University of Pretoria
Faculty of Health Sciences
School of Health Systems and Public Health

STAVUDINE-ASSOCIATED TOXICITY IN PATIENTS ON LOW-DOSE VERSUS HIGH-DOSE STAVUDINE IN AN HIV TREATMENT COHORT

By
Mmatsie Manentsa
Student No: 20099968

Submitted in partial fulfilment of the requirements for the degree
Master of Science (MSc) Epidemiology

November 2015

Supervisor
Dr. N.R.T. Ledibane

Co-Supervisor
Prof. P. Rheeder

SUMMARY

Background

Stavudine (d4T) is an antiretroviral drug used in developing countries for the management of HIV due to its efficacy and low cost. Concerns over its toxic side effects has led the WHO to recommend that it be phased out. The study aim was to ascertain whether a low, yet efficacious dose of d4T (20 mg twice daily) would lead to fewer incidences of toxicity.

Objectives

This study compared incidence of toxicity in patients on low dose d4T (20 mg) versus the higher doses (30 mg /40 mg).

Methods

A retrospective analysis using STATA 12 was conducted on 1086 patients in a South African HIV treatment cohort. They were stratified into 3 d4T dose groups; 20 mg (n=43); 30 mg (n=707); and 40 mg (n=336). Time to onset of toxicity was assessed using survival analysis. Toxicity incidence rates were estimated using Poisson regression. Cox models were used to determine risk factors.

Results

Median time to onset of toxicity was 217, 137 and 55 weeks for the d4T 20 mg, d4T 30 mg and d4T 40 mg groups, respectively. Toxicity incidence rates per 100 person-years were 43 (95%CI 25 - 76), 67 (95%CI 54 - 83) and 174 (95%CI 143 - 211), respectively. Patients on d4T 20 mg were less likely to develop toxicity compared to the other doses, hazard ratio 0.36 (95%CI 0.20 - 0.65). Female sex was a risk factor for toxicity, hazard ratio 1.58 (95%CI 1.25 – 2.00). Other identified risk factors included the presence of renal dysfunction as well as BMI.

Conclusion

d4T 20 mg twice daily led to fewer incidences of toxicity. Further clinical trials are needed to compare this drug dose to other antiretrovirals.

Key Words

Antiretroviral, HIV cohort, Stavudine, Cumulative toxicity, Dose comparison, Retrospective, Survival Analysis, South African

DECLARATION OF AUTHORSHIP

I, Mmatsie Manentsa, do hereby declare that the dissertation titled "Stavudine-associated Toxicity in Patients on Low-dose versus High-dose Stavudine in an HIV Treatment Cohort" submitted for the Degree MSc Epidemiology, is my own original work and has not been submitted before for a diploma or degree at any other tertiary institution.

Ethics Approval

Date of Approval: 26/02/2015

Reference number: 32/2015

Name Of Journal For Proposed Submission:

AIDS

Dr. Mmatsie Manentsa — 20 / 11 / 2015 — Date

Dr. NRT Ledibane (Supervisor) — 20 / 11 / 2015 — Date

Prof. P. Rheeder (Co-supervisor) — 20 / 11 / 2015 — Date

ACKNOWLEDGEMENTS

Firstly and foremost I would like to thank Dr. Neil Martinson without whom none of this would have been possible. His encouragement and efforts to help me further my studies will not be forgotten.

I would like to thank my supervisors, Dr. Neo Ledibane and Prof. Paul Rheeder for their enduring patience and assistance during this process. I appreciate that you always found time for me.

I would also like to thank Mr Kennedy Otwombe for the input he gave on the statistics when I was at times truly lost.

Lastly I would like to thank my family and friends whose support was unceasing. I thank them for all the countless times they had to listen to me complaining about my studies. They have kept me sane.

TABLE OF CONTENTS

LIST OF ACRONYMS

ABC	Abacavir
AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Treatment
ARV	Antiretroviral
ATP	Adenosine Triphosphate
AZT	Zidovudine
BID	Two times daily
BMI	Body Mass Index
d4T	Stavudine
DNA	Deoxyribonucleic Acid
EFV	Efavirenz
ETOX	A randomized study comparing the efficacy and tolerability of low-dose versus standard-dose stavudine in antiretroviral-naïve patients
HB	Haemoglobin
HIV	Human Immunodeficiency Virus
IQR	Interquartile Range
MNAR	Missing Not At Random
NDOH	National Department of Health
NGO	Non-Government Organisation
NRTI	Nucleoside Reverse Transcriptase Inhibitor
PEPFAR	President's Emergency Plan For AIDS Relief
PHRU	Perinatal HIV Research Unit
PMTCT	Prevention of Mother To Child Transmission
SD	Standard Deviation
TB	Tuberculosis
TDF	Tenofovir
UN	United Nations
UNAIDS	Joint United Nations Programme on HIV and AIDS
VL	Viral Load
WHO	World Health Organisation
3TC	Lamivudine

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE: INTRODUCTION

1.1 Background

Stavudine (d4T) is a nucleoside reverse transcriptase inhibitor antiretroviral (ARV) drug that has been used for the treatment of Human Immunodeficiency Virus (HIV) infection worldwide. The initial recommended dosage was 40 mg twice daily (bid), based on efficacy studies.[1] However, the long-term toxic effects of the drug led the World Health Organisation (WHO) to recommend decreasing the dose to 30 mg bid to improve its safety profile.[2] Eventually the WHO recommended that the drug be phased out of ARV treatment guidelines in favour of alternative safer drugs.[3]

Alternative ARV's such as Tenofovir (TDF), Zidovudine (AZT) and Abacavir (ABC) cost significantly more than d4T.[4,5] The increasing number of people living with HIV and Acquired Immune Deficiency Syndrome (AIDS), who need ARV treatment[6] means that those paying for Antiretroviral Treatment (ART) must explore novel approaches to ensure the sustainable provision of ARV's. The demand for inexpensive ARV's ensures that d4T continues to play a role in the ART programmes of most developing countries, including South Africa. As a result there have been calls to investigate potentially safer, lower dosages of d4T for both efficacy and safety.[7,8]

The overall aim of the study was to compare differences in cumulative toxicity among patients taking low dose d4T 20 mg versus the higher doses of 30 mg and 40 mg in a South African setting. This was done through comparisons of time to onset of toxicity stratified by dose, incidence rates of toxicity as well as through analysis of the risk factors for the development of toxicity. A retrospective review of an ARV treatment cohort of up to 6 years of follow-up was conducted. The analysis included patients who were initiated onto stavudine between 2004 and 2008.

1.2 Motivation

In South Africa, the debate about the use of d4T still continues despite the change in the ART guidelines, by the National Department of Health (NDOH), to have a TDF-based regimen as first line treatment since 2010.[9-11] d4T is still used as an alternative treatment option for patients who, due to anaemia and poor renal functions, cannot tolerate other ARV's such as AZT and TDF. It is also used as a substitute when these recommended ARV's are not available due to drug shortages.

The supply of TDF has at times been erratic with frequent reports of stock-outs in many health facilities across the country.[12-14] When these stock-outs occur HIV clinicians have been advised to switch patients to d4T temporarily as short term use of d4T is associated with less side effects.[13] Causes of these stock-outs are multifactorial and include poor supply chain management as well as increased demand.[12,15]

The demand for ARV's is set to increase with the change in eligibility criteria for initiation of ARV treatment, in the 2015 National HIV treatment guidelines, from the current standard of a CD4 count of < 300 cells/mm^3 to a CD4 count <500 cells/mm^3.[16] Moreover, as HIV incidence remains high[17], the proportion of people who will need treatment will escalate which will further put a strain on the country's ART programme budget due to the high cost of TDF.

The current price for d4T combined with 3TC is $43 per person per year, which is significantly less than $73 per person per year, for the preferred first line drug TDF combined with 3TC.[4] Therefore, due to its low cost and availability d4T cannot be completely abandoned. This has prompted the call for further investigations into the efficacy and safety of low dose formulations of d4T, as it seems that d4T will continue to play a role in the South African ART programme.[18]

There have been a few dose-ranging studies to ascertain safety and efficacy of low dose d4T (30/20 mg bid).[7] Most of these studies found that lower doses demonstrated the same antiviral activity as higher doses. The ETOX clinical trial (A randomized study comparing the efficacy and tolerability of low-dose versus

standard-dose stavudine in antiretroviral-naïve patients), found that patients on low dose d4T experienced less toxicity than those on standard doses.[19] Early dosing studies by Bristol-Myers Squibb found even lower rates of toxicity with d4T 20 mg (15%) than d4T 30 mg (21%).[21]

A study by McComsey et al in the United States looked at the effects of switching to low dose d4T (20 /15 mg bid) in patients who had developed signs of toxicity on standard doses (40 /30 mg bid).[22] The study showed that the lower doses improved mitochondrial toxicity at 48 weeks by decreasing lactate levels and increasing fat-mitochondrial deoxyribonucleic acid (DNA). Currently a clinical trial looking into low dose d4T (20 mg bid) versus TDF is underway in South Africa.[11] The limitation of this clinical trial is that it will not be able to assess long-term cumulative toxicity as the follow-up period is only 96 weeks.[9]

Little is known about the long-term cumulative toxicity of lower dose d4T as most studies have not followed-up patients beyond 48 to 96 weeks. Furthermore, the low dose of d4T 20 mg was used in a small proportion of patients who either had very low weight or renal dysfunction.[21] This analysis sought to ascertain whether there is a significant difference in the incidence of long-term toxicity between low dose formulations and standard doses. The lengthy follow-up period yielded more information than what is currently known on rates of cumulative toxicity of low dose d4T. This knowledge could help to determine if the use of d4T should be abandoned completely or if the use of low dose, inexpensive formulations should be pursued as a sustainable long-term strategy in the national ART programme.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

The 2013 UNAIDS report on the Global Aids Epidemic estimated that there are 35.3 million people living with HIV worldwide.[6] By 2012, a total of 9.7 million people living in low and middle income countries were on ARV treatment.[6] In 2012, 6.4 million South Africans were estimated to be living with HIV and 2 million of those have been initiated on ART.[23]

The roll-out of ARV's in the South African public health sector began in 2004 as outlined in the NDOH's Operational Plan for Comprehensive HIV and AIDS Care, Management and Treatment for South Africa.[24] At that time the national treatment guidelines recommended d4T 40 mg, lamivudine (3TC) and efavirenz (EFV) for first-line treatment of HIV.

In 2007 the WHO recommended that the dose of d4T should be reduced to 30 mg for adults and adolescents irrespective of body weight.[2] This was due to compelling evidence which showed that a reduced dosage of d4T is still effective in suppressing viral replication and also resulted in a lower incidence of toxic side effects.[2]

Growing concerns about d4T toxicity and the arrival of new and safer drugs on the market led to its disfavour by HIV clinicians. In 2010 WHO published the recommendation that d4T should be phased out of ART programmes due to the high rates of side effects associated with long-term use.[3]

2.2 Stavudine

d4T is a thymidine 2'-deoxynucleoside analogue belonging to the class of Nucleoside Reverse Transcriptase Inhibitors (NRTI). It has demonstrated antiviral activity against HIV-1 and HIV-2 through inhibition of the HIV reverse transcriptase enzyme. The drug inhibits the enzyme by competing with thymidine triphosphate, which is the natural substrate for viral DNA synthesis, ultimately leading to termination of the HIV DNA chain and thereby inhibiting replication of the virus.[25] In

early efficacy studies, patients on d4T monotherapy had lower rates of clinical progression to AIDS and longer survival compared to patients on AZT monotherapy.[1] As a result, d4T became the most widely used ARV in developing countries. It also requires less routine laboratory monitoring at initiation which contributes to the reduced overall cost of treatment.[8]

When the drug was first introduced in 1995, the recommended dosage was 40 mg twice a day for adults with body mass of ≥60kg's, and 30 mg twice a day for adults <60 kg.[26] At the time there were no other studies that proved the efficacy of other dosages.[18,26] Upon initiation the drug is well tolerated and causes less side effects such as nausea and diarrhoea compared to other ARV's.[8] However, prolonged use of the drug may lead to mitochondrial toxicity which can manifest as several toxic side effects including; peripheral neuropathy, lipodystrophy, hyperlactataemia, lactic acidosis, hepatic steatosis and pancreatitis.[8,27,28]

The main pathophysiologic mechanism by which d4T-associated toxic side effects occur is through inhibition of mitochondrial DNA replication by inhibiting the enzyme polymerase-γ (gamma).[29] This results in depletion of mitochondria which leads to decreased production of adenosine triphosphate (ATP) in cells, which causes an accumulation of lactic acid and adipocyte apoptosis.[30] The toxic side effects are dose-related and accrue over time. Certain factors such as increased age, female sex and high BMI (body mass index) are associated with an increased risk of developing toxicity.[8,31] Some of the side effects, such as peripheral neuropathy, are reversible when the dose is decreased or the drug is stopped; whereas for others (such as lipodystrophy) progression can be halted but is usually not reversible.[32]

Up to 20% of patients develop peripheral neuropathy, with age over 35 years and increasing height reported as risk factors.[8,30,33] Peripheral neuropathy is a sensation of pain, numbness and paraesthesia in the hands and feet. It may be accompanied by loss of vibration sense and decreased ankle reflexes.[33,34] Lipodystrophy is an abnormality in body fat distribution. It is a syndrome that incorporates lipohypertrophy (fat accumulation in the abdomen, breasts, neck and upper back) as well as lipoatrophy (depletion of fat from the face, arms, legs, hips and gluteus).[35]

Prevalence rates of lipodystrophy of 42,9% after two years of d4T treatment have been reported.[35]

Research by Domingo et al suggests that development of lipodystrophy is associated with high concentrations of intra-cellular d4T, which was dependent on the cumulative exposure to d4T and not the dose[33]. Other risk factors for development of this side-effect are, female gender and CD4 count >150 cells/mm^3.[8] A South African study reported that increased BMI at baseline was associated with a higher risk of developing lipodystrophy.[35]

Hyperlactataemia, defined as an elevated serum lactate of 2-5 mmol/L, is attributed to the female gender, increased BMI and increased age.[8] The presence of hyperlactataemia has been causally linked to the development of other d4T-associated toxicities such as peripheral neuropathy, pancreatitis, and lipodystrophy.[36] Hyperlactataemia can progress to lactic acidosis, a life-threatening condition with mortality rates as high as 60%.[31] Lactic acidosis is defined as a lactate level > 5 mmol/L pH <7.35, and/or bicarbonate (HCO3) <20 mmol/L and anion gap >20.[37]

Several studies have described high rates of d4T-associated toxicities in various ART programmes in Africa (*Table 1*).[33,38-40] Work done by Karara et al comparing tolerability and efficacy of d4T 30 mg vs d4T 40mg in Kenyan patients found cumulative incidences of toxicity of 11% at 6 months and 18% at 12 months. In Cameroon, Cournil et al describe overall prevalence rates or lipoatrophy of 7 to 24%.[42] An observational study from Rwanda found incidence rates of 52 per 1000 person-years for peripheral neuropathy; 20 per 1000 person-years for hyperlactataemia/lactic acidosis; and 47 per 1000 person-years lipoatrophy.[38] A follow-up cohort study conducted in Malawi found high levels of d4T-toxicity with an incidence rate of 26,7 per 100 person-years, in patients mostly on the 30 mg dose.[39] Prevalence of peripheral neuropathy was 21,3% and of lipodystrophy 14,7% in this study.

Reports from Southern African cohorts have also shown higher rates of hyperlactataemia and lactic acidosis compared with developed countries (*Table 1*).[31] One study from South Africa reported higher rates of treatment switching due to toxicity compared with other treatment cohorts.[43] These higher rates are linked to the fact that women make up a greater component of patients on treatment and they have a higher risk for developing these conditions.[40] A retrospective study in South Africa by Stead et al reported a high incidence of symptomatic hyperlactataemia, 17,5 case per 1000 person-years in which 91% of the patients were on d4T 40 mg bid; and high rates of lactic acidosis were reported at 12,3 cases per 1000 person-years.[28]

The high rates of d4T-associated toxicity that were reported in treatment cohorts around the world led to the WHO's recommendation in 2010, that countries should phase out the drug from their treatment guidelines and replace it with TDF or AZT.[3] However, the United Nations (UN) target to have 15 million people living with HIV/AIDS on ARV's has ensured that d4T still features prominently in ART programmes of low and middle income countries.[18,50] The drug's cost-effectiveness and availability in co-formulated, fixed-dose combinations are major drivers for its continued use. Moreover, as global funding for HIV is decreasing, many countries are facing the dilemma of whether to continue with d4T as first-line treatment or switch-over to safer yet costlier ARV's.[51]

Table 1: Stavudine-associated Toxicity in Low and Middle Income Countries

STUDY	COUNTRY	DOSE	TOXICITY	FREQUENCY*	RISK FACTORS
van Oosterhout et al.[39]	Malawi	30 mg bid	PN, Lipodystrophy	19.8, 11.4	↑ age
Cherry et al.[44]	Australia, Malaysia, Indonesia	30 mg bid	PN	42%, 19%, 34%	↑ age, ↑ Height
Phan et al.[45]	Cambodia	30 mg & 40 mg bid	PN, LA, Lipodystrophy	10.7% 0.5%, 37.1%	□□male gender, ↑ age, advanced HIV stage
van Griensven et al.[38]	Rwanda	30 mg & 40 mg bid	Severe PN, SH/LA, Lipodystrophy	8%, 3.1%, 7.2%	↑ weight, ↑ weight, female gender, duration of treatment
Mutimura et al.[46]	Rwanda	Not reported	Lipodystrophy	34%	Duration of treatment
George et al.[35]	South Africa	30 mg & 40 mg bid	Lipodystrophy	42.9%	Female gender
Geddes et al.[47]	South Africa	Not reported	LA	1.57%	Female gender, ↑ weight
Bolhaar et al.	South Africa	40 mg	SH/LA	1.33%	Female gender, ↑ BMI
Osler et al.[48]	South Africa	30 mg & 40 mg bid	SH/LA	N/A	Female gender, ↑ weight, ↑ BMI
Menezes et al.[49]	South Africa	30 mg & 40 mg bid	PN, SH, LA Lipodystrophy	17.1%, 5.75%, 3.3%, 7,3%	↑ BMI
Wadley et al.[33]	South Africa	30 mg bid	PN	57%	↑ age, ↑ Height

Adapted from WHO topics[8]
PN = Peripheral Neuropathy; SH = Symptomatic hyperlactataemia; LA = Lactic Acidosis
* Measured either as Incidence rate/ 100 person years or proportion in %

CHAPTER THREE: AIMS AND OBJECTIVES

3.1 Aim

The aim of the study was to compare time to onset of toxicity as well incidence of cumulative toxicity between low dose d4T (20 mg bid), and the previously recommended higher doses of d4T (30 mg bid and 40 mg bid). As well as to investigate the risk factors of toxicity in patients on d4T.

3.2 Hypothesis

The null hypothesis:
There is no difference in the time to onset of the first toxic side effects when using low doses of d4T (20 mg bid) compared to higher doses (40 mg and 30 mg bid).
$H_{0:}\ t_1 = t_i$ where i = 2,3

The alternative hypothesis:
There is a difference in the time to onset of toxicity between low dose d4T (20 mg bid) and higher doses (40 mg and 30 mg bid).
$H_{A:}\ t_1 \neq t_i$ where i = 2,3

3.3 Objectives

The objectives of the study were as follows:

I. To determine time to onset of the first stavudine-associated toxic side effect, which was defined as any of the following:
   - Peripheral neuropathy
   - Lipodystrophy
   - Hyperlactataemia
   - Lactic Acidosis

II. To compare incidence rates of toxicity between low dose (20 mg bid) stavudine versus the previously recommended higher doses (30 mg bid and 40 mg bid)

III. To determine the risk factors for developing stavudine toxicity

CHAPTER FOUR: METHODOLOGY

4.1 Study Design

A retrospective cohort analysis was performed of data that was collected from patients treated at an HIV clinic.

4.2 Study Setting

The study setting was an adult HIV treatment clinic located at the Chris Hani Baragwanath hospital, overseen by the Perinatal HIV Research Unit (PHRU). The clinic was established in 2004 with funding obtained from PEPFAR, to provide free medical treatment to the community of the peri-urban township of Soweto. A team of physicians and primary health care nurses with extensive HIV experience managed the patients at the clinic. The initiation of ARV's was doctor-led and based on the South African National Antiretroviral Treatment Guidelines developed by the NDOH.[52] By the end of 2010, over 2300 patients had been enrolled into the clinics ART programme.

4.3 Study Population

The study population comprised patients who were initiated on a d4T-containing regimen between January 2004 and December 2008. The entire cohort was followed-up until 31 December 2010. All eligible patients were included into the study even if they did not remain on d4T until the end of the follow-up period.

4.3.1. Eligibility Criteria

I. Inclusion Criteria

- Patients over 18 years of age
- ARV naïve patients

II. Exclusion Criteria:

- Patients under 18 years of age at time of initiation

- Patients previously initiated on ARV before the start of study period
- Patients on the Prevention of Mother to Child Transmission (PMTCT) programme

Only adults who were not yet on ARV's before the start of the study period were included to eliminate the possibility of toxicity having developed from other regimens the patients my have been previously exposed to. Patients on the PMTCT programme were excluded as they would only be on treatment for the duration of their pregnancy even if they were on d4T.

4.3.2 Sampling Method

The database of patients served as the sampling frame. The patients were stratified into three dose groups, namely d4T 20 mg, d4T 30 mg and d4T 40mg and all eligible patients in each group were included in the study.

4.3.3 Pilot Study

A small pilot study was conducted to determine the expected prevalence of toxicity in patients on d4T (see appendix 1). A retrospective record review was conducted on patients at another ARV clinic run by PHRU. A Total of 165 patients on d4T were analysed. Prevalence rates of toxicity for the d4T 20 mg, d4T 30 mg and d4T 40 mg groups were 45%, 60% and 61% respectively.

4.3.4 Sample Size

The statistical software STATA 12 (StataCorp. 2011. *Stata Statistical Software: Release 12*. College Station, TX: StataCorp LP) was used to compute the sample size. This sample size was estimated using Survival Analysis techniques.[53] In order to compare the groups d4T 20 mg vs. d4T 30 mg and d4T 20 mg vs. d4T 40 mg, the log-rank test was used as follows:[54]

$$\mathrm{N} = \frac{\mathrm{E}}{\mathcal{P}\mathrm{E}}$$

$$\mathrm{E} = \frac{1}{\lambda}\left(z_{1-\frac{\alpha}{\kappa}} + z_{1-\beta}\right)^2\left(\frac{\lambda\Delta+1}{\Delta-1}\right)^2$$

$$\mathcal{P}\mathrm{E} = 1 - \frac{S_1(t)+\lambda S_2}{1+\lambda}$$

Where N is sample size;

E is the number of events required;

p_E is the overall probability of an event;

$z_{1-\alpha/k}$ and $z_{1-\beta}$ are to be the quantiles of the standard normal distribution;

Δ is the hazard ratio; and

λ is the ratio of the two dose groups

Using data from a pilot study (see Appendix 1), we assumed:

- Expected probability of toxicity of 0.45 in the d4T 20 mg group
- Expected probability of toxicity of 0.6 in the d4T 30 mg group
- Expected probability of toxicity of 0.61 in the d4T 40 mg group
- A difference of 15% between d4T 20 mg vs. d4T 30 mg
- A difference of 16% between d4T 20 mg vs. d4T 40 mg

Due to the expected unequal sizes of the dose groups expected ratios for the sample size were estimated:

- Ratio of 9 for comparing d4T 30 mg: d4T 20 mg
- Ratio of 5 for comparing d4T 40 mg: d4T 20 mg

Assuming further a power of 80% and a 5% significance level, it was envisaged that the minimum required sample sizes to test for a difference per group were:
d4T 20 mg group n = 72
d4T 30 mg group n = 666
d4T 40 mg group n = 364

In the final analysis the required sample was not reached for some of the groups due to the exclusion criteria and missing data. A total of 1086 patients met the criteria to be included in the study resulting in the following sample size per group:

d4T 20 mg group n = 43

d4T 30 mg group n = 707

d4T 40 mg group n = 336

4.4 Data Management

A data extraction form developed by the researcher, was used to extract data from the password protected Excel-based (Microsoft 2010, Microsoft Excel. Redmond, Washington) dataset which consisted of anonymised patient details (See appendix 2). All patient identifiers were removed during extraction of the data. The data was then transferred to a Microsoft Excel spreadsheet using the double entry method and exported to STATA 12 for analysis.

4.5 Measurements

The outcome variable of interest was d4T-associated toxicity. Defined as the first recorded diagnosis of peripheral neuropathy, hyperlactataemia, lactic acidosis or lipodystrophy. The presence or absence of specific individual toxicities as well as all-cause toxicity were measured. Other variables used in the analysis were measured as described in *Table 2*. For variables such as renal dysfunction there is no evidence in the database to support how the diagnosis was made. Therefore, to avoid measurement error all the definitions of the variables and terms were used as coded in the original database.

4.6 Ethical Considerations

Ethical approval for the study was obtained from the University of Pretoria's Faculty of Health Sciences Research Ethics Committee on 26 February 2015 (Appendix 4) (reference number: 32/2015). The CEO of PHRU granted permission for the use of data (Appendix 3). The database was password-protected for security and only accessed by the researcher. All patient identifiers were removed during data abstraction to ensure anonymity of the study participants.

Table 2: Definition of Variables

Variable		Definition		
Age		Age in years at time of initiation calculated from date of birth <25 26-35 36-45 46-55 >55		
Sex		Male or Female		
Weight		Body weight in kilograms at initiation < 40 kg 40 – 59 kg ≥ 60 kg		
BMI		Body Mass Index at time of initiation ≤ 18,5 kg/m^2 18,5 – 24,5 kg/m^2 25-30 kg/m^2 >30 kg/m^2		
WHO HIV/AIDS Clinical Stage		Stage of HIV clinical disease at initiation of ART WHO Stage 1 WHO Stage 2 WHO Stage 3 WHO Stage 4		
Baseline CD4		CD4 count at initiation of ART <50 cells/mm^3 50-100 cells/mm^3 101- 200 cells/mm^3 201-350 cells/mm^3 >350 cells/mm^3		
Baseline VL		Viral Load at initiation of ART <400 c/ml 401-1000 1001 - 50000 50 001 – 100000 >100000		
Baseline Hb		Haemoglobin level at initiation >10 g/dl 7-10 g/dl <7 g/dl		
Renal dysfunction		Any diagnosis of renal impairment or renal failure at anytime during the follow-up period.	Yes	No
Co-trimoxazole Rx		On co-trimoxazole antibiotic as prophylactic treatment for opportunistic infections at initiation.	Yes	No
TB Treatment		A diagnosis of TB at anytime during the follow-up	Yes	No
Follow-up time on d4T		Duration of treatment on stavudine in weeks		
Toxicity	**All-cause**	The first recorded diagnosis of either peripheral neuropathy or hyperlactataemia or lactic acidosis or lipodystrophy	Yes	No
	Specific	Peripheral neuropathy: Pain and loss of sensation in the feet	Yes	No
		Hyperlactataemia: Elevated serum lactate levels	Yes	No
		Lactic Acidosis: Elevated serum lactate with signs of metabolic acidosis	Yes	No
		Lipodystrophy: fat loss and/or fat accumulation in distinct regions of the body	Yes	No

4.7 Statistical Analysis

A 95% Confidence Interval (CI) and significance level of alpha = 0.05 were set for all statistical analyses. Data was described using frequencies and proportions for the categorical variables. Medians, and interquartile ranges were used for continuous variables. As the baseline characteristics were not normally distributed the differences in proportions between the dose groups were assessed using the Kruskall Wallis test, which is a non-parametric test. Data was missing for some of the variables (*Table 3*). The variables WHO Stage and Co-trimoxazole had the highest rate of missing data of 15% and 38% respectively. The missingness of Co-trimoxazole was found to be missing not at random (MNAR) and the variable was dropped from further analysis

Time to d4T-related toxicity, was determined for all patients who experienced toxicity using Kaplan-Meier curves. Patients were censored if they had not developed toxicity by the end of the study period (31 December 2010). Those patients whose treatment was switched either by a change in their initial d4T dose or a change in their drug regimens were censored. Patients who died, transferred out, lost to follow-up, as well as those who had treatment interruption were censored. The log-rank test was used to compare the differences in time to onset of toxicity between the three dose groups.

Incidence rates of toxicity comparing the d4T 20 mg dose group against the d4T 30 mg and d4T 40 mg groups were calculated using Poisson regression modelling. The log of the follow-up time in years was used in the regression models. Initially all-cause toxicity was modelled amongst the different dose groups. Then, each specific toxicity was modelled against each dose group. Post-estimation X^2 tests were conducted to test if the differences were significant. All incidence rates were expressed per 100 person-years. Cox proportional hazards regression was used to determine predictor variables that may act as risk factors in the development of toxicity.

Before running the Cox regression models the assumption of proportional hazards was tested to determine whether the hazard functions across the dose groups were

proportional to each other across time for each predictor variable. This statistical test was conducted in STATA using scaled and unscaled Schoenfeld residuals. Unadjusted hazard ratios and 95% confidence intervals were determined for all predictor variables by univariate analyses. Only those variables with a p-value less than 0.2 were included in the initial multivariate model. Backwards selection was then used to eliminate variables, and only those variables found to have a p-value < 0.1 were included in the final model. The significance of the coefficients eliminated was tested using the Likelihood ratio test. Cox-Snell residuals were used to assess overall fit of the final model.

CHAPTER FIVE: RESULTS

5.1 Descriptive Analysis

The treatment cohort comprised more females (815; 77%) than males (245; 23%) (*Table 3*). The difference in proportions for sex between the three dose groups was not statistically significant (p = 0,4176). The median age at initiation of ART for the three dose groups was also similar (p= 0.8696). Body weight indices between the three dose groups did show significant differences with BMI in the d4T 40 mg group being much higher (26kg/m^2 p=0.0001). The entire study population had advanced immune suppression at initiation with a median CD4 count of 113 (IQR 53,5; 164). No differences between the dose groups were noted with a X^2 p-value of 0.3416.

Table 3: Characteristics of Study Participants

Variable (n)		**D4T 20 mg n= 43, (4%)**	**D4T 30 mg n=707, (65%)**	**D4T 40 mg n=336, (31%)**	**P-value**
Sex (1060) missing =26 (2%)	**Female**	30 (71%)	543 (78%)	242 (75%)	0.4176
	Male	12 (29%)	153 (22%)	80 (25%)	
Age (1043) missing = 43 (4%)	**Median**	34	35	34	0.8696
	IQR	30 - 44	30 – 39	30 - 41	
Weight (1076) missing = 10 (1%)	**Median**	55	56	68	0.0001
	IQR	51 - 64	50 – 64	62 - 65	
Height (1052) missing = 34 (3%)	**Median**	161	160	163	0.0001
	IQR	156 - 168	156 - 165	158 - 169	
BMI (1043) missing = 43 (4%)	**Median**	22	21	25	0.0001
	IQR	19.5 - 25	19 – 25	23 – 29	
	≤ 18,5 kg/m^2	7 (18%)	132 (19%)	11 (3%)	0.0001
	25 - 30 kg/m^2	22 (56%)	372 (54%)	123 (39%)	
	18,5 - 24,5 kg/m^2	7 (18%)	103 (15%)	125 (40%)	
	>30 kg/m^2	3 (8%)	81 (12%)	7 (18%)	

WHO Stage (927) missing = 159 (15%)*	**Stage 1**	13 (38%)	176 (29%)	85 (30%)	0.3960
	Stage 2	9 (26%)	146 (24%)	69 (24%)	
	Stage 3	9 (26%)	244 (40%)	126 (44%)	
	Stage 4	3 (9%)	41 (8%)	6 (2%)	
Baseline CD4 (1061) missing = 25 (2%)	**Median**	123	113	111	0.3416
	IQR	58 - 186.75	54 – 165	50.5 - 156.5	
	<50 cells/mm^3	8 (20%)	159 (23%)	82 (25%)	0.2468
	50-100 cells/mm^3	4 (10%)	149 (22%)	61 (18%)	
	101- 200 cells/mm^3	24 (60)	333 (48%)	172 (52%)	
	201-350 cells/mm^3	2 (5%)	41 (6%)	9 (3%)	
	>350 cells/mm^3	2 (5%)	10 (1%)	5 (2%)	
Baseline VL (1016) missing = 70 (6%)	**Median**	27630	55491	44575	0.1139
	IQR	5387 - 168911	17870 – 153441	16172-137650	
	<400 c/ml	5 (13%)	57 (9%)	25 (8%)	0.2056
	401-1000	0 (0 %)	7 (1%)	0 (0%)	
	1001 - 50000	19 (49%)	261 (39%%)	152 (48%)	
	50 001 – 100000	4 (10%)	105 (16%)	45 (14%)	
	>100000	11 (28%)	229 (35%)	96 (30%)	
Baseline Hb (1028) missing = 58 (5%)	**Median**	12.4	12	12.5	0.0017
	IQR	10.8 – 14.2	10.7 – 13.2	11.2 – 13.6	
	<7 g/dl	1 (3%)	7 (1%)	1 (1%)	0.0085
	7-10 g/dl	5 (13%)	101 (15%)	28 (8%)	
	>10 g/dl	32 (84%)	560 (84%)	293 (915)	
TB Treatment	**Yes**	2 (5%)	113 (16%)	36 (11%)	0.0132
	No	41 (95%)	590 (84%)	300 (89%)	
Renal Dysfunction Missing = 0	**Yes**	1 (2%)	30 (4%)	14 (4%)	0.8289
	No	42 (98%)	677 (96%)	322 (96%)	
Co-trimoxazole Rx Missing = 413 (38%)**	**Yes**	14 (56%)	265 (67%)	179 (71%)	0.2312
	No	11 (44%)	171 (23%)	73 (29%)	

KEY: n: Number of Study Participants; IQR: Inter-quartile Range
* WHO Stage missing completely at random, Littles Test p- value = 0.7567
**Co-trimoxazole Rx missing not at random, Littles Test p-value = < 0,0001

5.2 Survival Analysis

The median cumulative time on d4T for the 20 mg group was 110 weeks (IQR 52; 125), 66 weeks (IQR 26; 134) for the d4T 30 mg group and 46 weeks (IQR 30; 85) for the d4T 40 mg group (*Table 4*). More than half of the patients developed toxicity 661(61%): 120(21%) developed hyperlactataemia, 13(2%) lactic acidosis, 136(24%) lipodystrophy and 292(52%) peripheral neuropathy. The longest duration of follow-up time on d4T was 417 weeks. The duration of follow-up for d4T 20 mg and d4T 40 mg was 278 and 325 weeks respectively. The median time to development of any toxicity was 42 weeks. The differences in median time to onset of toxicity are depicted in the Kaplan Meier graph (*Figure 1*). The d4T 20 mg group had the longest median time to onset of toxicity (217 weeks). The log rank test showed a statistically significant difference between the dose groups ($p < 0,0001$).

Table 4: Time To Toxicity For Different d4T Dosages

Dose	**Duration of Time on d4T (weeks)**		**Median Time to Toxicity (weeks)**
d4T 20 mg	**Median**	110	217
	IQR	52 -125	
d4T 30 mg	**Median**	66	137
	IQR	26 - 134	
d4T 40 mg	**Median**	46	55
	IQR	30 -85	

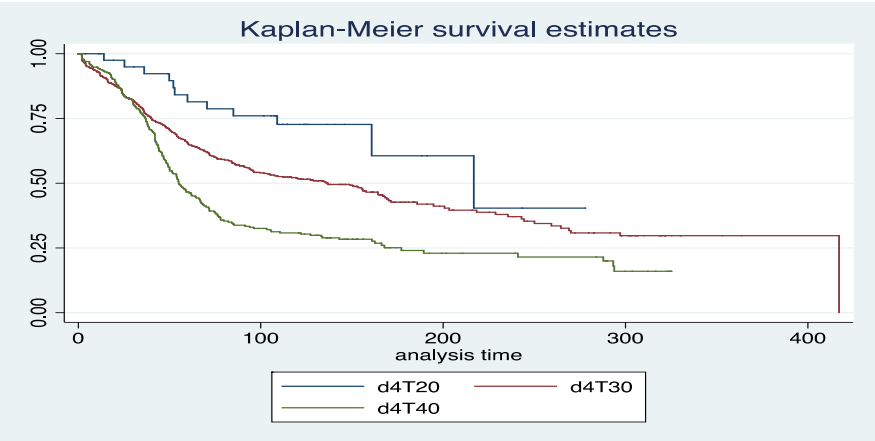

Figure 1: Kaplan Meier Graph depicting Time to Onset of Toxicity

5.3 Poisson Regression

The results from the Poisson regression showed that patients in the d4T 20 mg group had the lowest incidence of all-cause toxicity with rates of 43 per 100 person-years (95% CI 25 -76) (*Table 5*). In comparison, the rates for the d4T 30 mg group and d4T 40 mg group were 67 per 100 person-years (95% CI 55- 80) and 174 per 100 person-years (95%CI 143 - 211), respectively. The X^2 test of difference between the d4T 20 mg group and the d4T 30 mg group was significant (p = 0.019); as well as between the d4T 20 mg and d4T 40 mg groups (p= 0.0001). The comparison of the specific toxicities showed more variable results with wide and insignificant confidence intervals due to the small sample sizes (*Table 5*).

The d4T 20 mg group had the lowest incidence rates of peripheral neuropathy, 46 per 100 person-years (95% CI 18 - 116). The X^2 test of difference between this group and the d4T 30 mg and d4T 40 mg groups were not significant with p-values of 0.0965 and 0.1093, respectively. The comparison of lipodystrophy also showed similar results, even though the d4T 30 mg group had the highest incidence rates of 127 per 100 person-years (95% CI 92 - 174). The X^2 p-values were also insignificant, 0.7461 for d4T 20 mg vs. d4T 30 mg and 0.3400 for d4T 20 mg vs. d4T 40 mg.

The highest incidence rates for hyperlactataemia were in the d4T 40 mg group, 187 per 100 person-years (95% CI 132 - 266). The X^2 test of difference between the dose groups was also insignificant with p-values of 0.3321 and 0.1240. The d4T 20 mg group had the highest incidence rates of lactic acidosis: 597 per 100 person-years (95% CI 158 - 2256). The X^2 test of difference was significant compared to the d4T 30 mg group (p= 0.0011) and insignificant compared to the d4T 40 mg group (p= 0.1553).

Table 5: Incidence of Toxicity

	d4T 20 mg		d4T 30 mg		d4T 40 mg	
	(n), %	**Rate/100p yr (CI)**	**(n), %**	**Rate/100p yr (CI)**	**(n), %**	**Rate/100p yr (CI)**
Toxicity – all cause	(12) 28%	43 (25 - 76)	(335) 47%	67 (55 - 80)	(214) 64%	174 (143 - 211)
Peripheral neuropathy	(4) 33%	46 (18 - 116)	(186) 55%	107 (83 - 137)	(102) 48%	100 (78 -129)
Lipodystrophy	(5) 42%	125 (56 - 281)	(90) 27%	127 (92 - 174)	(40) 19%	76 (54 - 105)
Lactic Acidosis	(2) 17%	597 (158 - 2256)	(2) 1%	11(3 - 51)	(9) 5%	408 (126 - 1320)
Hyperlactataemia	(1) 8%	28 (4 - 217)	(59) 8%	59 (41 - 83)	(63) 29%	187 (132 - 266)

KEY: n: number; CI: Confidence Interval; p: person; yr: year

5.4 Cox Regression Models

The results of the test of proportional hazards confirmed that most of the variables met this criterion. However, the variable sex was found to violate the assumption of proportional hazards with a significant p-value of 0.002, even though it is a fixed covariate that is not expected to change over time. Furthermore, the variable is an important risk factor for d4T associated toxicity and stratifying the model according to sex would mean the effects of sex on toxicity would not be determined. The initial full model included d4T dose, BMI, sex, baseline CD4 count and renal dysfunction. Backwards elimination of variables found CD4 to be insignificant in the model with Likelihood Ratio Test p-value of 0.3954. Baseline BMI was kept in the model as the Likelihood Ratio Test p-value was 0.1014.

.

Table 6: Risk Factor for d4T-associated Toxicity

Variable (n)		Univariate Analysis		
		Crude HR	**P-value**	**95 % CI**
d4T Dose	**d4T 20 mg**	0.42	0.003	0.24 - 0.74
	d4T 30 mg	0.70	0.000	0.59 - 0.83
	d4T 40 mg	Reference		
Age	**<25**	0.69	0.278	0.35 - 1.36
	26 -35	0.83	0.521	0.46 - 1.48
	36 -45	0.75	0.335	0.42 - 1.35
	46 -55	0.96	0.889	0.51 - 1.78
	>55	Reference		
Sex (female vs. male)		1.14	0.000	1.08 - 1.21
WHO Stage	**WHO1**	Reference		
	WHO2	1.02	0.818	0.81 - 1.31
	WHO3	0.92	0.464	0.74 - 1.14
	WHO4	1.06	0.795	0.69 - 1.62
Baseline CD4	**<50 cells/mm^3**	1.41	0.340	0.69 - 2.89
	50-100 cells/mm^3	1.30	0.472	0.63 - 2.67
	101- 200 cells/mm^3	1.25	0.538	0.62 - 2.52
	201-350 cells/mm^3	1.74	0.121	0.80 - 3.78
	>350 cells/mm^3	Reference		
BMI	≤ 18,5 kg/m^2	Reference		
	25 - 30 kg/m^2	1.11	0.439	0.85 - 1.44
	18,5 - 24,5 kg/m^2	1.24	0.142	0.93 - 1.65
	>30 kg/m^2	1.68	0.001	1.22 - 2.30
Baseline VL	**<400 c/ml**	Reference		
	401-1000	1.10	0.852	0.40 - 3.05
	1001 - 50000	0.92	0.599	0.67 - 1.26
	50 001 – 100000	0.92	0.656	0.65 - 1.32
	>100000	0.92	0.889	0.66 - 1.26
Baseline Hb	**<7 g/dl**	1.09	0.839	0.45 - 2.63
	7-10 g/dl	0.85	0.211	0.65 - 1.11
	>10 g/dl	Reference		
TB Treatment (1082)		1.09	0.537	0.86 - 1.38
Renal Dysfunction (1086)		1.85	0.001	1.29 - 2.64
d4T Duration	**Time on d4T20**	1	0.807	0.99 - 1.00
	Time on d4T30	1	0.402	1.00 – 1.00
	Time on d4T40	Reference		

KEY: n: number; HR: Hazard Ratio; CI: Confidence Interval

The final model selected included d4T 20 mg, d4T 30 mg, d4T 40 mg (held as the reference), sex, BMI and renal dysfunction as risk factors for development of d4T associated toxicity (*Table 7*). Of the 3 drug doses the hazard ratio's show that the d4T 20 mg dose was 0.36 times less likely to cause toxicity as the reference dose (p = 0.003. Females were 1.58 times more likely to develop toxicity (p <0.001) than men in this analysis. The presence of renal dysfunction was also shown to be a significant risk factor as those patients were 1.88 times more likely to develop toxicity (p-value = 0.001). BMI as a categorical value was included in the final model even though the p-values were high, as the likelihood ratio test showed that the variable was still significant in the model.

Table 7: Multivariate Analysis: Final Model

Variable (n)		Multivariate Analysis (Final Model)		
		Adjusted HR	**P-value**	**95 % CI**
d4T Dose	**d4T 20 mg**	0.36	0.003	0.20 - 0.65
	d4T 30 mg	0.58	<0.000	0.48 - 0.71
	d4T 40 mg	Reference		
BMI	**≤ 18,5 kg/m^2**	Reference		
	25 - 30 kg/m^2	0.96	0.770	0.74 - 1.26
	18,5 - 24,5 kg/m^2	0.83	0.249	0.61 - 1.14
	>30 kg/m^2	1.23	0.230	0.88 - 1.71
Sex		1.58	<0.001	1.25 – 2.00
Renal Dysfunction		1.88	0.001	1.25 - 1.99

Goodness-of-fit testing of the model using Cox-Snell residuals showed that the model fit reasonably well. Figure 2 graphically displays the hazard function following the 45 degree line fairly closely with some deviation at large values of time.

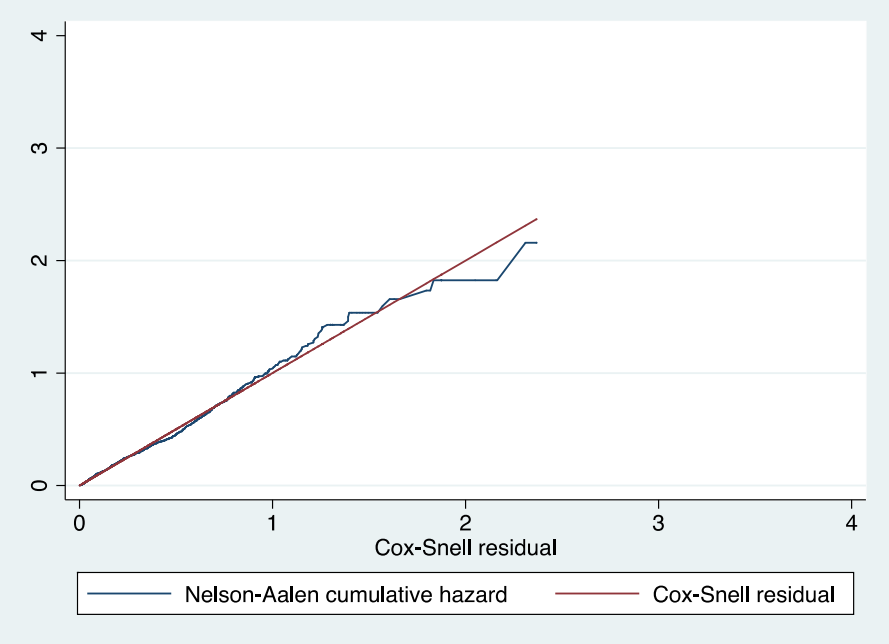

Figure 2: Cox-Snell Residuals

CHAPTER SIX: DISCUSSION

This retrospective data analysis aimed to compare the differences in cumulative toxicity between low dose d4T (20 mg bid) and the higher doses d4T (30 mg bid) and d4T (40 mg bid). The primary objectives were to determine the time to onset of toxic side effects for each of the dose groups, namely d4T 20 mg, d4T 30 mg and d4T 40 mg. Another important objective was to determine the differences in incidence rates of toxicity between the dose groups. Additionally, risk factors associated with the development of toxicity were identified.

Baseline characteristics of the study population were consistent with findings from other African treatment cohorts in reference to; the female preponderance, age and clinical stage at time of ART initiation.[20,38,42,49,55-57] The study population consisted largely of women with a mean age of 36 years (18-75 years). Baseline immunity at initiation was low in the population as patients were only started on treatment at a CD4 count ≤ 200 cells/mm^3.[52] The distribution of characteristics was equal amongst the dose groups except for the weight indicators.

BMI and weight were significantly higher in the d4T 40 mg group than in the other dose groups. This was due to the fact that patients were initiated onto d4T 40 mg when their weight was ≥60kg. Furthermore, as of June 2007 patients were no longer initiated onto d4T 40 mg which explains the smaller number of patients in this group compared to the d4T 30 mg group.[2] The d4T 20 mg group was also very small as few patients were on this dose because it was only used for patients who had very low weight or had renal dysfunction However, even with the smaller than expected sample size, rates of toxicity were still high in this cohort.

Toxicity is a fairly common complication of d4T treatment and the results of this study attest to this.[30,33,37-39,49] There were high levels of toxicity amongst patients with incidence rates of up to 174 per 100 person-years. A significant finding was that the d4T 20 mg dose group had the lowest incidence rates of toxicity compared to the higher dose groups. The results from the analysis of the specific toxicities did not show consistent results and lacked precision due to the small numbers in some of

the groups. Peripheral neuropathy was found to be the most common toxicity, which was consistent with other studies.[33,38,39,49]

The timing of toxicity was delayed for those on d4T 20 mg compared to the other dose groups. However the switching of patients from d4T 40 mg to lower doses would have reduced the length of follow-up time for patients in that dose group as such patients were censored at the time of switching. The longest duration of follow-up was 417 weeks for the d4T 30 mg group only. This was not a sufficient period to assess the long-term cumulative effects of d4T toxicity in the other does groups. However the length of follow-up was sufficient for identifying potential risk factors for development of toxicity.

Risk factor analysis for the causes of d4T-associated toxicity showed that being female had the highest hazard of developing toxicity. This sex-related difference has been noted in other studies and has been related to increased body mass in women at ART initiation.[30,37,45,60] Body mass indicators also proved to be a risk factor in this study, which was consistent with other studies.[38,57] Menezes et al found a strong association between weight and d4T-associated toxicity with BMI 25-30 kg/m^2 being a risk factor for toxicity.[49] Renal dysfunction was found to be a risk factor in the development of toxicity in this population. As d4T is eliminated through the kidney, patients with reduced creatinine clearance are not able to eliminate the drug effectively.[58,59]

6.1 Limitations of the Study

The use of secondary data has challenges which were encountered during this study.[64] Some of these challenges include poor quality of some of the variables; certain desired information was not collected; as well as lack of information on how certain variables were defined.

This analysis had the following limitations:

i. The required sample size that was estimated to reach a power of 80% was not obtained. There were also missing values in the dataset that further contributed to decreasing the sample size.

This affected the precision of some of the statistical tests that were performed, which was indicated by some of the wide confidence intervals seen in the results. The low sample size lead to an underpowered study that reduces the chance of the findings being true. The estimate of the size of the significant results may be exaggerated.

ii. Due to the clinical data being retrospectively collected, it was not possible to ascertain whether the clinical definitions of toxicity applied by clinicians involved in the care of patients were standardised.

iii. For the purposes of this study the first record of toxicity was taken as the start date of toxicity. The exact timing of adverse events may also not have been adequately determined as these patients would be seen at intervals and data would be recorded as and when they came for follow-up which could affect validity of these results.

iv. The first adverse event associated with toxicity was considered as an event irrespective of the grading or severity since this was not available.

CHAPTER SEVEN: CONCLUSION

The assessment of toxicity of low dose d4T compared to standard (higher) doses in this study has shown an increase in the development of toxicity in the study population on higher doses. Despite the limitations of secondary data analysis, the results provide evidence that the d4T 20 mg bid dose has a long-term better safety profile than the standard doses. Incidence rates of toxicity were lower for this group and the time to onset of toxicity was also significantly lower. The hazard ratios for developing toxicity on this dose were also lower than on the higher doses.

There is a justification for pursuing d4T 20 mg as an alternative first line treatment option for developing countries. The few studies conducted on d4T 20 mg have shown that it has efficacy which is similar to the higher standard doses with patients having equivalent survival and reaching virological suppression in the cohorts.[30]

This study has shown that people on low dose d4T had fewer incidences of toxicity. The use of a reduced d4T dose could lead to cost saving which could potentially lead to more people accessing ARV's.[65] A randomised controlled trial with a duration beyond 96 weeks that would compare low dose d4T against first line ARV's such as TDF is recommended. Furthermore this clinical trial should also focus on the risk factors for development of toxicity stratified by dose. This would give much clearer results on the benefits of low dose d4T.

REFERENCES

1. Spruance SL, Pavia AT, Mellors JW, Murphy R, Gathe J, Stool E, et al. Clinical efficacy of monotherapy with Stavudine compared with Zidovudine in HIV-infected, Zidovudine-experienced patients: A randomized, double-blind, controlled trial. Ann Intern Med. 1997;126(5):355-63.
2. World Health Organisation. Addendum to 2006 WHO guidelines on antiretroviral therapy for HIV infection in adults and adolescents. [Internet]. Geneva: World Health Organisation; 2007.[Internet] [cited 2015 Feb 04]. Available from: http://www.who.int/hiv/pub/arv/adult/en/.
3. World Health Organisation. Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach. – 2010 rev. [Internet]. [cited 2014 Feb 02]. Available from: http://www.who.int/hiv/pub/arv/adult2010/en/.
4. Frontières MS. Untangling the web of antiretroviral price reductions. Geneva, Switzerland: MSF Access Campaign; 2014. [Internet]. [cited 2015 Sep 17]. Available from: http://www.msfaccess.org/content/untangling-web-antiretroviral-price-reductions-17th-edition-%E2%80%93-july-2014.
5. World Health Organisation. March 2014 supplement to the 2013 consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. [Internet]. Geneva: World Health Organisation, 2014. [cited 2015 Aug 12]. Available from: http://www.who.int/hiv/pub/guidelines/arv2013/arvs2013upplement_march2014/en/.
6. WHO, UNAIDS, and UNICEF. Global report: UNAIDS report on the global AIDS epidemic 2013. [Internet]. Geneva: UNAIDS (2013). [cited 2015 Feb 02] Available from: http://www.unaids.org/en/resources/campaigns/globalreport2013/globalreport
7. Hill A, Ruxrungtham K, Hanvanich M, Katlama C, Wolf E, Soriano V, et al. Systematic review of clinical trials evaluating low doses of stavudine as part of antiretroviral treatment. Expert Opin Pharmacother. 2007;8(5):679-688
8. Toxicity of reduced and standard doses of d4T. [Internet]. [cited 2015 Feb 02]. Available from: www.who.int/hiv/topics/treatment/toxicity_d4t.pdf

9. Andrieux-Meyer I, Clayden P, Collins S, Geffen N, Goemaere E, Harrington M, et al. Why it's time to say goodbye to stavudine... everywhere. South Afr J HIV Med. 2012;13(1):17-9.
10. National Department of Health. Clinical Guidelines for the Management of HIV & AIDS in Adults and Adolescents. National Department of Health: Pretoria; 2010. [Internet]. [cited 2015 Sep 17]. [Available from: http://www.fidssa.co.za/Guidelines/2010_Adult_ART_Guidelines.pdf.
11. Venter WF, Innes S. Low-dose stavudine trials: A public health piority for developing countries. South Afr J HIV Med. 2012;13(1).
12. Medicine shortages –Finding local solutions to a global problem. S Afr Pharm J. 2013;80(10):45-7
13. Schowalter L, Conradie F. Approaches to Tenofovir and Abacavir drug shortages in South Africa: a guide for clinicians: forum. South Afr J HIV Med. 2012;13(2):56-7.
14. Stock outs in South Africa – A national crisis. [Internet]. [cited 2015 Aug 19]. Available from: http://stockouts.org/uploads/3/3/1/1/3311088/stop_stockouts_report_2013pdf_1.pdf.
15. Bateman C. Drug stock-outs: Inept supply-chain management and corruption. SAMJ. 2013;103(9):600-2
16. National Department of Health. National consolidated guidelines for the prevention of mother-to-child transmission of HIV (PMTCT) and the management of HIV in children, adolescents and adults. National Department of Health Pretoria; 2015. [Internet]. [cited 2016 Dec 16]. Available from: http://www.sahivsoc.org/practise-guidelines/national-dept-of-health-guidelines.
17. Sinxadi PZ, van der Walt J-S, McIlleron HM, Badri M, Smith PJ, Dave JA, et al. Lack of association between Stavudine exposure and lipoatrophy, dysglycaemia, hyperlactataemia and hypertriglyceridaemia: a prospective cross sectional study. AIDS Res Ther. 2010;7(1):23.
18. Hill A. d4T: keep it or abandon it? Asian Biomed. 2011;4(4):541.
19. Ribera E, Paradiñeiro J, Domingo P, Sauleda S, Luque S, Garcia-Arumi E, et al., editors. A Randomized study comparing the efficacy and tolerability of low dose versus standard-dose Stavudine in antiretroviral-naïve patients (ETOX Study). Proceedings of the 3rd IAS Conference on HIV Pathogenesis and

Treatment; 2005. [Internet]. [cited 2015 Oct 1] Available from: https://www.aids2014.org/Abstracts/A2176921.aspx.

20. Ogola BA, Oluka MO, Osanjo GO. Is it Time to Abandon Stavudine? The Safety and Tolerability of Low Dose Stavudine versus Zidovudine in Urban Kenya. Afr J Pharmacol Ther. 2013;2(2):38-47
21. Anderson RE, Dunkle LM, Smaldone L, Adler M, Wirtz C, Kriesel D, et al. Design and Implementation of the Stavudine Parallel-Track Program. The J Infect Dis. 1995;171 Suppl 2: S118-S122.
22. McComsey GA, Re VL, O'Riordan M, Walker UA, Lebrecht D, Baron E, et al. Effect of reducing the dose of Stavudine on body composition, bone density, and markers of mitochondrial toxicity in HIV-infected subjects: A randomized, controlled study. Clin Infect Dis. 2008;46(8):1290-6.
23. Shisana O, Rehle T, Zuma K, Simbayi L, Jooste S, Pillay-van-Wyk V, editors. South African national HIV prevalence, incidence and behaviour survey, 2012. [Internet]. [cited 2014 Feb 04]. Available from: http://www.hsrc.ac.za/en/researchdata/view/6871#sthash.GhSekIZZ.dpuf
24. Mbewu A, Simelela N, Makubalo L. Operational plan for comprehensive HIV and AIDS care, management and treatment for South Africa. Johannesburg: South African Department of Health. 2003.[Internet]. [cited 2015 Feb 04]. Available from: http://www.hst.org.za/publications/operational-plan-comprehensive-hiv-and-aids-care-management-and-treatment-south-africa
25. Zerit: EPAR - Scientific Discussion [Internet]. [cited 2015 Feb 02]. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000110/WC500049162.pdf
26. Martin JC, Hitchcock MJM, De Clercq E, Prusoff WH. Early nucleoside reverse transcriptase inhibitors for the treatment of HIV: A brief history of stavudine (D4T) and its comparison with other dideoxynucleosides. Antiviral Res. 2010;85(1):34-8.
27. Gardner K, Hall PA, Chinnery PF, Payne BAI. HIV treatment and sssociated mitochondrial pathology: Review of 25 years of in vitro, animal, and human studies. Toxicol Pathol. 2014;42(5):811-22.
28. Riddler SA, Anderson RE, Mellors JW. Antiretroviral activity of Stavudine (2′,3′-didehydro-3′-deoxythymidine, D4T). Antiviral Res. 1995;27(3):189-203.

29. Walker UA, Setzer B, Venhoff N. Increased long-term mitochondrial toxicity in combinations of nucleoside analogue reverse-transcriptase inhibitors. AIDS. 2002;16(16):2165-73.
30. Makinson A, Moing VL, Kouanfack C, Laurent C, Delaporte E. Safety of Stavudine in the treatment of HIV infection with a special focus on resource-limited settings. Expert Opin Drug Saf. 2008;7(3):283-293
31. Stead D, Osler M, Boulle A, Rebe K, Meintjes G. Severe hyperlactataemia complicating Stavudine first-line antiretroviral therapy in South Africa. Antiviral Ther. 2008;13:937-43.
32. Brinkman K. Stavudine in antiretroviral therapy: is this the end? AIDS. 2009;23(13):1727-9.
33. Wadley AL, Cherry CL, Price P, Kamerman PR. HIV Neuropathy Risk Factors and Symptom Characterization in Stavudine-Exposed South Africans. J Pain Symptom Manage. 2011;41(4):700-6
34. Schütz SG, Robinson-Papp J. HIV-related neuropathy: current perspectives. HIV/AIDS (Auckland, NZ). 2013;5:243
35. George JA, Venter WD, Van Deventer HE, Crowther NJ. A longitudinal study of the changes in body fat and metabolic parameters in a South African population of HIV-positive patients receiving an antiretroviral therapeutic regimen containing stavudine. AIDS Res Hum Retroviruses. 2009;25(8):771-81.
36. Bonnet F, Balestre E, Bernardin E, Pellegrin J-L, Neau D, Dabis F. Risk factors for hyperlactataemia in HIV-infected patients, Aquitaine Cohort, 1999-2003. Antivir Chem Chemother. 2005;16(1):63.
37. Pérez EH, Dawood H. Stavudine-induced hyperlactatemia/lactic acidosis at a tertiary communicable diseases clinic in South Africa. JIAPAC. 2010;9(2):109-12
38. Van Griensven J, Zachariah R, Rasschaert F, Mugabo J, Atté EF, Reid T. Stavudine- and nevirapine-related drug toxicity while on generic fixed-dose antiretroviral treatment: incidence, timing and risk factors in a three-year cohort in Kigali, Rwanda. T Roy Soc Trop Med H. 2010;104(2):148-53.
39. Van Oosterhout JJ, Mallewa J, Kaunda S, Chagoma N, Njalale Y, Kampira E, et al. Stavudine toxicity in adult longer-term ART patients in Blantyre, Malawi. PLoS ONE. 2012;7(7):e42029.

40. Castelnuovo B, Kiragga A, Kamya MR, Manabe Y. Stavudine toxicity in women is the main reason for treatment change in a 3-year prospective cohort of adult patients started on first-line antiretroviral treatment in Uganda. JAIDS. 2011;56(1):59-63.
41. Karara MW, Okalebo FA, Ng M, Ombega J, Guantai AN, Osanjo GO. Comparative tolerability and efficacy of stavudine 30 mg versus stavudine 40 mg in patients on combination antiretroviral therapy in Kenya. J AIDS HIV Res. 2010;2:024-31.
42. Cournil A, Coudray M, Kouanfack C, Essomba CN, Tonfack CAD, Biwolé-Sida M, et al. Short communication: Reduced dose of Stavudine and lipoatrophy in HIV-infected patients in Cameroon. Antivir Ther. 2010;15:1039-43.
43. Wood R. The role of Stavudine in the South African public sector antiretroviral programme: Should the perfect be the enemy of the good? South Afr J HIV Med. 2008;7(2):5.
44. Cherry C, Affandi J, Imran D, Yunihastuti E, Smyth K, Vanar S, et al. Age and height predict neuropathy risk in patients with HIV prescribed Stavudine. Neurology. 2009;73(4):315.
45. Phan V, Thai S, Choun K, Lynen L, Van Griensven J. Incidence of treatment-limiting toxicity with Stavudine-based antiretroviral therapy in Cambodia: a retrospective cohort study. PloS one. 2012;7(1):e30647.
46. Mutimura E, Stewart A, Rheeder P, Crowther NJ. Metabolic Function and the Prevalence of Lipodystrophy in a Population of HIV-Infected African Subjects Receiving Highly Active Antiretroviral Therapy. JAIDS. 2007;46(4):451-5.
47. Geddes R, Knight S, Moosa MYS, Reddi A, Uebel K, Sunpath H. A high incidence of nucleoside reverse transcriptase inhibitor (NRTI)-induced lactic acidosis in HIV-infected patients in a South African context: original article. SAMJ. 2006;96(8):722-4.
48. Osler M, Stead D, Rebe K, Meintjes G, Boulle A. Risk factors for and clinical characteristics of severe hyperlactataemia in patients receiving antiretroviral therapy: a case–control study. HIV Med. 2010;11(2):121-9
49. Menezes C, Maskew M, Sanne I, Crowther N, Raal F. A longitudinal study of Stavudine-associated toxicities in a large cohort of South African HIV infected subjects. BMC Infect Dis. 2011;11(1):244

50. Assembly UG. Political declaration on HIV/AIDS: intensifying our efforts to eliminate HIV/AIDS. Sixty-fifth Session New York: UNGA. 2011. [Internet]. [cited 2015 Feb 05]. Available from: http://www.unaids.org/sites/default/files/sub_landing/files/20110610_UN_A-RES-65-277_en.pdf.
51. Salaam-Blyther T. The President's Emergency Plan for AIDS Relief (PEPFAR): Funding Issues After a Decade of Implementation, FY2004-FY2013. Congressional Research Service, Washington, DC. 2012.[Internet]. [cited 2015 Feb 05]. Available from: https://www.fas.org/sgp/crs/misc/R42776.pdf.
52. National Department of Health. National antiretroviral treatment guidelines. Department of Health Pretoria; 2004. [internet]. [cited 2015 Feb 15]. Available from: http://southafrica.usembassy.gov/media/2004-doh-art-guidelines.pdf
53. Zhang D, Quan H. Power and sample size calculation for log- rank test with a time lag in treatment effect. Stat Med. 2009;28(5):864-79
54. Marchenko Y. Power analysis and sample-size determination in survival models with the new stpower command. [Internet] 2007. [cited 2015 Mar 14]. Available from: http://www.stata.com/meeting/6nasug/marchenko_boston07.pdf.
55. Brennan AT, Shearer K, Maskew M, Long L, Sanne I, Fox MP. Impact of choice of NRTI in first-line antiretroviral therapy: a cohort analysis of Stavudine vs. Tenofovir.TM & IH. 2014;19(5):490-8.
56. Hoffmann CJ, Charalambous S, Fielding KL, Innes C, Chaisson RE, Grant AD, et al. HIV suppression with Stavudine 30 mg versus 40 mg in adults over 60kg on antiretroviral therapy in South Africa. AIDS. 2009;23(13):1784.
57. Karara MW, Okalebo FA, Ng M, Ombega J, Guantai AN, Osanjo GO. Comparative tolerability and efficacy of Stavudine 30 mg versus Stavudine 40 mg in patients on combination antiretroviral therapy in Kenya. J AIDS HIV Res. 2010;2:024-31
58. Grasela DM, Stoltz RR, Barry M, Bone M, Mangold B, O'Grady P, et al. Pharmacokinetics of single-dose oral Stavudine in subjects with renal Impairment and in subjects requiring hemodialysis. Antimicrob Agents Chemother. 2000;44(8):2149-53.
59. Hurst M, Noble S. Stavudine. Drugs. 1999;58(5):919-49.

60. Guira O, Tiéno H, Yaméogo B, Diendéré AE, Korsaga N, Sagna Y, et al. Characteristics and factors associated with the clinical forms of lipoatrophy during highly active antiretroviral therapy in Ouagadougou, Burkina Faso. JIAPAC. 2014;13(2):184-7.
61. Teklay G, Legesse B, Legesse M. Adverse effects and regimen switch among patients on antiretroviral treatment in a resource limited setting in Ethiopia. J Pharmacovigilance. 2013;1(4):115
62. Forna F, Liechty CA, Solberg P, Asiimwe F, Were W, Mermin J, et al. Clinical toxicity of highly active antiretroviral therapy in a home-based AIDS care program in rural Uganda. JAIDS. 2007;44(4):456-62.
63. Suthar AB, Granich R, Mermin J, Van Rie A. Effect of cotrimoxazole on mortality in HIV-infected adults on antiretroviral therapy: a systematic review and meta-analysis. Bull World Health. 2012;90(2):128-38
64. Boslaugh S. An introduction to secondary data analysis. [Internet]. [cited 2015 Aug 2]. Available from: http://assets.cambridge.org/97805218/70016/excerpt/9780521870016_excerpt.pdf.
65. Vento S, Lanzafame M, Lattuada E, Cainelli F, Restelli U, Foglia E. Dose reduction of antiretrovirals: a feasible and testable approach to expand HIV treatment in developing countries. TM & IH. 2013;18(1):40-4.

APPENDICES

APPENDIX 1: Pilot Study

Introduction

In order to estimate the expected prevalence of toxicity amongst patients taking d4T a small exploratory pilot study was conducted. The data used was collected from Zuzimpilo Clinic, which is a fee-for-service HIV clinic based in downtown Johannesburg overseen by PHRU. Data was collected from patients' files and no identifiers such as patient names, file numbers or personal identity numbers were recorded. All patients who were above 18 years and had been initiated onto d4T between January 2007 and December 2011 and followed up until December 2012 were included in the analysis.

Demographics

A total of 165 patients had been initiated onto treatment during that period.

Variable	N	Mean	Std Dev	Min	Max
Age	165	35.5	7.6	20	64
Sex	165 [Female n= 66 (40%); Male n= 99 (60%)]				
Baseline CD4	164	191.4	175.2	1	876

Stavudine Dosages

As per National ART guidelines most patients were started on a triple combined ART regimen that included Stavudine (d4T). The d4T dosage was weight based with patients either receiving 20 mg, 30 mg or 40 mg twice daily.

The dosages were adjusted according to changes in the patient's weight and renal functions. As the d4T 20 mg dose was used mainly for patients with compromised kidney functions or very low weight. There were disproportionately fewer patients in this group than in the other 2 dose groups (d4T 30 mg and d4T 40 mg).

d4T Dose	N(%)
d4T20	11(7)
d4T30	100(60)
d4T40	54(33)

Toxicity

Toxicity is defined as the presence of peripheral neuropathy, lipodystrophy, hyperlactataemia or lactic acidosis in a patient since starting Stavudine (d4T) treatment. The first diagnosis of any of these conditions was considered as the start of toxicity. Ninety eight patients (59%) developed toxicity whilst on d4T. The following prevalence of toxicity was found in the cohort:

d4T Dose (initial)	Toxicity n(%)
d4T 20 mg	5(45%)
d4T 30 mg	60(60%)
d4T 40 mg	33(61%)

APPENDIX 2: Data Extraction Form

Date of Birth (yy/mm/dd)											
Sex		Female			Male						
Weight		Height			BMI						
CD4		VL			Hb						
WHO Stage	1		2		3		4				
D4T dose		Start			Stop						
Adverse event	Yes		No		Renal Dysfunction		Yes		No		
Co-trimoxazole	Yes		No		TB Diagnosis		Yes		No		
Toxicity1		Start		Treatment Change		Yes		No			
Toxicity2		Start		Treatment Change		Yes		No			
Toxicity3		Start		Treatment Change		Yes		No			
Toxicity4		Start		Treatment Change		Yes		No			
Toxicity5		Start		Treatment Change		Yes		No			
Toxicity6		Start		Treatment Change		Yes		No			
Toxicity7		Start		Treatment Change		Yes		No			
Toxicity8		Start		Treatment Change		Yes		No			
Lost to Follow-up		Date		Dead		Date					

APPENDIX 3: Letter of Permission For Data Use

Permission to access Records / Files / Database

To: Dr N Martinson
(CEO Perinatal HIV Research Unit)

Re: Permission to do research at Perinatal HIV Research Unit

I, Dr Mmatsie Manentsa, am a student at the University of Pretoria in the School of Health Systems and Public Health, under the supervision of Dr NRT Ledibane and Prof. P Rheeder.

I hereby request permission to access patient records from your institution for the purpose of a research study for the MSc Epidemiology degree.

The request is lodged with you in terms of the requirements of the Promotion of Access to Information Act. No. 2 of 2000.

The title of the study is: **Analysis of Stavudine-associated Toxicity in Patients on Low-dose versus High-dose Stavudine in an HIV Treatment Cohort**

I request access to the following information:

Access to the clinical files, record book and the database.

I intend to publish the findings of the study in a professional journal and/ or at professional meeting like symposia, congresses, or other meetings of such a nature.

I intend to protect the personal identity of the patients by assigning each patient a random code number.

I undertake not to proceed with the study until I have received approval from the Faculty of Health Sciences Research Ethics Committee, University of Pretoria.

Yours Sincerely

Dr Mmatsie Manentsa 16 September 2014

Permission to do the research study at this institution and to access the information as requested, is hereby approved.

Chief Executive Officer

Dr Martinson

Signature of the CEO

29/09/2014
Date

APPENDIX 4: Research Ethics Committee Approval Letter

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 20 Oct 2016.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 22/04/2017.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

26/02/2015

Approval Certificate
New Application

Ethics Reference No.: 32/2015

Title: Stavudine-associated Toxicity in Patients on Low-dose versus High-dose Stavudine in an HIV Treatment Cohort

Dear Dr Mmatsie Manentsa

The **New Application** as supported by documents specified in your cover letter for your research received on the 25/01/2015, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 25/02/2015.

Please note the following about your ethics approval:
- Ethics Approval is valid for 1 year.
- Please remember to use your protocol number (**32/2015**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

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

We wish you the best with your research.

Yours sincerely

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

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

☎ 012 354 1677 🖷 0866516047 deepeka.behari@up.ac.za http://www.healthethics-up.co.za
✉ Private Bag X323, Arcadia, 0007 - 31 Bophelo Road, HW Snyman South Building, Level 2, Room 2.33, Gezina, Pretoria