Virologic outcomes for tenofovir disoproxil fumarate based antiretroviral therapy regimens in Swaziland, 2013 – 2015.

by

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DECLARATION

I, Malvern Masango (student number 12206042), a masters student at the University of Pretoria school of health systems and public health. I do hereby declare that the following research project is my original work unless otherwise stated. The project report is submitted in partial fulfilment of my Masters of Science degree in epidemiology and has not been submitted anywhere else.

Name: Malvern Masango

Date: 10 August 2018

DEDICATION

This project is dedicated to my wife Nobukhosi and our daughter Chiedza who have been my pillar of support and my biggest cheerleaders. I would like to thank them for their patience for all the times that I had to be away or I was unavailable.

ABSTRACT

Introduction: The scale up of antiretroviral therapy (ART) in sub-Saharan Africa has seen a great reduction in morbidity and mortality related to human immuno-deficiency virus (HIV). Recent advances in ART has seen the introduction of more efficacious and safer regimens. As a result, the use of tenofovir disoproxil fumarate (TDF) based regimens have increased exponentially in the last ten years. However, few studies in the sub-Saharan Africa setting have sought to evaluate the comparative virologic efficacy and durability of the two first line TDF based regimens used in Eswatini (Swaziland), namely TDF + lamivudine (3TC) + efavirenz (EFV) and TDF + 3TC + nevirapine (NVP), (TDE and TDN respectively).

Methods: A retrospective cohort analysis was conducted of routinely collected patient data enrolled onto ART from three health facilities of the Shiselweni region, Eswatini between 2013 and 2015. The primary outcomes were viral non-suppression at 12 months and regimen modification, for any reason, during the follow-up period. Modified Poisson regression models with robust error variance were used to estimate relative risks (RR) and 95% Confidence Intervals (CI) of the association between potential risk factors and viral non-suppression at 12 months. Cox proportional hazard models were applied to estimate the hazard ratios (HR) and 95% CIs of the association between potential risk factors and regimen change during follow-up.

Results: Out of 1442 patients, 10.5% failed to achieve viral suppression by 12 months on ART. Patients on NVP based TDF regimens (NVP-TDF-3TC / TDN) had a 2-fold risk for viral non-suppression compared to those on EFV-TDF-3TC / TDE (adjusted RR: 2.03, 95% CI 1.31-3.15). Advanced disease (WHO stage III and IV) and poor adherence (<95%) were significantly associated with viral non-suppression (adjusted RR: 1.89, 95% CI 1.39-2.57 and adjusted RR: 3.87, 95%CI 2.83-5.29 respectively). Over a median follow-up time of 2.2 years, incidence of regimen change was 24.4 per 1 000 person-years (95% CI 19.5 - 30.5). Patients on a TDN had an approximately 6-fold risk of regimen modification compared to those on TDE combination, adjusted HR: 5.67, 95%CI 3.23 - 9.58.

Conclusions: Among adult patients on first line ART regimens, those on an NVP-based TDF regimen (TDN) had poorer virologic outcomes at 12 months and had higher risk of regimen modification compared to patients that were on an EFV-based regimen (TDE). Even in the advent of more durable and safer TDF based first line ART regimens, these data suggest EFV should remain the preferred non-nucleoside reverse transcriptase inhibitor.

Keywords: Viral suppression, Anti-retroviral therapy, regimen change Tenofovir disoproxil fumerate, Non-nucleoside reverse transcriptase inhibitor

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Lastly, I am grateful to the support I got from the University of Pretoria School of Health Systems and Public Health, my friends and relatives. This project would not have been a success without the input of each of the above.

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NOMENCLATURE

ABC	Abacavir
ART	Antiretroviral therapy
BMI	Body mass index
d4T	Stavudine
EFV	Efavirenz
FTC	Emtricitabine
HIV	Human immune-deficiency syndrome
HR	Hazards ratio
MSF	Médecins Sans Frontières
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
PI	Protease inhibitor
PMTCT	Prevention of mother to child transmission
RR	Risk ratio
SHIMS	Swaziland HIV incidence measurement survey
TDE	Tenofovir +Lamivudine +Efavirenz
TDF	Tenofovir disoproxil fumarate
TDN	Tenofovir +Lamivudine + Nevirapine
UNAIDS	Joint United Nations program on HIV/AIDS
VL	Viral load
WHO	World health organisation
3TC	Lamivudine

CHAPTER 1. INTRODUCTION

1.1 Background

Human Immunodeficiency Virus (HIV) remains one of the biggest global public health challenges of the 21st century despite tremendous gains made in the last decade. As of 2012, global estimates of people living with the virus stood at approximately 35 million, showing an increase from previous years.[1] This is as more people are put on life-long treatment and previously enrolled people live longer and achieve a near- normal life span. In the advent of antiretroviral therapy (ART), new infections fell globally by 33% from 3.4 million in 2001 to 2.4 million in 2012 while Acquired immunodeficiency Syndrome (AIDS) deaths have also fallen from 2.3 million in 2005 down to 1.6 million in 2012.[1] Although the epidemic is global, it has disproportionately affected sub-Saharan Africa with 26 million infections and 66% (1.4 million) of the world new infections in 2014.[2]

Swaziland, now known as Eswatini is a small Southern African country with a population of 1.3 million with the world`s highest HIV prevalence of 28.8% (220000 people) and a total of 11000 new infections in 2015.[3] Although incidence rate has been falling from 2.5% in 2011 to 1.8% in 2013 and was projected to further decline to 1.5% in 2015,[3,4] the prevalence has been steadily going up largely due to more people being put on ART and living longer.

Since the advent of ART in the mid-1990s, a lot of progress has been made not only in the efficacy and durability of combinations used but also in controlling the epidemic, improving quality of life and prolonging life expectancy to near normal. As incidence continues to fall, ambitious undertakings have been made to eliminate HIV by year 2030. Moving away from the previous guidelines of ART initiation based on CD4-count and World Health Organisation (WHO) staging eligibility criteria, new guidelines were introduced by the WHO and UNAIDS that recommend universal access to therapy by all HIV infected people regardless of CD4 or WHO stage.[5,6] Since 2010, WHO recommended the phasing out of stavudine (d4T) due to a high risk of irreversible mitochondrial toxicity side effects like lipodystrophy, peripheral neuropathy and lactic acidosis.[7] Due to their similar resistance profiles, in the majority of cases d4T was substituted by Tenofovir Disoproxil Fumarate (TDF). In Eswatini, Lamivudine (3TC) is used in the Nucleoside Reverse Transcriptase Inhibitors (NRTI) backbone of ART in the public sector and Emtricitabine (FTC) is not available. Nevirapine (NVP, in combination with the TDF+3TC backbone is administered as

a twice daily dose of 200mg per dose and not as a once daily dose as was practised in other settings. On the other hand, the regimen Tenofovir + Lamivudine + Efavirenz is available as a fixed dose combination (FDC) taken once a day with a standard EFV adult dose of 600mg for weight over 40kg and 400mg for weight below 40kg.

1.2 Research Problem

ART regimen efficacy and durability has always been a crucial aspect of HIV chronic care. In addition to non-adherence to treatment, sub-optimal drug regimens give rise to the emergence of drug resistance and the need to switch to second line. In most cases, this is often more costly, has higher pill burden and has more side effects. It is therefore important that new drug regimens continue to be evaluated for efficacy, durability and side effects' profile amongst different populations so as to guarantee patient safety and delay the emergence of drug resistance.

1.3 Justification of study

First line therapy is often the best chance for HIV infected clients to achieve viral suppression for as long as possible since current regimens are efficacious, pill burden is lower and side effects are fewer. The number of clients on second line ART has been shown to be on the increase in recent years. With scaling up of viral load monitoring, mathematical modelling predicts that by 2020, up-to approximately 16% of HIV infected clients on ART will be on second line rising up to almost 20% by 2030.[8] Since the recommendations to phase out d4T in 2010,[7] there has been a huge bias on the reliance on TDF for first line combinations in low to middle income countries including Eswatini.[9] It is crucial that new ART regimens be tested for efficacy if health system factors of emergence of drug resistance are to be minimised. While TDF+3TC+EVF has been shown to be as efficacious as the other existing first line combinations,[10] the same cannot be said for TDF+3TC+NVP. Although earlier indications have suggested the latter regimens could be inferior compared to TDF+3TC+EVF (and other first line regimens),[10,11] it nevertheless continues to be used as an alternative first line combination in resource limited settings.[5,9] Since some of the studies comparing NVP vs EFV regimens were done using FTC instead of 3TC, a study of the drug combinations available in Eswatini need to be performed within the Swazi context. With more and more people expected to be started on TDF based regimens for first line therapy, there is therefore a need to scrutinise the value of this regimen in the face of this possible inferiority. Moreover,

the efficacy of these regimens need to be tested against high baseline CD4 counts as the test and start strategy being rolled out mean more patients will be starting treatment with higher CD4 counts. A number of studies have examined the risk of death, program failure and substitutions due to side effects of the various regimens,[10,11] but very few studies have sought to look at the rate of viral suppression from the time of initiating therapy. This study therefore seeks to make a head to head comparison of the two TDF based first line regimens in use in Eswatini in terms of rate of viral suppression from the time of commencing therapy and durability before a substitution or switch to second line therapy.

1.4 Literature review

1.4.1 Indications and goals of ART

ART is now initiated in all HIV infected people regardless of CD4 or WHO stage as recommended by the WHO.[5] This was a culmination of a number of studies showing the benefits of early initiation of therapy at individual level.[12,13,14] This has seen the progressive increase in CD4 eligibility criteria for ART initiation from 200, 350, 500 cells/mm ^{3} and now to all HIV infected individuals regardless of CD4 cell count level. A systematic review and meta-analysis of Randomised Controlled Trials (RCT) and cohort studies showed a lower risk of mortality, progression to AIDS and death, as well as diagnosis of non-AIDS defining illnesses among patients initiating therapy with CD4 ≥350 cells/mm ^{3}.[12] The same study however showed an increased risk of major (grade 3 and 4) laboratory abnormalities among those starting ART with CD4 ≥350 cells/mm ^{3}.

The main goals of ART include 1) maximal and durable viral suppression, 2) restoration and preservation of immune function, 3) reduction of HIV associated morbidity, prolongation of duration and quality of life and 4) prevention of HIV transmission.[15,16] Viral suppression has been shown, among other clinical benefits, to delay or prevent the selection of drug resistance mutations.[16] At a public health level, early initiation of ART has been shown to reduce the risk of transmission of HIV infection.[5,17,18] The HPTN052 study for example showed that ART reduces the risk of HIV transmission in sero-discordant by more than 96%.[19]

1.4.2 First line antiretroviral therapy

First line ART therapy has for some time known to combine 2 NRTIs (backbone) and one Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI), tail in resource limited settings.[5] The backbone always includes either 3TC or FTC in combination with any one of TDF, Zidovudine (AZT) or Abacavir (ABC). The NNRTI tail is a choice between the NVP and EFV. In resource limited settings including Eswatini the public health approach is used in regimen selection. First line treatment is not individualised according to client baseline resistance profile but is standardised across all clients starting therapy. Since TDF has been shown to carry no extra risk of renal toxicity,[5,20] its use in resource limited setting with no consistent baseline renal function tests increased from around 2010 onwards. With documented high rates of transmitted resistance,[21,22] this public health rather than individual approach poses challenges with high risk of first line HIV drug resistance, hence the need to switch to second line early. In Malawi for example, rates of transmitted NNRTI resistance were found to be as high as 15%, while transmitted NRTI and PI resistance was less than 5% in 2009.[23] Patients get started on different regimens according to their baseline renal function, liver function, haemoglobin levels and presence or absence of psychiatric illness.

In the developed countries however, first line regimens consist of 2 NRTIs (backbone) and a third drug from any one of the following classes; an integrase strand transfer inhibitor (INSTI), a non-nucleoside reverse transcriptase inhibitor (NNRTI), or a protease inhibitor (PI) with a pharmacokinetic (PK) enhancer (booster) (cobicistat or ritonavir).[24] Unlike in limited resource settings where baseline genotypic resistance testing is not readily available, first line ART regimen is individualised depending on the patient baseline resistance pattern.[24]

1.4.3 TDF based regimen efficacy

Drug regimen efficacy among other things is important in ensuring viral suppression for as long as possible. First line drug regimens have been shown not to be equally efficacious. A retrospective study conducted in Nigeria showed that patients on NVP containing combinations were at a higher risk of virological failure compared to patients on EFV containing combinations.[25] Patients on TDF+3TC/FTC+NVP had approximately 1.6-fold

increase in risk of virological failure compared to TDF+3TC/FTC+EFV (aHR= 1.57 95%CI 1.35–1.83).[25] In this study, TDF+3TC/FTC+EFV was found to be the most efficacious combination against which all the other regimens were compared. This study however recruited treatment experienced clients and those whose previous drug exposure history was unknown therefore potentially introducing selection and contamination bias**.** The study was also a cross sectional view of viral load status, not factoring in the different follow-up times on ART of the participants. In an HIV drug resistance survey in Kwa-Zulu Natal, South Africa of adult participants on first-line ART for 12 – 15 months, being on EFV-based ART was associated with reduced risk of virological failure, compared to being on NVP-based ART (adjusted prevalence ratio (aPR) 0.31, 95% CI 0.14 – 0.70).[26] In this survey which recruited patients receiving (d4T/TDF/AZT + 3TC/FTC + EFV/NVP) regimens, those with prior first-line drug substitutions and treatment interruptions were also included. This study also showed that drug resistance mutations were common amongst those who were failing therapy, with 84% shown to have NNRTI mutations and about 75% having NRTI mutations. Similarly, differential virologic efficacy between EFV and NVP based combinations was reported in the AIDSRelief study, a multi-national retrospective study comparing 4 combinations (2 AZT and 2 TDF based combinations).[27] "On treatment analysis" found EFV based regimens achieved higher viral suppression rates compared to NVP regimens (OR 0.59, 95% CI 0.59 to 0.96). Again, this was a cross sectional study looking at a single viral load measurement at 14 months and treatment experienced participants were also included in the study. Some of the participants included had been previously exposed to single dose NVP which could increase the risk of failure in subsequent treatments thereby increasing the risk of a type 1 error. A study in Lusaka, Zambia showed increased risk of mortality among patients on TDF+3TC/FTC+NVP compared to TDF+3TC/FTC+EFV (aHR: 1.91, 95% CI: 1.09 to 3.34).[11] In this study though, the outcome of interest was death and not viral suppression. As participants were analysed using an intention-to-treat approach it was difficult to get a clear effect of each drug combination as their regimens were substituted during the course of treatment.

In contrast, other studies found no increased risk of virological failure associated with TDF+3TC/FTC+NVP compared to first-line EFV-based combinations. Labarga et al in a retrospective study found no difference in risk of virological failure between TDF+FTC+EFV and TDF+FTC+NVP.[28] Since FTC has been shown to have a longer half-life compared to

3TC, it is thought to confer a higher genetic barrier to resistance compared to 3TC. For example, the ATHENA study in Netherlands found that 3TC in combination with TDF and EFV was associated with an almost 2-fold higher risk of virological failure at week 48 compared to FTC in combination with the same backbone.[29] Annan et al, in another study showed no difference in efficacy between the NVP and EFV tail of a nucleoside backbone. It is worth noting that the majority of participants in this study were not on TDF+FTC backbone (3% on TDF+FTC+EFV and 0.5% on TDF+FTC+NVP) therefore possibly lacked power to establish that difference.[30]

1.4.4 Antiretroviral treatment failure

Since clinical and immunological surrogate markers have been shown to have poor sensitivity and specificity in diagnosing treatment failure,[5,31] viral load monitoring is the gold standard in monitoring response to ART. The need to roll out viral load monitoring cannot be overemphasized if treatment failure is to be promptly diagnosed and patients switched to second line. Factors that have been shown to be associated with viral suppression include age, sex, adherence to treatment, level of education, baseline CD4, haemoglobin (Hb) and ART drug combination.[25,32,33]

It is in light of all this evidence that the real efficacy of TDF+3TC+NVP ought to be investigated. The use of TDF based regimens is set to increase with the test-and-start strategy and this combination should be tested against other first line regimens in treatment naïve patients. Although some studies suggest that NVP regimens have a higher risk of modifications due to toxicity,[34] the safety profile of NVP in combination with TDF also needs to be fully understood.

1.5 Hypothesis

The ART regimen TDF+3TC+NVP is non-inferior to TDF+3TC+EFV in achieving viral suppression in treatment of naïve HIV patients in Shiselweni region, Eswatini.

1.6 Study aim

The aim of the study was to compare the virologic efficacy of the two first line TDF based ART regimens in treatment naïve HIV infected patients in Eswatini.

1.7 Study objectives

1. To compare viral suppression rates among HIV-infected patients on TDF+3TC+NVP versus those on TDF+3TC+EFV in Shiselweni region, Eswatini.
2. To compare the rate of regimen modification (substitution or switch) between TDF+3TC+NVP and TDF+3TC+EFV among HIV-infected patients on ART in Shiselweni region, Eswatini.
3. To determine factors associated with viral suppression among HIV-infected patients on first line ART in Shiselweni region, Eswatini.
4. To determine factors associated with regimen modification (substitution or switch) among HIV-infected patients on first line ART in Shiselweni region, Eswatini.

CHAPTER **2**. METHODOLOGY

2.1 Study design

A retrospective cohort analysis was performed on routinely collected data of all treatment naïve HIV infected adult clients initiating TDF-based first-line ART between 01 January 2013 and 31 December 2015.

2.2 Study setting

Participants were drawn from the southern region of Eswatini, Shiselweni region. This is one of the four administrative regions in the country covering approximately 3,790km^2, [35] with an estimated population of about 208000.[36,35] It is mainly a rural and homogenous population with farming as the main economic activity.

An estimated 31% of adults (age 18-49 years) live with HIV in the region as of 2011 according to the SHIMS study.[37] By 2015, the cumulative number of patients ever started on ART was 38172, with 24043 remaining active on ART.[35] The region is served by one regional hospital and two rural health centres and they in turn support a total of 15 clinics with HIV services. In 2012, the region with the support of Médecins Sans Frontières (MSF) piloted viral load monitoring, the gold standard for monitoring HIV infected clients on treatment.

In 2015, one of the health centres, Nhlangano piloted the test-and-start strategy where all HIV positive patients were started on ART regardless of CD4 cell count or WHO stage. The rest of the region adopted this strategy in 2016. The available combinations for first-line follow the national guidelines. TDE is the preferred combination while TDN, AZT+3TC+EFV/NVP, ABC+3TC+EFV/NVP and d4T+3TC+EFV/NVP are available as alternative combinations. Baseline tests done at initiation include CD4 count, liver and renal function tests (LFT and RFT respectively), full blood count and urinalysis. After initiation, patients were seen after 2 weeks to look out for early side effects, reinforce adherence (pill counts) and LFT is done if on NVP. Thereafter, patients were seen after another 2 weeks then monthly for at least 6 months. If the patient was stable and adherence was good, they were then seen 2 or 3 monthly. At every visit, pill count is done as a good surrogate measure of adherence,[38] in

addition to other clinical evaluations. Viral load was done every 6 months after starting therapy until viral suppression. Following 2 suppressed viral load tests, annual viral load was then performed. Detectable viral load in Shiselweni region was viral load greater than 100 copies/ml. Drug substitution was done for side effects and regimen switching for virological failure (2 viral load results greater than 1000 copies/ml done at least 3 months apart with optimum adherence). Genotypic tests were only available for second line treatment failure through an MSF funded program.

2.3 Study population and sampling

The study participants included all treatment naïve HIV positive clients who started ART between 1 January 2013 and 31 December 2015 from the 3 main health facilities in the Shiselweni region (1 regional hospital and 2 rural health centres). Patients were followed up until 31 August 2016 to give participants who started ART at the end of 2015 at least 6 months of follow up for viral load testing.

The power of this study was computed using the STATA version 13.1 (Stata Corp., College Station, TX, USA) power and sample size analysis function. With the total number of participants enrolled (1442), proportion of viral suppression in the controls (0.9) and in the experimental group (0.795), ratio of sample sizes, experimental to controls (79/1374), study power was computed for a one sided (non-inferiority) test and a significance level of 0.05. The total power of **0.84** obtained was satisfactory to detect differences in viral suppression between the two groups.

2.4 Study eligibility

Inclusion criteria

i. 18 years and older
ii. ART naïve: No previous exposure to ART including Prevention of Mother to Child Transmission (PMTCT).
iii. Initiated on either first-line TDF+3TC+EFV or TDF+3TC+NVP combinations
iv. Minimum follow up time of 6 months with at least one viral load test done

Exclusion criteria

i. Previous ART experienced clients including defaulters and previous PMTCT
ii. Clients less than 18 years old
iii. Clients transferred in
iv. Follow up time less than 6 months or viral load test not done.

2.5 Measurements and data sources

Primary data were routinely collected during client consultation at the time of enrolment onto ART and at all follow up clinical visits. Data were obtained through history taking, examination and laboratory tests. Data collected by clinicians and lay counsellors were recorded in the patient file (hard copy), in the electronic database at the health facilities and in the electronic database held by the Ministry of Health.

The primary data source for the study was the electronic database obtained from the Ministry of health in the form of an excel spread sheet. Variables not captured in this database, for example percentage adherence, or any missing data were sought and obtained from the physical chronic care files at the health facilities.

2.6 Definition of outcome

The main study outcome was viral non-suppression at the end of 12 months. Although viral load testing according to national guidelines was to be done every 6 months, in reality the actual time of viral load testing varied. For practical purposes, a viral load test was accepted if it was performed between 5 months and by the end of twelve months. Viral suppression for the purposes of this study is defined as first viral load result < 100 copies/ml.

A secondary outcome was regimen modification (either drug substitution or switch to second line therapy).

Table 1. Variable list

VARIABLE	Variable type and categories
1. Facility	Categorical: Hlatikhulu -1, Nhlangano -2, Matsanjeni - 3
2. Regimen	TDF+3TC+EFV - 0, TDF+3TC+NVP - 1
3. Age	Continuous
4. Sex	Male – 0, Female - 1
5. Baseline CD4	Categorical[1] : <350 – 2, (350 – 500) – 1, >500 - 0
6. Adherence	Categorical[2]: < 95% - 1, ≥ 95% - 0
7. Side effects	Binary: No Side effects reported – 0, At least one side effects reported - 1
8. Substitution or Switch	Binary: Yes – 1, No – 0
9. WHO stage	Categorical[3]: Stages 1 & 2 – 0, Stages 3 & 4 - 1
10. BMI	Categorical[4]: <18.5 – 2, (18.5 – 24.99) – 1, ≥ 25 – 0
11. Died	Binary: Yes – 1, No – 0
12. Follow up time	Continuous
13. Viral Suppression	Binary: Yes – 1, No – 0

1 - WHO CD4 categories of severity of immunosuppression, 2 - Poor vs good adherence respectively

3 - WHO stage categories of advanced vs less advanced HIV disease, 4 - WHO categories for BMI

2.7 Definition of terms

Viral suppression; viral load below 100 copies/ml

Optimal adherence; pill count greater than 95%

Treatment failure; two viral load values above 1000 copies/ml at least 3 months apart with optimum adherence. (For the purposes of this study, the term viral non-suppression will be used instead of treatment failure since only one viral load reading was considered)

Substitution; changing one or more drugs in an ART combination but within the same treatment line (first line)

Switch; drug substitution in a combination with change of treatment line e.g. from first to second line due to treatment failure.

2.8 Data Management

The dataset was received as an excel dataset from the Monitoring and Evaluation department of the Ministry of Health. The data were then extracted onto an excel record file with relevant variable list. Inconsistencies in the data set like age outliers, pregnant men and ART start date after end point date were cleaned using the paper based chronic care files at the facilities. Any gaps in the data were filled by referring to the chronic care file (hard copy) and the patient electronic database at the facilities. Due to clerical errors, some of the information in the hard copy chronic care files was not captured or was captured wrongly in the electronic data base.

Data were stored securely in a password protected personal computer and no names or personal identifier information were collected.

2.9 Statistical analysis

All analyses was done using STATA version 13.1 (Stata Corp., College Station, TX, USA).

2.9.1 Descriptive statistics

Descriptive statistics were presented as frequencies, proportions of baseline characteristics and of the outcome, incidence rates of regimen modification and time at risk for categorical and binary variables. Continuous variables were described using means and standard deviation for normally distributed variables whereas the median and inter-quartile range were computed to summarize non-normally distributed variables.

2.9.2 Inferential statistics

Relative risks for viral non-suppression (VL <100copies/ml) were estimated using modified Poisson regression with robust error variance since the outcome (viral non-suppression) occurred in more than 10% of the participants.[39] Additional analysis was performed using VL<1000copies/ml. Kaplan – Meier survival analysis was used to examine rates of regimen modification according to ART regimen and other co-variates. Differences between categories were compared using the log-rank test. Multivariate Cox proportional hazard regression models were used to estimate the hazard ratios for regimen modification. Person-time was accrued from the date of ART initiation until the earliest of 1) regimen modification ; 2) lost-to follow-up; 3) transfer-out; 4) death or 5) close of the data set (31 August 2016). Patients who transferred out during the follow-up period were censored at their last clinic visit date. Patients who were lost to follow-up or reported to have died during the follow-up period were censored at their last visit date to the clinic. LTFU was defined as a patient who has not reported to the health facility 90 days or more after their last scheduled visit date. Once declared LTU, patients were not readmitted into the study upon return into care. Those who interrupted treatment by less than 3 months continued in the study but adherence for each month interrupted was assigned 0%.

For inclusion into initial multivariate models (modified Poisson and Cox regression), we considered covariates that were significant at the 20% level in bivariate analysis as well as those deemed relevant to the outcome as a *priori*. Variables were removed manually one by one starting with those with highest p-values so as, finally, to construct a model with those variables that did have a statistically significant effect, or deemed relevant to the outcome. The results of the regression modelling were presented in a table form showing crude and adjusted risk ratios (to 2 decimal places), p-values and 95% confidence (CI) intervals. The proportionality assumption was checked using graphical methods and the global test was performed.

2.10 Ethical and legal considerations

The data used in this project were collected as routine data during client consultation and care therefore no participant consent was required. Authority to conduct the study and access data as well as ethical approval was sought and obtained from the Eswatini Research and Ethics committee of the Ministry of Health (MoH) in written form (Appendix A). Ethical approval

was sought and obtained from the University of Pretoria ethics committee. Data were used for research and publication purposes only and access to the data was restricted to the researcher, supervisors and statistician. No client names were collected but unique identifiers were used. No potential harm arose from this project and the outcomes of this study will be useful in determining the optimum ART combination within the local context and also add to the body of scientific evidence regarding ART combination efficacy. The data used in this study (excel, Epidata files) were stored securely.

Raw data will be kept for a minimum of 15 years after which they will be disposed according to the university regulations.

The researcher declares no known conflict of interests and is the sole funder of the project.

CHAPTER 3 RESULTS

Below is a description of the main results of this project. The selection of the study participants is described with the aid of a flow diagram showing the exclusions and the reasons for exclusion. Descriptive statistics are shown in table form while inferential statistics are shown in tables and graphs.

3.1 Selection of study participants

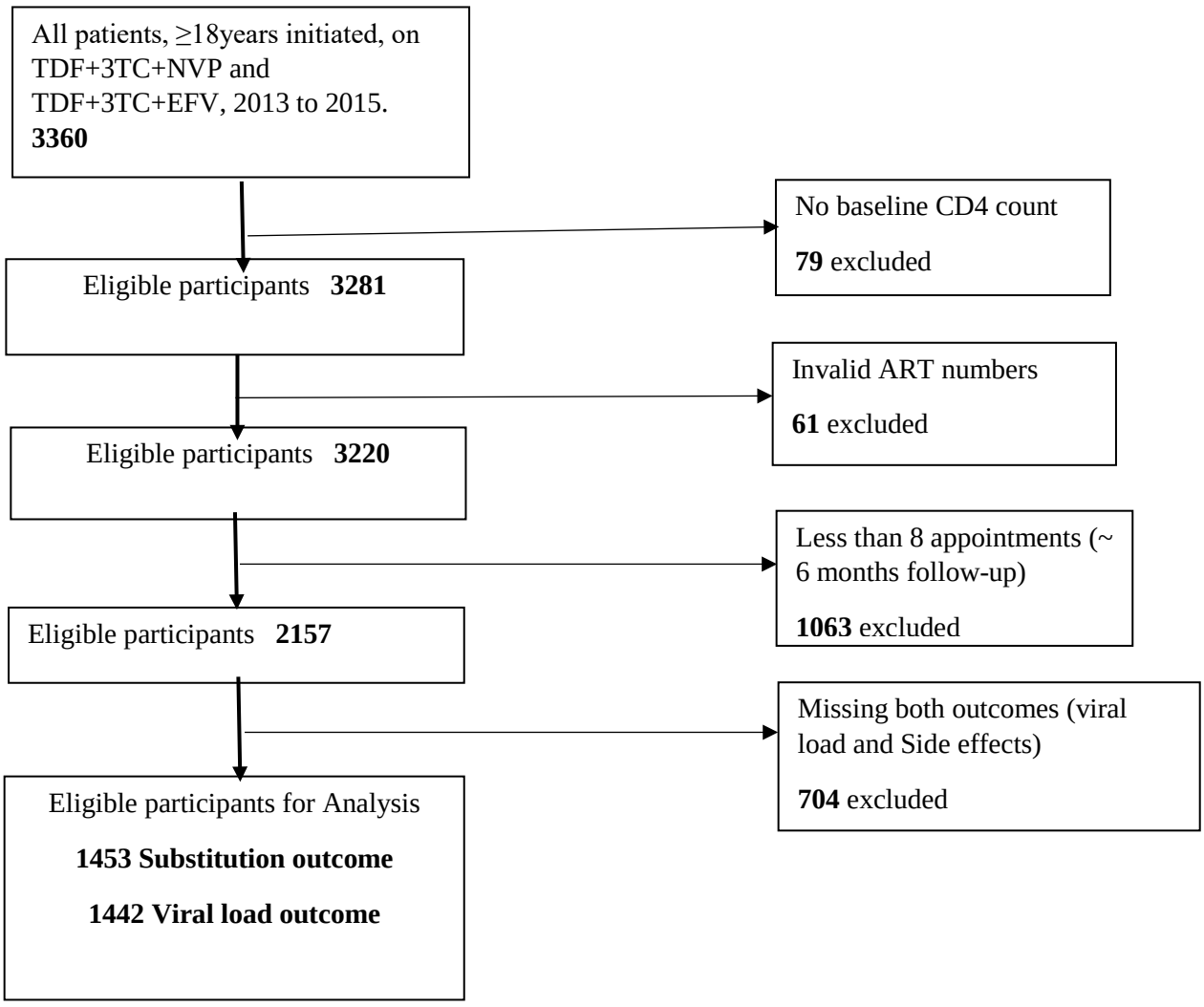

Figure 1. Participants flow chart; Selection of final study sample.

Figure 1 illustrates the selection of study participants from the master dataset from the Ministry of Health to the final study participants eligible for analysis. After applying the

inclusion criteria, 3360 patients were eligible for inclusion in the study. Participants were further exclude for a variety of reasons and 1453 were left for regimen modification outcome analysis and 1442 for viral suppression analysis.

3.2 Characteristics of the participants at initiation of ART

Table 2 is a summary of the baseline characteristics of the study participants. Nhlangano health centre contributed the bulk of the study participants (51.5%) while Matsanjeni health centre contributed the least (18%). Patients on Tenofovir + Lamivudine+ Efavirenz (TDE) were by far the majority (94.6%) while those on (Tenofovir + Lamivudine + Nevirapine) TDN were only 5.4% of the study population. There were more females than males (62% versus 38%), in general and within each regimen category. The median age at initiation of ART was 32 years (IQR 27-40). Most of the participants enrolled onto ART with a low baseline CD4 count (74.6% had CD4 count<350 cells/ml) with a median enrolment CD4 count of 248 (IQR 125-350). The majority of patients (73.4%) started treatment with less advanced HIV disease (stages I and II) while only 26.6% started treatment with advanced disease (stages III and IV). Only 9% of the patients were underweight (BMI< 18.5kg/m2).

Table 2. Baseline characteristics of the study participants initiating ART between 2013 and 2015.

Characteristic	**Total**	**TDE***	**TDN****
Total enrolled *(n)*	1453	1374 (94.6)	79 (5.4)
Facility			
Nhlangano *(n, %)*	748 (51.5)	685(49.9)	63(79.8)
Hlatikulu *(n, %)*	443 (30.5)	438(31.9)	5(6.3)
Matsanjeni *(n, %)*	262 (18.0)	251(18.2)	11(13.9)
Sex, n (%)			
Male	552 (38.0)	522(38.0)	30(38.0)
Female	901 (62)	852(62.0)	49(62.0)

Age at initiation (years), Median *(IQR)*	32 (27-40)	32 (27-40)	29 (25-36)
Baseline CD4 count, Median *(IQR)*	248 (125 -350)	249.5 (124-352)	213(133-318)
Baseline CD4 count, n (%)			
< 350	1084 (74.6)	1020(74.2)	64(81.0)
350 – 500	244 (16.8)	231(16.8)	13(16.5)
>500	125 (8.6)	123(9.0)	2(2.5)
WHO stage, n (%)			
I and II	1067 (73.4)	1013(73.7)	54(68.3)
III and IV	386 (26.6)	361(26.3)	25(31.7)
BMI, Median *(IQR)*	23.46 (20.68- 27.07)		
<18.5 (n, %)	131 (9.0)	126(9.2)	5(6.3)
18.5- 24.9 (n, %)	778 (53.5)	733(53.3)	45(57.0)
≥25 (n, %)	544 (37.5)	515(37.5)	29(36.7)

**TDE- Tenofovir + Lamivudine+ Efavirenz, **TDN - Tenofovir + Lamivudine + Nevirapine*

3.3 Main outcome events: Virological non-suppression and regimen change

Table 3 below shows a summary of the main outcome events. Overall viral non-suppression rate by the end of 12 months was 10.5% (89.5% suppressed) for viral load less than 100 copies/ml and 7% (93% suppression) for viral load less than 1000 copies/ml. Patients enrolled on TDE achieved 90.0% (n=1228) viral suppression while those enrolled on TDN achieved 79.5% (n=62) viral suppression.

Only 5.2% of patients had their regimen modified (drug substitution or switching to second line) by the end of the study period. The overall incidence rate of regimen modification was 24.4 per 1000 person-years after a total follow up time of 3159 person-years. Patients on

TDE had a regimen modification incidence rate of 19.2 per 1000 person-years compared to those on TDN with an incidence rate of 108.7 per 1000 person-years.

7 (0.5%) patients were reported dead by the end of the study period.

Table 3. Summary of main outcome events

Outcome	**Total**	**TDE**	**TDN**
Viral suppression/undetectable viral load (VL<100 copies/ml), n (%)	1,290 (89.5%)	1,228 (90.0%)	62 (79.5%)
Viral suppression (VL<1000 copies/ml)	1,341 (93.0%)	1,277 (93.6%)	64 (82.1%)
Regimen change; (%) incidence rate per 1000 person-years	(5.3%) 24.4	(4.2%) 19.2	(25.3%) 108.7

3.4 Predictors of viral non-suppression at 12 months

Table 4 shows (crude and adjusted) risk ratios (RR) for viral non-suppression (undetectable viral load) by ART regimen and other risk factors. In bi-variate analysis, TDN ART regimen (cRR= 2.06, CI 1.29 - 3.28, p-value 0.002), advanced HIV disease (WHO stage III and IV), cRR= 1.90, 95%CI 1.40 - 2.57, p-value <0.001 and poor adherence (<95%), cRR= 3.81, 95%CI 2.76 - 5.25, p-value <0.001 were significant risk factors for viral non- suppression. Gender, age at initiation, baseline CD4, BMI at initiation and presence or absence of side effects were not significantly associated with viral non-suppression.

Table 4. Predictors of viral non-suppression (detectable viral load): Univariate and multivariate models.

Characteristic	Univariate analysis		Multivariate analysis	
	*cRR (95% CI)	p-value	**aRR (95% CI)	p-value
Regimen				
TDE	1		1	
TDN	2.06 (1.29-3.28)	0.002	2.03 (1.31-3.15)	0.002
Facility				
Hlatikulu	1			
Nhlangano	0.77 (0.55-1.09)	0.144		
Matsanjeni	1.12 (0.75-1.69)	0.575		
Sex				
Male	1		1	
Female	0.86 (0.63-1.17)	0.335	1.02(0.75 -1.40)	0.894
Age at initiation (years)	1.10 (0.75-1.63)	0.621		
Baseline CD4 count				
>500	1		1	
350 – 500	1.23 (0.61-2.50)	0.558	1.18(0.61-2.30)	0.619
< 350	1.37 (0.74-2.55)	0.315	1.08(0.60-1.92)	0.800
WHO stage				
I, II	1		1	
III, IV	1.90 (1.40-2.57)	<0.001	1.89(1.39-2.57)	<0.001
BMI				
>25	1			

18.5- 25	1.14 (0.82-1.58)	0.440		
<18.5	1.40 (0.84-2.33)	0.201		
Adherence				
≥95%	1		1	
<95%	3.81 (2.76-5.25)	<0.001	3.87(2.83-5.29)	<0.001
Side effects				
No	1		1	
Yes	1.39 (0.84-2.32)	0.205	1.29(0.82-2.03)	0.268

**cRR= crude/unadjusted risk ratio, **aRR= adjusted risk ratio*

In the multi-variate model (Table 4), ART regimen, WHO stage category, and adherence remained significantly associated with viral non-suppression. The risk of viral non-suppression was twice as high for patients on TDN regimen compared to TDE (aRR 2.03, 95% CI 1.31 - 3.15, p-value 0.002). The risk of viral non-suppression was increased by 89% in patients that initiate therapy with advanced disease (WHO stage III and IV) compared to those that initiate with less advanced disease (WHO stage I and II), aRR 1.89, 95% CI 1.39 - 2.57, p- value < 0.001. Poor adherence (<95%) was also associated with an almost 4 times increase in risk of viral non-suppression compared to those with adherence greater than 95% (aRR 3.87, 95% CI 2.83 - 5.29, p- value 0.< 001. The rest of the variables were not found significantly associated with viral suppression.

Further, additional analysis were conducted using a different definition for virological non-suppression (VL >1000copies/ml). The estimates were largely similar to the analysis where a definition of VL> detectable was used.

Table 5. Predictors of virological failure (VL>1000 c/ml): Univariate and multivariate models

Characteristic	Univariate analysis			Multivariate analysis		
	cRR	95% CI	p-value	aRR	95% CI	p-value
Regimen						
TDE	1			1		
TDN	2.81	1.68- 4.72	<0.001	2.79	1.72-4.52	<0.001
Facility						
Hlatikulu	1					
Nhlangano	1.07	0.68- 1.68	0.776			
Matsanjeni	1.56	0.93 - 2.63	0.095			
Sex						
Male	1					
Female	1.10	0.74- 1.63	0.634			
Age at initiation (years)	1.11	0.68- 1.81	0.681			
Baseline CD4 count						
>500	1					
350 – 500	0.69	0.30-1.58	0.377			
< 350	1.03	0.53-2.01	0.920			
WHO stage						
I, II	1			1		
III, IV	1.96	1.34-2.86	<0.001	1.92	1.33-2.76	<0.001
BMI						
>25	1					

18.5- 25	1.03	0.68-1.55	0.888			
<18.5	1.38	0.74-2.59	0.307			
Adherence						
≥95%	1			1		
<95%	4.41	2.96-6.58	<0.001	4.501	3.06-6.64	<0.001
Side effects						
No	1			1		
Yes	1.51	0.81-2.80	0.195	1.38	0.79-2.42	0.256

**cRR= crude/unadjusted risk ratio, **aRR= adjusted risk ratio*

Table 5 above shows results from the univariate and multivariate models assessing the association between various risk factors and viral non- suppression (viral load greater than 1000 copies /ml). Similar results as those for viral load less than 100 were obtained. In univariate analysis, only ART regimen (p-value <0.001), WHO stage category (p-value 0.000) and adherence category (p-value <0.001) were found significantly associated with viral non-suppression.

In multivariate analysis, TDN regimen, advanced HIV disease and poor adherence remained significant risk factors for viral non-suppression. TDN (aRR 2.79, p-value <0.001), advanced HIV disease (aRR 1.92, p-value <0.001) and poor adherence (aRR 4.51, p-value <0.001) significantly increased the risk of viral non-suppression in the final model.

3.5 Regimen modification

3.5.1 Overall incidence of regimen modification

A total of 1453 patients were followed for a total of 3159.2 person-years for the regimen modification outcome analysis. The median follow-up time was 2.2 person-years and 77 regimen modifications were recorded, corresponding to an overall incidence of 24.4 per 1 000 person-years (95%CI 19.49 - 30.47).

Figure 2 below shows the Kaplan Meier curve of the time to regimen modification through-out the follow up period for all the study participants.

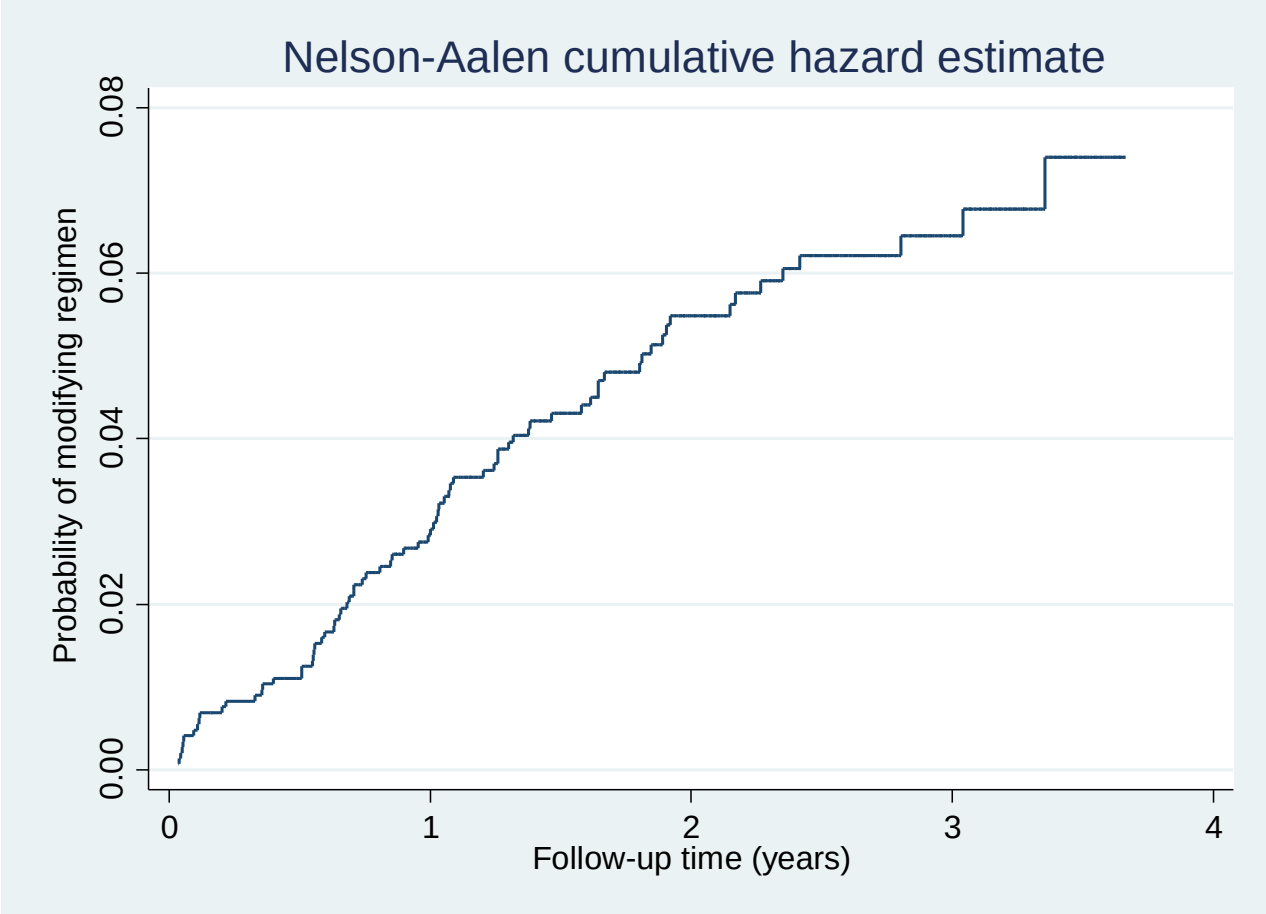

Figure 2. Cumulative hazard estimate; Probability of regimen modification over time)

3.5.2 Interval Incidence

Table 6 below shows the period incidence rates for regimen modification after starting ART. The highest incidence rates for regimen modification were between zero and 18 months after starting treatment and no regimen modification was recorded after 42 months of treatment.

Table 6. Interval incidence rate for regimen change

Time period(Months)	0 – 6	7- 12	13-18	19-24	25 - 30	31 - 36	37 –42	>42
Regimen Modification	16	24	18	11	5	1	2	0
Incidence rate (per 1000 person-years)	22.23	34.91	30.13	23.43	14.30	4.44	19.38	0

3.5.3. Cumulative hazard of regimen modification by ART regimen

Of the 79 patients on TDN regimen followed up for a total of 184.1 person-years, 20 regimen modifications were recorded (incidence rate of 108.65 per 1000 person years). On the other hand, 1374 patients on TDE had a follow-up time of 2975.15 person-years and recorded 57 regimen modifications (incidence rate of 19.16 per 1000 person-years). Patients on TDN regimen had a higher incidence rate of regimen modification compared to those on TDE (incidence rate ratio 5.67, 95%CI 3.23 - 9.58).

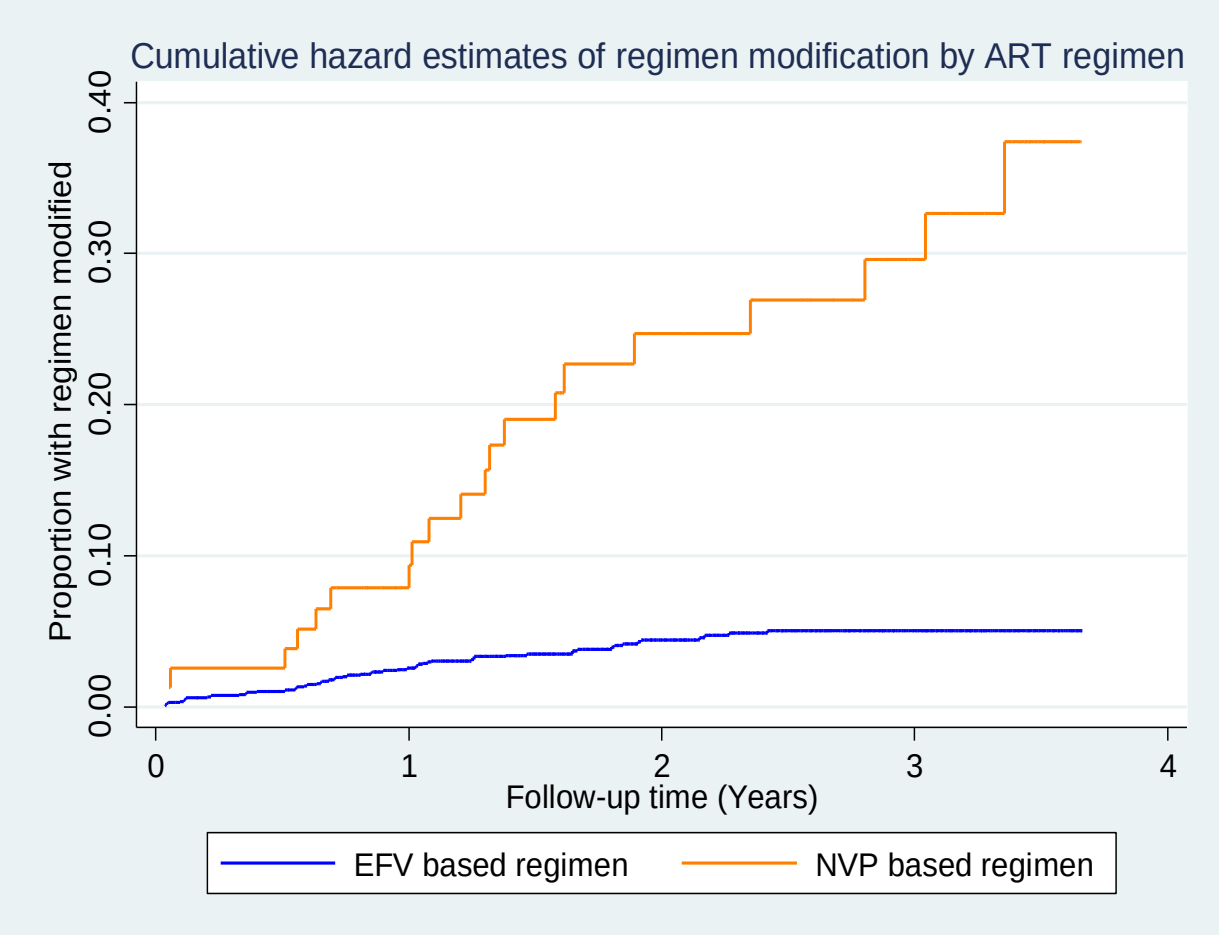

Figure 3. Cumulative hazard estimate for regimen modification by ART combination

Figure 3 above shows a cumulative hazard estimates for regimen change by baseline regimen. TDN regimen was associated with a significantly higher hazards for regimen change compared to TDE (log rank test, p-value <0.001).

3.5.4 Cumulative hazard of regimen modification by WHO stage category

Of the 386 patients enrolled onto treatment with either WHO stage III or IV, 29 patients had their regimen modified after a total follow up time of 839.63 person-years (incidence rate 34.54 per 1 000 person-years). The 1067 patients enrolled with WHO stage I or II had 48 regimen modifications after a follow up time of 2319.60 person-years (incidence rate 20.69 per 1 000 person-years). WHO stage III and IV was therefore was associated with a higher incidence of regimen modification (incidence rate ratio 1.67, 95%CI 1.01 - 2.70) compared to WHO stages I and II.

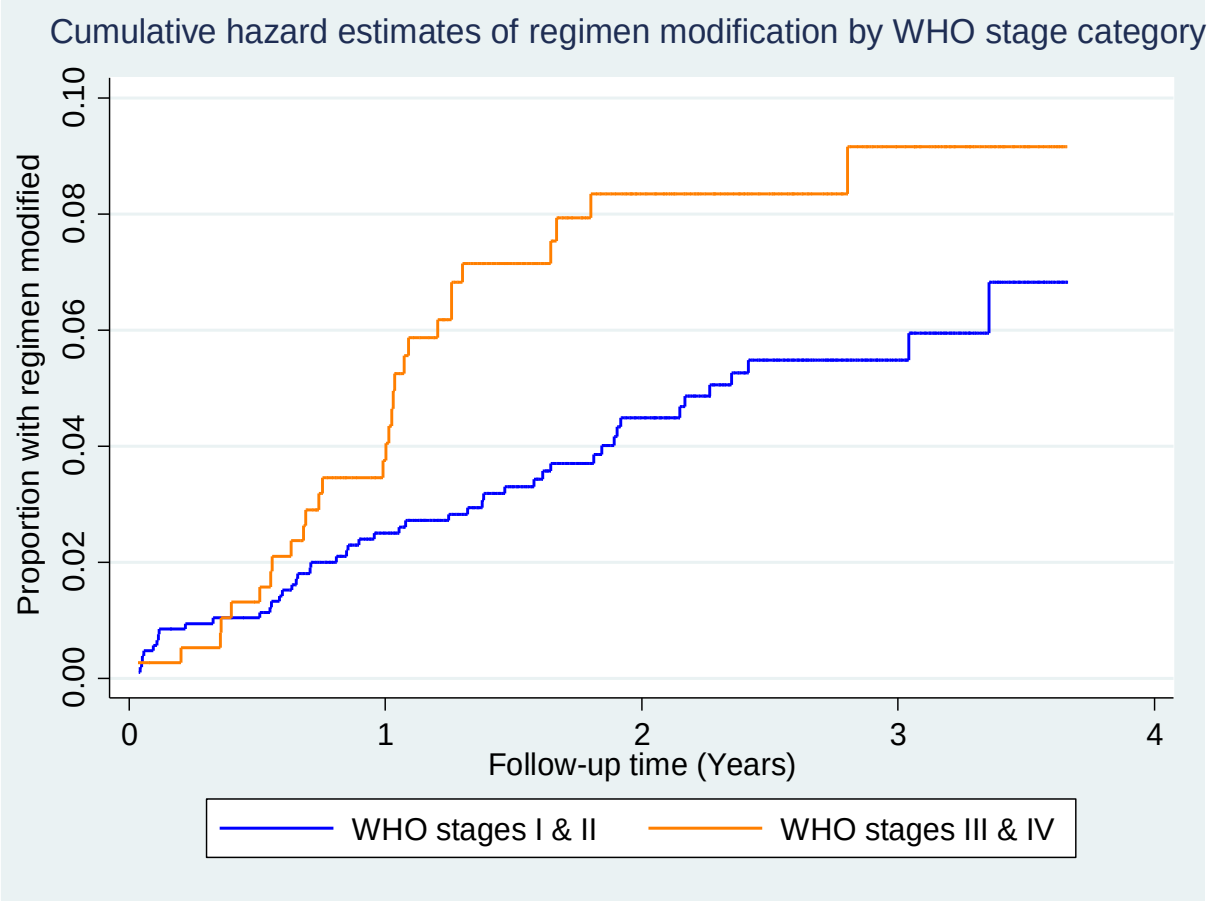

Figure 4. Cumulative hazard estimate for regimen modification by WHO stage category

Figure 4 above shows a Kaplan Meier plot of regimen modification by WHO category. The log rank test, (p-value =0.025) shows WHO stage I and II category to have a significantly better survival compared to WHO stage III and IV category.

3.5.5 Cumulative hazard of regimen modification by side effects category

A total of 55 regimen modifications were recorded among the 108 patients that reported any side effects over a follow up time of 160.93 person-years. This corresponds to an incidence rate of 341.77 per 1 000 person years. On the other hand, 22 regimen modifications were recorded among the 1345 patients that did not report any side effects over a total follow up period of 2998.30 person-years, corresponding to an incidence rate of 7.34 per 1 000 person days. The incidence rate ratio between the side effects and the no side effects groups was 46.58, 95% CI 27.94 - 80.21.

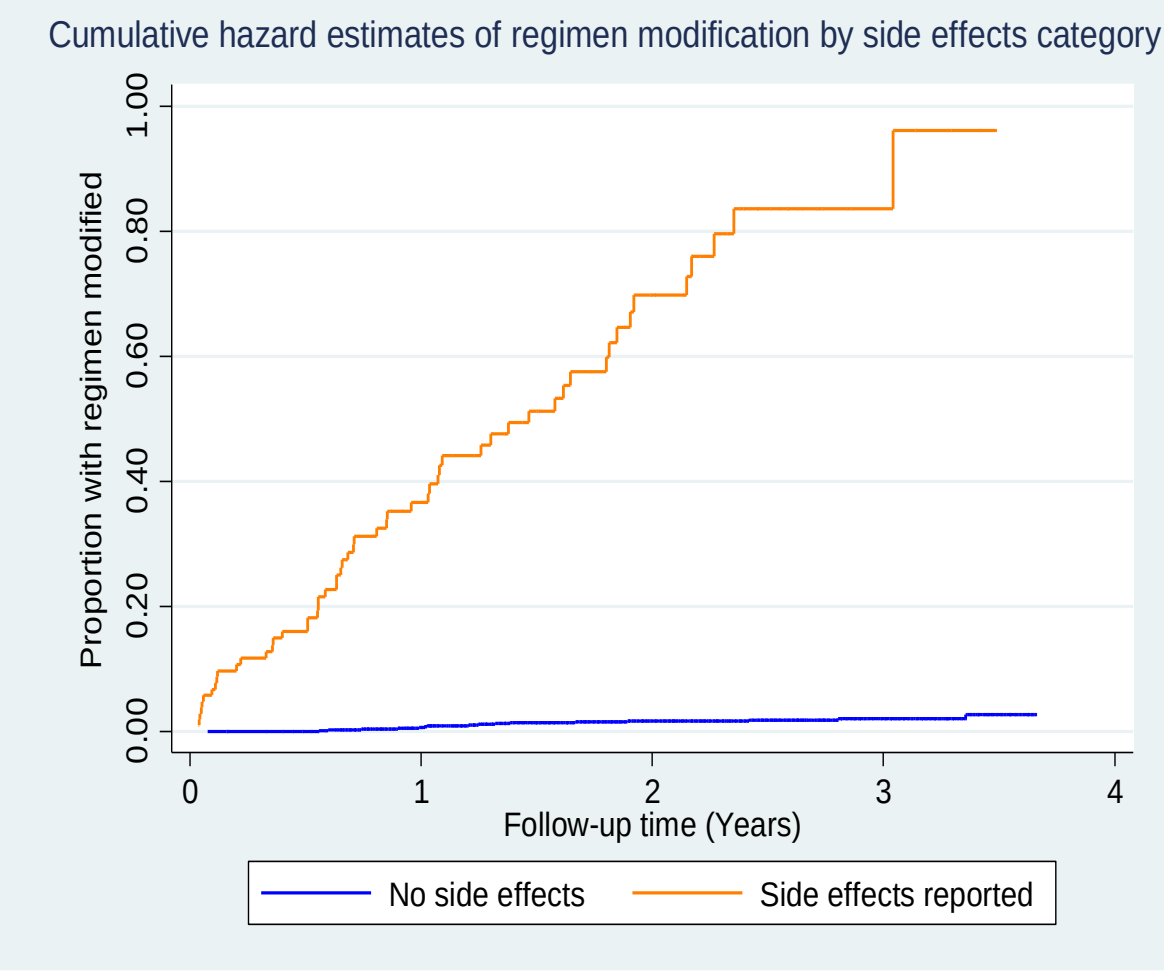

Figure 5. Cumulative hazard estimate for regimen modification by side effects category.

Figure 5 above shows the Kaplan Meier plot by side effects category. The patients who reported side effects showed a significantly higher incidence of regimen modifications compared to those that reported no side effects (log rank, p-value <0.001).

3.6 Predictors of regimen modification

3.6.1 In the univariate model, ART regimen, sex, WHO stage and side effects were significantly associated with regimen modification. Table 7 below shows the unadjusted and adjusted hazard ratios for regimen modification and various risk factors. Patients on TDN had almost 6 times the hazards of having their regimen modified compared to those on TDE (cHR 5.99, 95%CI 3.60-9.99, p-value < 0.001). Females had a 40% reduction in the hazard of regimen modification compared to males (cHR 0.60, 95%CI 0.38-0.94, p-value 0.025). Patients who started treatment with advanced disease (WHO stage III or IV) were 68% more likely to have their regimen modified compared to patients enrolled with less advanced (WHO stage I or II) HIV disease (cHR 1.68, 95%CI 1.06-2.67, p-value 0.027). Patients that experienced side effects during the study period were 45 times more likely to modify regimen compared to those that did not report any side effects (cHR 45.13, 95%CI 27.40-74.34, p-value <0.001)

Table 7. Predictors of Regimen modification: Univariate model

Characteristic	**Univariate analysis**		**Multivariate analysis**	
	*cHR (95% CI)	p-value	aHR (95% CI)	p-value
Regimen				
TDE	1		1	
TDN	5.99(3.60-9.99)	<0.001	4.49(2.68-7.53)	<0.001
Facility				
Hlatikulu	1			
Nhlangano	0.86(0.52-1.43)	0.565		
Matsanjeni	0.87(0.44-1.74)	0.699		
Sex				
Male	1		1	
Female	0.60(0.38-0.94)	0.025		

Age at initiation (years)	1.08(0.59-1.96)	0.807		
Baseline CD4 count				
>500	1		1	
350 – 500	0.93(0.27-3.16)	0.901		
< 350	1.83(0.67-5.02)	0.241		
WHO stage				
I, II	1		1	
III, IV	1.68(1.06-2.67)	0.027	1.66(1.04-2.64)	0.033
BMI				
>25	1			
18.5- 25	1.25(0.77-2.04)	0.374		
<18.5	1.19(0.52-2.76)	0.681		
Adherence				
≥95%	1			
<95%	1.35(0.62-2.94)	0.447		
Side effects				
No	1		1	
Yes	45.13(27.40-74.34)	<0.001	41.76(25.25-69.08)	<0.001

3.6.2 Multivariate analysis

In the multivariate analysis (Table 7), patients on TDN had over 4 and half times increased hazards of regimen modification compared to those on TDE (aHR 4.49, 95%CI 2.68-7.53, p-value < 0.001). Sex was no longer significantly associated with regimen modification. Patients that enrolled into therapy with advanced HIV disease had a 66% increase in hazards for regimen modification compared to those that enrolled with less advanced disease (aHR 1.66, 95%CI 1.04-2.64, p-value 0.033). Side-effects remained significantly associated with

regimen modification with those that reported side effects having over 40 times increase in hazards compared to those that did not report any side effects (aHR 41.76, 95%CI 25.25-69.08, p-value < 0.001). Sex was not significantly associated with regimen modification in the multivariate model.

3.7 Proportional hazard assumption test

The stphplot for sex, ART regimen, side effects, CD4 categories were fairly parallel hence satisfying the proportional hazards assumption. There was minor violation of the assumption with WHO stage and BMI categories. (Appendix C)

The global test for proportional hazards assumption was borderline (p-value 0.05). Appendix C shows the stphplots for various risk factors and the global test.

CHAPTER 4. DISCUSSION

4.1 Summary

The main objectives of the study were to compare the rates of viral non-suppression and regimen modification between an EFV and a NVP based TDF first line regimen in a Shiselweni cohort, Eswatini. Overall viral suppression rates of 89.5% (for undetectable viral load) and 93% (for viral load less than 1000 copies per ml) observed in this study were comparable to the viral suppression rates of 91.9% observed in the general Eswatini ART population (Swaziland HIV incidence measurement survey ,SHIMS 2).[40] The regimen modification incidence rate of 5.3% observed in this study was also largely similar to the regimen modification incidence rates of 3.9% seen in a similar study done at Mbabane government hospital, Eswatini.[34] NVP based regimen was consistently a strong risk factor associated with both virological non-suppression and regimen modification after controlling for sex, baseline CD4 count and WHO stage. While this study found EFV to be associated with a higher risk of regimen modification, findings from other studies were inconsistent.

4.2 Baseline characteristics

While the Eswatini population based HIV incidence survey used age group 15 years and above to estimate on treatment viral suppression rates for adolescents and adults,[40] our study recruited participants 18 years and above. Just like in our study, most viral suppression studies excluded children and treatment experienced participants.[27,43,44] There were more females than males in all studies reviewed, which is similar to the gender profile we observed in our study. Our study only recruited participants on a TDF + 3TC backbone but a number of reviewed studies included patients on FTC and even AZT in their combinations.[43,44,45]

Generally, almost three quarters of the patients started treatment with a CD4 count below 350 (median 248 IQR125-350). Despite the test and start strategy, most patients still started treatment with advanced HIV disease. More women started treatment with CD4 count above 500 compared to men (11.43% and 3.99 % respectively). Conversely, more men started treatment with low CD4 counts (<350) compared to women (84.42% and 68.59%

respectively). This is consistent with results from thither studies locally and internationally, and can be explained by the late health seeking behaviour by men. Women also got routinely offered HIV test during pregnancy and breastfeeding hence are likely to be started on treatment earlier compared to men.

4.3 Rate of viral suppression

Viral suppression rates differ from country to country and from time to time and their improvement has been one of the pillars in a three pronged approach (the 90-90-90 target) by WHO to control the HIV pandemic.[5] The viral suppression rates of 89.5% observed in this study were consistent with the national viral suppression rates shown in the Eswatini population based survey of 91.9% conducted in a similar time frame (2016-2017).[40] This supports the evidence that Eswatini has just about reached its target of 90% viral suppression among all patients taking ART. This does not give the full picture however since patients under 18 years were not part of this study. A study conducted in Eswatini showed that children (AOR 2.6, 95%CI 1.5–4.5) and adolescents (AOR 3.2, 95%CI 2.2–4.8) have higher viral non-suppression rates than the general population on ART.[45] A study in Uganda between 2014 and 2015 also reported similar viral suppression rates of 89% among patients on ART.[46] Similarly, a European study reported viral suppression rates of 92.3% among patients on TDE.[43] This study was however conducted much earlier (between 2009 and 2010) compared to our study. Our study findings were therefore consistent with local, regional and international viral suppression rates.

4.4 Predictors of viral non-suppression

Apart from ART regimen, other risk factors for viral suppression considered in this study included age, sex, baseline CD4 count, baseline WHO stage, baseline BMI, percentage adherence (pill count) and presence of side effects. TDN ART regimen, WHO stage III and IV, and poor adherence (95%) were associated with an increased risk of viral non-suppression.

A nevirapine based regimen consistently increased the risk (more than double) of viral non-suppression (both detectable and viral load greater than 1000 copies per ml) compared to EFV based regimen. Similar findings have been reported from similar studies.[47,27,28,] Results from those studies also showed poor viral suppression on other NVP based regimens even

with an AZT backbone. This seems to suggest an inferiority of NVP in combination with other anti-retroviral drugs and not specifically TDF. Single drug substitution in a three drug combination may have a significant effect on regimen efficacy as shown with 3TC and FTC.[47,43] A possible explanation could be differences in the pharmacodynamics and pharmacokinetics of the NNRTIs.

Advanced HIV disease (WHO stages III and IV) and poor adherence were associated with poor virologic outcomes. This was also reported in other studies in similar settings. [32,33] This has supported the move by WHO of universal access to ART for all HIV infected people regardless of WHO stage. It is interesting however to note that baseline CD4 count was not significantly associated with viral suppression. The importance of good adherence is well documented in chronic infectious disease care. It is for this reason that a single high viral load (>1000 copies/ml) is to be followed by a period of step-up counselling to optimise adherence before a repeat viral load test is performed.

4.5 Incidence of regimen modification

The overall regimen modification rate of 5.3% were observed in this study was comparable to another study done at Mbabane government hospital, Eswatini (3.9%).[34] The slightly higher rate seen in our study could be due to a longer follow-up period (over 3 years) compared to the Mbabane government hospital study (1 year). The rate observed in our study was however much lower than rates of regimen change observed in a Nigerian study (83%).[42] Notably, this study was conducted on patients initiating ART between 2004 and 2006. During those early days of ART, some of the drugs (d4T) used in the combination therapy were associated with a high rate of side effects. This confirms the safety and durability of ART regimen in use today as advancement in HIV and ART knowledge continues. The follow up time was also considerably longer (3-8years) than in our study (6 months to 42 months). Most of the regimen modifications (50%) were reported in the first 12 months of initiating treatment, while there were very few regimen modification after 24 months of initiating treatment. This is consistent with the fact that side effects tend to be seen early in treatment and was by far the commonest reason for regimen modification. The fewer regimen changes seen later in treatment suggest the emergence of treatment failure in addition to late side effects.

4.6 Predictors of regimen modification

NVP based regimen, WHO stage III and IV and presence of side effects were shown to increase the hazards of regimen modification. In a high TB prevalence setting like Eswatini, substitution of NVP by EFV upon diagnosis of TB could be part of the reason NVP is associated with regimen change. Since NVP was also associated with poor viral suppression, it also follows that patients on NVP were likely to fail first line ART regimen and to be switched to second line. Furthermore, patients on NVP had more side effects than on EVF (p-value 0.024), and were therefore more likely to have regimen modified. Therefore the higher rates of regimen modification seen with NVP based regimen could be due to TB co-infection, virological failure and more side-effects associated with NVP, among other factors. As shown earlier, WHO stages III and IV, may result in more regimen modifications through switching to second line as a result of treatment failure.

4.7 Strengths and limitations

The sample size and follow up times achieved in this study were large enough to show differences between the primary exposure variable (ART regimen) and the main outcome variable (viral suppression). The study was also able to look at two aspects of regimen comparison, that is, viral efficacy and regimen durability. Our study also recruited patients from the 3 main health service facilities with in the Shiselweni region thereby giving a fairly representative sample for the whole region.

While our study was conducted in real life clinical settings, and the results were largely consistent with similar studies locally and internationally, it had a number of challenges. As with all retrospective studies, the use of secondary data meant some significant data were missing. The exclusion of patients with missing data from the study could have introduced bias in the event that the patients with missing data were associated with either a particular ART regimen and/or a viral load or regimen change outcomes. Since this was routinely collected data, some of the data might not have been collected hence under estimating the association between viral suppression, regimen modification and side effects. Some variables that are potential risk factors were also not routinely collected and hence excluded in the analysis, for example tuberculosis infection. Other aspects of regimen efficacy like time to viral suppression and duration of viral suppression were not measured in this study.

4.8 Conclusion and recommendations

Although the estimates above have been largely consistent with findings from similar studies, their interpretation must be done with caution due to the limitations mentioned earlier. NVP based first line regimen was associated with viral non-suppression. It was also shown to be less durable in the chronic treatment of HIV. This combination should therefore be used with caution and where it cannot be avoided. Patients on this regimen may require closer monitoring to look out for virological failure. It may also be advisable to only use this regimen as a "bridging regimen" for patients that are being stabilised for more efficacious and durable regimens. Further research is recommended to evaluate the role of other risk factors like TB, time to viral suppression (with monthly viral load is taken as opposed to 6 monthly as is the current clinical practice) and to evaluate how long patients remain virally suppressed to arrive at a holistic comparison of these 2 regimens.

Among patients over 18 years of age on TDF based first line ART regimens, those on TDN regimen had poorer virologic outcomes at 12 months and had higher risk of regimen change compared to patients on TDE regimen. Even in the advent of more durable and safer TDF based first line ART regimens, EFV should remain the preferred non-nucleoside reverse transcriptase inhibitor.

4.9 Generalizability

Our study recruited patients from throughout the Shiselweni region and analysed a large sample. The study was done in a typical clinical setting guided by national treatment guidelines. The Swazi population is also fairly homogenous in demographic, socio-economic characteristics and HIV prevalence. Our findings were also similar to those from a wider, population based SHIMS 2 survey. For these reasons, our findings can be generalized to the wider Eswatini adult population outside the Shiselweni region (18 years and above). The same findings may however not be generalizable outside the Eswatini population or population under 18 years old where most of the above variables may not be similar.

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APPENDICES

Appendix A: Ethics Clearance

Research Protocol clearance certificate

Type of review	Expedited	X		Full Board			
Name of Organization	STUDENT						
Title of study	Virologic outcomes for tenofovir disoproxil fumarate based antiretroviral therapy regimens in Swaziland, 2013 – 2015						
Protocol version	1.0						
Nature of protocol	New	x		Amendment			
List of study sites	HLATHIKHULU HOSPITAL, NHLANGANO AND MATSANJENI HEALTH CENTRES						
Name of Principal Investigator	DR MALVERN MASANGO						
Names of Co- Investigators	N/A						
Names of steering committee members in the case of clinical trials	N/A						
Names of Data and Safety Committee members in the case of clinical trials	N/A						
Level of risk (Tick appropriate box)	Minimal				High		
	X						
Clearance status (Tick appropriate box)	Approved	X			Disapproved		
Clearance validity period	Start date	29/08/2017	End date		29/08/2018		
Signature of Chairperson							
Date of signing	29/088/2017						
Secretariat Contact Details	Name of contact officers	Ms Simangele Masilela					
	Email address	kaluamasi@gmail.com					
	Telephone no.	(00268) 24040865/24044905					

MINISTRY OF HEALTH / 9 SEP 2017 HEALTH RESEARCH REVIEW BOARD SWAZILAND

Approval Conditions

1	Implementation of approved version of protocol	✓					
2	Reporting of adverse events within 5 days of occurrence	✓					
3	Submission of progress reporting for multi-year studies	Yr 1	Yr 2	Yr 3	Yr 4	Yr 5	
		N/A	N/A	N/A	N/A	N/A	
4	Submission of end of project report (Hard copy)	✓					
5	Submission of end of project report (Soft copy)	✓					
6	Submission of data sets	✓					

List of reviewed documents

Ref.	Documents	Reviewed documents (tick appropriate box)
1	Completed application form	✓
2	Cover letter	✓
3	Evidence of administrative permission to conduct the research by involved institutions/sites (where applicable)	✓
4	Detailed current resume or curriculum vitae of Principal Investigator/s including Principal investigators declaration	✓
5	Summary resume or biography for other investigator(s)	✓
6	Evidence of approval/rejection by other Ethics Committees, including comments and requested alterations to the protocol, where appropriate.	
7	Research protocol (see outline in Annex 1)	✓
8	Questionnaires and interview guides (with back-translated versions where applicable)	✓
9	Case report forms (CRFs), abstraction forms and other data collection tools	✓
10	Participant/subjects Information Statement(s) (where applicable)	✓
11	Informed consent form(s) including photographic and electronic media consent statements.	
12	Advertisements relevant to the study (where applicable)	
13	Source of funding and detailed budget breakdown including material and incentives to participants if applicable	
14	Notification form for adverse effects/events.	
15	Proof of payment	
16	Proof of insurance cover for research subjects in clinical trials or where applicable	✓
17	Any other special requirements should be stated, if applicable	None

Appendix B: Hospital permission letters

Telephone: (09268) 2207 8260

Fax: (09268) 2207 9513

Nhlangano Health Centre

P.O. Box 29
Nhlangano
Swaziland

THE KINGDOM OF SWAZILAND

To whom it may concern

RE: PERMISSION TO CONDUCT STUDY AT NHLANGANO HEALTH CENTRE.

This serves to certify that Dr Malvern Masango has been granted permission to access ART clients' records at our institution subject to him bringing the ethics approval letter from the health Research Review board, Swaziland. His research topic is titled Virologic outcomes for Tenofovir Disproxil Fumerate based antiretroviral therapy regimens in Swaziland, 2013-2015 and is in partial fulfillment of his Masters in Epidemiology with the University of Pretoria.
We are interested in the findings and will be happy to get feedback of the study findings.

Yours faithfully

Acting senior medical officer

NHLANGANO HEALTH CENTRE SENIOR MEDICAL OFFICER 2017 -08- 30 P.O. BOX 29 NHLANGANO S400 SWAZILAND

Dr R Kashangura

Telephone: (+268 22176111)
Fax: (+268 2276004)

HLATIKULU HOSPITAL
P.O. BOX 20
HLATIKULU
SWAZILAND

THE KINGDOM OF SWAZILAND

25 AUGUST 2017

TO WHOM IT MAY CONCERN

RE: PERMISSION TO CONDUCT STUDY AT HLATIKULU GOVERNMENT HOSPITAL

This serves to certify that Dr Malvern Masango has been granted permission to access ART clients' records at the institution. This is in partial fulfillment of his Masters in Epidemiology with the University of Pretoria. His research topic is titled Virologic outcomes for Tenofovir Disproxil Fumerate based antiretroviral therapy regimens in Swaziland, 2013-2015 and it has been approved by the Health Research Review board at the Ministry Of Health Swaziland.

May you assist him accordingly.

HLATIKULU GOVERNMENT HOSPITAL SENIOR MEDICAL OFFICER 2017 -08- 25 P.O. BOX 20 HLATIKULU SWAZILAND

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Matsanjeni Health Centre
P. O. Box 143
Lavumisa
Swaziland

THE KINGDOM OF SWAZILAND

18 August 2017

TO WHOM IT MAY CONCERN

RE: PERMISSION TO USE MATSANJENI HEALTH CENTRE AS A STUDY SITE

This is to certify that Dr Malvern Masango has been given access to patient records at Matsanjeni Health Centre to conduct academic scientific study for his Masters in Epidermilogy "with the University of Pretoria. The research topic is titled "Virologic outcdomes for Tenofovir Disoproxil fumerate based antiretroviral therapy regiments in Swaziand, 2013-2015.

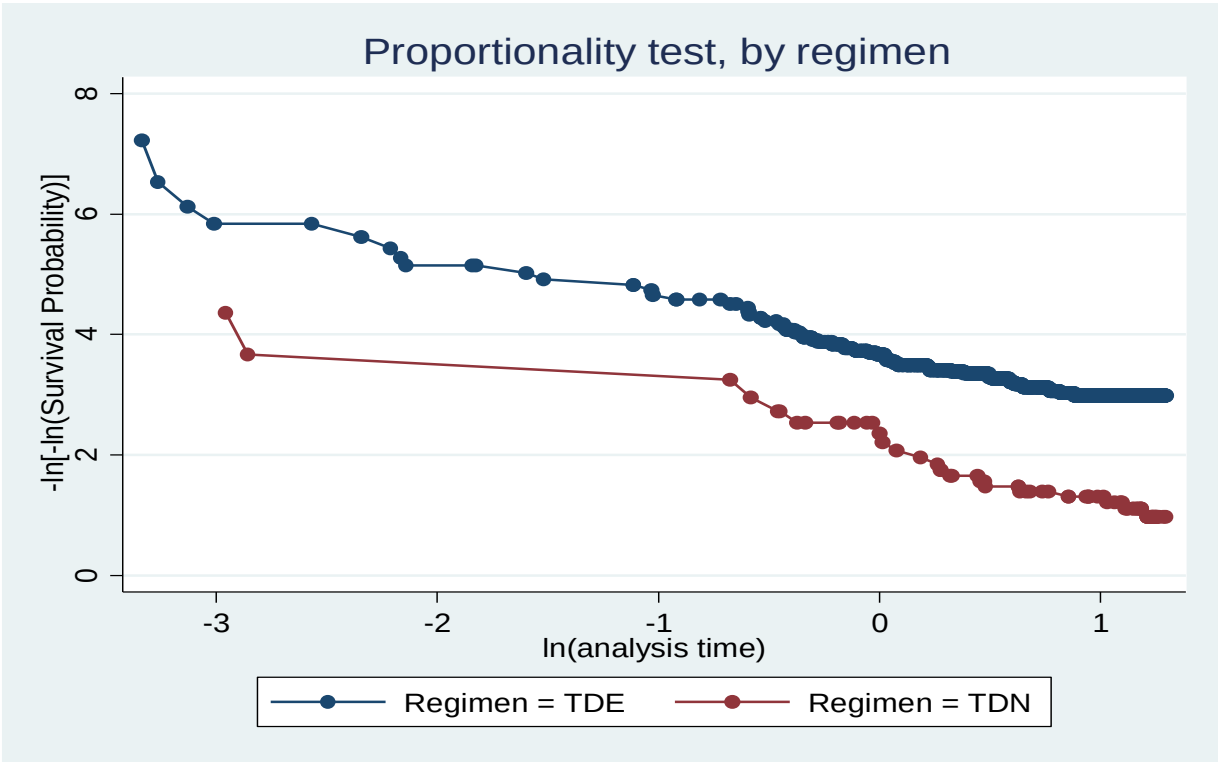
May you assist him accordingly

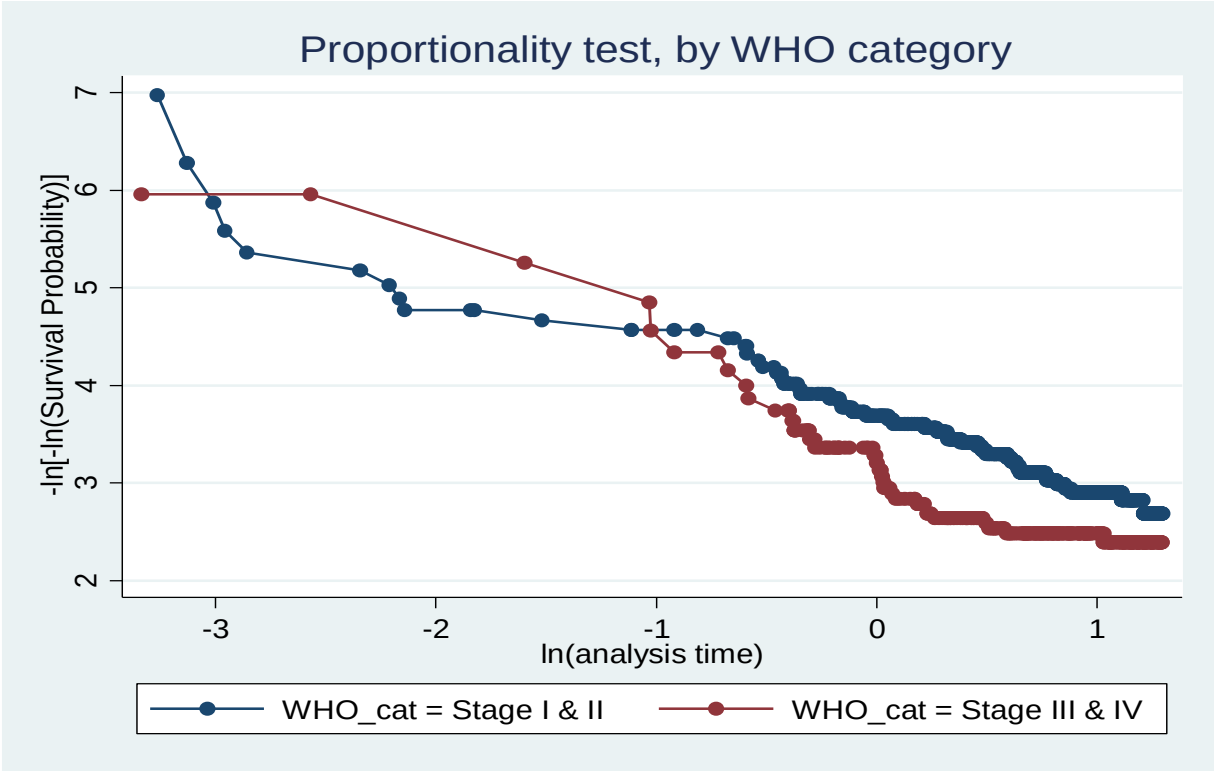
Yours faithfully

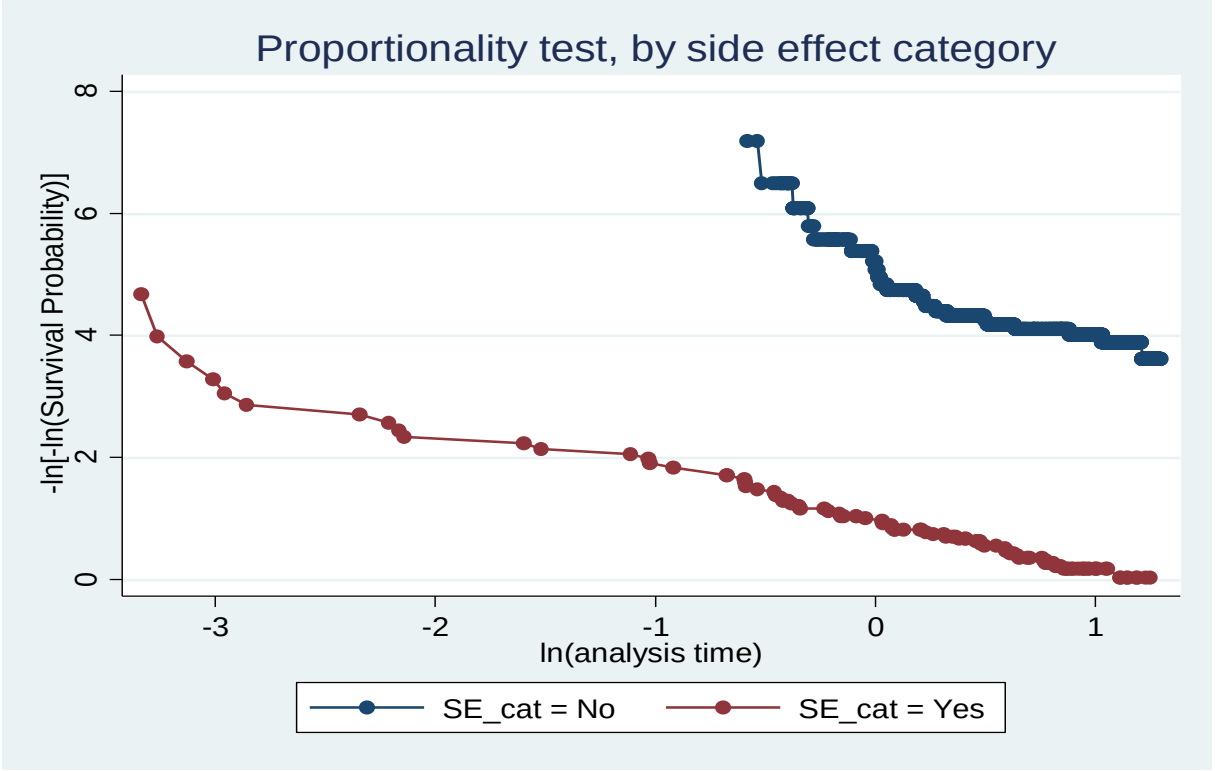
DR CLARA M. NYAPOKOTO
Senior Medical Officer
claramunyaradzi@gmail.com

MATSANJENI HEALTH CENTRE
P.O. Box 143,Lavumisa
18 AUG 2017
SENIOR MEDICAL OFFICER

Appendix C: Proportionality test




Proportionality test, by WHO category
-ln[-ln(Survival Probability)]
2
3
4
5
6
7
-3
-2
-1
0
1
ln(analysis time)
WHO_cat = Stage I & II
WHO_cat = Stage III & IV


Proportionality test, by side effect category
-ln[-ln(Survival Probability)]
0
2
4
6
8
-3
-2
-1
0
1
ln(analysis time)
SE_cat = No
SE_cat = Yes

estat phtest

Test of proportional-hazards assumption

Time: Time

---

chi2	df	Prob>chi2
global test	8.01	3	0.0459
