pill counts, social worker consult to identify social barriers to adherence etc. Some clinics may allow one member of the treatment team, while others create a committee comprised of different members of the treatment team to conduct an interview with the patient to get to the bottom of the situation and get the patient to commit to correcting the adherence problems identified. The method chosen is usually one that is more appropriate to a particular setting but need to be standardized as well as flexible enough to cater for a wide range of possibilities.

Knowledge of local practices (like cleansing rituals or habits with induced vomiting or diarrhea, ingestion of untested herbal or traditional concoctions), substance abuse, especially alcohol, that may adversely affect adherence or the pharmacodynamics and pharmacokinetics of ART should be incorporated into all adherence counseling sessions including targeted adherence intervention. It is crucial to note that the role of the health care worker is to assist the patient to identify and appreciate activities that hamper adherence and to facilitate correction of any such practices or behaviors.

Of the 14 (58.3%) patients who failed to suppress at 6 months, 5 (20.8%) managed to re-suppress on the same regimen, probably due to poor adherence which was subsequently addressed or corrected. Also, 4 (17%) patients proceeded to fail even a second line regimen. If adherence issues are not identified and addressed, patients will go on to fail any subsequent regimen as time progresses. High level adherence is imperative for any patient taking ART to stand a chance of adequate viral suppression and halt disease progression because even with 95% adherence, the incidence of virological failure is 2.4% and rises to 17.4% with 75% adherence (Bhattachayra, 2009).

The difficulty in objectively and accurately measuring patient adherence to treatment in resource-limited settings requires that issues affecting adherence be thoroughly addressed during the pre-ART adherence counseling sessions and reinforced regularly during routine ART care. This strategy seeks to prevent poor adherence rather than deal with it only when it happens. Pre-ART readiness was incorporated in the SA 2004 national ART guidelines with certain conditions having to be satisfied prior to ART initiation; for instance, drug literacy aims to equip the patient with basic information about HIV, the benefits and possible side-effects of ART and the consequences of poor adherence among other things. All this is done to try and minimize the chances of poor adherence.

Virological failure among patients on ART should be avoided since antiretroviral drug resistant virus was detected in more than 80% of South African patients who experienced virological failure of the first HAART regimen (Marconi et al, 2008). And with limited treatment options on offer, drug resistance poses a real threat to the ART program as a whole. 20% of patients re-suppressed following failure to suppress at their first viral load testing while 17% continued to fail even a second line regimen. Possible explanations were suggested for the suppression of genotypically drug resistant HIV by triple ART: first, HIV strains with multiple mutations that confer resistance to individual antiretroviral drugs may still be phenotypically sensitive to a combination of those drugs; second, multidrug resistant variants could have a comprised capacity to replicate; and thirdly, some patients harbouring drug resistant mutants could have hyper-susceptibility to NRTIs (Marcias et al, 2005).

The prevalence of virological failure of 8.9% observed in this study signifies high suppression rates, even though there is room for improvement as 100% suppression rate is possible with a combination of an efficient ART service with motivated and therefore adherent patients.

When it comes to comorbidity, the fewer number of patients identified to be on TB treatment in the study may point towards other challenges of either under-diagnosis of TB or poor integration of HIV and TB services whereby TB patients are treated in another facility or service point (as opposed to under one roof) given the high co-

infection rates (50 – 80%) reported in the country. The other possible explanation may be that patients are first treated for TB and only initiate ART once TB treatment has been completed, the practice which is not supported by literature or the SA national ART guidelines and therefore highly discouraged.

The lack of documentation of the diagnosis of virological failure, the reason for virological failure and a definite plan to correct or intensify adherence support puts patients at risk of failing any current antiretroviral treatment regimen including a subsequent regimen. It should be noted that it is the responsibility of every member of the treatment team to emphasize adherence at each visit, even if it's just asking the patient about when and how medications are taken.

5.2 LIMITATIONS OF THE STUDY

Since it was an observational study, interventions and sometimes even causality could not be assessed. Another limitation relates to having limited options for statistical computation as some statistics best suited for prospective studies are not possible in retrospective studies. And like any other retrospective study, there is almost always missing data which may affect analysis. The high rate of attrition may have distorted the true picture of what really happening.

Selection bias as a result of the methodology as well as the inclusions and exclusion criteria may provide some bias on the results. The lack of universal definitions or a standard glossary of terms used to describe treatment outcomes posed a challenge, for instance, transient rebounds of HIV plasma viremia, viral rebound and viral breakthrough all meant a viral load of more than 1000copies/ml preceded by an optimal viral suppression. Information or misclassification bias was minimized by the use of definitions used by the South African national ART guidelines. Bias from loss to follow up could not be controlled but the design of the study was to select a uniform sample in which loss to follow up did not occur. The follow up period could have been longer but was limited due to the restrictions of resources (manpower and time).

5.3 CONCLUSION

It was a challenge to keep patients in care but those that remained in care had good treatment outcomes with only 8.9% developing virological failure proving the effectiveness of the program. Patients who fail to suppress at their first viral load testing require more attention in the form of targeted adherence support as they were more likely to develop virological failure. The decentralization of ART services to the PHC level with active patient tracing is expected to improve the retention in care while continuous administrative, logistic and clinical support is more likely to improve the quality of care. And as the South African national ART program aspires to initiate more people on treatment, an equivalent amount of effort should be directed towards improving and sustaining a high quality of care at all ART providing sites.

5.4 RECOMMENDATIONS

- ART providing facilities should be set up to allow for easy monitoring and evaluation of the program with **complete capturing of information** including all treatment outcomes.
- A **standard glossary of terms** to define concepts that will assist in the monitoring of the program is imperative, for instance loss to follow up (after how long can a patient be classified as loss to follow up in routine clinical patient management) and viremia (viral rebound, viral blip) and their significance.
- **Standard operating procedures**, to operationalize the national guidelines, with a clear step by step guide of the minimum activities to be completed before ART start, on routine ART care and what to do when certain outcomes occur. Especially since ART care is shifted to health care staff with less expertise.
- **Further research** will need to be commissioned on the **loss to follow-up** to determine how long they were in care and the treatment outcomes at the time of defaulting and the reasons for defaulting. Even a **longer study period** may bring some new insights into the programme performance. It is also imperative for the

province to encourage and support operational research in various other clinics so as to be able to assess province-wide programme performance. All ART facilities have to keep proper clinical case records to allow for monitoring and evaluation of their performance.

- The building of **local capacity** to provide continuous training, support and clinical mentorship. Empowering facilities by conducting in-service training and distributing good practices to all facilities.
- Strengthening and continued support of **counseling services** as they are central to the success of any ART program.
- Strengthening the **community-based services** and **outreach (patient tracer) services** to account for all the patients lost to follow up.
- **Good record-keeping** is pivotal for: routine care as it allows for continuity of care and for continuous monitoring and evaluation of the program which forms part of the quality assurance.

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APPENDICES

Appendix A

Table A1. Baseline Characteristics

Unique patient Id	
Age	
Sex	
Baseline CD4 cell count	
Baseline Body weight	
Stage of disease	
Initial regimen	
Haemoglobin	

Table A2. Follow-up characteristics

	CD4 count	cell	Viral load	Body weight	Current Regimen	*Outcome/Endpoint
6 months						
12 months						
18 months						
24 months						
30 months						
36 months						

*Virological failure, Virological suppression, Viral rebound, Viral blip, Re-suppression

Reason(s) for outcome or endpoint occurring

Appendix B

Enrolment at the clinic

Enrolment into the programme followed the National Department of Health (NDoH) 2004 ART guidelines, which were based on the 2002 WHO recommendations for scaling up ART in resource-poor settings (Bekker, 2006). The medical criteria for adult eligibility included those with a CD4 cell count of <200 cells/µl irrespective of WHO stage or WHO stage IV disease irrespective of CD4 cell count (NDoH, 2004) .

Following referral from where VCT and the CD4 cell count was done, the standard schedule of visits was as follows: First visit [staging, exclude tuberculosis, pregnancy etc. and treatment of intercurrent illnesses and collection of baseline bloods, full blood count (FBC), liver function tests (LFT) or just aspartate transaminase (AST) and alanine transaminase (ALT) depending on the condition of the patient, and rapid plasma reagent (RPR), and co-trimoxazole prescribed for those with CD4 cell counts <200 cells/µl or dapson where co-trimoxazole is contraindicated] and then the patient was referred to the dietician (for nutritional assessment and support), the social worker as indicated and to the clinic-based counselor for drug literacy and adherence counseling.

The counsellor conducted three consecutive counselling sessions on dates agreed to with the patient (usually within 5 to 10 days), at least one of which included the patient's treatment supporter. After the third counselling session, the patient was referred back to the doctor who made a follow up on presenting problems in the first visit, reviewed baseline results and assessed the patient's willingness and readiness to take ART adherently. Baseline viral load testing was not performed. Most patients are initiated on ART within five to 10 days of enrolment at the HIV clinic

Clinical services

The clinical services were provided by Mankweng Hospital's Department of Family Medicine doctors in a 50/50 partnership (in terms of staff contribution, both clinical and administrative) with the Foundation for Professional Development (FPD), a non-governmental organization. The Department of Family Medicine trains junior doctors and medical interns, and that training allowed them rotate through and treat patients in the HIV Unit for some exposure to ambulant HIV medicine. There was a separate pediatric HIV clinic run by the Department of Pediatrics for children below 13 years, after which they were transferred to the adult side of the clinic to continue their treatment.

Appendix C

Regimens and follow-up schedule

This was performed according to the South African National Antiretroviral Treatment Guidelines 2004: first-line ART comprises of stavudine 30mg 12hourly, lamivudine 150mg 12hourly and either efavirenz 600mg at night (or 400mg if body weight <40 kilograms) or nevirapine 200mg daily for the first 2 weeks, increasing to 200mg 12hourly if the ALT is normal. The second-line regimen comprises of zidovudine 300mg 12hourly, didanosine 250mg daily (400mg if body weight > 60kg) and lopinavir/ritonavir 400/100mg 12hourly.

Drug substitutions in the first-line regimen were instituted for toxicity, drug interactions with mostly anti-tuberculosis medications, pregnancy etc. Regimen change was based largely on virological failure (consecutive viral loads of >5000 copies/ml at least 3 months apart). Adherence was stepped-up by the doctor and the lay-counsellor when viral rebound is detected. After ART initiation, patients are seen at 2 weeks, for those on Nevirapine, for safety bloods (ALT and AST) and then at one month and then monthly thereafter until 6 months. Monitoring bloods were done at 6 months and included CD4

count and viral load measurement. With good treatment response (increasing CD4 cell count and viral loads < 400 copies/ml), patients were referred back to their local clinics (down-referral) with a repeat prescription to return at a date corresponding to the 6 month visit for the 6 monthly bloods. In addition to the scheduled clinic appointments, patients had open access to the clinic whenever they were not well even though there is a booking system which assisted in controlling numbers and identification of defaulters. A secure supply of medication was maintained by the provincial department of health and all consultations, treatment and investigations were offered free of charge to the patients.

Appendix D

Laboratory services

Plasma HIV-1 RNA (viral load) and blood CD4 cell count were performed every 6 months after treatment initiation and safety bloods at baseline, according to the 2004 South African Antiretroviral Treatment guidelines, and as indicated. All laboratory tests were performed by the National Health Laboratory Services (NHLS). Plasma HIV-1 RNA was assayed using nucleic acid sequence-based amplification procedure (NASBA), expressed as the viral load count in copies per millilitre and a log value, and CD4 cell counts were performed by flow-cytometry and expressed as absolute CD4 cell count in cells/ μ l and the percentage CD4 cells of Total lymphocytes.

Appendix E

MEDUNSA Research & Ethics committee Clearance Certificate



MEDUNSA RESEARCH & ETHICS COMMITTEE

CLEARANCE CERTIFICATE

P O Medunsa
Medunsa
0204
SOUTH AFRICA

MEETING: 04/2011

Tel: 012 - 521 4000

PROJECT NUMBER: MREC/H/53/2011: PG

Fax: 012 - 560 0086

PROJECT :

Title: Virological failure among adult HIV positive patients 3 years after starting antiretroviral treatment at Mankweng hospital, Limpopo Province, RSA

Researcher: Dr MA Lekoloana
Supervisor: Prof NZ Nyazema
Co-supervisor: MP Kekana
Department: Public Health
School: Health Sciences
Degree: MPH

DECISION OF THE COMMITTEE:

MREC approved the project.

DATE: 13 May 2011


PROF GA OGUNBANJO
CHAIRPERSON MREC

Note:

- i) Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee.
- ii) The budget for the research will be considered separately from the protocol. PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.

Appendix F

Department of Health's permission to conduct the research



LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF HEALTH

Enquiries: Selamolela Donald

Ref: 4/2/2

22 July 2011
Lekoloane MA
University of Limpopo
Sovenga
0727

Greetings,

Re: Permission to conduct the study titled: Virological failure among adult HIV positive patients 3 years after starting antiretroviral treatment at Mankweng hospital

1. The above matter refers.
2. The permission to conduct the above mentioned study is hereby granted.
3. Kindly be informed that:-
 - Further arrangement should be made with the targeted institutions.
 - In the course of your study there should not be any action that will disrupt the services
 - After completion of the study, a copy of the report should be submitted to the Department to serve as a resource
 - You should be prepared to assist in the interpretation and implementation of study recommendations where possible

Your cooperation will be highly appreciated

Head of Department
Department of Health
Limpopo Province

18 College Street, Polokwane, 0700, Private Bag x9302, POLOLKWANE, 0700
Tel: (015) 293 6000, Fax: (015) 293 6211/20 Website: <http://www.limpopo.gov.za>

Appendix G

Ethics Committee Clearance Certificate, UL, Polokwane Mankweng Hospital Complex



LIMPOPO

PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF HEALTH & SOCIAL DEVELOPMENT

**ETHICS COMMITTEE
CLEARANCE CERTIFICATE
UNIVERSITY OF LIMPOPO
POLOKWANE MANKWENG HOSPITAL COMPLEX**



PROJECT NUMBER : PMREC-26/2011

TITLE : Virological failure among adult HIV positive patients 3 years after antiretroviral treatment at Mankweng hospital, Limpopo Province, RSA

RESEARCHER : Dr M.A Lekoloana

ALL PARTICIPANTS:

Supervisor : Prof. N.Z Nyazema

Co-supervisor : Mr M.P Kekana

DATE CONSIDERED : 08 September 2011

DECISION OF COMMITTEE

- Recommended for approval

DATE : 12 September 2011


PROF A J MBOKAZI
Chairperson of Polokwane Mankweng
Hospital Complex Ethics Committee



NOTE: *The budget for research has to be considered separately. Ethics committee is not providing any funds for projects.*