

A retrospective descriptive investigation into the clinical profile of HIV patients in the North West Province switched to third-line regimens

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Abstract

South Africa has the largest human immunodeficiency virus (HIV) epidemic in the world, with the number of people living with HIV increasing to 7.06 million by mid-2017. In October 2014, the Joint United Nations Programme on HIV/AIDS (UNAIDS) implemented the 90-90-90 targets. This means 90% of people living with HIV should know their HIV status, 90% of people diagnosed with HIV should receive antiretroviral therapy (ART) and 90% of those receiving ART should be virally suppressed by the end of 2020 to end the epidemic as a public threat by 2030.

The number of patients experiencing virological failure will inevitably increase due to greater access and prolonged exposure to antiretroviral therapy (ART); this may increase the need for third-line antiretroviral therapy (TLART) in a resource-limited setting such as South Africa.

The main overarching aim of this all-inclusive retrospective descriptive investigation was to create a typical clinical profile of adult patients initiated onto TLART in the North West Province of South Africa by reporting and describing the demographics, certain clinical variables and viral resistance patterns recorded from patient health records.

Approval to conduct this study was obtained from the North West Department of Health, Policy, Planning, Research, Monitoring and Evaluation Directorate, Mahikeng, on 13 January 2017. Ethics approval was obtained from the North-West University, Health Research Ethics Committee, with approval number NWU-00340-16-A1, on 13 March 2017 with a protocol amendment to replace and add an additional study-site, approved on 25 May 2017.

All recorded data were analysed by means of descriptive statistics, with median (interquartile range, IQR: 25th to 75th) used for variables without a normal distribution and percentages (frequencies) used for categorical variables. The Stanford University HIV Drug Resistance Database was used to interpret the clinical significance of drug resistant mutations identified in the study population.

Twenty-one adult patients were included in this cohort, 17 were females and four males, located in three districts and four hospitals of the North West Province. The median age at HIV diagnosis was 34 years (IQR: 30.25-36.75) and 46 years (IQR: 40-48.50), respectively, at TLART initiation.

At baseline, a median CD4 count and viral load (VL) of 68.50 cells/ μ L (IQR: 40.75-127.75) and 98 000 copies/ml (IQR: 45 569-820 000), were reported respectively. At failure of first-line ART, the CD4 count and VL were 79.00 cells/ μ L (IQR: 26.5-124) and 100 000 copies/ml (IQR: 42 085-248 852), and at the end of second-line ART failure the CD4 count and VL were 119 cells/ μ L (IQR: 61.25-201.25) and 73 196 copies/ml (IQR: 26 210-197 007), respectively.

Clinical monitoring markers documented included, serum creatinine, haemoglobin, mean corpuscular volume (MCV), alanine transferase (ALT) and total cholesterol at baseline and at first- and second-line ART failure. Some of these results, however, were incomplete due to missing data in patient health records, thus drawing any valuable conclusion to contribute to the description of a typical clinical profile was not possible.

The median duration from HIV diagnosis to first-line ART initiation was 101 days (IQR: 36.50-366.75), on first-line ART it was 1 269 days (IQR: 765-2 343), on second-line ART 1 512 days (IQR: 706-2 096) and between second-line ART failure and TLART initiation, 71 days (IQR: 57.5-126).

The first-line ART regimen, most often initiated was (n=11) lamivudine (3TC), stavudine (d4T) and efavirenz (EFV) which occurred prior to 2010 and the PI-based second-line ART regimen was (n=7) lamivudine (3TC), zidovudine (AZT) and ritonavir boosted lopinavir (LPV/r).

The resistance mutations most prevalent for protease inhibitors (PIs) (n=20) were M46I (75%), V82A (65%) and I54V (65%). The median number of total PI resistance mutations (major and minor) found per patient was three mutations (IQR: 2-3). The resistance mutations most prevalent for nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs) (n=18) were M184V (89.47%), D67N (31.58%), M41L (26.31%) and T215Y, and V118I (21.05%) and for non-nucleoside reverse transcriptase inhibitors (NNRTIs) (n=17): K103N (47.01%) and G190A (23.52%).

High-level of resistance most prevalent in this cohort for PIs were against nelfinavir/r (85.7%), indinavir/r (81.0%), lopinavir/r (76.2%), fosamprenavir/r (66.7%) and atazanavir/r (57.1%); NRTIs were emtricitabine (95.2%), lamivudine (95.2%) and zidovudine (52.4%); NNRTIs were nevirapine (76.2%) and efavirenz (71.4%).

A constant low CD4 count and detectable VL >50 copies/mL throughout the duration of ART were found in this study population. The duration between second-line ART failure and TLART initiation found in this study may contribute to an increased number of PI resistance mutations, after a median duration of 71 days (IQR: 58-126).

High-level cross-resistance between PIs and a combination of major and minor PI resistant mutations were found in most patients in this study population, but even with limited data numbers, certain markers were identified and used to create an initial clinical profile of the patients. Results from this study can form the basis for larger studies in South Africa, to create more successful TLART management and care programmes.

One of the limitations of this retrospective descriptive investigation was the missing data at certain time-points, as the majority of the retrospective data dated back to 2004 and the National ART guidelines changing several times with regards to the regimen compositions, efficacy and toxicity tests and frequencies of monitoring intervals.

This study highlighted important demographic and clinical characteristics that may improve the management and care of HIV patients and possibly prevent patients from failing their respective ART regimens. It also highlighted the importance of individualised HIV management and care programs in each healthcare facility, intensified adherence counselling and earlier drug resistance testing for improved and more effective and optimised ART in the future.

Keywords: HIV, Human immunodeficiency virus, third-line antiretroviral therapy, treatment failure, drug resistance patterns, salvage therapy

Opsomming

Suid-Afrika het die grootste menslike immuniteitsgebreksvirus- (MIV-) epidemie in die wêreld, waar die hoeveelheid mense wat met MIV gediagnoseer is, toegeneem het tot 7.06 miljoen teen die middel van 2017. In Oktober 2014 het die Gemeenskaplike Verenigde Nasiesprogram vir MIV/VIGS (UNAIDS) die 90-90-90-teiken geïmplementeer. Dit impliseer dat 90% van mense hul MIV-status moet ken, 90% wat met MIV gediagnoseer is antiretrovirale terapie (ART) behoort te ontvang en dat 90% van diegene wat ART ontvang teen die einde van 2020 viraal-onderdruk moet wees en dat die epidemie, as 'n publieke bedreiging, teen 2030 uitgewis moet wees.

Die aantal pasiënte wat onsuksesvolle virale onderdrukking ondervind, sal noodwendig toeneem vanweë verbeterde toegang en verlengde blootstelling aan ART; dit mag ook die aantal pasiënte wat derde-linie-ART (DLART) in 'n hulpbron beperkte omgewing soos Suid-Afrika benodig verhoog.

Die oorhoofse doel van hierdie alles-insluitende retrospektiewe en beskrywende ondersoek was om 'n tipiese kliniese profiel, van 'n DLART-geïnisieerde volwasse pasiënt in die Noordwes Provinsie van Suid-Afrika te skep, deur die demografiese besonderhede, kliniese veranderlikes en virale weerstandigheidspatrone te rapporteer wat in pasiënte se medieserekords aangeteken was.

Etiese goedkeuring is ontvang van die Noordwes Departement van Gesondheid se direktoraat vir Beleid, Beplanning, Navorsing, Monitering en Evaluering, Mahikeng, op 13 Januarie 2017. Etiese goedkeuring is ook op 13 Maart 2017 van die Etiekkomitee vir Gesondheidsnavorsing van die Noordwes-Universiteit ontvang (goedkeuringsnr. NWU-00340-16-A1), met 'n finale wysiging aan die protokol om 'n studieperseel weg te laat en 'n ander by te voeg, wat goedgekeur is op 25 Mei 2017.

Alle gedokumenteerde data is geanaliseer deur middel van beskrywende statistiek, met die gebruik van 'n mediaan (interkwartielverspreiding, IKV: 25^{ste} tot 75^{ste}) vir veranderlikes sonder 'n normaalverspreiding en persentasies (frekwensies) gebruik vir kategorieëse veranderlikes. Die Stanford Universiteit se MIV Geneesmiddelweerstandigheidsdatabasis was gebruik om die kliniese relevansie van geneesmiddel-weerstandige mutasies in die studiegroep te identifiseer.

Een-en-twintig pasiënte was ingesluit in hierdie studiegroep, 17 vrouens en vier mans, afkomstig vanuit drie distrikte en vier hospitale binne die Noordwes Provinsie. Die mediaan-ouderdom ten tye van diagnose was 34 jaar (IKV: 30.25-36.75) en 46 jaar (IKV: 40-48.50) met DLART inisiëring.

Die CD4-seltelling en virale lading (VL) was onderskeidelik 68.50 selle/ μ L (IKV: 40.75-127.75) en 98 000 kopieë/ml (IKV: 45 569-820 000) by basislyn. Tydens die eerste-linie mislukking was die mediaan CD4-seltelling en VL onderskeidelik 79 selle/ μ L (IKV: 26.5-124) en 100 000 kopieë/ml (IKV: 42 085-24 8852), en tydens die tweede-linie mislukking was die mediaan CD4-seltelling en VL onderskeidelik 119 selle/ μ L (IKV: 61.25-201.25) en 73 196 kopieë/ml (IKV: 26 210-197 007).

Kliniese moniteringsmerkers wat gedokumenteer was, het ingesluit serumkreatinien (SCr), hemoglobien (Hb), gemiddelde korpuskulêre volume (GKV), alanien transferase (ALT) en totale cholesterolvlakke by basislyn en tydens eerste-linie en tweede-linie ART mislukking. Sekere van hierdie resultate was egter onvolledig weens verlore data in pasiënte se mediese rekords, sodat geen waardevolle gevolgtrekking gemaak kon word om by te dra tot die beskrywing van 'n tipiese kliniese profiel nie.

Die mediaan-tydverloop tussen MIV diagnose en die inisiëring van eerste-linie-ART was 101 dae (IKV: 36.50-366.75), op eerste-linie ART 1 269 dae (IKV: 765-2 343), op tweede-linie ART 1 512 dae (IKV: 706-2 096) en tussen die mislukking van tweede-linie-ART en inisiëring van DLART was 71 dae (IKV: 57.5-126).

Die eerste-linie-ART kombinasie wat die meeste gebruik was (n=11), was lamivudien (3TC), stavudien (d4T) en efavirens (EFV) en dit was voor 2010 en die tweede-linie proteaseinhibeerder-gebaseerde regime (n=7) wat die meeste gebruik was, was lamivudien (3TC), zidovudien (AZT) en ritonavir-versterkte lopinavir (LPV/r).

Die weerstandigheidsmutasies met die hoogste voorkoms vir die protease inhibeerders (PI's) was M46I (75%), V82A (65%) en I54V (65%). Die mediaan vir die totale aantal PI weerstandigheidsmutasies (belangrik en gering) gevind per pasiënt was drie mutasies (IKV: 2-3). Die weerstandigheidsmutasies met die hoogste voorkoms vir die nukleosied- en nukleotied-trutranskriptase-remmers- (NTTRs-) geneesmiddelgroep (n=18) was M184V (89.47%), D67N (31.58%), M41L (26.31%), T215Y en V118I (21.05%) en vir die nie-nukleosied-trutranskriptase-remmer- (NNTTR-) geneesmiddelgroep (n=17): K103N (47.01%) en G190A (23.52%).

Hoë-vlak-weerstandigheid met die hoogste voorkoms vir die PIs was teen nelfinavir/r (85.7%), indinavir/r (81.0%), lopinavir/r (76.2%), fosamprenavir/r (66.7%) en atazanavir/r (57.1%); NTTRs: emtrisitabien (95.2%), lamivudien (95.2%) en zidovudien (52.4%) en die NNTTR's: nevirapien (76.2%) en efavirens (71.4%).

'n Konstante lae CD4-seltelling en waarneembare VL >50 kopieë/mL gedurende die duur van ART was teenwoordig in hierdie studiepopulasie. Die tydsduur tussen tweede-linie-ART mislukking en inisiëring van DLART in hierdie studiegroep kon bydra tot 'n verhoging in die aantal PI-weerstandigheidsmutasies, na 'n mediaan-tydsverloop van 71 dae (IKV: 58-126).

Hoë-vlak-kruisweerstandigheid tussen PIs en 'n kombinasie van belangrike en geringe PI-weerstandige-mutasies was gevind in die meeste van die pasiënte in hierdie studiepopulasie, maar selfs met die beperkte data, kon bepaalde merkers geïdentifiseer en geïnkorporeer word, om 'n aanvanklike kliniese profiel van die pasiënte te skep. Die resultate van die studie kan die basis vorm van groter studies in Suid-Afrika, ten einde meer suksesvolle DLART bestuurs- en sorgprogramme te skep

Een van die tekortkominge van die retrospektiewe en beskrywende ondersoek was die verlore data by sekere tydsintervalle. Meeste van die retrospektiewe data, dateer sover terug as 2004 en die nasionale ART riglyne het sedertdien al 'n paar maal verander met betrekking tot die behandelingskombinasies, effektiwiteits- en toksisiteitstoetse en die frekwensie van moniteringsintervalle.

Hierdie studie het die aandag gevestig op belangrike demografiese en kliniese kenmerke wat die bestuur en sorg van MIV pasiënte kan verbeter en moontlik die oorskakeling na verskillende ART behandelings kan beperk. Ten slotte het hierdie studie die belangrikheid uitgelig van geïndividualiseerde MIV-bestuurs- en sorgprogramme vir gesondheidsorgfasiliteite, geïntensifiseerde meëwerkendheidsberading en vroeër toetsing vir weerstandigheid vir verbeterde en meer effektiewe en geoptimaliseerde ART in die toekoms.

Sleutelwoorde: MIV, Menslike immuniteitsgebreksvirus, derde-linie-antiretrovirale terapie, mislukte terapie, geneesmiddel-weerstandigheidspatrone, reddingsterapie

Conference Proceedings

Parts of the results obtained from the current study were presented as follows:

- a) Rudman, C., Viljoen, M., Rheeders, M. **HIV drug resistance patterns for adult patients on third-line ART in the North West Province since 2004-2017.**

This presentation was presented as a podium presentation at the South African Annual Pharmacology Conference held in Bloemfontein, South Africa 1-4 October 2017.

The abstract is presented in **Addendum A**.

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List of Abbreviations

3TC:	Lamivudine
ABC:	Abacavir
ADR:	Adverse drug reaction
AIDS:	Acquired immune deficiency syndrome
ART:	Antiretroviral therapy
ARV:	Antiretroviral
ATP:	Adenosine triphosphate
ATV:	Atazanavir
AZT:	Zidovudine
CCR5:	Chemokine co-receptor subtype-5
CNS:	Central nervous system
CrCl:	Creatinine clearance
CYP:	Cytochrome
DNA:	Deoxyribonucleic acid
d4T:	Stavudine
ddl:	Didanosine
DKK:	Dr Kenneth Kaunda
DRV:	Darunavir
DTG:	Dolutegravir
EFV:	Efavirenz
ETR:	Etravirine
EVG:	Elvitegravir
eGFR:	Estimated glomerular filtration rate
FDA:	Food and Drug Administration
FPV:	Fosamprenavir
FTC:	Emtricitabine
GART:	Genotypic antiretroviral resistance testing
GFR:	Glomerular filtration rate
HAART:	Highly Active Antiretroviral Therapy
Hb:	Haemoglobin
HIV:	Human immunodeficiency virus
HIV-1:	Human immunodeficiency virus type 1
HIV-2:	Human immunodeficiency virus type 2
HREC:	Human Research Ethics Committee
IDV:	Indinavir
INSTI:	Integrase strand transfer inhibitor
IQR:	Interquartile range
LPV:	Lopinavir
MVC:	Maraviroc

NDoH:	South African National Department of Health
NFV:	Nelfinavir
NHLS:	National Health Laboratory Services
NNRTI:	Non-nucleoside reverse transcriptase inhibitor
NRTI:	Nucleoside and nucleotide reverse transcriptase inhibitor
NVP:	Nevirapine
NWDoH:	North West Department of Health
NWU:	North-West University
PI:	Protease inhibitor
r:	Ritonavir
RAL:	Raltegravir
RCT:	Randomised controlled trials
RNA:	Ribonucleic acid
RPV:	Rilpivirine
SCr:	Serum creatinine
SIV:	Simian immunodeficiency virus
SPSS:	Social science computing cooperative statistics
START:	Strategic timing of antiretroviral therapy
SQV:	Saquinavir
TDF:	Tenofovir
TLART:	Third-line antiretroviral therapy
TPV:	Tipranavir
UNAIDS:	Joint United Nations Programme on HIV/AIDS
USA:	United States of America
VL:	Viral load
WHO:	World Health Organization

Chapter 1: Introduction

1.1 Introduction

This chapter will reflect on the problem statement of the study, study objectives, the structure of the dissertation and the contributions made by the authors of the manuscripts presented in this dissertation.

1.2 Problem statement

Human immunodeficiency virus (HIV) is considered as a global pandemic with an estimated 36.7 million people diagnosed as HIV positive at the end of 2016 (UNAIDS, 2016b). Sub-Saharan Africa has the highest prevalence of HIV positive people in the world and accounts for 25.8 million or 69% of the global HIV infected population (UNAIDS, 2013). South Africa, part of sub-Saharan Africa, has the largest epidemic in the world with 7.06 million people living with HIV during middle 2017 (UNAIDS, 2016a). This represents 12.6% of the total South African population and 18.0% of its adult population, aged between 15 and 49 years (StatsSA, 2017).

The number of people in South Africa having access to antiretroviral therapy (ART) increased in 2015 from 4.02 million to 6.19 million (UNAIDS, 2017), resulting in South Africa again having the largest ART programme in the world as for the last couple of years (SANAC, 2015; NDoH, 2015; NDoH, 2016). This increase in access to ART can also be seen in the number of people globally having access to ART increasing from 17.1 million to 19.5 million (UNAIDS, 2016b).

Managing the largest HIV epidemic in the world the South African National Department of Health (NDoH), through the Strategic Plan for South Africa and the operational plan for comprehensive HIV and AIDS Management, Care and Treatment for South Africa, introduced the first National ART guidelines in South Africa in 2004 (NDoH, 2004). These ART guidelines were revised in 2010, 2013, December 2014 and April 2015 to reflect the dynamic and rapidly changing world of management and care of HIV.

Currently less than 15% of patients in Africa are receiving a protease inhibitor (PI)-based second-line ART regimen containing a ritonavir-boosted PI (Wallis *et al.*, 2010). The proportion of resistance to first- and second-line ART will increase due to increased access to ART, a longer duration of exposure to antiretroviral (ARV) drugs and non-adherence (Boulle *et al.*, 2010; Madec *et al.*, 2013; Rawizza *et al.*, 2013) leading to an increased need for third-line ART (TLART) regimens. A clear response to this ever changing field of management and care of HIV can be seen with the December 2014 revision of the ART guidelines and the full implementation TLART by the South African NDoH.

Third-line ART or salvage ART includes drugs such as etravirine (non-nucleoside reverse transcriptase inhibitor, NNRTI), darunavir (PI), dolutegravir and raltegravir (integrase strand transfer inhibitors, INSTI) that achieved reasonable virologic suppression in clinical trials and showed a relative low-level of resistance, but are five times more expensive than first-line ART in the South African context (Arastéh *et al.*, 2009; Meyer-Rath *et al.*, 2017; Steigbigel *et al.*, 2010; Yazdanpanah *et al.*, 2009).

The World Health Organization (WHO) indicated in 2013 that national programmes should conduct studies and develop policies for patients receiving TLART with multidrug-resistance after second-line ART failure (WHO, 2013) as this “fatal” multidrug-resistance to second-line ART was emphasized recently in the literature (Magambo *et al.*, 2014; WHO, 2013).

Published data on the clinical outcomes and efficacy of TLART for the majority of HIV-infected patients in Africa are limited due to the limited number of patients initiated on TLART and the relative short duration of time since TLART were implemented.

Third-line ART was included in the South African National ART guidelines of 2013, but not fully implemented due to modalities. In the updated National ART guidelines of December 2014 the TLART regimens were fully implemented with updated policies and management models (NDoH, 2013; NDoH, 2014).

Antiretroviral therapy failure and resistance to ART are becoming an enormous challenge to the South African healthcare system due to the high percentage of patients exposed to ART for a number of years as well as patient adherence and compliance that are not always at a satisfactory level (Meintjes *et al.*, 2015; Wallis *et al.*, 2010).

Initiating more and more patients on TLART might pose significant challenges in a resource-limited setting such as South Africa and a number of factors will prevent patients from accessing TLART:

- Third-line ART is five times more expensive than first-line ART and ranged from R 848.85 to R 1 894.16 per month in the public sector on 2 November 2017 (de Villiers, 2017);
- Resistance testing according to the National Health Laboratory Services (NHLS) is R 5 029.13 per assay on 21 September 2017 (Marais, 2017);
- Managing TLART requires more skilled human resources;
- Managing TLART is time-consuming, due to individual care and adherence counselling;
- Centrally managing patients receiving TLART at the NDoH, calls for demanding management and coordination;
- Non-adherence to ART is a long existing problem and will continue in the future (Conradie *et al.*, 2012; Khan *et al.*, 2014; Wallis *et al.*, 2010) and;
- Medication errors occur that cause drug-drug interactions and a rise in resistance to ART (WHO, 2013).

In a resource-limited country like South Africa, the accurate clinical assessment of patients and their treatment is crucial as TLART is very expensive and may become unsustainable as the trend of patients living with HIV are ever increasing and switching to TLART will become more frequent and apparent (Meyer-Rath *et al.*, 2017; UNAIDS, 2013; Wallis *et al.*, 2010).

Initial descriptive clinical information will enable healthcare workers to assess, evaluate and identify indicators and possible early warning signs that may assist in shortening the administrative process to initiate patients onto other regimens and to also possibly prevent patients from initially failing their respective first- and second-line regimens. To our knowledge no published information on clinical profiles of adult patients initiated on TLART in the North West province are available yet. This study may highlight important clinical or demographic characteristics in this province and will contribute to the optimisation of healthcare management and care of HIV patients in general.

1.3 Study objectives

The general predominant goal of this study was to describe the typical clinical profile of adult patients initiated on TLART with regards to certain clinical and demographical characteristics, resistance patterns and to identify possible factors contributing to ART failure.

1.3.1 Primary objectives

1.3.1.1 Literature objectives

- To describe and evaluate the literature regarding first-, second-, and TLART and reasons for drug switches in the respective regimens.
- To define the mechanism of ARV drug resistance and describe ARV resistant mutations most commonly reported nationally and internationally and;
- To describe possible clinical indicators influencing the switching process from first-line ART to TLART.

1.3.1.2 Empirical objectives

- To describe monitoring test results from patient health records at baseline and at annual intervals for haematology (full blood count and CD4 count), virology (viral load, VL), chemical pathology (serum creatinine, liver function tests and lipids) and ARV drug resistance and mutations.
- To describe patient demographics such as age, gender, duration from HIV-diagnosis to ART initiation, duration on first- and second-line ART and reasons for switching ART regimens (VL failure, side effects etc.).
- To create a typical clinical profile of TLART patients in the North West Province initiated onto TLART.

1.3.2 Secondary objectives

- To describe possible risk factors that could be associated with regimen changes
- To provide recommendations for possible clinical improvements in the management and care of ART patients, to prevent ART failure and subsequently reduce the number of patients switching to TLART

1.4 Structure of dissertation

The structure of this dissertation is presented in an article form, with all chapters having their own reference list provided at the end of each chapter. Chapters are arranged as follows:

Chapter 1: Introduction

Chapter 2: Literature review

Chapter 3: Research methodology

Chapter 4: Manuscript A

Chapter 5: Additional results and discussions not covered in manuscript A

Chapter 6: Discussions, conclusions and recommendations

Addendum: A

Addendum: B

Addendum: C

Addendum: D

Addendum: E

Addendum: F

Addendum: G

Addendum: H

1.5 Contribution of authors to the manuscript in this dissertation

Roles and responsibilities of authors involved in this study dissertation and manuscript are provided in **Table 1-1**.

Table 1-1: Contributions from authors

AUTHOR	AFFILIATION	ROLE
Mr C Rudman (MSc Student)	PharmaCen, Centre of Excellence for Pharmaceutical Sciences (PharmaCen), Division of Pharmacology, North-West University (NWU).	First author, preparation of study proposal, collection of clinical data, statistical analyses in coordination with Statistical Consultation Services, NWU, writing dissertation and manuscripts.
Associate Professor M Viljoen (Supervisor)	PharmaCen, Centre of Excellence for Pharmaceutical Sciences, Division of Pharmacology, NWU (2016). Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, University of the Western Cape (2017)	Conceptualisation of study and proposal, liaison with Ethics (HREC and NWDoH), guidance and input of dissertation and co-author of manuscript.
Dr M Rheeders (Co-supervisor)	PharmaCen, Centre of Excellence for Pharmaceutical Sciences, Division of Pharmacology, NWU.	Guidance and input of congress presentation, proposal, dissertation and co-author of manuscript.

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Chapter 2: Literature Review

2.1 Introduction

This literature review provides an overview of HIV disease and progression, the pharmacology of the most important ART regimens, mechanisms of ARV resistance and an overview of HIV resistance within the South African context.

2.2 HIV epidemiology

The HIV epidemic ascended from African primates, after zoonotic infections with simian immunodeficiency virus (SIV). HIV-1 forms part of the mammalian family of retroviruses known as lentiviruses, represented in three separate transmission events from chimpanzees (group M, N and O) and one from gorillas (group P) (see **Figure 2-1**) (Maartens *et al.*, 2014; Sharp & Hahn, 2011).

The HIV epidemic, as seen globally in the twenty-first century, is related to HIV-1 (group M) dating back from approximately 100 years ago. The SIV primarily confined to western Africa is associated with HIV-2 (Flexner, 2011; Maartens *et al.*, 2014). As a genetically diverse virus, HIV-1 (group M) can be divided into nine subtypes identified as A-D, F-H and K, with subtype C having the highest prevalence in Africa and India, accounting for 48% of all HIV infections globally in 2007 (Hemelaar *et al.*, 2011; Maartens *et al.*, 2014).

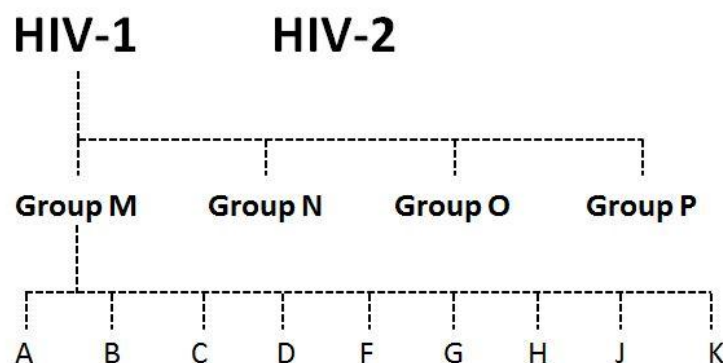


Figure 2-1: HIV types and strains

Human immunodeficiency virus was discovered in humans in 1983 by Barre-Sinoussi et al. The authors suggested that the retrovirus could be the cause of AIDS-related illnesses, after recovering a reverse transcriptase-containing virus from the lymph node of a patient suffering from persistent lymphadenopathy (Montagnier *et al.*, 1987).

It took a year after discovery, to classify the virus as a lentivirus, which evolved to create a gradual onset of clinical symptoms and cause chronic infection in both human and nonhuman primate hosts (Chiu *et al.*, 1985; Gonda *et al.*, 1985; Flexner, 2011; Levy, 1993; Levy *et al.*, 1985).

Dedicated research, over the last decade, into understanding HIV led to remarkable progress in the approach to ART by understanding its pathology, and how the virus and the host are affected by chemotherapeutic agents (Inciardi & Williams, 2005).

Thirty-four years since the discovery of HIV in humans, it is considered a global pandemic with an estimated 36.7 million people being diagnosed as HIV positive at the end of 2016 and since the start of the HIV epidemic, over 35 million people died of AIDS-related illnesses (UNAIDS, 2017). In 2016 alone, there were 1.8 million new HIV infections and an estimated 1 million deaths (UNAIDS, 2017). Since 2005, there is no country in the world not affected by this pandemic (Inciardi & Williams, 2005).

In October 2014, the Joint United Nations Programme on HIV/AIDS (UNAIDS) implemented the 90-90-90 targets. This means 90% of people living with HIV should know their HIV status, 90% of people diagnosed with HIV should receive ART and 90% of those receiving ART should be virally suppressed by the end of 2020 and to end the epidemic as a public threat by 2030. Currently an estimated 19.5 million people have access to antiretroviral therapy globally in order to keep patients virally suppressed (UNAIDS, 2014; UNAIDS, 2017).

In 2013, the WHO emphasised that national programmes in countries affected by this pandemic should develop policies and management protocols for more effective treatment programmes (WHO, 2013), as increased ARV exposure and access to ART will ultimately lead to more and more patients failing their respective first- and second-line ART as further drug resistance develops (Magambo *et al.*, 2014).

South Africa has the highest global burden and remains the HIV epicentre with an estimated 7.06 million people living with HIV in mid-2017, accounting for 18% of the total adult population, aged between 15 and 49 years, and 12% of the total population (StatsSA, 2017;

UNAIDS, 2014). This resulted in South Africa having the largest ART management and care programme in the world for the last couple of years (SANAC, 2015).

It is important for NDoH's and healthcare providers to keep up with current and relevant scientific knowledge in the ever-changing HIV environment, in order to provide effective management and care to people living with HIV.

2.3 HIV pathogenesis

The HIV life cycle is diverse and complex, targeting activated CD4 T lymphocytes to gain entry into the host cell via interactions with CD4 and chemokine co-receptors (CCR5 and CXCR4) (Maartens *et al.*, 2014). Two molecules, external glycoprotein gp120 and the transmembrane protein gp41, are of importance during the HIV entry process and can be found as spikes on the surface of the envelope of HIV-1 (Eckert & Kim, 2001; Platt *et al.*, 2005; Ray & Doms, 2006) (see **Figure 2-2**).

External protein gp120 binds to the CD4 cell membrane during the entry process by binding to the CD4+ receptor, allowing subsequent fusion of the gp41 protein with the chemokine co-receptors on the cell membrane. This attachment activates an irreversible conformational change, due to covalent bonds, completing the entry process into the host cell with the viral core disassembling (Eckert & Kim, 2001; Ray & Doms, 2006).

After fusion and transmission of the virus over the membrane of the host cell, full-length viral RNA enters the cytoplasm within minutes of pore formation, where viral RNA is transcribed by the virus into viral DNA through the release of the enzyme, reverse transcriptase (Eckert & Kim, 2001; Flexner, 2011; Platt *et al.*, 2005). Reverse transcription of viral RNA into DNA is characterised as extremely error prone and introduces, on average, one to ten mutations for every viral genome transcribed, as reverse transcriptase does not contain any proofreading activity (Arts & Hazuda, 2012; Coffin, 1997).

At the centre of HIV infection the viral protein, integrase, in combination with host DNA repair enzymes inserts the viral DNA into gene-rich and transcriptionally active sites of the chromosomal DNA of the CD4 host; an integrase binding host factor (lens epithelium-derived growth factor) facilitates the integration process. At this point, the host cell is transformed into a potential virus producer (Ciuffi *et al.*, 2005; Mitchell *et al.*, 2004; Scherdin *et al.*, 1990; Schröder *et al.*, 2002; Simon *et al.*, 2006; Turlure *et al.*, 2006).

At completion of integration, viral proteins are transported and assembled in close proximity to the cell membrane of the host. The virus then takes advantage of the vesicular sorting pathway (ESCRT-I, II, III), normally used to mediate budding of endosomes into

multivesicular bodies, to egress from the host cell (Bieniasz, 2006; Martin-Serrano *et al.*, 2003). Viral protease produces mature and infectious virions through the cleavage of the Gag-Pol poly-protein and since cytoplasmic molecules of the host cell are incorporated into the newly formed virus, virions contain specific characteristics of the host cell produced and can later determine the phenotype of the virus in different ways (Cantin *et al.*, 2005; Coffin, 1997; Zhu *et al.*, 1993).

Another characteristic of HIV is the high level of viral production and turnover in treatment-naïve patients, with the number of productively infected lymphoid cells at roughly 10^7 to 10^8 copies per millilitre with a turnover rate of $\sim 10^{10}$ cells/d (Haase, 1999; Ho *et al.*, 1995; Perelson *et al.*, 1996; Wei *et al.*, 1995). As previously alluded, the reverse transcriptase process transcribing viral RNA into DNA is extremely error prone and each viral genome may generate a large number of viral mutations (Arts & Hazuda, 2012).

These two characteristics of high viral production and the number of errors in each viral genome replication cycle may contribute to the already complex genetic diversity of HIV, resulting in a more complex antiretroviral treatment and care programme for the future.

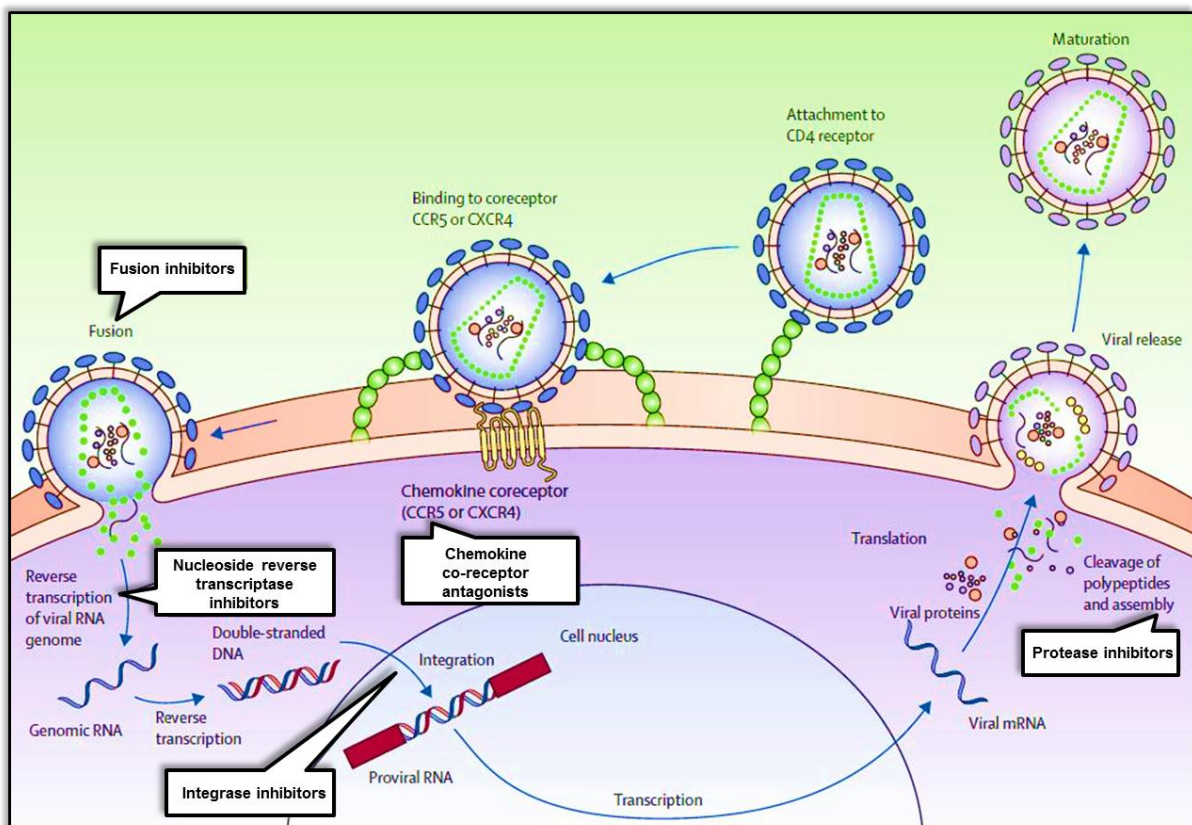


Figure 2-2: HIV life cycle illustrating the active binding sites for antiretroviral agents
Adapted from Walker and colleagues, 2014.

Human immunodeficiency virus is known to establish chronic and persistent infection with a steady onset of clinical symptoms requiring antiretroviral therapy for continued viral suppression and subsequently, a decline in morbidity and mortality (Flexner, 2011; Simon *et al.*, 2006).

Only a few HIV-1 treatment options of ART existed prior to 1996, with the management and care of AIDS-related illnesses mainly focusing on prophylaxis against common opportunistic infections (Arts & Hazuda, 2012). The first ARV deemed effective against HIV-1 was the nucleoside reverse transcriptase inhibitor zidovudine (azidothymidine, AZT), synthesised by Horwitz in 1964 (Flexner, 2011).

When AZT was approved in 1987 for the management and care of HIV infection, based on the results of a small randomised clinical trial (Fischl *et al.*, 1987; McLeod & Hammer, 1992), it led the way for other compounds and classes, such as the nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase strand transfer inhibitors (INSTIs), fusion inhibitors and entry inhibitors, to be developed and implemented for ART in the twenty-first century.

Antiretroviral therapy for the management of HIV-1 evolved from the monotherapy used in the early 1990's, with the approval of the NRTIs, to the administration of more than one ARV in combination therapy, also known as highly-active-antiretroviral-therapy (HAART). The latter reduced the morbidity and mortality of AIDS related illnesses (Collier *et al.*, 1996; D'Aquila *et al.*, 1996; Staszewski *et al.*, 1996).

2.4 Antiretroviral therapy

2.4.1 Nucleoside and nucleotide reverse transcriptase inhibitors

Nucleoside and nucleotide reverse transcriptase inhibitors were the first antiretroviral class approved by the FDA for the treatment of HIV (Young, 1988). Compounds found in this class are administered as prodrugs that can only exert antiretroviral activity after host cell entry and phosphorylation by cellular kinases (Furman *et al.*, 1986; Hart *et al.*, 1992; Mitsuya & Broder, 1986; Mitsuya *et al.*, 1985; St Clair *et al.*, 1987).

All agents in this class are known as nucleosides with tenofovir being the only nucleotide, requiring two additional phosphates to reach full-activity (Flexner, 2011).

Nucleoside and nucleotide reverse transcriptase inhibitors are effective ARVs inhibiting the enzyme reverse transcriptase, used by HIV to transcribe viral RNA into DNA. Terminating further DNA chain elongation, resulting in viral suppression, is achieved by the lack of a 3'-hydroxyl group at the sugar moiety of the NRTI, preventing the development of a 3'-5'-phosphodiester bond between the incoming natural 5'-deoxynucleoside triphosphates and the NRTIs (Waller & Sampson, 2013).

Termination of DNA chain elongation can occur at either the synthesis of RNA-dependant DNA or DNA-dependent DNA (Balzarini *et al.*, 1989; Cheng *et al.*, 1987; Richman, 2001).

Currently there are seven NRTIs available in South Africa: tenofovir (TDF), lamivudine (3TC), emtricitabine (FTC), abacavir (ABC), zidovudine (AZT), stavudine (d4T) and didanosine (ddI) (SAMF, 2016; Meintjes *et al.*, 2017).

Side effects of the NRTI class are often considered severe enough to cause withdrawal of therapy. Unwanted effects are believed to be related to the inhibition of mitochondrial enzymes, with reduced formation of adenosine triphosphate (ATP) and may include the following: neutropenia and anaemia, gastrointestinal side effects (nausea, vomiting and diarrhoea), insomnia and headache, myalgia or myositis, hepatomegaly with steatosis that may be potentially life-threatening and peripheral neuropathy and pancreatitis (see **Table 2-1**) (SAMF, 2016; Waller & Sampson, 2013).

Patients initiated on d4T generally experienced drug-specific side effects of peripheral neuropathy, with a prevalence of approximately 12% at the then recommended dose of 40mg twice daily (Hurst & Noble, 1999), with an even higher prevalence in patients with underlying HIV-related neuropathy or in those using other neurotoxic drugs. Stavudine use is associated with fat wasting syndrome or lipoatrophy and lactic acidosis and has therefore largely been phased out due to these toxicities (Calmy *et al.*, 2009).

Patients initiated on AZT generally experience drug-specific side effects of fatigue, malaise, myalgia, nausea and insomnia, but these usually resolve after a few weeks of treatment. More severe side effects, occurring in patients with advanced HIV disease and a low CD4 count, associated with AZT are bone marrow suppression, anaemia and granulocytopenia (Flexner, 2011).

Patients initiated on TDF may experience episodes of acute renal failure and Fanconi's syndrome, which is associated with a decline in the estimated creatinine clearance after long durations of treatment.

Patients initiated on TDF should be regularly monitored for renal function as the TDF dosage should be reduced if renal impairment is present. Tenofovir is generally well tolerated with few significant side effects reported (Gallant & Moore, 2009).

Lamivudine and emtricitabine, as chemically equivalent ARVs, are known to be the least toxic in the NRTI class, with few significant side effects reported (Saravolatz & Saag, 2006).

2.4.2 Non-nucleoside reverse transcriptase inhibitors

Non-nucleoside reverse transcriptase inhibitors consist of a variety of chemical substrates that bind and induce the formation of a hydrophobic pocket, proximal to the p66 subunit of HIV-1 reverse transcriptase, inducing a conformational change in the three-dimensional structure of the binding site and reducing the activity of polymerase (Arts & Hazuda, 2012; Flexner, 2011; Kohlstaedt *et al.*, 1992; Spence *et al.*, 1995; Tantillo *et al.*, 1994).

Compounds found in the NNRTI class do not require intracellular phosphorylation to exert antiretroviral activity and are only active against HIV-1 and not HIV-2, as the binding site for NNRTIs is virus-strain-specific (Harris & Montaner, 2000).

Currently there are four NNRTIs available in South Africa: efavirenz (EFV), nevirapine (NVP), rilpivirine (RPV) and etravirine (ETR) (SAMF, 2016; Meintjes *et al.*, 2017).

Class specific side effects include rashes during the first four weeks of therapy that are generally mild and self-limited, but cases of life-threatening Stevens-Johnson syndrome have been reported with the use of nevirapine, efavirenz and etravirine (Flexner, 2011; Michael, 2005). Fat accumulation was also reported with long-term use of NNRTIs (Calmy *et al.*, 2009) and fatal hepatitis can be associated with nevirapine use (see **Table 2-1**) (Michael, 2005).

Patients initiated onto EFV generally experience central nervous system (CNS) side effects, with up to 53% reporting some CNS or psychiatric side effect after the first dose that may last for several hours; more severe effects may take weeks to subside. Rashes can also be seen within the first few weeks of treatment and were reported in up to 27% of adult patients; rare cases of Stevens-Johnson syndrome have also been reported and may be life-threatening (Flexner, 2011; Michael, 2005).

Those initiated onto NVP will frequently experience drug-specific rash occurring in ~16% of patients and they may also experience mild papular or macular eruptions within the first six weeks of treatment (Flexner, 2011).

2.4.3 Protease inhibitors

Protease inhibitors are peptide-like substances competitively inhibiting virus specific aspartyl protease, responsible for the maturation of the virion to an active form through the binding of the viral gag and gag-pol polyprotein precursor, with the main target site being the N-terminal side of proline residues between proline and phenylalanine (Flexner, 2011; Miller, 2001; Park & Morrow, 1993). The PIs do not have an effect on the human aspartyl protease as it only contains one polypeptide chain (Flexner, 2011).

All the PIs can cause toxicity through metabolic drug interactions by inhibiting CYP3A4 at therapeutic concentrations, with ritonavir considered as the most potent enzyme inhibitor (Flexner, 2011; Piscitelli & Gallicano, 2001). Significant drug-drug interactions have been seen for drugs metabolised by hepatic metabolism, as potent enzyme inhibitors and inducers will alter the therapeutic concentration and clinical efficacy of the PIs (SAMF, 2016).

It is common practice to combine the PIs with a sub-therapeutic dose of ritonavir to exploit the pharmacokinetic properties of this drug and improve the bioavailability significantly, as it widely differs for all PIs (Flexner, 2000).

Currently there are seven PIs available in South Africa: atazanavir (ATV), lopinavir (LPV), darunavir (DRV) fosamprenavir (FPV), indinavir (IDV), saquinavir (SQV) and ritonavir (r). Ritonavir is used as a pharmacokinetic enhancer and in combination with most available PIs (SAMF, 2016; Meintjes *et al.*, 2017).

Gastrointestinal side effects for PIs are common and include nausea, vomiting and diarrhoea, and usually subside within four weeks of initiating therapy. Metabolic disturbances including lipodystrophy, insulin resistance, fat redistribution causing buffalo hump, hyperlipidaemia and breast enlargement may be caused by the inhibition of regulatory proteins in adipocytes. Other side effects include hepatic dysfunction and pancreatitis (see **Table 2-1**) (Flexner, 2011; SAMF, 2016).

As lopinavir and ritonavir (LPV/r) is used as a co-formulation, the major side effect reported during treatment is gastrointestinal, including loose stools, diarrhoea, nausea, and vomiting as major symptoms. Gastrointestinal side effects may be reduced if LPV/r is taken with meals. The most common laboratory irregularities experienced during treatment include elevated total cholesterol and triglycerides, but it is unclear whether the latter side effects are due to ritonavir, lopinavir or the co-formulation as both these drugs exert the same side effects (Flexner, 2011).

2.4.4 Integrase strand transfer inhibitors

The HIV-1 enzyme, integrase, is successfully inhibited by the integrase strand transfer inhibitors (INSTIs) that were approved for antiretroviral therapy in 2007 (Espeseth *et al.*, 2000; Hazuda *et al.*, 2004a; Hazuda *et al.*, 2004b). Chromosomal integration is an important characteristic for the life cycle of the retrovirus, allowing viral DNA to remain inactive or latent in the nucleus of the host cell for an extended period.

As the first approved INSTI, raltegravir prevents the formation of covalent bonds between the viral and host DNA by interfering with divalent cations in the catalytic core of the enzyme (Hicks & Gulick, 2009). The mechanism of action for INSTIs is now well defined and is the only ARV class that interacts with two important characteristics of the retrovirus in which (1) it only binds between integrase and the viral DNA specific complex and (2) by interacting with two magnesium metal ion cofactors in DNA and the integrase active site (Arts & Hazuda, 2012; Grobler *et al.*, 2002).

Currently there are only two INSTIs available in South Africa: raltegravir (RAL) and dolutegravir (DTG). The use of DTG has been highlighted in 2017 and is expected to make its appearance as part of first-line ART in the South African public health sector in 2018 (Meintjes, 2017; South African Government News Agency, 2017).

According to the SINGLE trial (Study ING114467), dolutegravir is seen as superior to efavirenz, with a higher resistance barrier and a better tolerability profile. The SINGLE trial compared a DTG/ABC/3TC to an EFV/TDF/FTC regimen in treatment-naïve patients in Australia, Europe and North America (n=833) (Walmsley *et al.*, 2015; Walmsley *et al.*, 2013).

Common side effects of INSTIs include headache and other central nervous system effects, GI upset, hepatitis, and rash. Rhabdomyolysis is often associated with raltegravir in rare cases and insomnia with dolutegravir (see **Table 2-1**) (Hicks & Gulick, 2009; Meintjes *et al.*, 2017).

2.4.5 Entry inhibitors

HIV-1 uses several host proteins to enter a cell, causing a set of intricate events leading to membrane fusion and release of viral RNA into the cytoplasm of the host cell. Currently there are only two drugs available in this class for HIV-1 treatment: namely maraviroc and enfuvirtide, both having different mechanisms of action (Flexner, 2011).

Maraviroc is classified as a CCR5 blocker and is the only approved ARV targeting a host protein. Maraviroc is only active against CCR5-tropic strains of HIV and blocks the binding of the gp120 protein on the outer envelope of HIV, to the CCR5 chemokine receptor on the host cell. Maraviroc is usually well tolerated with rash, hepatitis, fever, abdominal pain, dizziness, cough and musculoskeletal symptoms reported as the only side effects, but are seen as rare (see **Table 2-1**) (Flexner, 2011; Meintjes, 2017).

Enfuvirtide is classified as a fusion inhibitor and its unique mechanism of action involves preventing a six-helix bundle to be formed, critical for membrane fusion and viral entry into the host cell. This unique mechanism of action is the reason enfuvirtide retains its susceptibility against viruses resistant towards other ARVs (Flexner, 2011). Common side effects seen with enfuvirtide are injection-site specific reactions as the only route of administration approved is parenteral. Side effects including pain and erythema affect about 98% of patients and 80% of patients develop nodules or cysts (Dando & Perry, 2003).

Table 2-1: Common adverse drug reactions of antiretroviral drugs available in South Africa.

Drug name	Drug class	Common and/or severe adverse drug reactions
Tenofovir (TDF)	NtRTI	Renal failure , nausea, tubular wasting syndrome, reduced bone density
Lamivudine (3TC)	NRTI	Anaemia*
Emtricitabine (FTC)	NRTI	Palmar hyperpigmentation
Abacavir (ABC)	NRTI	Hypersensitivity reaction
Zidovudine (AZT)	NRTI	Anaemia, neutropenia , GI upset, headache, lipoatrophy, myopathy, hyperlactataemia or steatohepatitis**
Stavudine (d4T)	NRTI	Peripheral neuropathy, lipoatrophy, pancreatitis, hyperlactataemia or steatohepatitis***, dyslipidaemia
Didanosine (ddI)	NRTI	Peripheral neuropathy, pancreatitis , nausea, diarrhoea, hyperlactataemia or steatohepatitis***
Efavirenz (EFV)	NNRTI	Central nervous system symptoms, rash, hepatitis , gynaecomastia
Nevirapine (NVP)	NNRTI	Rash* , hepatitis*
Rilpivirine (RPV)	NNRTI	Rash*, hepatitis* , central nervous system symptoms*
Etravirine (ETR)	NNRTI	Rash*, hepatitis*
Atazanavir (ATV)	PI	Unconjugated hyperbilirubinaemia (visible jaundice in minority of patients), dyslipidaemia*, renal stones*, hepatitis*
Lopinavir/ritonavir (LPV/r)	PI	GI upset, dyslipidaemia, hepatitis
Darunavir (DRV)	PI	GI upset, rash, dyslipidaemia, hepatitis*
Saquinavir (SQV)	PI	GI upset**, hepatitis, hyperglycaemia, dyslipidaemia
Fosamprenavir (FPV)	PI	GI upset, hyperglycaemia, fatigue, paraesthesia, headache, skin eruptions
Indinavir (IDV)	PI	Crystalluria, nephrolithiasis, unconjugated hyperbilirubinaemia, lipodystrophy syndrome, hyperglycaemia
Raltegravir (RAL)	INSTI	Headache, central nervous system symptoms, GI upset, hepatitis*, rash*, rhabdomyolysis*
Dolutegravir (DTG)	INSTI	Insomnia, headache, central nervous system symptoms, GI upset, hepatitis*, rash*
Maraviroc (MVC)	CCR5 blocker	Rash*, hepatitis*, fever*, abdominal pain*, cough*, dizziness*, musculoskeletal symptoms*

ADR, adverse drug reaction; **NtRTI**, nucleotide reverse transcriptase inhibitor; **NRTI**, nucleoside reverse transcriptase inhibitor; **NNRTI**, non-nucleoside reverse transcriptase inhibitor; **PI**, protease inhibitor; **INSTI**, integrase strand transfer inhibitor; **CCR5**, C-C chemokine receptor type 5.

(* , rare potential/uncommon), (** , medium potential), (***, high potential) Life-threatening ADR`s indicated in bold.

Adapted from Meintjes et. al 2017

2.5 Antiretroviral therapy use in South Africa

As HAART, implemented in 1996, remains the only effective treatment strategy against HIV, the NDoH applied it in the public sector as part of its ART roll-out programme, with the implementation of the first National ART guidelines in 2004 through the Strategic Plan for HIV and AIDS and the operational plan for Comprehensive HIV and AIDS Management, Care and Treatment for South Africa (Clavel & Hance, 2004; NDoH, 2004).

The National ART guidelines have since been updated in 2010, 2013, December 2014 and April 2015, to reflect the dynamic and rapidly changing environment of HIV management and care (NDoH, 2015). An updated version of the National ART guidelines are also expected to be implemented in 2018 in the public sector, in order to keep up with the progress of ART, newer ARVs and to improve and optimise the safety and efficacy of ART in South Africa (Meintjes, 2017).

2.5.1 Therapeutic goals of antiretroviral therapy

The South African HIV Clinicians Society listed the main objectives of ART in South Africa as (Meintjes, 2017):

- deliver maximal and long-lasting viral load suppression;
- repair and preserve a patient's immune system;
- decrease HIV associated infectious and non-infectious morbidity;
- prolong a patient's life expectancy and improve their quality of life;
- prevent further transmission of HIV;
- limit adverse drug reactions to ART.

Suppressing HIV replication completely with good adherence to sustainable and well tolerated ART, for as long as possible, is the only way of achieving the objectives listed above. Keeping these prolonged viral suppression goals in mind will increase the CD4 count of HIV positive patients, thus decreasing their risk of HIV-associated morbidity and mortality (Meintjes *et al.*, 2017).

Collaborative analysis of cohort studies indicated that patients with good adherence to ART have near normal life expectancies, indicating the effectiveness of ART implemented in the national consolidated guidelines of South Africa (Johnson *et al.*, 2013).

2.5.2 First-line antiretroviral regimens

First-line regimens consist preferably of two ARVs from the NRTI group and one from the NNRTI group, used as combination therapy, with a PI only included if the use of a NNRTI is contra-indicated.

The current recommended first-line ART regimen for all adult patients in the public sector of South Africa is the fixed-dose combination implemented in April 2013 and includes TDF + 3TC or FTC + EFV (NDoH, 2013; NDoH, 2015). All patients on d4T must be switched to TDF, as this drug has been phased out of the ART programme of South Africa. If contra-indications for EFV are present, it should be substituted with NVP or LPV/r and if contra-indications for TDF are present this should be substituted with ABC (NDoH, 2015).

2.5.3 Second-line ART regimens

Second-line ART regimens in South Africa consist of two NRTIs and one PI. A boosted PI is usually included as a combination of low-dose ritonavir with lopinavir, which acts as a pharmacokinetic enhancer that will increase the lopinavir plasma concentration (Flexner, 2000). Second-line regimes consist of AZT + 3TC + LPV/r (when failing a TDF-based first-line ART regimen), or TDF + 3TC or FTC + LPV/r (when failing a d4T or AZT-based first-line ART regimen) (NDoH, 2015).

2.5.4 Third-line ART regimens

Third-line ART (TLART) for adults can only be initiated in South Africa by the Adult group of the Peer Review Committee for TLART, managed by the NDoH, and only after virological failure is experienced on NRTIs, NNRTIs and if a patient has documented PI resistance after at least one year of treatment on a PI-based second-line ART regimen (NDoH, 2015).

As non-adherence to ART, specifically PI-based second-line regimens, is one of the main reasons for ART failure, adherence should be first addressed through counselling, support and education and should be seen as the main objective of improvement by healthcare providers in South Africa (Khan *et al.*, 2014; Meintjes *et al.*, 2014; Wallis *et al.*, 2010).

If a patient does achieve adequate viral suppression, after intensified interventions by a healthcare provider for three consecutive viral load measurements (VL <1000 copies/ml), a genotypic antiretroviral resistance test (GART) should be performed, with the help of the National Health Laboratory Service (NHLS), to determine if the patient has documented resistance to their respective ARVs (Meintjes *et al.*, 2014; NDoH, 2015).

Genotypic antiretroviral resistance tests are performed to determine the best suitable TLART regimen on which the respective patient should be initiated.

After patients have been approved by the Adult group of the Peer Review Committee for TLART, they will be managed on a named patient basis by the NDoH, which centrally manages each patient's medication by sending the individual drugs from the different suppliers/manufactures to the primary healthcare facility that manages the rollout of TLART. This is done to ensure each patient receives optimal ART, which also incorporates adherence counselling as a main priority (NDoH, 2015).

In the South African context, there is no empirical or generic TLART and regimen choices should be made individually according to the genotypic resistance test results (Jamaloodien, 2014).

As there is no generic regimen for TLART, a few crucial factors to be taken into account include: older generation NNRTIs should not be used in TLART as they will not exert any viral suppressive properties, PIs with the broadest spectrum should be selected of which darunavir shows the highest promise, drugs from the INSTI class are the most preferred ARVs for TLART as this entirely new class has a low possible risk for cross-resistance and a favourable tolerability profile (Meintjes *et al.*, 2017).

If TLART failure is evident in a patient, maraviroc can be used as a last resort, but only after a tropism test demonstrates susceptibility to the CCR5 chemokine co-receptor as maraviroc is extremely costly (Meintjes *et al.*, 2014). In May 2016 a case of drug resistance to TLART in South Africa was published, indicating that maraviroc was the only available option for treatment after a tropism assay predicted that a CCR5 antagonist is likely to be effective (Rossouw *et al.*, 2016).

2.6 HIV disease progression and monitoring

The evaluation of treatment outcomes, in HIV positive patients receiving ART, is done by measuring and interpreting two key clinical prognostic indicators, namely CD4 cell counts (or CD4 % in children) and HIV RNA levels, also referred to as viral load (VL). These two indicators, used as a function of immune improvement in patients, are of utmost importance in evaluating a patient's clinical outcomes and response to ART in HIV disease progression and monitoring (Govender *et al.*, 2014).

2.6.1 CD4 counts

CD4 cells, also known as T-helper cells, a subpopulation of lymphocytes, are the human body's immune response coordinators. CD4 cells assist B cells to produce antibodies, as well as increase cellular immune responses to antigens that might be present in the body. CD4 cells are the main target of HIV, with the destruction of these cells leading to a loss in an immune response against HIV (WHO, 2007).

Within hours of being exposed to HIV, it will infiltrate and infect the CD4 cells in a patient, leading to active viral replication within the healthy CD4 cells. Virions are then released from the infected cells, enabling them to infect uninfected CD4 cells and thereby constituting the life cycle of the virus (Simon *et al.*, 2006).

In 2015, the WHO announced the Universal Test and Treat (UTT) policy for all newly diagnosed HIV positive patients to be initiated on ART as soon as they are ready and within two weeks of the CD4 count being done. The NDoH announced in May 2016 that they will also implement the UTT policy, enabling the South African guidelines to provide ART to all HIV positive patients, irrespective of their CD4 count (Child, 2016; Pillay, 2016).

Patients with a reduced CD4 count of <200 cells/ μ L and suffering from severe immunosuppression are at higher risk of opportunistic infections and related mortalities and should be initiated on ART within a week of diagnoses and patient counselling (Meintjes *et al.*, 2017).

A reduction in the CD4 count indicates a reduction in the immune system's capability and capacity to protect the body against pathogens such as HIV and other opportunistic infections (Ford *et al.*, 2015). Good adherence to ART will result in a CD4 count increase of >50 cells/ μ L within the first few weeks post viral suppression (Meintjes *et al.*, 2017).

2.6.2 Viral loads

The measurement of plasma HIV RNA levels (viral load, VL) can be defined as the number of copies of HIV genetic material per millilitre (mL) plasma (Pebody, 2017). An undetectable VL can be defined as the cut-off point below which the HIV virions cannot be reliably detected in the blood of an infected patient and is one of the main goals of ART that should be maintained throughout (Meintjes *et al.*, 2017).

Long-lasting, quiescent, memory T cells harbour infectious HIV DNA that is integrated into the host chromosome, indicating that the likelihood of HIV being eradicated with ART would not be possible in the near future (Chun *et al.*, 1998; Finzi *et al.*, 1997). Undetectable VL values are therefore not an indication of a patient being HIV free, as the VL test does not take into account the virions in the lymph nodes, gut or genital fluids, but is an indication that the virions cannot mutate or develop resistance (Meintjes *et al.*, 2017; Pebody, 2017).

Viral load “blips” occur when a patient has an undetectable VL that increases to a low, but detectable VL of <1000 copies/mL, before returning to undetectable levels. This is not an indicator for virological failure and most patients are followed up with a subsequent VL test (Kieffer *et al.*, 2004; Meintjes *et al.*, 2014; Pebody, 2017).

Viral load monitoring is used in South Africa to decide when to switch from a first- or second-line ART regimen, after virological failure is experienced, rather than using immunological and clinical failure as an indication (Tucker *et al.*, 2014). Viral load monitoring is seen as an important aspect for the success of ART (Tucker *et al.*, 2014).

Currently, viral load at baseline is not used in South Africa to decide if ART should be initiated in treatment naïve patients and is only implemented as a monitoring tool to assess treatment outcomes. Once a patient is diagnosed as HIV positive, viral load will be measured at baseline and three months after initiation of ART and is used as an indicator of adherence. Viral load monitoring at baseline is one of the important aspects of the anticipated new guidelines to be re-introduced in 2018. Several patients with a high VL at baseline might not achieve full viral suppression, even if adherence is 100%, but would have had a >2 log₁₀ decrease from the baseline value (Meintjes *et al.*, 2017).

Viral load should be monitored at set intervals, according to the National ART guidelines, for patients experiencing virological failure on a first-line ART regimen with any VL >50 copies/mL requiring adherence counselling and interventions. Viral loads <400 copies/mL should be monitored 12 monthly, however if the VL is between 400–1000 copies/mL, it should be repeated after six months and if >1000 copies/mL, it will require two monthly repeated testing until a desired VL is achieved (NDoH, 2015).

2.7 Initiation of ART

In South Africa, HAART was only made available to the public sector with the implementation of the National ART guidelines in 2004. This guideline indicated that patients were only eligible for ART initiation if their CD4 count was <200 copies/mL, or if they were classified as WHO stage IV (NDoH, 2004). The National ART guidelines were updated in 2010 and 2015 and patients were only eligible for ART initiation if their CD4 count was <350 copies/mL and ≤500 copies/mL, respectively (NDoH, 2010; NDoH, 2015).

This meant patients with CD4 counts higher than the eligibility criteria, were initiated on a prophylaxis regimen containing co-trimoxazole until their respective CD4 count decreased to the level of eligibility indicated above (NDoH, 2004; NDoH, 2010; NDoH, 2013).

Two studies, conducted by the INSIGHT START Study Group (International Network for Strategic Initiatives in Global HIV Trials - Strategic Timing of Antiretroviral Treatment) and the TEMPRANO ANRS 12136 Study Group (early antiretroviral treatment and early isoniazid prophylaxis against tuberculosis in HIV-infected adults, March 2008 to January 2015) investigated the effect of immediate initiation of ART and differing treatment initiation until the eligibility criteria was met.

The INSIGHT START study was conducted on 4685 patients in 35 countries between April 2009 and May 2015, and the TEMPRANO ANRS study was conducted in the Ivory Coast (n=2056) between March 2008 and January 2015, with both studies unanimously concluding that significant benefits were found for patients immediately initiated on ART irrespective of their CD4 count (Grarup *et al.*, 2015; Group, 2015).

The above-mentioned studies reconfirm the decision by the WHO to implement the UTT policy, which was also adopted by the NDoH in order to reduce the number of virological failure episodes experienced in South Africa and globally, to improve life-expectancies, morbidity and mortality of patients.

2.8 Durations on ART

Typical demographical and clinical characteristics of a TLART patient is very limited in South Africa, but according to a study conducted in the private sector, through the Aid for AIDS programme, on 152 patients initiated onto TLART between July 2007 and December 2011, the median duration for the respective patients on ART prior to initiation TLART was 8.9 years (IQR: 6.9–10.4) (Meintjes, 2015). The duration spent on ART will inevitably increase in the near future as patients live longer and management and care policies improve (Palella Jr *et al.*, 1998; Zaidi *et al.*, 2013).

Longer durations spent on ART however creates yet another problem if patients are not managed properly as patients are kept on a failing first- or second-line ART regimen for too long, with literature reporting that 24 months on a failing second-line ART will result in a median of five (IQR: 0–6) PI mutations being formed (Lee *et al.*, 1998).

A Nigerian cohort estimated that patients would develop 0.6 PI-mutations for every six months they were maintained on a failing second-line ART regimen (Rawizza *et al.*, 2013). ARV resistance is not seen as an all-or-nothing occurrence, but increases over longer duration of treatment (Barbour *et al.*, 2002; Richman, 1990; Ross *et al.*, 2000).

The use of ART in South Africa has proven to be remarkably effective in managing the progression of HIV and increased the life expectancy of many HIV positive patients (Palella Jr *et al.*, 1998; Zaidi *et al.*, 2013), but these benefits are to no avail if the development of resistance continues (DeGruttola *et al.*, 2000; Ledergerber *et al.*, 1999).

2.9 Antiretroviral therapy failure

Antiretroviral therapy failure is broadly defined as ART not being able to suppress and sustain the VL of a patient below 1000 copies/mL, when measured at two consecutive measurements (with adherence support between the two measurements) taken two to three months apart (NDoH, 2014). Viral “blips” and other factors can influence the result of the VL tests as referred to earlier in section 2.6.2.

The most common reason for treatment failure remains poor adherence to ART, and regimen switching should only be considered after intensified adherence counselling has been done (Flexner, 2011; Wallis *et al.*, 2010).

Causes of non-adherence can include but is not limited to the following (Meintjes *et al.*, 2014):

- Inadequate knowledge regarding treatment, although adherence rates are much higher when compared to other chronic diseases;
- Side effects of ARVs, such as nausea, loss of appetite and bad dreams;
- Mental illness and depression (the prevalence is twice as high in HIV-patients);
- Poverty and food insecurity;
- Difficulties in the workplace including shift working and the inability to attend clinic appointments;
- Substance abuse;
- Stigma that manifest in tablets being found and clinic and pharmacy visits becoming known;
- Social problems in general.

Other contributing factors include the use of single-dose nevirapine for the prevention of mother-to-child-transmission (PMTCT) and when the child does get infected at a later stage, interactions between drugs that decrease the concentration of ARV's and subsequently increase resistance to ART (Kuhn *et al.*, 2012; WHO, 2013).

Non-adherence associated problems with second-line ART failure, specifically in resource-limited settings such as South Africa, are that patients often do not own a refrigerator which is considered a necessity for the earlier thermolabile LPV/r soft gel capsule formulations. Long distances travelled to and from healthcare facilities, resulting in missed appointments or medication refills, are also considered a problem (El-Khatib *et al.*, 2010).

The current national consolidated guidelines provide clear guidance for the management and assessment of first- and second-line ART failure. Patients failing on second-line ART are increasing with longer durations of exposure, non-adherence and better access to ART causing an increase in the number of patients failing second-line ART (Meintjes *et al.*, 2015).

2.10 Regimen switching

Switching from first- to second-line ART regimens in the public sector of South Africa is based on the National ART guidelines, with the updated version of April 2015 currently in use.

Switching from first-line ART can be considered if a patient has experienced virological failure (VL >1000 copies/mL) on two occasions, two to three months apart, despite good adherence. Patients receiving TDF-based first-line ART should also be tested for hepatitis B before withdrawal of TDF, as it is associated with an extreme hepatitis flare. A patient's cholesterol should also be checked, especially before switching to a LPV/r-based second-line ART regimen (NDoH, 2015).

The following guidance (see **Figure 2-3**) for the management and assessment of first-line ART failure should be followed, as prescribed by the NDoH, 2015.

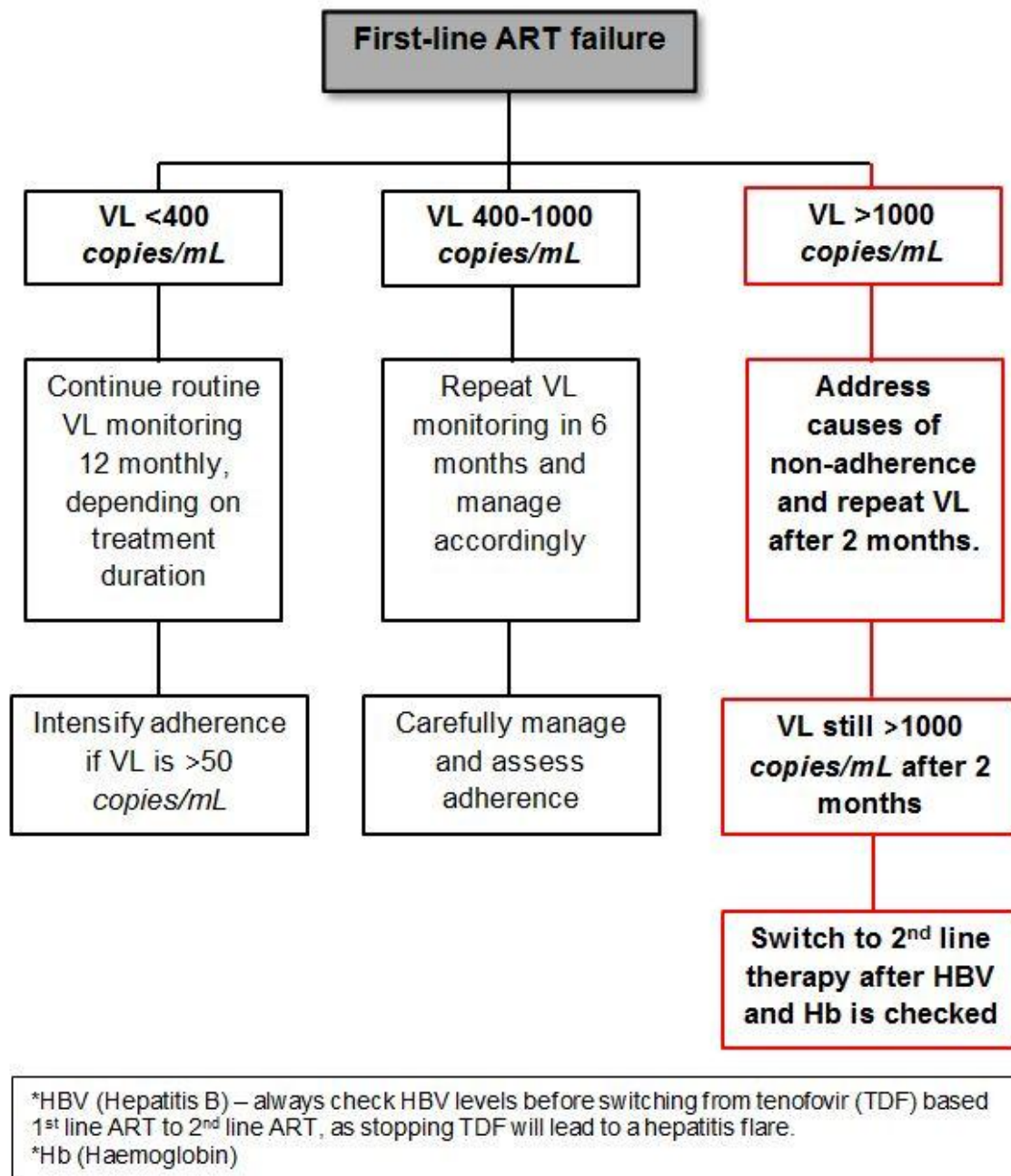


Figure 2-3: First-line ART failure algorithm. Adapted from, the National ART guidelines, 2015

The following guidance (see **Table 2-2**) for switching from first- to second-line ART regimens should be followed as prescribed by the NDoH, 2015

Table 2-2: Recommended second-line ART for patients failing first-line ART

Virological failure on first-line ART:	Recommended second-line ART:
Failing on a TDF-based regimen	AZT + 3TC + LPV/r AZT + TDF + 3TC + LPV/r (if HBV co-infected)
Failing on a d4T- or AZT-based regimen	TDF + 3TC or FTC + LPV/r
Dyslipidaemia, cholesterol >6 mmol/L or LPV/r associated diarrhoea	Switch LPV/r to ATV/r
Anaemia and renal failure	Switch to ABC

Taken from, the National ART guidelines for the prevention and management of HIV in children, adolescents and adults (NDoH, 2015).

Patients failing on second-line ART will be centrally managed by the Adult group of the Peer Review Committee for TLART, within the public sector of South Africa, to enable each to receive individual management and care (NDoH, 2015). Patients can only be switched from second-line ART if they received their respective PI-based regimen for at least one year and after patients received intensified adherence counselling with tolerability and drug-drug interactions checked in advance (Meintjes, 2017; NDoH, 2015).

Resistance to a PI-based second-line ART is unlikely to develop within 12 months as resistance mutations found in South African studies were infrequent for patients failing their respective second-line ART (Van Zyl *et al.*, 2013; Wallis *et al.*, 2010). Second-line ART failure can be attributed to adherence problems on first-line ART, which carried over when a patient was initiated onto second-line ART, in both cases non-adherence leads to resistance development against ART (Fox *et al.*, 2016).

It is only after all the latter protocols have been followed that a patient is eligible for GART to be performed to identify the specific resistance mutations for each ARV drug and determine which ARVs to use in TLART (Meintjes *et al.*, 2014).

The following guidance for the management and assessment of second-line ART failure as prescribed by the NDoH, 2014 is reflected in **Figure 2-4**.

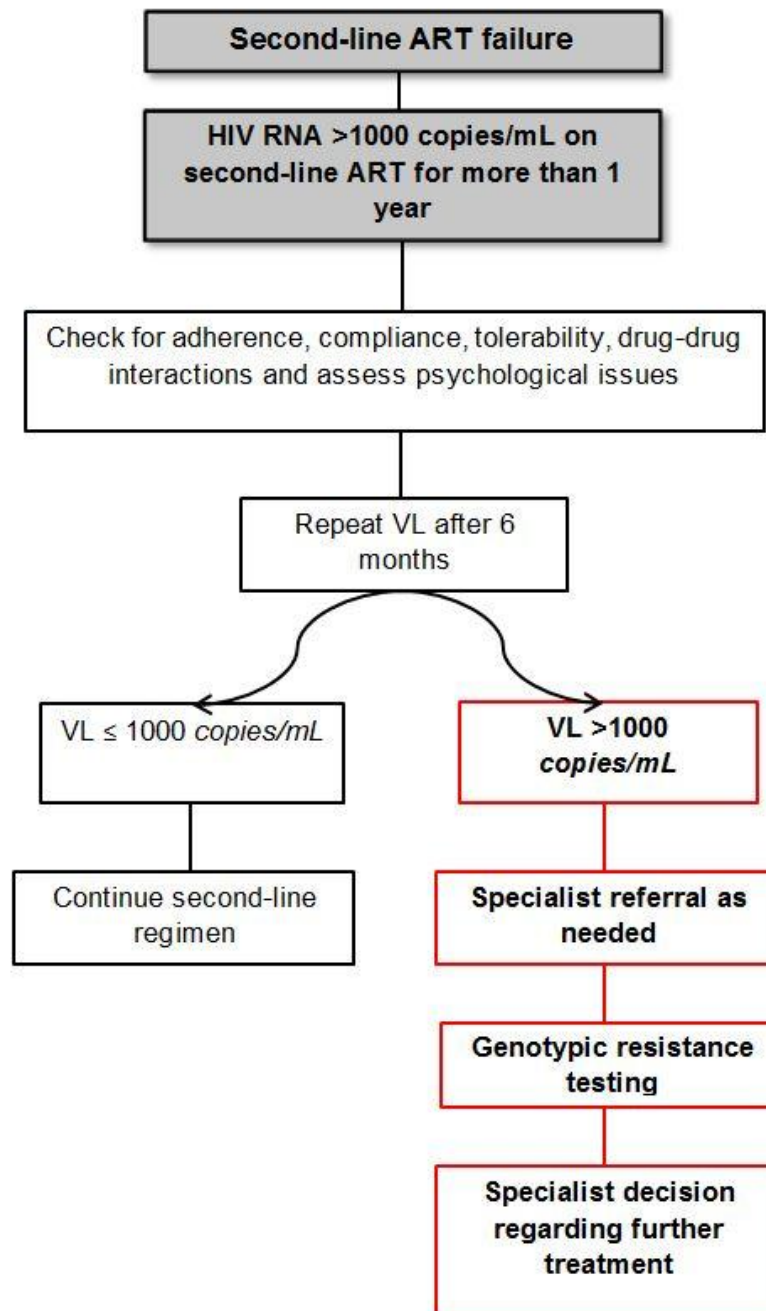


Figure 2-4: Second-line ART failure management algorithm. Adapted from, the National ART guidelines 2015.

2.11 Resistance development

The first resistant mutation variant was discovered and isolated not long after the introduction of AZT in 1987 (Erice *et al.*, 1993), with the first transmission of an AZT-resistant mutation reported in 1992 (Grant *et al.*, 2002; Little *et al.*, 2002; Yerly *et al.*, 1999). In the last two decades of HAART, trends indicate a clear increase in the development of resistant mutations (Clavel & Hance, 2004; Grant *et al.*, 2002; Little *et al.*, 2002).

To understand the development of HIV resistance to ARV drugs, two specific concepts regarding the HIV replication process needs to be understood. The first concept is that HIV can be characterised by a high rate of virus production and turnover. Untreated HIV patients usually have a virus production rate of approximately 10^7 to 10^8 cells/ mL plasma and a CD4 cell turnover rate of 10^{10} cells/day, which is a clear indication of the high-rate of HIV infection that takes place in an untreated patient (Clavel & Hance, 2004; Ho *et al.*, 1995; Perelson *et al.*, 1996; Wei *et al.*, 1995).

The second concept of resistance development is that reverse transcription of viral RNA to DNA is extremely prone to generate base substitution errors in one mutation that can be formed for every 1 000 to 10 000 nucleotides synthesised, implying that 10 mutations may be generated during each replication cycle (Ho *et al.*, 1995; O'Neil *et al.*, 2002; Perelson *et al.*, 1996; Wei *et al.*, 1995). Literature further suggests that after infection a rapid increase in HIV replication can be seen alongside a striking induction of immune response after which the VL will decrease to the so-called set point (Maartens *et al.*, 2014; Stacey *et al.*, 2009)

This high rate of HIV mutation production highlights the highly complex and diverse mixture of viral quasi-species present in patients, only differing by one or more amino acid substitutions. In some cases it is possible for one amino-acid substitution to produce a high level of resistance, with the most common example being mutation M184V creating complete high-level resistance to 3TC (Clavel & Hance, 2004). This highly resistant mutation can emerge within weeks and necessitates the use of multidrug or combination ARV therapy (Coffin, 1995).

Resistance in the context of HAART is only possible if HIV continues to replicate, while ARVs are insufficient and unable to suppress viral reproduction, allowing positive selective pressure to be exerted on variants that have a decreased susceptibility to ARVs. Resistance to HAART is the consequence and not the cause of initial treatment failure (Clavel & Hance, 2004).

2.12 Mechanisms of HIV resistance

Different mechanisms of viral resistance have been identified for different classes of antiretroviral agents. Resistance mechanisms discussed in this section are applicable to the NRTIs, NNRTIs, PIs and cross-resistance between and within these classes respectively.

2.12.1 Nucleoside and nucleotide reverse transcriptase inhibitor resistance

The NRTI analogues block the synthesis of viral DNA from viral RNA by interfering with the reverse transcriptase process needed for replication of HIV. After phosphorylation the analogues formed are incorporated into the emerging chain of viral DNA, but due to the lack of a 3' hydroxyl group on the formed NRTI derivatives no further additional nucleotides can be attached to the viral chain of DNA, stopping the synthesis of viral DNA (Clavel & Hance, 2004).

Nucleoside and nucleotide reverse transcriptase inhibitor drug resistance, known as “impairment of analogue incorporation”, form resistance by preventing reverse transcriptase to incorporate the analogue, formed during phosphorylation, into the viral DNA allowing it to be formed without any obstructive effect from the NRTI (Clavel & Hance, 2004).

Drug combinations, including thymidine analogues, AZT and d4T, result in resistance mutations formed by the removal of the nucleoside analogue from the terminated DNA chain, and can promote resistance to almost all NRTIs, including TDF (Coakley *et al.*, 2000; Larder & Kemp, 1989; Picard *et al.*, 2001; Shafer *et al.*, 1996).

Amino acid substitution, with regard to mutation M184V, includes the replacement of methionine with valine at position 184 of reverse transcriptase. This replacement of a valine, consisting of a different side-chain than methionine, prevents the proper incorporation of lamivudine triphosphate at the catalytic site, creating high-level resistance to lamivudine (Boucher *et al.*, 1993; Sarafianos *et al.*, 1999).

The most clinically significant NRTI resistance mutations globally are M184V, K65R, TAM's and Q151M complex.

Mutation M184V is clinically relevant and is primarily selected by 3TC and FTC, reducing the susceptibility of the latter by >100 fold, and by ABC and ddI, causing low-level resistance. Mutation M184V causes, in contrast, an increase in the susceptibility to AZT, d4T and TDF, slowing down the formation of resistance (Harrigan *et al.*, 2000; Melikian *et al.*, 2012; Miller *et al.*, 2000; Whitcomb *et al.*, 2003).

Mutation K65R is selected by ABC, TDF, d4T and ddI. The susceptibility of TDF, ddI and ABC is reduced two fold and d4T's susceptibility is reduced 1.5 times; it is also suggested that mutation K65R is more likely to emerge in HIV-1C subtypes. Viral populations containing both K65R and M184V provide significant evasion to NRTI activity towards regimens containing ABC, TDF or d4T, thus despite relative low level resistance formed, it is highly clinically relevant (García-Lerma *et al.*, 2003; Hawkins *et al.*, 2009; Wainberg *et al.*, 1999).

Thymidine Analogue Mutations (TAM's) are nonpolymorphic mutations primarily selected by the thymidine analogues, d4T and AZT, and reduce the susceptibility of the latter by facilitating primer unblocking, also known as nucleotide excision. The main mutations classified as TAM's are M41L, D67N, T215Y and L210W (Arion *et al.*, 2000; HIVDB, 2017; Meyer *et al.*, 1998; Meyer *et al.*, 2002).

Mutation M41L and mutation T215Y usually occur in combination and confer high-level resistance to both AZT and d4T and low-level resistance to ABC, TDF and ddI. Mutation D67N usually reduces the susceptibility of AZT and d4T (HIVDB, 2017).

Mutation Q151M complex is known as a multi-nucleoside reverse transcriptase inhibitor mutation and occurs in combination with accessory mutations A62V, V75I, F77L and F116Y, causing intermediate-level resistance to 3TC, FTC and TDF (HIVDB, 2017; Margot *et al.*, 2015; Melikian *et al.*, 2012; Shafer *et al.*, 1994).

2.12.2 Non-nucleoside reverse transcriptase inhibitor resistance

The NNRTIs are small molecules that bind with a strong affinity to a hydrophobic pocket located near the catalytic domain of reverse transcriptase by inhibiting the flexibility of the enzyme to suppress DNA formation (Esnouf *et al.*, 1997).

The most clinically significant NNRTI resistance mutations globally are K103N, G190A, A98G and Y188L.

K103N is a nonpolymorphic mutation selected by NVP and EFV, reducing the susceptibility of the latter fifty-and twenty-fold each (Rhee *et al.*, 2004; Zhang *et al.*, 2007). Mutation A98G is a relatively nonpolymorphic mutation selected by patients receiving EFV and NVP, and which reduces the susceptibility of these drugs two- to threefold (Rhee *et al.*, 2004; Zhang *et al.*, 2007).

Mutation G190A is a nonpolymorphic mutation selected for patients receiving NVP and EFV. If G190A is found alone, it reduces the susceptibility of NVP and EFV by >fiftyfold and tenfold respectively. It does not seem to be selected by ETR or RPV or to reduce their susceptibility in the absence of other NNRTI mutations (Melikian *et al.*, 2013; Rhee *et al.*, 2004; van Westen *et al.*, 2013).

Mutation Y188L is known as a nonpolymorphic two-base pair mutation selected by NVP and EFV, resulting in high-level resistance and causing a decrease in susceptibility of both drugs by >fiftyfold. Mutation Y188L also reduces the susceptibility of RPV fivefold, but has minimal effect on the susceptibility of ETR (Bachelier *et al.*, 2000; Reuman *et al.*, 2010; Rhee *et al.*, 2004).

2.12.3 Protease inhibitor resistance

The main mechanism of action of this class is its ability to end the functionality of HIV protease. The function of HIV protease is to split large polyprotein precursors, releasing the structural proteins and enzymes required for the assembly of infectious viral particles. Without these proteins and enzymes, viral particles are produced but they are not mature or infectious. Protease inhibitors are specifically designed to bind with maximal affinity to the protease and occupy more space in the active binding site than natural substances (Clavel & Hance, 2004).

Resistance to protease inhibitors are formed by amino acid substitutions in the active binding site or at distant binding sites that directly or indirectly alter the point of contact between the protease inhibitors and the protease enzyme, reducing the affinity for the enzyme (Condra *et al.*, 1995; Kaplan *et al.*, 1994; Niu *et al.*, 1996). Cross-resistance in the PI drug class is generally observed and is due to the wide overlap between the combinations of mutations and the similarities in chemical structure of the PIs (Hertogs *et al.*, 2000; Race *et al.*, 1999; Schapiro *et al.*, 1999).

The most clinically significant PI resistance mutations globally are V82A, I84V, M46I and L76V.

V82A is classified as a nonpolymorphic substrate-cleft mutation selected by indinavir (IDV) and LPV, subsequently causing a reduction in susceptibility. This mutation is also associated with cross-resistance to NFV and ATV, causing a decrease in susceptibility to SQV and FPV, when in combination with other PI-resistance mutations (Condra *et al.*, 1996; Rhee *et al.*, 2010; Van Zyl *et al.*, 2013).

Mutation I84V is a nonpolymorphic substrate-cleft mutation and results in a decrease in susceptibility to all the available PIs (Condra *et al.*, 1996; Rhee *et al.*, 2003; Vermeiren *et al.*, 2007). Mutation M46I is a nonpolymorphic mutation primarily selected by NFV, IDV, ATV, FPV and LPV and occurs in about 20% of PI-treatment experienced patients. This mutation also occurs in combination with mutations V32I, I47V, L76V, I84V and L90M (Condra *et al.*, 1996; Garriga *et al.*, 2007; Varghese *et al.*, 2013).

Mutation L76V is selected by IDV, DRV and LPV and decreases the susceptibility of these drugs and of FPV, but increases the susceptibility of ATV, TPV and SQV (Rhee *et al.*, 2010; Vermeiren *et al.*, 2007; Young *et al.*, 2010).

2.12.4 Cross-resistance

Cross-resistance can be defined as resistance to a drug that HIV has never been exposed to, and is the result of mutations that have been selected by the use of other drugs. It therefore takes only a few additional amino acid substitutions to synthesise highly resistant variants (Clavel, 2004).

In a South African study conducted at Tygerberg Academic Hospital (n=490), in patients failing their respective LPV/r-based second-line ART, intermediate or high-level resistance to LPV were reported in 45 patients, intermediate or high-level resistance to ATV in 36 and DRV resistance in 23 patients. This phenomenon is seen as cross-resistance as patients in this study population were ATV and DRV treatment naïve, but still showed reduced susceptibility. The low prevalence of cross-resistance in this study can be attributed to the relative short duration of second-line failure, as virological failure was classified as a repeated VL of >1000 copies/ml and a higher prevalence of cross-resistance can be expected with a longer duration of ART failure (Van Zyl, 2013).

2.13 Resistance in a South African context

Research done in the South African context has shown that resistance patterns follow the same trends as seen globally.

In a cross-sectional study conducted in the public sector of South Africa (n=788) in patients experiencing virological failure to their respective first-line NNRTI-based regimens, most patients failed a TDF based backbone regimen (74.4%) in combination with EFV (82.1%) after 36 months of treatment. The most prevalent NRTI mutations found were: M184V/I (82.7%) and K65R (45.8%). The most prevalent NNRTI mutations were: K103N (48.9%) and V106M (34.9%) (Steegen *et al.*, 2017).

A study conducted in two large public hospitals in Johannesburg on 75 patients in 2009 experiencing virological failure on a PI-based second-line ART regimen, the most prevalent mutations found in the NRTI drug class were M184V (50%), TAM's (M41L, L210W and T215Y) (33.3%) and Q151MM complex (3.3%) mutations. The major NNRTI mutations were K103N (21%) and V106M (12%) and the major PI mutations found in the study population were M46I, L76V, V82A, I54V and L90M, with 89% of patients having one or two minor resistance mutations (Wallis *et al.*, 2010).

A study conducted on 152 patients in the private sector of South Africa between July 2007 and December 2011, on patients initiated onto TLART indicated the most prevalent NRTI mutations were M184V (55.3%), K65R (4%), L74V (7.3%), Q151M (2.6%) and the T69 insertion (0.6%), with the most prevalent major PI mutations being M46I/L, L76V, V82A, D30N, V32I, I47V/A, I50L, I54V and L90M (Meintjes *et al.*, 2015)

These three studies conducted by Steegen *et al.*, 2017, Wallis *et al.*, 2011, and Meintjes *et al.*, 2015 indicated the resistant mutation variants present were similar in all three studies, conducted at three different stages of HIV for the three different ARV drug classes used in the South African context. These studies show the genetic variability of HIV and are seen as important contributions, as the identification of HIV resistant mutation variants have not been done on a large scale in South Africa.

2.14 Genotypic resistance testing

Resistance testing consists of two main assays available, genotypic assays, to detect the presence of mutations and phenotypic assays, to measure the susceptibility of the virus to various ARVs (Clavel & Hance, 2004). In a resource limited setting, such as South Africa, genotypic antiretroviral resistance tests (GART) in the public sector is primarily reserved for patients experiencing virological failure on a second-line PI-based ART regimen and who have not shown viral suppression in response to a PI and after intense adherence counselling has been done (NDoH, 2014).

In South Africa, sequence-based resistance testing can only be performed at five facilities capable of performing these tests, with only Tygerberg Academic Hospital, Cape Town, and the Helen Joseph Hospital, Johannesburg, able to conduct testing on a large scale (Conradie *et al.*, 2012).

Performing a genotypic resistance test requires ethylenediaminetetra-acetic acid (EDTA), prepared whole blood or plasma, and the samples should be from patients currently on the failing regimen, or taken within 4 weeks of discontinuation, to ensure the resistant mutation variant does not become undetectable, as the 'wild-type' HIV is more viable than the resistant mutation and outgrows it (Conradie *et al.*, 2012; Meintjes *et al.*, 2014).

Resistance tests available in South Africa only evaluate reverse transcriptase and protease genes, because as mutations, these genes cause resistance to NRTIs, NNRTIs and PIs (Conradie *et al.*, 2012). In South Africa, resistance testing for the HIV-1 protease and reverse transcriptase genes is performed with polymerase chain reaction (PCR) amplification and sequencing (Manasa *et al.*, 2012).

2.15 Third-line ART cost

Third-line ART in South Africa is considered to be five times more expensive than first-line ART, with prices ranging from R 848.85 to R 1894.16 per month per patient in the public sector in November 2017 (de Villiers, 2017; Meyer-Rath *et al.*, 2017).

An increase in the number of patients in South Africa receiving ART will inevitably result in an enormous financial burden on the economy, as the national budget for 2014/2015 was an estimated USD350 million for the >3 million people receiving ART in the public sector. In mid-2016, the number of patients receiving ART increased to 3.4 million with ~145 000 patients receiving second-line ART and >700 receiving TLART, adding to South Africa's status as the world largest ARV consumer, using more than a quarter of the global ARV production (Venter *et al.*, 2017).

2.16 References

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Chapter 3: Research Methodology

3.1 Introduction

This chapter reports on the research methodology used in this study. Included are the ethical considerations, study design, study population, study sites, justification of study sites, data collection process and statistical analysis.

3.2 Ethical considerations

3.2.1 Approval from respective committees

Approval from the Human Research Ethics Committee (HREC) of the North-West University, Potchefstroom, was obtained for this study on 13 March 2017 (NWU-00340-16-A1). An amendment to exclude Job Shimankana Tabane Provincial Hospital and to include Mahikeng, Bophelong Provincial Hospital was approved by the HREC on 25 May 2017.

Approval from the North West Department of Health, Policy, Planning, Research, Monitoring and Evaluation (PPRME) directorate, Mahikeng, was obtained for this study on 13 January 2017. This approval granted access to all public healthcare records (healthcare files and electronic databases) for the specific study participants.

Permission to conduct the research study in the Dr Kenneth Kaunda district was obtained from Dr C van Deventer, Chairperson of the Dr Kenneth Kaunda Research Committee, on 31 January 2017.

Goodwill permission from the CEO of the Joe Morolong Memorial Hospital, Dr Z.A. Esterhuizen, was obtained on 23 January 2017 to continue with the research study in their facility.

Goodwill permission from the Clinical Manager of the Potchefstroom Hospital, Dr M. Shakung, was obtained on 16 February 2017 to continue with the research study in their facility.

Goodwill permission from the Clinical Manager of the Klerksdorp/Tshepong Hospital Complex, Dr N.D. Leburu, was obtained on 24 February 2017 to continue with the research study in their facility.

Goodwill permission from the Senior Clinical Manager of Mahikeng/Bophelong Hospital Complex, Dr T.N.C. April, was obtained on 4 May 2017 to continue with the research study in their facility.

All relevant approvals and goodwill permission letters are reflected under Addendum E

3.2.2 Confidentiality and anonymity

As a retrospective descriptive investigation, this study did not require any informed consent as the aim was not to recruit any patients or have direct contact with them. The NWU-HREC, therefore, approved a waiver of personal consent for this investigation in terms of section 3.2.2 (page 27) of the Ethics in Health Research 2nd edition (NDoH, 2015). Patients from whom data were captured will not receive any feedback on conclusions made from statistical analysis, but feedback will be provided to the NWU-HREC (annual monitoring report), NWDoH and the four main referral hospitals. Non-disclosure agreements were signed by all researchers and collaborators involved in this study, with none of the latter having any personal relations with any of the patients involved in the study.

The integrity of the data was protected by keeping hard copies of collected data in locked cabinets and electronic data on a password protected computer and internet cloud. Personal patient information, such as names, addresses, contacts details, etc., have not been captured onto the case report forms ensuring that the anonymity of patients is kept by eliminating personal identifiers linked to an individual.

Only a patient's date of birth and gender were incorporated into a patient-specific code used for transferring data from hard to electronic copies, assuring the integrity of transferred data was maintained. After double-checking and 20% re-entering of data the patient-specific code was blacked-out with a permanent marker, to ensure no patient could be traced in any way.

3.3 Study design

This study was designed as a retrospective descriptive investigation that reported on clinical variables and demographic details from patient health records. These clinical characteristics and demographic details were used to determine the progression from HIV diagnoses to the initiation of third-line antiretroviral therapy (TLART) in HIV-infected individuals in the North West Province of South Africa.

This research study was based on all patients already approved, by the Adult group of the Peer Review Committee for Third-Line ART, to receive TLART in the three selected districts of the North West Province as of December 2014 (see **3.5**).

3.4 Study population

An all-inclusive sample of all available adult patients on TLART in the North West Province was included in this study therefore no sample size calculation was performed. The number of patients on TLART was small and limited, which was due to the National Antiretroviral Treatment Guidelines only incorporating TLART in the March 2013 guidelines and only becoming readily available in the public sector with the December 2014 guidelines because of modality problems.

According to the National Antiretroviral Treatment Guidelines of 2014, TLART can only be initiated by the Adult group of the Peer Review Committee for Third-line ART, centrally managed by the NDoH (NDoH, 2014). After a patient has experienced virological failure on an NRTI and NNRTI first-line ART regimen, and has documented resistance to a PI-based second-line ART regimen, they could be considered for TLART and a medical doctor should then apply for TLART at the Peer Review Committee.

3.4.1 Inclusion criteria

All adult patients already approved and initiated on TLART by the Adult group of the Peer Review Committee for Third-line ART in three districts of the North West Province, were included in this research study from 2004 until the date of data collection at the respective facilities in 2017. The Adult group of the Peer Review Committee for Third-line ART had to approve the patients for TLART and allocate them a TLART number before they could be selected and included as participants in the study population.

3.4.2 Exclusion criteria

The exclusion criteria for this research were:

- All HIV positive children initiated onto TLART by the Paediatric group of the Peer Review Committee for Third-Line ART.
- Patients not yet approved by the Adult group of the Peer Review Committee for Third-line ART even though a medical doctor had already applied for TLART.
- If medical records were not available for the approved TLART patient.

3.5 Study sites

South Africa consists of nine provinces with the North West Province being the sixth largest. The North West Province consists of four districts, namely Dr Kenneth Kaunda (DKK), Dr Ruth S. Mompati, Ngaka Modiri Molema and the Bojanala district. This study was conducted in three of the four districts. The Job Shimankana Hospital in the Bojanala district, also being a main referral hospital, was not included in this research study as it became apparent, after communication with the hospital management, of which no patients were approved for TLART during this study's duration timeline and a protocol amendment were made and approved by NWU HREC.

The study was conducted in the following main referral hospitals that provided TLART:

- Potchefstroom Hospital - Tlokwe/Ventersdorp* local municipality, part of DKK.
- Klerksdorp-Tshepong Hospital Complex - City of Matlosana local municipality, part of DKK.
- Mahikeng/Bophelong Hospital Complex - Mahikeng local municipality, part of Ngaka Modiri Molema.
- Joe Morolong Memorial Hospital (Vryburg Public Hospital) - Naledi (Vryburg) local municipality, part of Dr Ruth S. Mompati.

*Tlokwe/Ventersdorp local municipality is in a name changing process but at the time of publishing, it was still known as the Tlokwe/Ventersdorp local municipality.

3.6 Justification with regards to chosen study sites:

The above-mentioned study sites were included in this study to enable the researcher to investigate all the patients on TLART in the North West Province. The following were contributing factors:

- This study would empower the provincial healthcare community with better knowledge and understanding of HIV management of TLART patients within the North West Province.
- The researchers involved in this study have long-standing relationships with the relevant healthcare providers in the main referral hospitals in the North West Province. These healthcare providers provided valuable knowledge regarding the healthcare system in their respective healthcare facilities.
- Third-line ARVs are centrally dispensed and managed at the main referral hospitals within a province.

3.7 Data collection process

3.7.1 Retrospective data collection

Appointments were made beforehand with specific role players (doctors, clinical managers and pharmacy managers) in the respective facilities to make arrangements and discuss logistics regarding the data collection process. Data collection for this study was of a retrospective nature, with access only to patient healthcare files and electronic records.

Upon arrival at a main referral hospital, the names and dates of birth of each TLART approved patient were accessed from either the pharmacy or clinical manager responsible for these patients using the hospital computer system Rx Solutions®. Using this information, files could be retrieved from the hospital's archives to capture the retrospective data onto the case report form specifically developed by the main researcher as a data collection tool for this study (**see** Addendum D).

The following variables were recorded:

- Main referral hospital
- Gender
- Age
- Height
- Weight
- Date of HIV diagnosis
- Date of first-line ART initiation
- Date of first-line ART failure
- Date of second-line ART failure
- Date of third-line ART initiation
- Full ART history
- Drug switches within respective regimens
- Reasons for switches within respective regimens
- Antiretroviral drug susceptibility, by means of phenotypic resistance testing
- Resistance mutations, at TLART, by means of genotypic resistance testing
- CD4 counts
- Viral loads
- Laboratory report of clinically relevant markers (chemical pathology, haematology and virology)
- Hospitalisation and other opportunistic infections
- TB co-infection
- Other side-effects

All variables were captured as per the National Antiretroviral Treatment Guidelines of 2014 of South Africa. Intervals used for VL and CD4 variables were 0, 3, 6, and 12 months, or as close to the respective interval. As these patients were non-compliant most of the time, measurements at set intervals in practice, as stipulated in the guidelines, are near impossible and all values were taken on the date documented in a patient's health records.

3.7.2 Data collection tool: Case report form

A study-specific case report form (CRF), developed by the main researcher for data to be captured and transferred to a Microsoft Office Excel® spreadsheet, were used to ensure hard and electronic copies of the recorded data could only be accessed by the researchers (**see** Addendum D).

Only a patient's date of birth and gender were recorded to ensure anonymity throughout the data collection process; no other personal information that could identify a patient was recorded. The date of birth and gender alongside a number and the hospital's initials were then incorporated into a patient-specific code to enable the researchers to transfer the captured data accurately from the case report form to the Microsoft Office Excel® spreadsheet. After double-checking and 20% re-entering of data, the code was blacked-out on the hard copies.

3.8 Statistical analysis

Descriptive statistics were calculated by means of IBM Statistical Package for the Social Sciences (SPSS®) version 24 with the assistance of a statistician at the School for Computer, Statistical and Mathematical Sciences, Faculty of Natural Sciences, North-West University, Potchefstroom.

Median (interquartile range, IQR: 25th to 75th) was used for variables without a normal distribution and percentages (frequencies) were used for categorical variables. The Stanford University HIV Drug Resistance Database was used to interpret the clinical significance of drug-resistant mutations identified in the study population.

3.9 References

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Chapter 4: Manuscript A

In this chapter, a manuscript titled:

“A retrospective descriptive investigation of adult patients receiving third-line antiretroviral therapy in the North West Province”

is presented. The manuscript will be submitted to AIDS Research and Therapy as a full length research paper and was prepared according to the specific Instructions to the Author included in this dissertation as Addendum B.

Title page

A retrospective descriptive investigation of adult patients receiving third-line antiretroviral therapy in the North West Province

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Abstract

Background: South Africa has the largest human immunodeficiency virus (HIV) epidemic in the world, with the number of people living with HIV increasing from 4.02 million in 2002 to 7.06 million during middle 2017. Patients experiencing virological failure will also increase due to greater access and prolonged exposure to antiretroviral therapy (ART); this may further increase the need for third-line ART (TLART) in a resource-limited setting such as South Africa. The aim of this study was to describe clinical characteristics and resistance patterns of all adult patients who were initiated on TLART in three districts of the North West province.

Methods: The study was an all-inclusive retrospective descriptive investigation. Demographical and clinical variables were recorded from patient health records from 2004 to 2017 and analysed with descriptive statistics.

Results: 21 Patients were included in this cohort, 17 females and four males, with a median age of 46 years (IQR: 40–49) when initiated on TLART and 36 years (IQR: 31–41) when diagnosed HIV positive. The median duration from HIV diagnosis to first-line initiation was 101 days (IQR: 37–363), on first-line ART 1,269 days (IQR: 732–2,455), on second-line 1,512 days (IQR: 706–2,096) and between second-line failure and third-line ART, initiation was 71 days (IQR: 58–126). High-level resistance most prevalent (n=21) for PI's were nelfinavir/r (85.70%), indinavir/r (81.0%), lopinavir/r (76.20%), fosamprenavir/r (66.70%) and atazanavir/r (57.10%); NRTI's: emtricitabine (95.20%), lamivudine (95.20%) and zidovudine (52.40%) and NNRTI's: nevirapine (76.20%) and efavirenz (71.40%). Resistance mutations most prevalent for PI's (n=20) were M46I (75%), V82A and I54V (65%), NRTI's (n=18) were M184V (89.47%), D67N (31.58%), M41L (26.31%), and NNRTI's (n=17) were K103N (47.01%) and G190A (23.52%).

Conclusions: The results provided a description of the characteristics and resistance patterns of the limited number of patients on TLART in the North West Province. Resistance data reported for cross-resistance on PI`s not previously used for ART was alarming. Better opportunities for larger studies to investigate and establish a typical clinical profile of a TLART patient, to improve and optimise HIV management and care in the future, would become available as more patients are initiated on TLART.

Keywords

HIV, Human immunodeficiency virus, third-line antiretroviral therapy, drug resistance patterns, salvage therapy

Background

Human immunodeficiency virus (HIV) infection remains a global pandemic, with an estimated 36.7 million people diagnosed HIV positive globally at the end of 2016 [1, 2]. UNAIDS set out the 90–90–90 targets, where 90% of people living with HIV should know their HIV status, 90% of the people diagnosed with HIV should receive antiretroviral therapy (ART) and 90% of those receiving ART should be virally suppressed, these goals should be achieved by the end of 2020 [3].

The number of people globally having access to ART significantly increased in one year from 17.1 million to 19.5 million at the end of 2016 [2]. South Africa, however, has the biggest epidemic in the world [3] with an estimated 7.06 million HIV positive people in the middle of 2017, which represents 12.6% of the total population and 18.0% of its adult population aged between 15 and 49 years [4, 5].

The South African National Department of Health (NDoH) implemented the first ART guidelines in 2004 through the Strategic Plan for HIV and AIDS and the operational plan for Comprehensive HIV and AIDS Management, Care and Treatment for South Africa [6] as part of its public healthcare system and ART roll-out programme. This resulted in South Africa managing the largest ART programme in the world for a number of years, with 3.4 million people remaining on ART at the end of 2016 [7-9].

In 2013, the WHO emphasised that policies should be developed by national programmes for patients receiving third-line ART (TLART), as increased antiretroviral (ARV) exposure and longer duration of ART would inevitably lead to more and more patients failing their first- and second-line ART due to drug resistance, increasing the need for more effective regimens for treatment-experienced patients [10-12].

The NDoH of South Africa implemented TLART regimens for patients failing their respective protease inhibitor (PI) -based second-line regimens and not achieving viral suppression for more than one year [13]. These TLART regimens in South Africa are about five times as expensive as first-line ART [14, 15]. It is therefore of utmost importance to limit the number of patients on TLART and optimise patient management through policies, especially in a resource-limited setting.

Methods

Aim

The aim of this investigation was to describe and report on the clinical characteristics and HIV resistance patterns of all available adult patients who were initiated on TLART in three districts of the North West Province in South Africa.

Design

This study was an all-inclusive retrospective descriptive investigation of all TLART approved adult patients.

Population

This investigation was conducted in four main referral hospitals in three districts (Dr Kenneth Kaunda (DKK), Dr Ruth S. Mompoti and Ngaka Modiri Molema) of the North West Province in South Africa. Inclusion criteria for this study were all adult patients (≥ 18 years) approved and already on TLART between December 2014 and 1 June 2017.

Patients had to experience virological failure and be on their respective PI-based second-line ART regimens for at least 12 months, with confirmed PI-resistance by means of a genotypic antiretroviral resistance test (GART) performed by the National Health Laboratory Service of South Africa, before TLART could be initiated.

Data

The following demographical data were captured: date of birth and gender.

Clinical markers (CD4 and viral loads) for disease progression were captured at baseline and at first- and second-line ART failure.

Time duration (in days) between HIV diagnosis and ARV initiation, duration on first- and second-line ART regimens and time spent between second-line failure and TLART-initiation were also recorded.

GART results, when patients experienced second-line ART failure, were also collected with the level of resistance for PI's, NRTI's and NNRTI's captured as susceptible, potential low-level, intermediate and high-level resistance.

Data collection

The data collection took place from 15 March to 31 May 2017. Data were anonymously and retrospectively captured onto a case report form, from TLART approved patient health records. These health records were accessed by the responsible pharmacist at the respective referral hospitals and data were captured onto a case report form by the researcher.

Statistical analysis

Descriptive statistics were calculated by means of IBM Statistical Package for the Social Sciences (SPSS®), version 24, with the assistance of a statistician. Median (interquartile range, IQR: 25th to 75th) was used for variables without a normal distribution and percentages (frequencies) were used for categorical variables. The Stanford University HIV Drug Resistance Database was used to interpret the clinical significance of drug resistant mutations identified.

Results

Twenty-four patients (adults and children) were approved for TLART at the end of February 2017; only 21 adult patients met the set inclusion criteria, with three paediatric patients being excluded. The median age at HIV diagnosis for the study population was 34 years (IQR: 30.25–36.75) and 46 years (IQR: 40–48.50) at TLART initiation. The 21 patients consisted of 17 females with a median age of 46 years (IQR: 40–49) and four males with a median age of 44 years (IQR: 41.50–46.50) at TLART initiation. (**Table 4-1**)

For this study population, the median baseline CD4 count was 68.50 cells/μL (IQR: 40.75–127.75), at first-line ART failure the median CD4 count was 79 cells/μL (IQR: 26.50–124) and at second-line ART failure the median CD4 count was 119 cells/μL (IQR: 61.25–201.25). (**Table 4-1**)

The median baseline viral load (VL) was 98,000 copies/ml (IQR: 45,569–820,000), the median VL at first-line ART failure was 100,000 copies/ml (IQR: 42,085–248,852) and the median VL at second-line ART was 73,196 copies/ml (IQR: 26,210–197,007). (**Table 4-1**)

Patients spent a median time of 101 days (IQR: 36.50–366.75) between HIV diagnosis and ART initiation, 1,269 days (IQR: 765–2,343) on first-line ART, 1,512 days (IQR: 706–2,096) on second-line ART and after failing their second-line ART regimen, patients waited a median additional 71 days (IQR: 57.50–126) before the approval and initiation of TLART. (**Table 4-1**)

The first-line ART regimens were recorded for 17 patients. Lamivudine (3TC), stavudine (d4T) and efavirenz (EFV) were the regimen most often initiated (n=11) followed by 3TC, tenofovir (TDF) and EFV (n=4), one patient was initiated on 3TC, zidovudine (AZT) and EFV and another on emtricitabine (FTC), TDF and EFV. One patient had missing data and three patients were initiated on ritonavir-boosted lopinavir (LPV/r) reserved for second-line ART, after failure of first-line ART in adults in South Africa [6, 13, 16]. (**Table 4-1**)

The PI-based second-line ART regimens were recorded for 19 patients. A 3TC, AZT and LPV/r based second-line regimen was used in seven patients, a 3TC, TDF and LPV/r based second-line regimen in five patients and a AZT, didanosine (ddI) and LPV/r based second-line regimen in three patients. Other second-line regimens were 3TC, abacavir (ABC) and LPV/r (n=2), 3TC, d4T, AZT and LPV/r (n=1) and lastly EFV and LPV/r (n=1). Two patients had missing data. (**Table 4-1**)

ARV drugs respectively included in different combinations for TLART were NRTI`s: FTC, 3TC, TDF, AZT and d4T; NNRTI: etravirine (ETR); PI: darunavir/r (DRV/r); integrase strand transfer inhibitor`s (INSTI`s): dolutegravir (DTG); raltegravir (RAL) (**Table 4-1**).

Table 4-1: Demographic and clinical characteristics of patients initiated onto TLART

Characteristic		Median (IQR)
Demographics		
Female gender		17
Male gender		4
Age at diagnoses (years)		34 (30.25–36.75)
Age at TLART initiation (years)		46 (40–48.50)
Clinical		
CD4 at baseline (cells/ μ L)		68.50 (40.75–127.75)
CD4 at first-line failure (cells/ μ L) ^a		79 (26.50–124)
CD4 at second-line failure (cells/ μ L)		119 (61.25–201.25)
VL at baseline (copies/ml)		98,000 (45,569–820,000)
VL at first-line failure (copies/ml) ^a		100,000 (42,085–248,852)
VL at second-line failure (copies/ml)		73,196 (26,210–197,007)
Durations (days)		
Durations between HIV diagnoses and ART initiation ^b		101 (36.50–366.75)
Duration on first-line ART ^c		1,269 (765–2,343)
Duration on second-line ART ^d		1,512 (706–2,096)
Duration between second-line failure and TLART initiation ^d		71 (57.50–126)
ARV regimens		
First-line regimens ^c		
	(n=11)	3TC/d4T/EFV
	(n=4)	3TC/TDF/EFV
	(n=1)	3TC/AZT/EFV
	(n=1)	FTC/TDF/EFV
Second-line regimens ^d		
	(n=7)	3TC/AZT/LPV/r
	(n=5)	3TC/TDF/LPV/r
	(n=3)	AZT/ddI/LPV/r
	(n=2)	3TC/ABC/LPV/r
	(n=1)	3TC/d4T/AZT/LPV/r
	(n=1)	EFV/LPV/r
ARV's used in TLART		
NRTI:		FTC, 3TC, TDF, AZT, d4T
NNRTI:		ETR
PI:		DRV/r
INSTI:		DTG, RAL

21 patients were approved for TLART and included into study population.

Numbers represent: Median (IQR: 25th – 75th) unless otherwise indicated

CD4 count minimum during 2004-2017 ART history = 3 cells/ μ L, maximum = 590 cells/ μ L.

^a Three patients not initiated onto first-line ART regimens (n=18)

^b Three patients had missing data (n=18)

^c Three patients not initiated onto first-line ART and one patients had missing data (n=17)

^d Two patients had missing data (n=19)

Major mutations found within the PI drug class with the highest prevalence (n= 20) were M46I (n=15; 75%), V82A (n=13; 65%) and I54V (n=13; 65%). Minor PI mutations found within this drug class (n=18) were L10F (n=9; 50%), L33F and T74S (n=5; 27.7%). **(Table 4-4)** Drug resistance mutations in the NRTI drug class with the highest prevalence (n=19) were M184V (n=17; 89.47%), D67N (n=6; 31.58%), M41L (n=5; 26.31%) and T215Y and V118I (n=4; 21.05%). (Table 4-3) Mutations found within the NNRTI drug class with the highest prevalence (n=17) were K103N (n=8; 47.01%) and G190A (n=4; 23.52%). **(Table 4-2)**

Protease inhibitors (n=21) presenting with high-level resistance were nelfinavir/r (NFV) (n=18; 85.71%), indinavir/r (IDV/r) (n=17; 80.95%), LPV/r (n=16; 76.19%), fosamprenavir/r (FPV/r) (n=14; 66.66%) and atazanavir/r (ATV/r) (n=12; 57.14%). None of the TLART patients had a high-level resistance to DRV/r. The following NRTI`s (n=21) presented with high-level resistance: 3TC (n=20; 95.24%), FTC (n=20; 95.24%), ABC, AZT, d4T and ddI (n=9; 42.86% each) and TDF (n=6; 28.57%). The following NNRTI`s (n=21) presented with high-level resistance: nevirapine (NVP) (n=16; 76.19%) and EFV (n=15; 71.43%). Details on NRTI, NNRTI and PI mutations, with the level of resistance to each ARV in each pharmacological class, are presented in Tables 4-2, 4-3 and 4-4.

Table 4-2: Drug resistance for NRTIs

Resistance level	Low-level %	Intermediate-level%	High-level %	Susceptible %
Lamivudine	4.76	0	95.24	0
Abacavir	47.62	9.52	42.86	0
Zidovudine	4.76	0	42.86	52.38
Stavudine	9.52	0	42.86	47.62
Didanosine	47.62	9.52	42.86	0
Emtricitabine	4.76	0	95.24	0
Tenofovir	9.52	14.59	28.57	47.62

Patients with NRTI mutations (n=19)

Major mutations for NRTI`s: M184V (**89.47%**), D67N (**31.58%**), M41L (**26.31%**), T215Y, V118I (**21.05%**), L210W, K219R (**15.8%**), V75I, T215F, Q151M, L74V, L74L, L74I, K70R, K70N, K65R, F77L, F116Y, D67D, A62V (**10.53%**), Y115F, V75M, T69T, T69S, T69D, M184M, K70E, K219Q, K219E, F77F, D67G (**5.3% each**)

Table 4-3: Drug resistance for NNRTIs

Resistance level	Low-level %	Intermediate-level %	High-level %	Susceptible %
Efavirenz	4.76	14.29	71.43	9.52
Etravirine	57.14	14.29	4.76	23.81
Nevirapine	4.76	9.52	76.19	9.52
Rilpivirine	38.10	19.05	19.05	23.81

Patients with NNRTI mutations (n=17)

Major mutations for NRTI's: K103N (**47.01%**), G190A (**23.52%**), E138A, A98G (**17.65%**), Y188L, V108I, P225H, K238N, K103R, K103K, K101S, H221Y (**11.80%**), Y181C, V318F, V179V, V179I, V179D, V108V, V106M, V106I, N348N, N348I, K238S, K103S, K101R, K101P, K101H, K101E, H221H, F227L, E138G, E138E (**5.90% each**)

Table 4-4: Drug resistance for major and minor PIs

Resistance level	Low-level %	Intermediate-level %	High-level %	Susceptible %
Atazanavir/r	14.29	28.57	57.14	0
Darunavir/r	19.05	33.33	0	47.62
Fosamprenavir/r	19.05	14.29	66.66	0
Indinavir/r	4.76	14.29	80.95	0
Lopinavir/r	14.29	9.52	76.19	0
Nelfinavir/r	0	14.29	85.71	0
Saquinavir/r	19.05	57.14	19.05	4.76
Tipranavir/r	33.33	38.10	4.76	23.81

Patients with major PI mutations (n=20), minor PI mutations (n=18)

Major PI mutations: M46I (**75%**), V82A, I54V (**65%**), L76V (**40%**), I84V (**20%**), V82V, V82S, V82C, V32I, M46M, M46L, L90M, L76L, I50V, I50L, I47A (**5%**)

Minor PI mutations: L10F (**50%**), T74S, L33F (**27.7%**), L24I, A71V, A71T (**17%**), L10I, K20T (**11%**), L89V, L89M, L89I, L10V, L10L, K43T, K20I, F53L, F53F, A71I, A71A (**5.55% each**)

The total median number of PI resistance (major and minor) found per patient was three mutations (IQR: 2–3). The median number (n=20) of major PI resistant mutations presented per patient was three (IQR: 3–4) and the median number (n=18) of minor PI resistant mutations presented per patient was two (IQR: 2–3). The median number of NRTI resistant mutations presented per patient was found to be three (IQR: 2–7) and the number of NNRTI resistant mutations presented per patient was three (IQR: 2–4).

Discussion

The number of patients on TLART will increase as more and more patients develop resistance to second-line ART therapy [12, 17, 18]. The financial burden the five times more expensive TLART [19, 20] may place on a resource-limited country is enormous and any intervention that can prevent this is of value.

This was the first study, to our knowledge, that attempted to describe the characteristics and identify possible trends in the clinical profile of TLART patients in the North West Province, taking into account certain clinical and demographical variables and resistance data. The majority (81%) of this study population were female, which also reflects the difference in prevalence of HIV infection amongst males and females in South Africa (4.10 million females compared to 2.93 million males) [17], that woman have on average a 60% higher risk of HIV infection [21] and can further be partially attributed to the female population accounting for 51.10% of the total South African population [4].

Patients in our study population had a median age of 36 years (IQR: 30.25–36.75) at HIV diagnosis, that were similar to other much larger studies conducted in 2011 in Soweto, South Africa, and a study in eight cities of the United States of America (USA) during 2000-2009, with a median age of 34 years (IQR: 28–41) and 38 years (IQR: (30–46) respectively at HIV diagnosis [22, 23]. Patients in the INSIGHT START group (n=4685), conducted in 35 countries, had a median age of 36 years (IQR: 29–44) at diagnosis [20]. Patients in this age range are in their reproductive age (15–49 years) and are more prone to be sexually active and thus at greater risk of acquiring HIV infection [4, 22].

The median age of TLART initiated patients in our study population was 46 years (IQR: 40–49), indicating a median time difference between HIV diagnoses and TLART initiation of at least 7.5 years on ART before they were initiated onto TLART.

Published data on demographics of patients on TLART are limited, but a South African study reported a median age of 44 years (IQR: 39.8–48.0) when patients were initiated onto TLART and the median duration of ART was 8.9 years (IQR: 6.9–10.4) since HIV diagnoses [23]. It is also worth mentioning that the aforementioned study was conducted in the private sector, with patients enrolled in the Aid for AIDS programme with salvage therapy being readily available from July 2007, contrary to the public sector enrolling TLART only from March 2013 [24].

A median CD4 count at baseline of 69 cells/ μ L (IQR: 41–128) was found for patients in this cohort. This was much lower than that found in studies conducted in South Africa between March and October 2011 (n=4,793 patients), in the USA between 2000 and 2009 (n=1,223 patients) and in China between January 2006 and December 2012 (n=388,496 patients); median CD4 counts at baseline in the latter studies were found to be 364 (IQR: 238–542), 299 (IQR: 100–498) and 294 (IQR: 130–454) respectively [18, 19, 22].

Results from our study population were similar to prior studies in KwaZulu-Natal, South Africa, conducted between 2011 and 2014 (n=223) with a median baseline CD4 count of 94 cells/ μ L (IQR: 2–384) [25] and another South African study [26] conducted at five study sites, from January 2000 to July 2008 (n=19,645), with a median baseline CD4 count of 96 cells/ μ L (IQR: 40–159). This finding can partially be attributed to the 101 days (IQR: 37–363) spent before ART was initiated due to the National ART guideline recommendations at that time.

It is important to note that baseline data for this descriptive investigation was recorded from as early as 2004, when it was standard care for HIV positive patients to be first initiated onto co-trimoxazole prophylaxis treatment until they were eligible for ART when their CD4 count was <200 cells/ μ L (2004–2010 guidelines), <350 cells/ μ L (2013 guidelines) and <500 cells/ μ L (2014–2015), as recommended by the National ART guidelines of that time [6, 13, 16, 24, 27].

Two randomised controlled trials, START (strategic timing of antiretroviral therapy, 2009) and TEMPRANO ANRS 12136 (early antiretroviral treatment and early isoniazid prophylaxis against tuberculosis in HIV-infected adults, March 2008 to January 2015), addressed the optimal time to initiate ART in HIV-infected individuals and both studies were consistent; significant clinical benefit can be seen from initiating ART immediately in patients with a CD4 count > 500 cells/ μ L, rather than waiting for a patient's CD4 count to reach a minimum threshold [20, 28-30].

Further studies illustrated that in order to improve the time lost in follow-up appointments missed by newly initiated ART patients, patients can safely be initiated on ART immediately after HIV diagnosis, irrespective of their CD4 count [31-33]. This strategy has since been implemented in the latest South African guidelines as the “test and treat” strategy [33, 34].

A South African study conducted in nine HIV clinics between 2004 and 2014 identified age, gender, a constant low CD4 count during ART and appointments missed during the first six months (non-adherence) as the strongest predictors of ART failure [35]. In this study, the median CD4 count in our study population was very low at 79 cells/ μ L (IQR: 28–122) during first-line ART failure and at second-line ART failure 119 cells/ μ L (IQR: 61–201), indicating a constant low CD4 count for the total median duration on ART.

The lowest CD4 count reported in this study population over the total duration of ART (HIV diagnosis to TLART-initiation) was 3 cells/ μ L, although non-adherence could not be reliably evaluated with the data retrospectively captured from the patient health records, therefore it can be concluded that a sustained low CD4 count could also be associated with ART failure.

Taking into account the latter CD4 results and conclusions made, it is important to note the median baseline VL of 98,000 copies/ml (IQR: 45,569–820,000) was much higher compared to a South African cohort of 4,793 patients between March and October 2011 at 13,000 copies/ml (IQR: 2,050–98,171). Their CD4 count at baseline was also much higher compared to this study population [36].

The median VL in this study population at second-line ART failure was 73,196 copies/ml (26,210–197,007) and is similar to a study by Meintjes et al. in 152 private sector TLART patients, during July 2007 and December 2011, with a median VL at second-line ART failure of 82,831 (IQR: 20,060–233,778) [23].

Due to the inclusion criteria of this study, a patient had to have documented resistance to a PI-based second-line ART regimen for more than 12 months before they could be initiated onto TLART. It could be expected that 100% of the patients experienced high-level resistance to ≥ 1 PI, and 95.23% had ≥ 1 major PI mutation at the time GART was performed.

The accumulation of PI mutations is closely linked to the time spent on a failing second-line ART regimen. Condra (1998) reported that 24 months of failure on second-line ART therapy resulted in a median of 5 (IQR: 0–6) PI mutations (major and minor) [35]. Another author, from a Nigerian cohort, estimated that patients developed 0.6 PI mutations for every six months they were maintained on a failing second-line regimen [10]. It is important to remember that PI resistance requires consecutive accumulation of mutations with on-going exposure to a non-suppressive PI-based ART regimen [34].

In this study, the total median duration on second-line ART was 1512 days (IQR: 706–2096). According to South Africa's National ART guidelines, a patient experiencing virological failure on a PI-based second-line ART regimen should be checked for tolerability and drug-drug interactions, and receive intensified adherence counselling before the VL can be repeated after 180 days [13]. Taking into account the median additional time prior to TLART initiation (71 days (IQR: 57.5-126)) and adding the above mentioned 180 days could have resulted in 251 days (8.96 months) spent on a failing second-line ART regimen before TLART was initiated.

The total median number of PI resistance (major and minor) found per patient in this study population was three mutations (IQR: 2–3) at the time of GART. The trend of 24 months, resulting in five PI mutations [35], can be seen in our results and thus 8.4 months resulted in approximately 1.87 PI mutations during this additional time.

The importance of shortening the period between identifying second-line ART failure and initiating TLART is vital to minimise the number of PI mutations formed.

The three major PI mutations found in this study population were M46I (75%), V82A and I54V (65%). M46I is a nonpolymorphic mutation selected for IDV, NFV, FPV, ATV and LPV and usually has a prevalence of 20% in treatment-experienced patients. M46I furthermore presents alone or in combination with mutations V32I, L76V, I84V and L90M [37], which can be seen in the data, with M46I occurring 15 times (n=20) and the latter mutations also being present once each in the study population. We also found a high prevalence of V82A and I54V mutations (65%) that do not usually present in combination with M46I, usually occurring in combination with M46V, not present in this study population [36].

Mutation V82A is a nonpolymorphic substrate-cleft mutation, largely selected by IDV and LPV and reduces the susceptibility of these PI`s, but is furthermore linked with cross-resistance to ATV and NFV. Lastly, mutation I54V is also a nonpolymorphic mutation, largely selected by IDV and LPV and decreases the susceptibility of all the PI`s, except for DRV/r [36]. This is also partially the reason why 100% of the patients in this cohort were initiated on DRV as part of their TLART, as this cohort had a 0% high-level resistance, 19% low-level resistance, 33% intermediate resistance and 48% susceptibility to DRV.

In this study, PI`s with the highest level of resistance were NFV (86%), IDV (81%), LPV (76%), FPV (67%) and ATV (57%) even though these patients were only treatment-experienced on LPV/r. PI`s share similar chemical structures, therefore cross-resistance is often observed and clarifies the observation of patients experiencing high-level resistance to PI`s they were never previously initiated on [37].

Considering the latter, we acknowledge that all the mutations reflected in Tables 2, 3 and 4 synergistically and holistically affected the level of resistance found in this study population, but the clinical effect of the mutations, as described in the Stanford University HIV Drug Resistance Database, can clearly be seen in the data of this study population and is a valuable contribution to the resistance mutation data on TLART patients in South Africa.

Protease inhibitor mutations most prevalent in our study population were similar to a Nigerian study conducted in 61 patients failing their respective second-line ART between 2004 and 2011, even though virological failure was defined as two consecutive VL measurements of >1000 copies/ml [10]. Major PI mutations found in Meintjes et al. 2015 for patients initiated onto TLART in the private sector of South Africa were also in agreement with most of the major PI mutations found in this study population, with exceptions to mutations V82V, M46M and L76L [23].

Resistance mutations for the NRTI`s with the highest prevalence (n=19) were M184V (89.47%), D67N (31.58%), M41L (26.31%). Mutation M184V is a clinically significant mutation primarily selected by 3TC and FTC and reduces the susceptibility of both these drugs by >100 fold, but in contrast causes an increase in the susceptibility to AZT, d4T and TDF.

Mutation M184V also decreases the rate at which AZT, d4T and TDF resistance occurs [36]. The high prevalence of mutation M184V found in this study population can be attributed to the 17 patients who were initiated on a 3TC based first-line ART regimen (n=20) and 14 patients on a 3TC based second-line ART (n=16). It is clear the M184V mutation had a significant effect on 3TC and FTC causing 95% high-level resistance, but M184V may have contributed to the increased susceptibility to AZT (52%), d4T and TDF (48%) although other factors may have had an impact. The occurrence of the most prevalent NRTI mutations found in this study population was also relevant in the Nigerian cohort [10].

High-level resistance within the NNRTI's (n=21) were found for NVP (n=16; 76.19%) and EFV (n=15; 71.43%) and mutations most prevalent for NNRTI's (n=17) were K103N (47.01%) and G190A (23.52%). Mutation K103N is a nonpolymorphic mutation that primarily selects for NVP and EFV and reduces the susceptibility of these drugs by fifty- and twentyfold respectively. Mutation G190A is also a nonpolymorphic mutation that primarily selects for NVP and EFV. Major NNRTI mutations found in a Nigerian cohort, for patients failing their second-line ART between 2004-2011, related with the mutations found in this study population with mutation K103N (46.62%) and G190A (26.23%) with the highest prevalence. Mutations Y181C also had a high prevalence in the Nigerian cohort, but not so (5.90%) in this study population.

It was not clear why 14% (n=3) of this study population were initiated on a first-line regimen containing LPV/r, considering that this compound has been reserved for second-line ART in adults in South Africa since 2004 [6, 13, 16, 24, 27]. This might be an indication that the National ART guidelines are not always followed in the public sector and that medication errors may play a role in ART outcomes. The most prevalent regimen used for first-line ART in this study population was 3TC/d4T/EFV, which relates with the findings of a literature review study of seven cohorts conducted in KwaZulu-Natal over a ten year period, 2003 to 2013 [38]. This was, however, expected as in 2004, 3TC/d4T/EFV was the initial regimen of choice [6].

Limitations of this study were that adherence could not be investigated retrospectively and as it was an all-inclusive sample, only a small number of patients were included due to the limited number of patients initiated on TLART in the North West Province and in South Africa. Data from patient health records recorded from 2004 were not always available and missing data, due to the retrospective nature of the study.

Conclusions

An increase in the number of TLART patients in the future will place a significant financial and infrastructural burden on a resource-limited setting such as South Africa [10]. This small descriptive study contributed and improved the knowledge on the clinical characteristics and resistance patterns of TLART patients in order to improve and optimise HIV management and care of these patients in a resource-limited setting, such as the North West Province, in South Africa.

The major NRTI mutations identified were M184V, D67N and M41L, major NNRTI mutations were K103N and G190A and the major PI mutations were M46I, V82A and I54V, working synergistically to form drug resistance to ART. The high number of cross-resistance found for PI's never before used by the study population is alarming and might decrease the available ART options for the future.

This study also highlighted the median 71 days between documented second-line ART failure and the initiation of TLART and the enormous burden this system is already facing in the early phases of TLART.

The importance of more frequent and earlier GART's, although costly, is also highlighted with patients spending long durations on failing ART regimens.

List of abbreviations

3TC:	Lamivudine
ABC:	Abacavir
AIDS:	Acquired immune deficiency syndrome
ART:	Antiretroviral therapy
ARV:	Antiretroviral
ATV:	Atazanavir
AZT:	Zidovudine
D4T:	Stavudine
ddl:	Didanosine
DKK:	Dr Kenneth Kaunda
DRV:	Darunavir
DTG:	Dolutegravir
EFV:	Efavirenz
ETR:	Etravirine
FPV:	Fosamprenavir
FTC:	Emtricitabine
GART:	Genotypic antiretroviral resistance test
HIV:	Human immunodeficiency virus
IDV:	Indinavir
INSTI:	Integrase strand transfer inhibitor
IQR:	Interquartile range
LPV:	Lopinavir
NDoH:	South African National Department of Health
NNRTI:	Non-nucleoside reverse transcriptase inhibitor
NRTI:	Nucleoside reverse transcriptase inhibitor
NVP:	Nevirapine
PI:	Protease inhibitor
r:	Ritonavir
RAL:	Raltegravir
RCT:	Randomised controlled trials
SPSS:	Social science computing cooperative statistics
START:	Strategic timing of antiretroviral therapy
TDF:	Tenofovir
TLART:	Third-line antiretroviral therapy
UNAIDS:	Joint United Nations Programme on HIV/AIDS
USA:	United States of America
VL:	Viral load
WHO:	World Health Organization

Ethical approval

Ethical approval was obtained from the Human Research and Ethics Committee of the North West University (REF: NWU-00340-16-A1) on 13 March 2017.

Author contributions

CR and MV conceptualised the study; CR collected data for analysis; CR and MR conducted data analysis, and interpretation of data was conducted by all the authors. CR drafted the manuscript, which was finalised with contributions from both MR and MV. The final manuscript was approved by all authors.

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Competing interests

The authors declare they have no competing interests.

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Chapter 5: Additional Results and Discussion

5.1 Introduction

This chapter covers the results and discussion from one primary and one secondary objective not included in manuscript A. These results report on the influence of ART on clinically relevant markers found in monitoring results (chemical pathology), and possible factors that may have contributed to regimen changes.

A summary of all the captured data for this cohort is presented in Addendum G, but although all the specific variables mentioned in Chapter 3 were captured, not everything documented was clinically relevant or had the power for statistical analyses in this study population.

It is important to mention that data presented in this chapter were scarcely documented in patient health records, thus making any scientifically sound conclusion not possible. The results that follow can only be interpreted as observations and descriptions found in this study population and therefore can only be viewed as a small contribution to the overall clinical profile of a typical TLART patient.

Serum creatinine, haemoglobin, mean corpuscular volume (MCV), liver function (ALT) and cholesterol test results were captured at baseline and at first- and second-line ART failure. Possible risk factors associated with regimen changes were also captured at first- and second-line ART failure and are presented as the most prevalent reasons for regimen switches, including reported side effects.

5.2 Results

It is important to note that the majority (15 of 21) of the study population were diagnosed with HIV prior to 2010 and that different ART regimens were initiated since the official roll-out in 2004, with the various monitoring and frequencies of testing, also varying since 2004. The introduction of a TDF-based regimen as part of the preferred first-line ART in adults, was only implemented from 2010 onwards. A summary of the changes in the National ART guidelines from 2004 to 2015 is presented in Addendum G.

The results captured in Addendum G, but not discussed due to small numbers, are height, weight, some laboratory results, hospitalisation, other opportunistic infections, TB co-infection and WHO clinical staging.

Serum creatinine

At baseline, nine serum creatinine (SCr) levels were documented. The median SCr at baseline was 72 $\mu\text{mol/L}$ (IQR: 58-80), with one patient experiencing elevated serum creatinine. The normal range for SCr is 49-90 $\mu\text{mol/L}$ according to the WHO laboratory test results.

At first-line ART failure, 14 serum creatinine levels were documented. The median serum creatinine at first-line ART failure was 76 $\mu\text{mol/L}$ (IQR: 62-93.75) with four elevated serum creatinine levels recorded.

Eight patients were initiated onto a TDF based first-line ART regimen or were switched to TDF within first-line ART, nine patients did not receive a TDF-based regimen, three patients were initiated onto a LPV/r based regimen, considered as second-line ART, and one had missing data. Three patients experienced elevated serum creatinine while on a TDF-based regimen.

At second-line ART failure, 18 serum creatinine levels were documented. The median serum creatinine at second-line ART failure was 70.50 $\mu\text{mol/L}$ (IQR: 68.25-87.50) with four elevated serum creatinine levels recorded.

Ten patients were initiated onto a TDF-based second-line ART regimen or were switched to this respective compound within second-line ART, whilst eleven patients did not receive a TDF-based second-line regimen. Two patients experienced elevated serum creatinine in conjunction with TDF.

Haemoglobin and mean corpuscular volume

At baseline, 11 haemoglobin levels and mean corpuscular volumes (MCV) were documented. Eight patients presented with low haemoglobin and three with low MCV. The median baseline haemoglobin level was 11.70 g/dL (IQR: 10.25-12.10) and the median MCV was 85.60 fL (IQR: 81-87.85). The normal range for haemoglobin is 12.10-16.30 g/dL and for MCV 83.0-101.0 fL according to the WHO laboratory test results.

At first-line ART failure, ten haemoglobin levels and MCVs were documented. Six patients presented with low haemoglobin and one presented low MCV. The median first-line ART failure haemoglobin was 11.20 g/dL (IQR: 10.57-12.40) and the median MCV was 90.85 fL (IQR: 87-93.15), with two patients experiencing elevated levels of MCV. Zidovudine was used in three patients and none experienced low haemoglobin or high MCV in conjunction with AZT use.

At second-line ART failure, 20 haemoglobin levels and MCVs were documented. Twelve patients presented with low and one with high haemoglobin levels and six patients presented with increased MCV. The median haemoglobin level was 11.80 g/dL (IQR: 10.90-13.33) and the median MCV was 97.45 fL (IQR: 94.23-101.53). Zidovudine was used in 12 patients, with four experiencing elevated MCV in conjunction with AZT use.

Alanine transaminase and total cholesterol

The alanine transaminase (ALT) and total cholesterol data captured at baseline and first-line ART failure were incomplete due to the different tests and monitoring frequencies implemented since 2004. This data were inconsistent and thus statistically unreliable due to the small number of data available in patient health records (**see** Addendum G).

At second-line ART failure, 15 ALT test results were captured with all but one patient achieving normal ALT levels. The median ALT at second-line ART failure was 16 U/L (IQR: 12-21). Only 12 total cholesterol values were captured at second-line ART failure, with the median total cholesterol level at second-line ART failure being 3.71 mmol/L (IQR: 3.18-4.03). The normal ranges for ALT are between 7 and 35 µ/L and should be <5.20 mmol/L for total cholesterol, according to the WHO reference ranges (WHO, 2011).

Factors, other than virology failure, that could have possibly contributed to first- and second-line ART failure are listed in Table 5-1.

Table 5-1: Possible factors contributing to switching ART, other than virological failure.

Reason for change in first-line ART		Reason for change in second-line ART	
	n ^a		n ^b
Stavudine phased out	4	Diarrhoea	5
Renal failure	1	Renal failure	3
Rash	1	Stavudine dysplastic features	1
Incorrect regimen initiated	1	Dizziness	1
Oedema	1	Resistance detected	1
Peripheral neuropathy	1		
Anaemia	1		
Lipodystrophy	1		
Pregnancy	1		

^a Reasons for first-line ART failure were not indicated in 12 patients

^b Reasons for second-line ART failure were not indicated in 15 patients

More than one reason could be documented for one patient

5.3 Discussion

Serum creatinine, urea, uric acid and hormone metabolites are classified as metabolic waste products excreted by the nephron, with the quantity of these substances in the urine reflecting the integrated function of the nephron. An increase in the serum creatinine level is an indication of reduced glomerular filtration and an indication of renal failure (Urbschat *et al.*, 2011).

Tenofovir use is associated with a decrease in creatinine clearance after months of treatment and is therefore responsible for an increase in serum creatinine levels in patients using TDF (Gallant & Moore, 2009). De Waal *et al.*, 2017 reported that a decline in kidney function due to TDF is associated with a baseline eGFR lower than 90 ml/min, low CD4 counts, geriatric patients and low bodyweight.

To calculate the creatinine clearance using the Cockcroft-Gault equation was not possible as data on patient weight in this study population were not recorded in the patient health records that were available, even though this is the preferred method of calculating renal function (Gupta *et al.*, 2005) and the modified Cockcroft-Gault calculation is used to assess if an adult patient can be initiated on a TDF-based regimen if it is >50 ml/min (NDoH, 2010-2015)

The median serum creatinine level in this study population was 72 $\mu\text{mol/L}$ (IQR: 58-80), 76 $\mu\text{mol/L}$ (IQR: 62-93.75) and 70.50 $\mu\text{mol/L}$ (IQR: 68.25-87.50) at baseline, first- and second-line ART failure, respectively, and is considered to be well within the normal serum creatinine range of 49-90 $\mu\text{mol/L}$.

There were, however, 37.50% and 20% of the patients during first- and second-line ART failure respectively, who experienced elevated serum creatinine levels with the use of TDF, therefore renal function should be monitored closely for those receiving TDF-based regimens as it might be a risk factor requiring regimen switches especially when baseline renal function is low (Karras *et al.*, 2003).

Haemoglobin is found in the red blood cells of a patient and is combined with oxygen to be made available to tissue in the human body at a later stage. Haemoglobin testing as part of full blood count is therefore used to monitor the status of haemoglobin in a patient's red blood cells and can indicate if a patient is experiencing anaemia (Harris *et al.*, 2014). The normal ranges for haemoglobin levels in the human body differ between male and female patients, with pregnancy also influencing the test results (WHO, 2011) (see **Table 5-2**).

Table 5-2: WHO reference ranges for anaemic haemoglobin values in adults (WHO, 2011).

	Mild	Moderate	Severe
Adult females	11-11.90 g/dL	8-10.90 g/dL	< 8 g/dL
Adult males	11-12.90 g/dL	8-10.90 g/dL	< 8 g/dL

Various factors can lead to anaemia, but literature had shown that HIV in itself can be a major cause of anaemia; patients diagnosed with HIV had considerably lower levels of haemoglobin than healthy controls (Obirikorang and Yeboah, 2009). It has also been reported that severe anaemia is manifested in 25% of HIV patients, using AZT at a dose of 1500 mg/day (Beck, 2008). Only five of the 21 patients in this study population received AZT as part of their respective ART regimen.

The median haemoglobin level in this study population was 11.7 g/dL (IQR: 10.25-12.10) at baseline, 11.20 g/dL (IQR: 10.57-12.40) at first-line ART failure and 11.80 g/dL (IQR: 10.9-13.33) at second-line ART failure, indicating mild anaemia within this population.

A median baseline haemoglobin level of 11.40 g/dL was found in a study conducted in Uganda and Zimbabwe (n=3 314), in 2015, prior to patients initiated onto an AZT-based first-line ART regimen and correlates with the findings in this study population at baseline of 11.70 g/dl. The authors also concluded that the characteristics of the population may have led to the low haemoglobin levels (Ssali *et al.*, 2006). It is, however, difficult to conclude from the limited data in this study population, whether the low levels of haemoglobin were due to HIV infection itself or due to the use of AZT.

Liver disease is one of the most common causes of mortality in HIV patients. The authors indicated higher ALT levels in HAART-experienced patients, than in HAART-naïve patients and that a higher ALT level could be attributed to viral hepatitis, opportunistic infections, current CD4 count, other ARVs and the male gender (Shiferaw *et al.*, 2016).

A number of ARVs, especially nevirapine and most PIs, have an influence on liver enzymes and cholesterol levels respectively, thus at first-line ART failure, it is important to measure cholesterol and triglyceride levels before second-line ART can be initiated. No abnormal levels of ALT were seen in this study population, with a median ALT of 16 U/L (IQR: 12-21) documented at second-line ART failure, which is well within the normal range of 7-35 U/L. This finding can again be skewed by the limited data found and because most of the patients were female, with the male gender more likely to be associated with abnormal ALT levels (Shiferaw *et al.*, 2016).

Protease inhibitors are known to increase the total cholesterol level in a patient, and it is indicated in literature that a combination of ARVs are synonymous with the development of dyslipidaemia. The PIs and stavudine (d4T) are more associated with higher total cholesterol and LDL levels (Aberg *et al.*, 2009) and is the reason why cholesterol levels should be checked before and after second-line ART is initiated.

With limited available data, no increase in cholesterol levels were observed in this small study, with a total median cholesterol level of 3.71 mmol/L (IQR: 3.18-4.03) found for patients failing their respective second-line ART and which is well below the desired 5.20 mmol/L threshold. The small number of patients and limited data could again possibly skew the results. No comparison over time could be made due to incomplete data at baseline and first-line ART failure and can also be attributed to the different ART guidelines implemented at different stages since 2004.

A number of reasons for ART failure and regimen switches, other than virological failure, were mentioned in Table 5-1. No specific written reasons for regimen switches were supplied for 57.14% of the patients on first-line ART and 71.40% of the patients on second-line ART, apart from failing regimen. It can be deduced that some of the factors that possibly contributed to change in ART regimens, could be linked to adverse effects of ARVs used.

Stavudine formed part of first-line ART in South Africa from 2004 to 2010 (NDoH, 2004) and it is also during this time that the majority of this study population were on first-line ART. Stavudine has been associated with fat-wasting syndrome or lipoatrophy as one of the main adverse effects and was therefore largely phased out in South Africa. Peripheral neuropathy, pancreatitis, hyperlactataemia and dyslipidaemia are also adverse effects attributed to stavudine (Calmy *et al.*, 2009).

The PIs are mostly associated with diarrhoea. The incidence of diarrhoea was reported as 7 to 28 % for LPV/r, 9 to 14% for darunavir/r, 3 to 14% for efavirenz and 9 to 16% for TDF. Diarrhoea decreases the absorption of ARVs thus reducing efficacy and can also increase non-adherence to ARVs which can contribute to treatment failure (Dikman *et al.*, 2015). It is thus not surprising that 23.80% of this study population presented with diarrhoea and that the latter is documented as one of the reasons for regimen change. In treatment naïve patients, before widely used ART, diarrhoea was mostly associated with infectious pathogens. Since the implementation of advanced ART in the 21st century, a decrease in these forms of diarrhoea was seen, but drug related diarrhoea increased (Dikman *et al.*, 2015).

5.4 Conclusions

The data collected for this part of the study were very inconsistent and limited due to unavailability and incomplete patient health records. It should also be kept in mind that it was a retrospective study with the majority of the results, especially first-line failure results were from 2004-2010, when different guidelines were followed. It is, however, an important and known observation from the results that a comparable high number of patients (all on LPV/r) presented with diarrhoea.

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Chapter 6: Discussion, Conclusion and Recommendations

The creation of an initial clinical profile, with the limited data available on TLART patients in the North West Province of South Africa, is seen as a valuable contribution by this all-inclusive retrospective descriptive investigation of 21 adult patients. This clinical profile can form the basis for larger studies in South Africa to highlight important clinical and demographical characteristics that may contribute to the optimisation of healthcare management and care of HIV patients in general.

The outlined primary and secondary objectives, as set out in Chapter 1 (**1.3.1** and **1.3.2**), were successfully achieved and included:

- a literature review describing and evaluating ART regimens (first-, second- and third-line regimens), clinical indicators influencing regimen switches and the mechanisms for resistance development;
- recording of certain haematology, virology and chemical pathology results at baseline, annual intervals and at the time of regimen switches;
- recording of ARV drug resistance phenotype and the prevalence of mutations (genotype);
- the evaluation of patient demographics (i.e. age and gender) at baseline and at TLART initiation;
- the investigation of the duration between HIV-diagnosis and ART initiation, duration on first- and second-line ART, duration between second-line ART failure and TLART initiation;
- to create a typical clinical profile of an adult TLART patient in the North West Province;
- the identification of possible risk factors that can influence regimen changes;
- the provision of possible recommendations for improvements on TLART management and care.

6.1 Discussion and conclusions

The number of patients on TLART will increase as more and more patients inevitably develop resistance to second-line ART (Boulle *et al.*, 2010; Madec *et al.*, 2013; Rawizza, 2013). This is the first study, to our knowledge, that attempted to create a typical clinical profile of patients failing their respective second-line ART in the North West Province of South Africa. Hereafter follows a brief summary of the results found in this cohort, which can assist in creating such a profile.

In the study cohort of 21 adult patients initiated onto TLART, 17 were females and four were males. The median age of these patients at HIV diagnoses was 34 years (IQR: 30.25-36.75) and 46 years (IQR: 40.00-48.50), respectively, when initiated on TLART. A median CD4 count at baseline of 68.50 cells/ μ L (IQR: 40.75 – 127.75) and a median baseline VL of 98 000 copies/ml (IQR: 45 569-820 000) were reported. The median CD4 count and VL at second-line ART failure were 119 cells/ μ L (IQR: 61.25-201.25) and 73 196 copies/ml (IQR: 26 210-197 007), respectively.

The median duration from HIV diagnosis to first-line ART initiation was 101 days (IQR: 36.50-366.75), the duration on first-line ART was 1 269 days (IQR: 765-2 343) and on second-line ART, 1 512 days (IQR: 706-2 096). The median duration between second-line ART failure and TLART initiation was 71 days (IQR: 57.5-126).

The first-line ART regimen most often initiated at 64.70% were 3TC, d4T and EFV and the PI-based second-line ART regimen at 36.84% were 3TC, AZT and LPV/r. The resistance mutations most prevalent for PIs were M46I (75%), V82A (65%) and I54V (65%); NRTIs M184V (89.47%), D67N (31.58%), M41L (26.31%) and T215Y and V118I (21.05%) and NNRTIs K103N (47.01%) and G190A (23.52%). There was a high-level of cross-resistance detected between the PIs in these patients.

Based upon these results, a patient failing second-line ART in the North West Province of South Africa could present with the following clinical profile:

There is an 81% chance the patient being female, diagnosed with HIV at the age of 36 years and receiving ART for at least seven years and six months. The CD4 count for this patient at baseline could possibly be <100 cells/ μ L and VL around 98 000 copies/ml. The duration between documented second-line ART failure and TLART initiation could be approximately 71 days. This patient might experience a high-level of cross-resistance to different PIs and could possibly present with the following major PI mutations: M46I, V82A and I54V.

Three patients with no psychiatric illnesses were initiated on a LPV/r-based regimen even though in South Africa it is reserved for second-line ART in adults (NDoH, 2015). This is an indication that universal implementation of the National ART guidelines is not always followed and is also reflected in the literature (Van Zyl *et al.*, 2013). The latter could be seen as a cause for premature treatment failure.

The long duration between second-line ART failure and TLART initiation is also important, as literature indicated an increase in PI mutations the longer a patient is kept on failing second-line ART (Lee *et al.*, 1998; Rawizza *et al.*, 2013).

The value of initial descriptive clinical characteristics could enable healthcare workers to assess, evaluate and identify indicators and possible early warning signs that may assist in strengthening the gap between administrative logistics and to initiate patients earlier onto alternative more effective ART. This could limit the number of patients failing their respective first- and second-line regimens and contribute to the optimisation of healthcare management and care of HIV patients.

6.2 Limitations

This was an all-inclusive sample of 21 adult patients, it is therefore difficult to make generalised conclusions and it is important to see the findings and recommendations only as indications for larger studies to verify and generalise.

As this study was a retrospective descriptive investigation, the researchers did not have any control over the availability and correctness of the collected data. Some data on critical time-points could not be recorded and contributed to incomplete patient profiles. The majority of the retrospective data, date back to 2004 when the ART program was first rolled-out and the ART guidelines have since changed several times with regards to the regimen compositions, efficacy and toxicity tests and frequencies of monitoring intervals.

Scientifically sound data on patient adherence could also not be measured, due to the retrospective nature of the available data in the study. Patient adherence to ART is seen as a significant indicator for treatment success or failure and should be taken into consideration in future studies (Johnson *et al.*, 2013; Magambo *et al.*, 2014).

6.3 Recommendations

Valuable and descriptive results were reported in this small study population, but it is recommended that the findings be verified in larger study populations in South Africa, as these will help to understand fully all aspects involved with the management and care of TLART patients and generalise the outcomes for implementation purposes. This study may form a platform for future studies to further expand on.

Important aspects that could influence the successful outcome of ART were highlighted: universal implementation of the National ART guidelines were sometimes not followed, missing data in patient health records could influence the effective monitoring of ART, the lack of motivation for regimen changes and a constant low CD4 count throughout ART could be possible risk factors contributing to treatment failure on first- and second-line ART.

Updated management strategies and protocols for TLART applications, is needed as the time spent between second-line ART failure and TLART initiation is a clear indication of the infrastructural burden that is placed on the Third-line Review Committee, as the number of patients failing their respective second-line ART will inevitably increase in the future (Boulle *et al.*, 2010; Madec *et al.*, 2013; Rawizza *et al.*, 2013).

All these factors highlighted the importance of effective and optimised HIV management and care and it's recommended that HIV management strategies should be individualised for facilities, but actual implementation should take place at patient care-level within the Wellness programme. Investment into well-trained staff with experience and important follow-up, on patients not adhering to their appointment dates, needs to be address in each environment. This will not only improve the quality and efficacy of patient care but also the ability to collect scientifically sound data for important research on managing this "untreatable" disease of the 21st century (Magambo *et al.*, 2014).

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Addendum A: Conference Proceedings

HIV drug resistance patterns for adult patients on third-line ART in the North West Province since 2004-2017.

Christian Rudman¹, Michelle Viljoen^{1, 2}, Malie Rheeders¹

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- ² Pharmacology and Clinical Pharmacy, School of Pharmacy, Faculty of Natural Sciences, University of the Western Cape, Bellville, South Africa.

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Introduction and Aim: South Africa has the largest human immunodeficiency virus (HIV) epidemic in the world with the total number of people living with HIV increasing from 4.02 in 2002 to 7.03 million in 2016. The number of patients that will experience virological failure and subsequently treatment failure in the future will increase due to improved access to antiretroviral therapy (ART) and a longer duration of exposure to ART. This will increase the need to access third-line ART. This study aimed to improve the knowledge on ART failure and the development of resistance in nucleoside reverse transcriptase inhibitors (NRTI's), non-nucleoside reverse transcriptase inhibitors (NNRTI's) and protease inhibitors (PI's) in the North West province.

Methods: The study was an all-inclusive retrospective descriptive investigation of third-line approved patients in 3 districts in the North West Province performed during April-May 2017. Demographical and clinical variables were recorded from patient health records from 2004 until 2017. Captured data were statistically analysed to generate descriptive statistics (mean $\pm$ SD) of the third-line approved patients in the North West Province.

Results: Data of twenty-one patients were recorded and analysed. In this cohort, 17 females and four males had an average age of 45.19 ± 7.26 years at the time of data capturing. The mean duration from HIV diagnosis to first-line initiation was 197 ± 232 days. Mean duration on first-line ART was $1\,362 \pm 996$ days and on second-line $1\,269 \pm 796$ days. Alarming the average duration between second-line failure and third-line application approval was 91.38 ± 73 days. The following resistance mutations were found to be the most prevalent for each drug class: NRTI's (n=18); M184V (89.47%), D67N (31.58%), M41L (26.31%), Q151M and K65R (10.52%). Mutations for NNRTI's (n=17) were; K103N (47.01%) and G190A (23.52%). Major PI's (n=20); M46I (75%), V82A and I54V (65%).

Antiretroviral drugs experiencing high level resistance in the NRTI class were; Emtricitabine (95.20%), Lamivudine (95.20%) and Zidovudine (52.40%). For the NNRTI's; Nevirapine (76.20%) and Efavirenz (71.40%) and high level resistance for the PI's; nelfinavir/r (85.70%), indinavir/r (81.0%), lopinavir/r (76.20%), fosamprenavir/r (66.70%) and atazanavir/r (57.10%).

Conclusions: These HIV drug resistance patterns were also found in studies conducted in other parts of South Africa. The high resistance to PI's, which was not used in treatment, is alarming and need further investigation. Non-adherence and ART side-effects can be highlighted as possible reasons for treatment failure. This study highlighted the importance of intensified support for adherence counselling and more frequent drug resistance testing.

Key words: North West Province, HIV, drug resistance, mutations, third-line regimen

Addendum B: Instructions to the Author (AIDS Research and Therapy)

Manuscript A has been prepared for online submission to AIDS Research and Therapy as a full length research article according to the specified Instructions to the Author - research article.

Detailed specifications for a publishing a research article in Aids Research and Therapy can be found using the following website URL:

<https://aidsrestherapy.biomedcentral.com/submission-guidelines/preparing-your-manuscript/research-articles>A brief overview of

Addendum C: Permission to Include Manuscript A in Dissertation

Included in Addendum C, are permission letters from:

- Prof Michelle Viljoen (study promotor and co-author of Manuscript A);
- Dr Malie Rheeders (study co-promotor and co-author of Manuscript A)

to include Manuscript A into the final dissertation as it is part of the requirements for fulfilment of the MSc degree.

Permission letter Prof Michelle Viljoen.



17 November 2017

The Examiner
MSc Thesis of C Rudman (23530804)

Dear Examiner

**Permission: Mr C Rudman to Include Manuscript in his
Dissertation for Examination Purposes for a MSc Degree**

As study promotor and co-author on the manuscript to be submitted in the near future, understanding that this was co-authored by Mr C Rudman, I hereby approve that the manuscript be included in Chapter 4 as part of the requirements for fulfilment of the MSc degree, and that this manuscript be submitted for examination of Mr Rudman's dissertation.

The title of the manuscript is as follows:
A retrospective descriptive investigation of adult patients receiving third-line antiretroviral therapy in the North West Province

I trust that you will find this in order.

Yours sincerely



Prof Michelle Viljoen
Pharmacology and Clinical Pharmacy

Permission letter Dr Malie Rheeders.



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Pharmacology
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14 November 2017

The Examiner
MSc Thesis of Mr C Rudman

Dear Examiner

Permission to Mr C Rudman to Include Manuscripts in his Thesis for Examination Purposes for an MSc Degree

As study co-promoter and author on the manuscript to be submitted in the near future, understanding that this was co-authored by Mr C Rudman, I hereby approve that the manuscript be included in Chapter 4 as part of the requirements for fulfilment of the MSc degree, and that this manuscript be submitted for examination of Mr Rudman thesis.

The title of the article is as follows:

A retrospective descriptive investigation of adult patients receiving third-line antiretroviral therapy in the North West Province

I trust that you will find this in order.

Yours sincerely

A handwritten signature in black ink, appearing to read 'M Rheeders'.

Dr Malie Rheeders
Senior Lecturer of Pharmacology

Do not type here

Addendum D: Case Report Form

A case report form (CRF), was specifically developed by the main researcher for the retrospective collection of data from patient health records at the respective research sites. An example of the CRF is presented in this Addendum D.

PATIENT CODE:

DEMOGRAPHIC INFORMATION				
Gender (M/F)				
Date of Birth (dd/mm/yy)				
Length (meters)				
Weight (kg)				
Ethnicity				
IMPORTANT DATES				
HIV diagnoses			First-line	
ARV initiation		Second-line		
ARV REGIMEN HISTORY				
First-line ART initiated				
Medication			Dosage	
1)				
2)				
3)				
4)				
Switches within regimen				
Medication	Dosage		Reason for switch	
1)				
2)				
Date regimen failed	Duration			
Second-line ART initiated				
Medication			Dosage	
1)				
2)				
3)				
4)				
Switches within regimen				
Medication	Dosage		Reason for switch	
1)				
2)				
3)				
Date regimen failed	Duration			

Third-line ART initiated by Third-line Review Committee				
Medication	Dosage			
1)				
2)				
3)				
4)				
5)				
6)				
ARV Resistance Profile				
Drug	Low	Intermediate	High	
Nucleotide and Nucleoside Reverse Transcriptase Inhibitors Resistance				
1)				
2)				
3)				
4)				
5)				
6)				
7)				
8)				
9)				
10)				
11)				
12)				
13)				
14)				
15)				
16)				
Non-Nucleotide Reverse Transcriptase Inhibitors Resistance				
1)				
2)				
3)				
4)				
5)				
Protease Inhibitors Resistance				
1)				
2)				
3)				

RETROSPECTIVE DESCRIPTIVE CASE REPORT FORM

DATE OF DATA COLLECTION:

[illegible]

PATIENT CODE:

RETROSPECTIVE DESCRIPTIVE CASE REPORT FORM

DATE OF DATA COLLECTION: _____

[illegible]

RETROSPECTIVE DESCRIPTIVE CASE REPORT FORM		PATIENT CODE:											
DATE OF DATA COLLECTION: _____													
ADHERANCE REMARKS BY COUNSELOURS AND OTHER FACTORS (stock-out's, appointment adherence, pill counts, other illnesses etc.)													

Addendum E: Ethical clearance

Ethics clearance letters contained in Addendum E include:

- 1) Approval letter from the Human Research Ethics Committee (HREC) of the North-West University, Potchefstroom with reference number NWU-00340-16-A1.
- 2) An approval letter from NWU HREC for an amendment to include a fourth study site (Mahikeng, Bophelong Provincial Hospital).
- 3) Approval from the North West Department of Health, Policy, Planning, Research, Monitoring and Evaluation (PPRME) directorate, Mahikeng.
- 4) Permission letter from Dr C van Deventer, Chairperson of the Dr Kenneth Kaunda Research Committee.
- 5) Goodwill permission from the CEO of the Joe Morolong Memorial Hospital, Dr Z.A. Esterhuizen.
- 6) Goodwill permission from the Clinical Manager of the Potchefstroom Hospital, Dr M. Shakung.
- 7) Goodwill permission from the Clinical Manager of the Klerksdorp/Tshepong Hospital Complex, Dr N.D. Leburu.
- 8) Goodwill permission from the Senior Clinical Manager of Mahikeng/Bophelong Hospital Complex, Dr T.N.C. April.

1.



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Faculty of Health Sciences
Health Sciences Ethics Office for Research,
Training and Support
Health Research Ethics Committee (HREC)

Tel: 018-285 2291
Email: Wayne.Towers@nwu.ac.za

13 March 2017

Prof M Viljoen
Pharmacology-PharmaCen

Dear Prof Viljoen

**APPROVAL OF YOUR APPLICATION BY THE HEALTH RESEARCH
ETHICS COMMITTEE (HREC) OF THE FACULTY OF HEALTH SCIENCES**

Ethics number: NWU-00340-16-S1

Kindly use the ethics reference number provided above in all correspondence or documents submitted to the Health Research Ethics Committee (HREC) secretariat.

Study title: A retrospective descriptive investigation into the clinical profile of HIV-patients in the North West Province switched to third-line regimens

Study leader/supervisor: Prof M Viljoen

Student: C Rudman

Application type: Single study

Risk level: Medium

You are kindly informed that your application was reviewed at the meeting held on 20/10/2016 of the HREC, Faculty of Health Sciences, and was approved on 13/03/2017.

The commencement date for this study is 13/03/2017 dependent on fulfilling the conditions indicated below. Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation up to a maximum period of three years when extension will be facilitated during the monitoring process.

After ethical review:

Translation of the informed consent document to the languages applicable to the study participants should be submitted to the HREC, Faculty of Health Sciences (if applicable).

The HREC, Faculty of Health Sciences requires immediate reporting of any aspects that warrants a change of ethical approval. Any amendments, extensions or other modifications to the proposal or other associated documentation must be submitted to the HREC, Faculty of Health Sciences prior to implementing these changes. Any adverse/unexpected/unforeseen events or incidents must be reported on either an adverse event report form or incident report form at Ethics-HRECIncident-SAE@nwu.ac.za.

A monitoring report should be submitted within one year of approval of this study (or as otherwise stipulated) and before the year has expired, to ensure timely renewal of the study. A final report must be provided at completion of the study or the HREC, Faculty of Health Sciences must be notified if the study is temporarily suspended or terminated. The monitoring report template is obtainable from the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-Monitoring@nwu.ac.za. Annually a number of studies may be randomly selected for an external audit.

Please note that the HREC, Faculty of Health Sciences has the prerogative and authority to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.

Please note that for any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the HREC, Faculty of Health Sciences. Ethics approval is required BEFORE approval can be obtained from these authorities.

The HREC, Faculty of Health Sciences complies with the South African National Health Act 61 (2003), the Regulations on Research with Human Participants (2014), the Ethics in Health Research: Principles, Structures and Processes (2015), the Belmont Report and the Declaration of Helsinki (2013).

We wish you the best as you conduct your research. If you have any questions or need further assistance, please contact the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-HRECApply@nwu.ac.za.

Yours sincerely



Prof Wayne Towers
HREC Chairperson



Prof Minrie Greeff
Ethics Office Head

Current details: (13210572) C:\Users\13210572\Documents\HREC\HREC - Applications\2016 Applications\Applications 09-20 October 2016\NWU-00340-16-S1(M Vlijoen-C Rudman)\NWU-00340-16-S1(M Vlijoen-C Rudman)-AL\NWU-00340-16-S1(M Vlijoen-C Rudman)-AL.docm
13 March 2017

File reference: 9.1.5.3

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Faculty of Health Sciences
Health Sciences Ethics Office for Research,
Training and Support
Health Research Ethics Committee (HREC)

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Email: Wayne.Towers@nwu.ac.za

25 May 2017

Prof M Viljoen
Pharmacology-PharmaCen

Dear Prof Viljoen

APPROVAL OF YOUR AMENDMENT REQUEST BY THE HEALTH RESEARCH ETHICS COMMITTEE (HREC) OF THE FACULTY OF HEALTH SCIENCES

Ethics number: NWU-00340-16-A1

Kindly use the ethics reference number provided above in all correspondence or documents submitted to the Health Research Ethics Committee (HREC) secretariat.

Study title: A retrospective descriptive investigation into the clinical profile of HIV-patients in the North West Province switched to third-line regimens

Study leader/supervisor: Prof M Viljoen

Student: C Rudman

The rebuttal submitted to the feedback from the Health Research Ethics Committee (HREC) as well as the documentation submitted by the applicant in response to the previous correspondence provided by the HREC, for the amendment request to the single study entitled "A retrospective descriptive investigation into the clinical profile of HIV-patients in the North West Province switched to third-line regimens" has been reviewed by the HREC and has been approved.

Please inform us immediately if there are any amendments required to your study.

We wish you the best as you conduct your research. If you have any questions or need further assistance, please contact the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-HRECAppl@nwu.ac.za.

Yours sincerely

A handwritten signature in black ink, appearing to read 'Wayne Towers', written in a cursive style.

Prof Wayne Towers
HREC Chairperson

A handwritten signature in black ink, appearing to read 'Minrie Greeff', written in a cursive style.

Prof Minrie Greeff
Ethics Office Head

3.

	health Department of Health North West Province REPUBLIC OF SOUTH AFRICA	3801 First Street New Office Park MAHIKENG, 2735	Enq: Ntshabang Mapogo Tel: 018 391 4504 NMapogo@nwps.gov.za www.nwps.health.gov.za
	POLICY, PLANNING, RESEARCH, MONITORING AND EVALUATION		

Name of researcher : Mr. C. Rudman
North West University

Physical Address : Building G23, Office 321
 (Work/ Institution) North West University Potchefstroom
2520

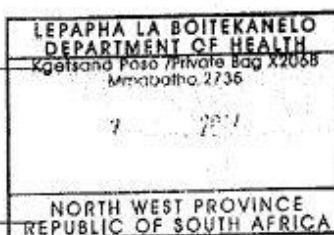
Subject : Research Approval Letter- A retrospective descriptive investigation
 into the clinical profile of HIV-patients in the North West Province
 switched to third-line regimens.

This letter serves to inform the Researcher that permission to undertake the above mentioned study has been granted by the North West Department of Health. The Researcher is expected to arrange in advance with the chosen facilities, and issue this letter as proof that permission has been granted by the Provincial office.

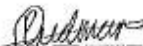
This letter of permission should be signed and a copy returned to the department. By signing, the Researcher agrees, binds him/herself and undertakes to furnish the Department with an electronic copy of the final research report. Alternatively, the Researcher can also provide the Department with electronic summary highlighting recommendations that will assist the department in its planning to improve some of its services where possible. Through this the Researcher will not only contribute to the academic body of knowledge but also contributes towards the bettering of health care services and thus the overall health of citizens in the North West Province.

Kindest regards


 Dr. FRM Reichel
 Director: PPRM&E



17/03/2017
 Date


 Researcher

22/03/2017
 Date




 Healthy Living for All

4.



health
Department of
Health
North West Province
REPUBLIC OF SOUTH AFRICA



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0823257608

Family Medicine Dr Kenneth Kaunda District

January 2017

A retrospective descriptive investigation into the clinical profile of HIV-patients in the North West Province switched to third-line regimens"

Due to the fact that this this research has been through all the formal processes and has received consent from the necessary committees, it meets with the Dr Kenneth Kaunda districts requirements and it is proposed that it be allowed to proceed.

Regards



Claire van Deventer
Chairperson DKK Research committee

5.



18 May 2017

Dr. M. Viljoen
Pharmacology
NWU

Att: Mr. C. Rudman

Re: GOODWILL PERMISSION

We hereby grant final goodwill permission for your intended study, Ethics number NWU-00340-16S1.
This permission is subject to final approval by the North West Department of Health Policy, Planning,
Research, Monitoring and Evaluation Unit.

Thank You



Dr. ZA Esterhuizen
CEO

6.



TO: Christian Rudman
MSc Pharmacology- NW University (Potchefstroom Campus)

FROM: Dr M Shakung
Clinical Manager

DATE: 16 February 2017

SUBJECT: A retrospective descriptive investigation into the clinical profile of HIV-patients in the North West Province switched to third-line regimens"

The above mentioned request bears reference .

You request to conduct research on the clinical profile of HIV-patients in the North West Province switched to third-line regimens has been approved. Kindly contact the hospital Pharmacy manager(Ms F Motara) for all logistics needed for your research.

The hospital will appreciate feedback from your research

Sincerely

Dr M Shakung
Clinical Manager
Potchefstroom Hospital



A RETROSPECTIVE DESCRIPTIVE INVESTIGATION INTO THE CLINICAL PROFILE OF HIV-PATIENTS IN
THE NORTH WEST PROVINCE SWITCHED TO THIRD-LINE REGIMENS"

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7.



health
Department of
Health
North West Province
REPUBLIC OF SOUTH AFRICA

*All correspondence
to be directed to:*
The Clinical Manager
Dr. David Leburu
K/T Hospital Complex
Private Bag A14
KLERKSDORP
1470

Tel: (018) 406 4740
Fax: (018) 462 1923
DLeburu@nwpg.gov.za
SB@nwpg.gov.za

CLINICAL MANAGER'S OFFICE

24th February 2017

To : Mr. Christian Rudman
MSc Pharmacology
NW University (Potchefstroom Campus)

From : Dr. N.D. Leburu
Clinical Manager
K/T Hospital Complex

**RE : GOODWILL PERMISSION TO CONDUCT A RETROSPECTIVE
DESCRIPTIVE INVESTIGATION INTO THE CLINICAL PROFILE OF HIV-
PATIENTS IN THE NORTH WEST PROVINCE SWITCHED TO THIRD-LINE
REGIMEN**

We are hereby grant conditional goodwill permission for your intended study, Ethics number NWU-00340-16S1.

As a Hospital Complex we expect a formal feedback on the outcome of the study as it may have an important influence on our operations.
This permission is subject to a final approval by the North West Department of Health Policy, Planning, Research, Monitoring and Evaluation Unit.

Regards,



Dr. N.D. Leburu
Clinical Manager
Klerksdorp/Tshepong Hospital Complex

8.



04 May 2017

Christian Rudman
MSc Pharmacology
NW University (Potchefstroom Campus)

A retrospective descriptive investigation into the clinical profile of HIV-patients in the North West Province switched to 3rd line regimens

Your email dated 2 May 2017 and the permission to conduct this research in our institutions granted by the Policy, Planning, Research, Monitoring and Evaluation (PPRME) Directorate dated 17 Mar 2017 bear reference.

This communication serves as a "Goodwill Permission Letter" approving your request to conduct this research in our institution in line with the overall permission rules, guidelines and applicable policies as stated by the PPRME.

Please contact Mr. Smith (Pharmacy Manager) for further assistance.

We request you to avail Research outcomes and/or findings to the hospital both electronically and signed hard copy form upon completion.

Best Regards

[Signature] 04 May 2017
Dr. TNC April
Senior Clinical Manager



Healthy Living for All

Addendum F: Proofreading Work Certificate

Gill Smithies

Proofreading & Language Editing Services

59, Lewis Drive, Amanzimtoti, 4126, Kwazulu Natal

Cell: 071 352 5410 E-mail: moramist@vodamail.co.za

Work Certificate

To	Christian Rudman
Address	Centre of Excellence for Pharmaceutical Sciences, Division of Pharmacology, North-West University
Date	15/11/2017
Subject	A retrospective descriptive investigation of adult patients receiving third-line antiretroviral therapy in the North West Province.
Ref	GS/CR/01

I, Gill Smithies, certify that I have proofed the following thesis,
**A retrospective descriptive investigation of adult patients receiving
third-line antiretroviral therapy in the North West Province,**
to the standard as required by NWU, Potchefstroom Campus.

Gill Smithies

Addendum G: Summary Statistics of all Variables

Addendum G contains all the relevant data captured from the patient health records of the 21 approved and initiated TLART patients, in three districts of the North West Province of South Africa.

The variables include:

1. Patient demographics
2. CD4 counts at baseline, first-line and second-line ART failure
3. VL values at baseline, first-line and second-line ART failure
4. Level of susceptibility to each antiretroviral drug, respectively
5. Resistance mutations present per patient
6. Antiretroviral drug regimens and durations between regimen switches
7. Serum creatinine at baseline, first-line and second-line ART failure
8. Alanine transferase and total cholesterol at baseline, first-line and second-line ART failure
9. Haemoglobin and mean corpuscular volume at baseline, first-line and second-line ART failure
10. WHO clinical staging, hospitalisation, tuberculosis co-infection and other opportunistic infections

Patient demographics

Patient number	M=1 F=2	Date of birth	Age HIV diagnosed	Age at TLART initiation	Duration between HIV diagnosis and ART initiation	Duration on first-line ART	Duration on second-line ART	Duration between second-line ART failure and TLART initiation	Median weight per patient, since diagnosis - TLART initiation	Length
1	2	1969/12/16	38	47	430	1 841	657	106	88.90 (IQR: 77.70-94.40)	161
2	1	1974/07/14	36	42	41	1 066	738	140	53.90 (IQR: 51.25-54.24)	176
3	2	1971/06/01	34	45	35	1 161	2 158	314	48.50 (IQR: 44.55-52.00)	152
4	1	1968/05/30	N/A	48	N/A	N/A	580	140	58.90 (IQR: 57.15-66.60)	178
5	1	1977/01/24	33	40	731	699	774	25	58.00 (IQR: 56.00-60.90)	172
6	2	1963/12/20	40	53	357	2 694	1 512	68	98.15 (IQR: 88.57-100.16)	154
7	2	1962/04/26	44	54	75	1 710	1 602	35	54.40 (IQR: 50.78-61.15)	157
8	2	1980/12/28	25	36	34	883	2 343	114	66.95 (IQR: 65.40-70.00)	157
9	2	1971/03/26	30	46	370	N/A	5 026	138	N/A	N/A
10	2	1985/12/25	19	31	35	1 581	2 255	63	N/A	N/A
11	2	1977/09/04	30	39	35	1 269	2 015	55	67.70 (IQR: 66.65-71.75)	N/A
12	2	1967/12/20	N/A	49	N/A	N/A	N/A	N/A	N/A	N/A
13	2	1973/12/03	31	43	63	2 844	614	165	66.00 (IQR: 64.00-68.00)	165
14	2	1956/08/27	N/A	60	N/A	N/A	N/A	N/A	N/A	N/A
15	2	1976/09/20	34	40	139	442	709	71	59.50 (IQR: 59.00-63.50)	167
16	2	1968/06/26	37	48	730	447	1 745	108	N/A	N/A
17	2	1969/09/08	36	47	112	2 705	1 182	62	53.30 (IQR: 50.82-54.82)	165
18	2	1971/02/04	32	46	443	2 343	2 034	39	N/A	N/A
19	1	1971/02/05	35	46	90	2 819	703	38	55.50 (IQR: 54.25-57.00)	172
20	2	1983/08/03	29	33	182	765	604	60	N/A	N/A
21	2	1961/02/15	46	56	34	483	2 219	110	N/A	N/A
Mean			33.83	45.19	218.66	1 514.82	1 551.05	97.42		164.66
SD			6.19	7.08	235.45	883.14	1 076.24	66.93		8.65
Median			34	46	101	1 269	1 512	71		165
IQR(25 th -75 th)			30.25-36.75	40-48.50	36.50-366.75	765-2 343	706-2 096	57.50-126		157-172
n=	21	21	18	21	18	18	19	19	13	12

Potchefstroom Hospital (n=6), Klerksdorp-Tshepong Hospital Complex (n=13), Joe Morolong Memorial Hospital – Vryburg (n=1), Mahikeng Hospital Complex (n=1)

CD4 counts at baseline, first-line and second-line ART failure

Patient number	Baseline (cells/ μ L)	Fist-line ART failure (cells/ μ L)	Second-line ART failure (cells/ μ L)
1	83	185	184
2	41	29	144
3	46	79	389
4	40	N/A	25
5	N/A	119	419
6	30	108	152
7	69	62	59
8	7	90	62
9	N/A	N/A	266
10	29	10	10
11	70	370	147
12	68	N/A	N/A
13	N/A	129	92
14	201	N/A	71
15	169	8	53
16	52	24	93
17	114	163	100
18	N/A	N/A	138
19	219	8	3
20	192	79	153
21	N/A	N/A	497
Average	89	97.53	157.85
SD	67.58	93.93	139.86
Median	68.50	79	119
IQR _(25th-.75th)	40.75-127.75	26.50-124	61.25-201.25
n=	16	15	20

Viral load values at baseline, first-line and second-line ART failure

Patient number	Baseline (copies/mL)	Fist-line ART failure (copies/mL)	Second-line ART failure (copies/mL)
1	6 300	42 969	33 865
2	60 139	24 074	6 230
3	98 000	100 000	43 676
4	145 317	N/A	311 055
5	N/A	1 856 745	4 390
6	16 000	297 704	191 571
7	16 000	320 297	56 278
8	820 000	3 500	79 391
9	N/A	N/A	16 458
10	82 500	200 000	67 000
11	31 000	43 133	20 506
12	4 777 992	N/A	N/A
13	N/A	41 202	27 303
14	83 395	N/A	1 111 280
15	4 755 255	5 492 381	90 359
16	820 000	36 000	232 794
17	2 000 000	51 511	120 807
18	N/A	N/A	213 315
19	390 000	182 561	262 582
20	N/A	190 757	22 929
21	N/A	N/A	129 719
Average	940 126.53	592 188.93	152 075.40
SD	1 641 658.58	1 431 141.28	244 209.28
Median	98 000	100 000	73 196
IQR(25 th -75 th)	45 569-820 000	42 085-248 852	26 210-197 007
n=	15	15	20

Level of susceptibility to each antiretroviral drug, respectively

Patient number	3TC	ABC	AZT	D4T	Ddl	FTC	TDF	IDV/r	LPV/r	NFV/r	SQV/r	TPV/r	EFV	ETR	NVP	RPV	ATV/r	DRV/r	FPV/r
1	3	1	4	4	1	3	4	3	3	3	2	1	3	1	3	3	3	4	3
2	3	1	4	4	1	3	4	3	3	3	2	2	3	1	3	2	3	4	1
3	3	3	3	3	3	3	3	3	3	3	2	4	2	1	3	1	2	2	3
4	3	2	4	1	2	3	1	3	3	3	2	1	3	1	3	1	2	1	3
5	3	3	3	3	3	3	3	3	2	3	1	4	3	2	3	2	2	4	2
6	1	3	3	3	3	1	2	3	3	3	3	4	3	1	3	1	3	4	3
7	3	1	4	4	1	3	4	3	3	3	2	1	1	4	2	4	3	4	2
8	3	1	1	4	1	3	4	3	3	3	2	2	3	1	3	3	3	2	3
9	3	1	4	4	1	3	4	3	3	3	2	1	3	2	3	3	3	4	3
10	3	3	3	3	3	3	3	3	3	3	3	3	4	4	4	4	3	2	3
11	3	1	4	4	1	3	4	2	1	2	1	4	4	4	4	4	3	4	1
12	3	1	4	4	1	3	4	3	3	3	2	1	3	4	1	4	2	1	3
13	3	3	3	3	3	3	2	2	1	3	2	4	3	1	3	1	1	4	1
14	3	1	4	4	1	3	4	3	3	3	2	1	3	1	3	1	2	1	3
15	3	1	4	4	1	3	4	1	1	2	1	1	3	1	3	1	1	4	1
16	3	3	3	3	3	3	3	3	3	3	2	2	2	1	3	2	3	4	2
17	3	2	4	1	2	3	1	3	3	3	3	2	2	4	2	4	3	2	3
18	3	3	3	3	3	3	2	3	3	3	3	2	3	3	3	3	3	2	3
19	3	3	3	3	3	3	3	3	3	3	4	2	3	1	3	1	2	2	3
20	3	1	4	4	1	3	4	2	2	2	1	4	3	1	3	1	1	1	3
21	3	3	3	3	3	3	3	3	3	3	2	2	3	2	3	2	3	2	3
Low	1	10	1	2	10	1	2	1	3	0	4	7	1	12	1	8	3	4	4
Intermediate	0	2	0	0	2	2	3	3	2	3	12	7	3	3	2	4	6	7	3
High	20	9	9	9	9	18	6	17	16	18	4	1	15	1	16	4	12	0	14
Susceptible	0	0	11	10	0	0	10	0	0	0	1	6	2	5	2	5	0	10	0
n=	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21

Resistance profile (Low-level resistance = 1; Intermediate-level resistance = 2; High-level resistance = 3; Susceptible to ARV = 4)

Resistance mutations present per patient

Patient number	NRTI resistant mutations	NNRTI resistant mutations	Major PI resistant mutations	Minor PI resistant mutations
1	M184V, V118I	Y118L	M461, V82A, 154V	K20T, L10F
2	M184V, K219R, V188I	A98G, E138G, K103N, P225H	M461, V82S, 154V	K20I
3	A62V, D67N, F116Y, F77L, K219E, K65R, Q151M, V75I	G190A, V106I	M461, V82A, 150V	L24I, L33F, L89V
4	M184V, K219R, K70E	H221Y, K103N, K238N, V108I	M461, V82A, 154V, L76V	A71V, L10F
5	M184V, M41L, T215F, D67N, L74V, V118I	G190A, K101E	M461, V82A	T74S, L10L, L10F
6	M41L, T215Y, D67N, D67D, L74I, L74V, L74L	F227L, V106M, V179D	M461, V82A, 154V	K43T, L10F, L24I
7	M184V, V118I	V318F	M461, V82A, V82V, 154V	T74S
8	M184V, K70R, T69T, T69S	E138A, Y188L, V179I, V179V	None	None
9	M184V	K103N, K101P, K101S	M461, 154V, V82A	L10F, L89M, L89I
10	M184V, M184M, T215Y, M41L, L210E, D67N	None	M461, 154V, 184V, L76V, V82C	A71T, L10I
11	M184V	None	150L, V82A	L33F, A71T, A71A, A71I, A71V
12	M184V	K103N	M461, 154V, L76V, V82A	L10F
13	M184V, K70N, M41L, T215Y, V75M, D67N, D67D, L74I, L74L, F77L, F77F	K103N, V108I, V108V, H221Y, H221H, K238S, K238N	L90M	None
14	None	None	V82A, L76V, 154V	L10V, F53F, F53L
15	M184V	K103K, K103N, K103R, K103S, E138A, E138E, N348I, N348N	154V	T74S
16	M184V, D67G, L210W, M41L, T215Y	K103K, K103R, K101S, K101R, E138A, G190A	154V, M46L, V82A	L24I, L33F, T74S, L10I
17	M184V, K70N, K219R	P225H	M461, 184V, L76V	L10F
18	M184V, D67N, K219Q, K70R, T215F, T69D	G190A, K101H, Y181C	154V, L76V, M461, V82A	L10F, L33F
19	M184V, A62V, G116Y, K65R, Q151M, V75I, Y115F	A98G, K103N	M461, V32I, 147A	K20T, A71V
20	M184V	A98G, K103N	M461, M46M, L76V, L76L	None
21	None	None	M461, V82A, 154V, L76V	L10F, L33F, T74S

Antiretroviral drug regimens and durations between regimen switches

Patients number	Time before ART (days)	First-line ART	Duration on first-line ART (days)	Second-line ART	Duration on second-line ART (days)	Duration between second-line ART failure and TLART initiation (days)	TLART initiated on
1	430	D4T/3TC/EFV 3TC/TDF/EFV	1841	ABC/3TC/LPV/r	657	106	FTC/TDF/DRV/r
2	41	3TC/TDF/EFV FTC/TDF/EFV	1066	3TC/EFV AZT/3TC	738	140	FTC/TDF/DRV/r
3	35	D4T/3TC/EFV AZT/3TC/EFV	1161	Ddl/TDF/LPV/r 3TC/TDF/LPV/r/AZT	2158	314	AZT/3TC/ETR/DRV/r /RAL
4	N/A	EFV/LPV/r EFV/TDF/FTC	N/A	TDF / LPV/r	580	140	AZT/3TC/DRV/r
5	731	FTC/TDF/EFV	699	ABC/3TC	774	25	FTC/TDF/DRV/r/ DTG
6	357	D4T/3TC/EFV 3TC/TDF/EFV	2694	ABC/TDF/LPV/r	1512	68	FTC/TDF/DRV/r
7	75	AZT/3TC/EFV	1710	3TC/TDF/LPV/r	1602	35	FTC/TDF/DRV/r
8	34	D4T/3TC/EFV	883	AZT/ddl/LPV/r	2343	114	FTC/TDF/DRV/r
9	370	AZT/ddl/LPV/r	N/A	AZT/3TC/LPV/r	5026	138	FTC/TDF/DRV/r
10	35	D4T/3TC/EFV	1581	AZT/3TC/LPV/r	2255	63	FTC/TDF/ETR/DRV/r/ RAL
11	35	D4T/3TC/EFV	1269	AZT/3TC/LPV/r	2015	55	FTC/TDF/DRV/r
12	N/A	AZT/D4T/3TC/ LPV/r	N/A	AZT/3TC/TDF/LPV/r	N/A	N/A	FTC/TDF/DRV/r/RAL
13	63	3TC/TDF/EFV 3TC/TDF/NEV	2844	3TC/TDF/LPV/r	614	165	FTC/TDF/DRV/r/RAL
14	N/A	N/A	N/A	N/A	N/A	N/A	FTC/TDF/DRV/r/RAL
15	139	3TC/TDF/EFV	442	AZT/3TC/LPV/r	709	71	3TC/TDF/DRV/r
16	730	D4T/3TC/EFV	447	AZT/3TC/LPV/r 3TC/TDF/LPV/r	1745	108	FTC/TDF/ETR/DRV/r/ RAL
17	112	D4T/3TC/TDF	2705	3TC/TDF/LPV/r	1182	62	AZT/3TC/DRV/r/DTG
18	443	D4T/3TC/EFV	2343	3TC/TDF/LPV/r	2034	39	FTC/TDF/DRV/r/DTG
19	90	D4T/3TC/EFV FTC/TDF/EFV	2819	AZT/3TC/LPV/r	703	38	AZT/3TC/LPV/r/ DRV/r / DTG
20	182	3TC/TDF/EFV	765	AZT/3TC/LPV/r AZT/D4T/LPV/r	604	60	FTC/TDF/DRV/r/DTG
21	34	D4T/3TC/EFV	483	AZT/ddl/LPV/r ABC/3TC/TDF	2219	110	D4T/3TC/DRV/r/ RAL
Mean	218.66		1514.82		1151.05	97.42	
SD	235.45		883.14		1076.24	66.93	
Median	101		1269		1512	71	
IQR(25th-75th)	36.50-366.75		765-2343		706-2096	57.50-126	
n=	18	20	17	20	19	19	21

Serum creatinine at baseline, first- and second-line ART failure (Normal range: 49-90 µmol/L)

Patient number	Baseline	First-line failure	TDF used	Second-line failure	TDF used
1	N/A	72	Yes	70	No
2	80	98	Yes	59	No
3	N/A	N/A	No	N/A	Yes
4	N/A	65	N/A	84	Yes
5	N/A	108	Yes	124	No
6	N/A	62	Yes	58	Yes
7	75	80	No	69	Yes
8	N/A	N/A	No	86	No
9	N/A	N/A	N/A	71	No
10	72	84	No	70	No
11	51	48	No	58	No
12	91	N/A	N/A	N/A	Yes
13	N/A	62	Yes	68	Yes
14	N/A	N/A	N/A	65	No
15	33	97	Yes	88	No
16	N/A	N/A	No	109	Yes
17	85	82	No	N/A	Yes
18	N/A	N/A	No	76	Yes
19	71	54	N/A	70	No
20	43	36	Yes	155	No
21	N/A	176	No	97	Yes
Average	66.77	80.28		82.05	
SD	19.87	34.19	Yes= 7	25.46	Yes= 10
Median	72	76	No= 9	70.50	No= 11
IQR (25th-75th)	58-80	62-93.75	N/A= 5	68.25-87.50	
n=	9	14		18	

Alanine transferase and total cholesterol at baseline, first- and second-line ART failure (Normal ranges: 7-35 μ L & <5.2mmol/L)

Patient number	Baseline		First-line failure		Second-line failure	
	ALT	Total cholesterol	ALT	Total cholesterol	ALT	Total cholesterol
1	N/A	N/A	N/A	N/A	13	4.88
2	N/A	N/A	44	N/A	26	3.88
3	N/A	N/A	N/A	N/A	21	3.93
4	129	N/A	109	N/A	120	2.82
5	N/A	N/A	16	N/A	16	N/A
6	N/A	N/A	22	N/A	8	3.24
7	N/A	N/A	N/A	N/A	13	2.81
8	N/A	N/A	N/A	N/A	14	3.03
9	N/A	N/A	N/A	N/A	8	3.56
10	N/A	N/A	N/A	N/A	N/A	N/A
11	N/A	N/A	N/A	N/A	31	4.33
12	N/A	N/A	N/A	N/A	21	N/A
13	N/A	N/A	N/A	N/A	N/A	N/A
14	N/A	N/A	N/A	N/A	N/A	N/A
15	N/A	N/A	42	N/A	N/A	4.93
16	N/A	N/A	N/A	N/A	11	N/A
17	N/A	N/A	N/A	N/A	17	3.86
18	N/A	N/A	N/A	N/A	9	3.54
19	N/A	N/A	N/A	N/A	N/A	N/A
20	11	N/A	23	N/A	18	N/A
21	N/A	N/A	N/A	N/A	N/A	N/A
Average	70	N/A	42.66	N/A	23.06	3.73
SD	83.43	N/A	34.43	N/A	27.60	0.71
Median	70	N/A	32.50	N/A	16	3.71
IQR (25 th -75 th)	40.50-99.50	N/A	22.25-43.50	N/A	12-21	3.18-4.03
n=	2	0	6	0	15	12

Haemoglobin and MCV at baseline, first- and second-line ART failure (Normal ranges: 12.1-16.3 g/dL and 83.0-101.0 fL)

Patient number	Baseline		First-line failure		AZT used	Second-line failure		AZT used
	Haemoglobin	MCV	Haemoglobin	MCV		Haemoglobin	MCV	
1	12.2	91.6	N/A	N/A	No	14.3	99.7	No
2	N/A	N/A	13.9	89.1	No	11.6	96.8	Yes
3	13.6	83	13.3	108.2	No	12.5	92.8	No
4	N/A	N/A	12.4	93.3	N/A	14.7	105.1	No
5	N/A	N/A	12.4	85.7	No	14.5	92.2	No
6	9.9	79	N/A	N/A	No	11.8	94.9	No
7	11.9	84.4	N/A	N/A	Yes	11	94.7	No
8	N/A	N/A	N/A	N/A	No	12	103.1	Yes
9	N/A	N/A	N/A	N/A	Yes	11	119.3	Yes
10	11.7	78.6	10.5	92	No	8.7	89.2	Yes
11	9.8	86.8	N/A	N/A	No	14.6	106.7	Yes
12	10.6	89.1	N/A	N/A	Yes	N/A	N/A	Yes
13	N/A	N/A	7.2	59.6	No	13	88.5	No
14	N/A	N/A	N/A	N/A	N/A	9.7	101.2	N/A
15	11.48	75	10.9	86.3	No	12.7	98	Yes
16	N/A	N/A	N/A	N/A	No	5.8	98.3	Yes
17	7.4	85.6	11.5	105.2	No	16.6	96.9	No
18	N/A	N/A	N/A	N/A	No	10.6	92.8	Yes
19	13	85.7	N/A	N/A	No	11.6	95	Yes
20	12	88.9	10.8	89.7	No	7.6	102.5	Yes
21	N/A	N/A	7.8	92.7	No	11.8	98.1	Yes
Average	11.23	84.33	11.07	90.18		11.80	98.29	
SD	1.72	5.06	2.18	13.08	Yes= 3	2.57	6.97	Yes= 12
Median	11.7	85.6	11.2	90.85	No= 16	11.8	97.45	No= 8
IQR (25 th -75 th)	10.25-12.1	81-87.85	10.57-12.40	87-93.15	N/A= 2	10.9-13.32	94.22-101.5	N/A= 1
n=	11	11	10	10		20	20	

WHO clinical staging, Hospitalisation, TB co-infection and other Opportunistic infections

Patient number	WHO clinical staging	Hospitalisation	Reason	TB infection	Other Opportunistic infections
1	No	No	N/A	No	N/A
2	No	No	N/A	No	N/A
3	No	No	N/A	No	Genital Herpes
4	No	Yes	Acute gastroenteritis	Yes	N/A
5	No	Yes	Cataract removal	Yes	Hepatitis B
6	No	Yes	Pneumonia	Yes	Herpes Zoster & Oral Candidiasis
7	No	No	N/A	No	Genital Herpes
8	No	No	N/A	No	N/A
9	No	No	N/A	No	N/A
10	No	Yes	Pneumonia	No	N/A
11	No	No	N/A	Yes	Viral Hepatitis
12	No	No	N/A	No	N/A
13	No	Yes	Epilepsy and Meningital TB	Yes	N/A
14	No	N/A	N/A	No	N/A
15	No	Yes	Pulmonary TB and Acute gastroenteritis	Yes	N/A
16	No	No	N/A	No	N/A
17	No	Yes	Extra pulmonary TB	Yes	N/A
18	No	No	N/A	No	Herpes Zoster & Tinea Corporis
19	No	Yes	Multi-drug resistant TB complications	Yes	N/A
20	No	No	N/A	No	Genital Herpes
21	No	No	N/A	No	N/A
n=	21	20	8	21	7

Addendum H: Summary of changes in National ART guidelines

Addendum H contains a brief overview of changes made to the South African National ART guidelines for adolescents and adults from 2004 to 2015. This summary is an overview of changes in the following aspects:

- CD4 cell counts;
- First-line ART regimens;
- Second-line ART regimens;
- Third-line ART regimens.

This summary was adapted from Van Graan *et al.*, 2016 dissertation and contains information as found in NDoH, 2004, 2010, 2013, 2014 and 2015.

Year	CD4 cell count (cells/ μ L)	1 st line ART	2 nd line ART	3 rd line ART
2004	Start ART in all HIV positive patients with a CD <200 cells/ μ L, irrespective of WHO staging	<u>Woman with guaranteed contraception:</u> d4T+3TC+EFV <u>Woman with no guaranteed contraception:</u> d4T+3TC+NVP	AZT+3TC+LPV/r	TLART not implemented in public sector
2010	Start ART in all HIV positive patients with a CD <200 cells/ μ L, irrespective of WHO staging	<u>All new patients:</u> d4T+3TC/FTC+EFV or TDF+3TC/FTC+NVP	<u>Failing d4T based ART:</u> TDF+3TC/FTC+LPV/r <u>Failing TDF-based ART:</u> AZT+3TC+LPV/r	Specialist referral possible but not implemented due to modality issues
2013	Start ART in all HIV positive patients with a CD <350 cells/ μ L, irrespective of WHO staging	TDF+FTC/3TC+EFV (as fixed dose combination) <u>EFV contra-indicated:</u> TDF+FTC/3TC+NVP <u>TDF contra-indicated:</u> AZT+3TC+EFV/NVP <u>TDF & AZT contra-indicated:</u> d4T+3TC+EFV/NVP <u>TDF, AZT & d4T contra-indication:</u> ABC+3TC+EFV/NVP	<u>Failing TDF-based ART:</u> AZT+3TC+LPV/r <u>Failing d4T-based ART:</u> TDF+3TC/FTC+LPV/r	Specialist referral and genotype resistance testing with supervised care. TLART drugs used: raltegravir, darunavir and etravirine

Addendum H

		<u>Currently on d4T-based ART:</u> TDF+FTC/3TC+EFV		
2014	Start ART in all HIV positive patients with a CD <500 cells/μL, irrespective of WHO staging, Prioritise those with CD4 <350 cells/μL	TDF + 3TC/ FTC + EFV (FDC) No patient must be on d4T (phased out) <u>Contraindication to EFV:</u> TDF + FTC/ 3TC + NVP or LPV/r <u>TDF contraindication:</u> Creatinine clearance of <50 mL/min ABC+ 3TC + EFV/NVP	<u>Failing: TDF-based first-line regimen:</u> AZT+3TC+LPV/r Or TDF + 3TC + LPV/r (If HBV co-infected) Failing: d4T or AZT-based first line regimen: TDF + 3TC/ FTC + LPV/r	Expert consultation with genotypic resistance testing and supervised care
2015	Guideline were revised in April 2015 with changes only made to ART regimens for pregnant and breastfeeding woman			