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Declaration:

I, Daniel Brozin, do hereby declare that this research report is my unaided work.

It is being submitted for the degree of Master of Medicine in the branch of Internal Medicine. This research report is submitted in the publishable format as recognized by the Faculty of Health Sciences. I further declare that this work has not been submitted for any other examination or degree at this or any other University.

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Signed on the 23 January 2024

Dedication:

This research paper is dedicated to the unwavering support of my family. My amazing wife Sarah, our boys Jo, Benny and Davey, our parents Robbie, Lee, Mark and Andy, and our dog Liz Lemon.

Publications and presentations arising from this research report:

The author intends to publish this research report in a reputable, peer-reviewed journal, currently being prepared for the South African Journal of Infectious Diseases

Ethical considerations:

Permission for this study was granted before data collection commenced by Dr. JML Tsitsi

(Head of Department Internal Medicine, Chris Hani Baragwanath Academic Hospital), the Medical Advisory Committee (MAC), and hospital (see Appendix) management at this institution as well as the Human Research Ethics Committee (Medical) at the University of the Witwatersrand HREC Ref No:M23/09/88. National Research Database Reference Number. *GP_202308_092*

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Thank you to the patients for allowing us to utilize their information for research.

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CHAPTER 1: Protocol and Extended Literature Review

CHAPTER 1

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A Review of HIV-positive Patients at Chris Hani Baragwanath Academic Hospital on Third line Antiretroviral Therapy

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Literature Review

Treatment failure on a second line regime: an emerging challenge

In South Africa's battle against the Human Immunodeficiency Virus/Acquired Immune Deficiency Virus **(**HIV/AIDS) epidemic, the ability of patients to readily access antiretroviral therapy (ART), has led to a significant reduction in mortality due to AIDS associated diseases.

As per the Joint United Nations Program on HIV/AIDS (UNAIDS), there are approximately 7.5 million people in Sub-Saharan Africa with HIV/AIDS. Of this subset, approximately 7 million people are aware of their status, with 5.5 million people currently on ART, and approximately 5 million people with an undetectable viral load. As we have more patients who are on first-line ART for a longer period, we are experiencing an increasing rate of treatment failure, with patents having to undergo regime switches, often to Protease Inhibitor (PI) based second line regimens, As a result of more patients being on PI-based regimens for longer periods of time, a subset of patients with virological failure on second-line treatment has emerged. These patients require more costly and clinically challenging third-line therapy regimens (1) . As one spends a longer period of time on a particular regime, in the setting of non-compliance, which will create a 'non-suppressed state' or low level viraemia, the inevitability of an accumulation of mutations to standard ART will increase. Studies done investigating the reasons for treatment failure in the South African context have provided many explanations concerning the above-mentioned issue.

A study done by Onoya et al (2) has identified that the primary reason for patients failing a particular ART regime is more than likely poor patient adherence to medication, and not necessarily the presence of significant mutations against current ART, echoing the findings of previous studies done in Sub-Saharan Africa, (SSA) highlighting the effect that non compliance to treatment has had on treatment failure and the subsequent necessity to change regimes. It is also noted that factors such as younger age, a lower socio economic status (SES), unemployment and the perceived stigmatization of HIV/AIDS, play a smaller but significant role in patients failing a treatment regime .

Baseline Resistance Profiles: An African Perspective

Multiple studies were done throughout the African continent investigating resistance profiles in patients failing ART regimes. A study by Kukabu *et al.* (3) analyzing patients with virological failure in Namibia, brought to light the issue of patients not disclosing prior diagnosis and/or ART initiation, and therefore being misidentified as 'treatment naïve' and thus treated ' appropriately ', according to local guidelines." The reasons for this non-

disclosure of medical information were myriad, with stigmatization, religious beliefs, and complex psycho-social issues involved (4). Based on this study, a treatment option used in Namibia in dealing with this patient subset was to aggressively switch failing patients to a third-line regime, as drug resistance test (DRT) kits were often unavailable.

Nanfack et al (5) investigated drug susceptibility in HIV-positive patients living in Cameroon. This study revealed a drug resistance mutation rate of up to 91.3% in patients on second-line PI therapy. The outcome of this study gave further evidence to support the knowledge that as the duration of PI exposure increases drug resistance will subsequently increase. Thus, time on a PI-based regime correlated with the chance of failure on that regimen.

Data from Uganda has revealed that in a population studied with virological failure, not only were the more common Nucleoside Reverse Transcriptase Inhibitor (NRTI) and non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) mutations present, but as more patients were enrolled onto a PI-based , 2^{nd} line treatment regime, a prevalence of up to 30% of protease inhibitor mutational changes was noted (6).

When looking into resistance patterns of South African patients failing second line ART , it is noted that PI resistance is not often the major obstacle in treatment failure , rather it is driven by patient non-compliance, this issue will unfortunately lead to ‘ accumulation of mutations ‘ (7) as more and more patients are exposed to second line treatment. As noted in other SSA countries in which HIV subtype C is the most commonly found genotype , most patients are found to have minor PI mutations , which if left to accumulation , will only result in PI resistance a significant duration of time after initiation .

<u>Clinical and laboratory reviews of patients on third line treatment</u>

In South Africa, it is estimated that ten percent of patients per annum on first-line ART will experience treatment failure and require a subsequent switch to second-line ART (8) . As previously described, this will result in an increased number of patients requiring third-line ART, should their second line treatment fail.

Medications currently used in third-line treatment regimens include etravirine (ETR) , boosted darunavir (DRV) and dolutegravir (DTG) (9). Third line regimens are designed individually, based on the patient’s individual drug resistance profile.

Data emerging from both South Africa and abroad have shown promising results about the utilization of third-line ART as salvage therapy (10). Chimbele *et al*. (11) provided significant insight into the outcomes of patients on third-line ART living in Zimbabwe. Results of this study revealed equivocal outcomes concerning a reduction of HIV viral load to studies done internationally, despite limited access to drug resistance testing in the public sector patient subset, as compared to those with private health care resources.

A study by Meintjies *et al.* (12) evaluating the role of salvage third-line ART therapy with regard to patient viraemia, within the privately funded healthcare sector in South Africa, noted a virological suppression rate of 87 %. These outcomes will likely be a representation similar to that of the public health care system, as medications used in third line regimes are becoming more analogous as drug availability expands. A paragon for the previously stated was noted in a cohort studied from Helen Joseph Hospital, a public sector hospital in Johannesburg South Africa, showed a viral load suppression rate (below 400 copies per milliliter) at a one year follow up of 83% in patients on salvage third-line treatment (1). These results mirror those found in the literature from other developing countries, such as those done in Brazil, with a trial consisting of 221 patients and Mexico , with a trial consisting of 219 patients (13).

This study is hoping to improve the knowledge gap with regard to third-line treatment of HIV in the South Africa context.

Study Aims and Objectives

Study aim

This study aims to document the patterns of baseline antiretroviral treatment and resistance of a patient at the time of third-line application, as well as viral suppression outcomes, to contribute to knowledge around the efficacy of these treatments.

Study objectives

1. To describe the patient cohort of patients on third-line Antiretroviral Therapy at the CHBAH ART clinic

2. To describe baseline CD_4 and Viral Load patterns before starting third-line ART, as well as after starting third-line ART to document rates of virological suppression 3. To assess compliance to third-line ART by reviewing medication collection from pharmacy, where collection of script is a surrogate for compliance
4. To describe the baseline resistance profile of patients in our dataset. 5. To describe retention in care of patients on third-line ART (this is assessed as documentation of clinic visit at three months and six months

Methodology

Study design, setting and population

A retrospective, descriptive analysis of all adult patients attending the Chris Hani Baragwanath Academic Hospital HIV clinic currently on a third-line ARV treatment regime. No time frame will be defined, as every patient transitioned to third-line ART at CHBAH will be included. Data will be collected for the period of ten years, starting from 1st July 2013 to 30th June 2023. Our sample size estimate is 200 patients. Patients will be identified using the third-line clinic database, which includes patient name, hospital number and therapy. Once identifying the patients for inclusion, the files will be extracted from Nthabiseng clinic files.

Inclusion criteria:
Patients over 18 years of age
Ever been on third-line ART

Exclusion criteria:
Baseline resistance profiles unobtainable

Data Collection

Data will be collected using Microsoft Excel, and will be collected from the database of the ART clinic.

Data Analysis

Descriptive statistics for continuous variables will be presented as mean ± standard deviation or median and interquartile ranges for normal or skewed data respectively.

Categorical data will be reported using proportions. Categorical variables will be compared between groups using the X2 test. Continuous variables will be compared between groups using Students unpaired t-test, if normally distributed and the Mann Whitney U test if skewed.

Univariate and Multivariate regression analysis will be the principle method used to determine the factors associated with ADR and resistance profiles.

Ethical approval

The WITS HREC will be approached to obtain ethics approval for this study. Permission to access and utilise the data will be sought from both Chris Hani Baragwanath Academic Hospital and the Division of Internal Medicine, and the head of Infectious Diseases. Patient identification details will not be included on the datasheet, ensuring patient anonymity. All patient data will be on a highly secure password-protected single-user laptop computer. Only the primary author and supervisors will have access to patient data.

Limitations

Retrospective nature therefore data is limited to what has been documented in source notes. Any missing files , or files with critical clinical information missing, will not be used in our study.

Funding

This study will require minimal funding and will include stationary, which will be covered by the student. No additional funding from the hospital will be required.

Timing

	Jan 2023	Feb 2023	March 2022	April 2023	May 2022	June 2023	July 2023	Aug 2023	Sept 2023	Dec 2023	Jan 2024	Feb 2024	Mach 2024	April 2024	Aug 2024
Literature Review															
Protocol															
Protocol Assessment															

Ethics application															
Data Collection															
Data analysis															
Write up thesis															
Submission															

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Extended literature review:

The widespread availability of antiretroviral therapy (ART) in the Republic of South Africa (RSA) has led to a significant reduction in mortality due to Human Immunodeficiency Virus/Acquired Immune-deficiency Syndrome (HIV/AIDS) related disease. Due to various factors, both patient- and disease-related, treatment failure on first-line ART, and subsequent switching to a second-line ART, is becoming a more prevalent occurrence (1). This in turn increases use of third-line ART (TLART) in the management of HIV/AIDS.

As per the Joint United Nations Program on HIV-AIDS (UNAIDS) there are approximately 7.5 million people in Sub-Saharan Africa (SSA) with HIV-AIDS. Of this subset, approximately 7 million people are aware of their status, with 5.5 million people currently on ART, and approximately 5 million people with an undetectable viral load (VL) (2). As more patients are on first-line ART for a longer period, RSA is experiencing an increased rate of treatment failure, whereby patients have to undergo regime switches, often to Protease Inhibitor (PI) based second-line regimes. As a result of more patients on PI-based regiments for longer periods of time, a subset of patients with virological failure on second-line treatment has emerged. These patients require costly and clinically challenging third-line therapy regimes (1). As one spends a longer period of time on a particular regime, in the setting of non-compliance, with a median time of 17 months on treatment, which will create a 'non-suppressed state' or low-level viraemia, the inevitability of an accumulation of mutations to standard ART will increase. Studies performed investigating the reasons for treatment failure in the South African context have provided many explanations concerning the above-mentioned issue, including age, gender and socioeconomic status (3).

In RSA, of the 5.5 million patients on ART, it is estimated that around 3.4 million patients are on first-line therapy, 1.5 million patients are on second-line treatment, and the remainder on TLART (4). Data suggests that patients on second-line have one-year failure rate of up to 25% on their new regime (5). This is due to multiple factors, with the most likely being repeat nonadherence to medication and a collection of mutations precluding them from second-line therapy (5). This will invariably lead to more patients requiring salvage TLART, which will cost an estimated R16 846 per patient, as opposed to the R1 649 for the commonly used first-line ART regime in RSA as at end of 2023 (6).

As RSA is one of the only SSA countries to provide TLART, and at a substantial cost to the

healthcare system, a rigorous inclusion criterion has been put in place to ensure that the patients are put onto TLART are optimized (1). Our current policy, as per the South African HIV Clinicians Society, for a patient to qualify for TLART requires the patient to meet the following criterion (7): firstly, for patients on second-line ART for >2years with 2 or 3 VL readings of >1000 copies within a 6 month period, despite satisfactory adherence counselling, a genotypic drug resistance test must be performed; secondly, documented PI resistance should be present before switching to TLART; and thirdly, these patients will then undergo an intensive review by a panel of experts in order to ascertain the need and required drug regime for the patient (6). Our current third-line medications available in the public sector are darunavir (DRV), dolutegravir (DTG), raltegravir (RAL) and etravirine (ETR).

A major issue in the battle against HIV/AIDS in RSA is patient compliance to medication. When dealing with HIV/AIDS, a compliance rate of >90% is required to obtain long term viral suppression (8). If adequate compliance goals are not maintained, not only are both morbidity and mortality due to HIV/AIDS related causes increase, but a significant rise in viral mutations and drug resistance is noted (9).

Malassiata *et al.* (10) have shown that as ART drug regimes become more complex with regard to dosing schedules, adherence to the medication regime declines, with patients receiving up to four different medications at a staggered dosing schedule. This is a cogent issue regarding TLART, as patients are required to utilize more intricate dosing schedules (11). A method that has shown success in countering this problem inherent to TLART is intensive patient counselling, both prior and post initiation of treatment (3). Studies performed in the Western Cape (12) showed that of all patients on second-line ART with an unsuppressed viraemia, 68% of patients were able to avoid TLART and become ‘re suppressed’ on their current regime with adherence counselling used as the only intervention.

As per Onoya *et al.* (13), a fundamental cornerstone to the development of ART regime failure may be based on poor patient compliance to a given treatment schedule. A critical component to providing our patients with a full bouquet of clinical services, is the availability of dedicated TLART counsellors to aid in patient understanding of what may oft times be a complicated drug regime. Whether or not a patient in our cohort received the above counselling will be a critical data point in our study.

Studies from SSA including RSA and Zimbabwe (14) have shown promise regarding both a

reduced viraemia, and improved CD_4 count, in patients on salvage third-line ART regimes. In our cohort we will describe baseline CD_4 and VL patterns before TLART initiation, as well as three months and six months post TLART initiation, to document rates of virological suppression and immune reconstitution.

Multiple studies throughout the African continent have investigated resistance profiles in patients failing ART regimes (15) as one of the potential factors in virological failure. As we have reviewed the resistance profiles of all patients in our dataset, our cohort will provide a South African visage to this evolving problem.

Drug resistance plays a significant role on the impact of patients maintaining a lower than detectable viral load, despite adequate compliance (16). This will result in an increased morbidity and mortality from HIV/AIDS related causes and impact our gains in the battle with this virus (17).

Drug resistance in RSA is usually to those in the non nucleoside reverse transcriptase inhibitors (NNRTI) and nucleoside reverse transcriptase inhibitor class (NRTI) as these are commonly used within our first-line ART regime (18). These studies also noted a high rate of thymidine synthase mutations (TAM) in patients exposed to zidovudine (AZT), which may lead to cross mutations in other NRTI drugs (18).

With regard to PI resistance in RSA, levels are lower than those of NRTI/NNRTI (19), which may be due to the fact that these medications are prescribed less, as they make up the backbone of second-line therapy. It is also noted that PI drugs have a higher genetic barrier to resistance than NNRTI and NNRTI drugs (20).

An interesting phenomenon to note, is that compared to PIs, NNRTI and NRTI have a more significant adherence-resistance relationship (21). This means that as adherence to these medications wanes, drug resistance will increase and mutations will accumulate. In contrast, PI mutations are seen at moderate and high adherence levels (22), which may be due to their inherent genetic barrier to resistance.

It has been shown in RSA based studies that a crucial factor in HIV drug resistance, are the relative lack of HIV drug resistance in ART naïve patients as opposed to those patients

‘pretreated’ with an NRTI or NNRTI based regime (16). What can be inferred from this is the importance of patient compliance to treatment and retention of patient care.

Our studies investigated the drug resistance profile of our CHBAH TLART cohort, as well as any previous ART exposures of our patients.

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CHAPTER 2: Proposed Manuscript

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A Review of HIV-positive Patients at Chris Hani Baragwanath Academic Hospital on Third-line Antiretroviral Therapy

Abstract

Introduction: A review of patient characteristics on Third Line Antiretroviral Therapy (TLART) at Chris Hani Baragwaneth Academic Hospital (CHBAH).

Study aim

The aim of this study is to conduct a review of patients on third line antiretroviral therapy in a tertiary hospital in Soweto, South Africa.

Study objectives

1. To describe the patient cohort of patients on third-line Antiretroviral Therapy at the CHBAH ART clinic
2. To describe baseline CD_4 and Viral Load patterns before starting third-line ART, as well as after starting third-line ART to document rates of virological suppression
3. To assess compliance to third-line ART by reviewing medication collection from pharmacy, where collection of script is a surrogate for compliance
4. To describe the baseline resistance profile of patients in our dataset.
5. To describe retention in care of patients on third-line ART (this is assessed as documentation of clinic visit at three months and six months

Methods:. A retrospective, inferential analysis of adult patients attending the TLART clinic, Chris Hani Baragwanath Academic Hospital

Results: All 172 patients on third-line ART at CHBAH were included in this study. Two thirds were female. The median age was 47.9 years [46.2-49.5]. Half of the cohort had previous opportunistic infections (54.2%). There was a high attendance at clinic three months post TLART initiation (95.9%). Evidence of prescription refill from pharmacy at one month post TLART initiation was also high (163/172, 95.9%). The mean baseline (prior to TLART initiation) CD_4 count was 264.2 (216.8) cells/mm^3, and median baseline viral load (VL) was 26500 copies/million (3750-127000). At three months post TLART initiation, the mean CD_4 was 322.0 (211.9) cells/mm^3, and at 6 months 367.8 (222.6) cells/mm^3, with the median VL at three months post TLART initiation of 151 (27.5-1110) copies/million, and the median VL at 6 months being 59

(19-265) copies/million. The majority of the cohort had resistance to lamivudine (3TC)

(81%), 35.5% to tenofivir (TDF),75.2% to emtricitanine (FTC) and 88% to abacavir (ABC). The resistance to nucleoside reverse transcriptase inhibitors (NRTI) was as high as 81.3 % and lopinavir (LPV) resistance was 81.3%. Darunivir (DRV) resistance was noted in 34.2% of patients in our cohort.

Conclusion: Our study showed a robust reduction in patients VL, and an improved CD_4 count at different time intervals post TLART initiation. Resistance profiles gleaned from this data set were in keeping with ARV drug resistance throughout Sub Saharan Africa.

Key Words:

Human Immunodeficiency Virus (HIV)

Antiretroviral Treatment Failure

Third-line ART

South Africa

Resistance profile

Submissible article:

Background:

The widespread availability of antiretroviral therapy (ART) in the Republic of South Africa (RSA) has led to a significant reduction in mortality due to Human Immunodeficiency virus /Acquired immune-deficiency Syndrome (HIV/AIDS) related disease. Due to various factors, both patient- and disease-related treatment failure on first-line ART, and subsequent switching to a second-line ART is becoming a more prevalent occurrence (1). This in turn increases use of third-line ART(TLART) in the management of HIV/AIDS.

As per the Joint United Nations Program on HIV-AIDS (UNAIDS) there are approximately 7.5 million people in Sub-Saharan Africa with HIV-AIDS. Of this subset, approximately 7 million people are aware of their status, with 5.5 million people currently on ART, and approximately 5 million people with an undetectable viral load (VL)(2). As more patients are on first-line ART for a longer period, RSA is experiencing an increased rate of treatment failure, whereby patients have to undergo regime switches, often to Protease Inhibitor (PI)

based second-line regimes. As a result of more patients on PI-based regiments for longer periods of time, a subset of patients with virological failure on second-line treatment has

emerged. These patients require costly and clinically challenging third-line therapy regimes (1). As one spends a longer period of time on a particular regime, in the setting of non compliance, which will create a 'non-suppressed state' or low-level viraemia, the inevitability of an accumulation of mutations to standard ART will increase.

In RSA it is estimated that around half a million patients are on TLART (4). At the time of writing, TLART has an estimated cost of R16 846 per patient, as opposed to the R1 649 for the commonly used first-line ART regime in RSA (6).

As RSA is one of the only SSA countries to provide TLART, and at a substantial cost to the healthcare system, a rigorous inclusion criterion has been put in place to ensure that the patients are put onto TLART are optimized (1). Our current policy, as per the South African HIV Clinicians Society, for a patient to qualify for TLART requires the patient to meet the following criterion (7):firstly, for patients on second-line ART for > 2years with 2 or 3 VL readings of >1000 copies within a 6 month period, despite satisfactory adherence counselling, a genotypic drug resistance test must be performed; secondly, documented PI resistance should be present before switching to TLART; and thirdly, these patients will then undergo an intensive review by a panel of experts in order to ascertain the need and required drug regime for the patient (6). Our current third-line medications available in the public sector are darunavir (DRV), dolutegravir (DTG), raltegravir (RAL) and etravirine (ETR).

A major issue in the battle against HIV/AIDS in RSA is patient compliance to medication. When dealing with HIV/AIDS, a compliance rate of >90% is required to obtain long term viral suppression (8). If adequate compliance goals are not maintained, not only are both morbidity and mortality due to HIV/AIDS related causes increase, but a significant rise in viral mutations and drug resistance is noted (9).

As per Onoya *et al.* (13), a fundamental cornerstone to the development of ART regime failure may be based on poor patient compliance to a given treatment schedule. A critical component to providing our patients with a full bouquet of clinical services, is the availability of dedicated TLART counsellors to aid in patient understanding of what may oft times be a

complicated drug regime. Whether or not a patient in our cohort received the above counselling will be a critical data point in our study.

Studies from SSA including RSA and Zimbabwe (14) have shown promise regarding both a reduced viraemia, and improved CD_4 count, in patients on salvage third-line ART regimes. Multiple studies throughout the African continent have investigated resistance profiles in patients failing ART regimes (15) as one of the potential factors in virological failure. Drug resistance in RSA is usually to those in the NNRTI and NRTI class, as these are commonly used within our first-line ART regime ((18), and lower levels of resistance to PI than NRTI/NNRTI (19).

Our studies investigated the drug resistance profile of our CHBAH TLART cohort, as well as any previous ART exposures of our patients.

Study objectives

1. To describe the patient cohort of patients on third-line Antiretroviral Therapy at the CHBAH ART clinic
2. To describe baseline CD_4 and Viral Load patterns before starting third-line ART, as well as after starting third-line ART to document rates of virological suppression 3. To assess compliance to third-line ART by reviewing medication collection from pharmacy, where collection of script is a surrogate for compliance
4. To describe the baseline resistance profile of patients in our dataset. 5. To describe retention in care of patients on third-line ART (this is assessed as documentation of clinic visit at three months and six months

Methods

A retrospective, descriptive analysis of all adult patients attending the Chris Hani Baragwanath Academic Hospital HIV clinic, in Soweto, South Africa, currently on a

TLART. All patients who transitioned to third-line ART at CHBAH were included. Data was collected for the period of ten years, starting from 1st July 2013 to 30th June 2023. Our sample size was 172 patients. Patients were identified using the third-line clinic database, which

included patient name, hospital number and therapy. Once identified for inclusion, the files were extracted from Nthabiseng clinic archives. The inclusion criteria consisted of patients over the age of 18 on TLART, and we excluded any patients for whom baseline resistance profiles were unobtainable. Patients within this cohort had qualified for TLART via the National TLART committee, patients still awaiting authorization to initiate TLART were excluded from this study.

Outcome measures

The primary aim was to review the patients on TLART, with specific attention to changes in CD_4 count and viral load (VL), compliance to medication, as assessed by prescription refills reported by the TLART pharmacy, presence of previous opportunistic infections (OI) and retention of care, specifically at three and six months post initiation of TLART.

Data analysis

Data was analyzed using Stata 16.1 (Statcorp, Texas, USA). Descriptive statistics for continuous variables were presented as means (standard deviations) or medians (interquartile ranges) for normal or skewed data respectively. Categorical data were reported using proportions. Univariate and regression models were generated using age, sex, baseline CD_4 count, baseline VL and previous OI as predictor variables for resistance to the various ARVs. Where age and baseline CD_4 count were statistically significant, they were categorized by decade and $CD_4 \leq$ 200 copies/ml and $CD_4 >$ 200 copies/ml respectively to further analyse which associations were significant. Multivariate analyses were then conducted to ascertain if these associations were still significant. Receiver operating characteristic (ROC) curves were then used to assess the performance of the multivariate models.

Categorical variables were compared between groups using the X^2 test. Continuous variables were compared between groups using Students Unpaired t-test, if normally distributed, and the Mann Whitney U test if skewed. Univariate and Multivariate regression analysis were the principal method used to determine the factors associated with resistance profiles.

Ethical Approval

The Human Research Ethics Committee, University of Witwatersrand was approached to obtain ethics approval for this study (M23/09/88). Permission to access and utilize the data were sought from Chris Hani Baragwanath Academic Hospital, the Division of Internal

Medicine, as well as the head of Infectious Diseases. Patient identification details were not included on the datasheet, ensuring patient anonymity. All patient data were on a highly secure password-protected single-user laptop computer. Only the primary author and supervisors had access to patient data. The research has been registered on the National Health Research Database, *GP_202308_092*.

Results

Patient Demographics and baseline data

Within our dataset of 172 patients on TLART, 114 (61.3%) were female, as noted in Table 1. The mean (SD) [95% CI] age of our patients was 47.9 (11.4) years old. Median (IQR) time since HIV diagnosis was 14.1 (10.4-16.5) years and mean (SD) [95%CI] time on TLART was 4.3 (2.9) years. In our dataset, 91 (54.2%) of patients had experienced previous opportunistic infections, which included previous meningitis and/or lung disease. Most, 163 (95.9%) of the patients in the dataset had evidence of clinic attendance three months post initiation of TLART, with 162 (95.9%) patients showing evidence of prescription refill from pharmacy post TLART, both measures used as surrogates for compliance.

Virological/immunological outcome data

The mean baseline (prior to TLART initiation) CD_4 count for patients in our study was 264.2 (SD 216.8) cells/mm^3, and the median (IQR) baseline VL (prior to TLART) was 26500 copies/million [3750-127000], as seen in table 1. At three months post TLART, the mean CD_4 count was 322 (211.9) cells/mm^3 and the median VL was 151 copies/million [277.5- 1110]. At six months post TLART the mean CD_4 count was 367.8 (222.6) cells/mm^3 and the median VL was 59 copies/million [19-265].

Medication Exposure and profile

The most used ARTs in our cohort prior to TLART were lamivudine (3TC) (96.5%), zidovudine (AZT) (79.5%), and combination Alluvia (LPV/r) 172 (100%) (as seen in table 2). This was followed by efavirenz (EFV) 122 (71.4%), emtracitibine (FTC) 63 (36.8%), abacavir (ABC) 52 (39.6%), with stavudine (D4T), atazanavir (ATZ), neviripine (NVP) and didanosine (DDI) used in <30% of our cohort. No patients were exposed to dolutegravir (DTG) as either first- or second-line therapy. With regard to the current regimes being utilized by our patients, 153 (88.9%) were on darunavir (DRV), with 7 (4.1%) on etravirine (ETR). The other drugs most frequently used as part of a combination third-line regime

were TDF in 169 (98.3%), ritinovir 163 (94.8%), DTG 105 (60.1%) and 3TC 110 (64.0%). AZT, ABC and LPV were used in <2% of patients as a third-line combination.

Resistance profile

In our dataset, resistance to NRTI, NNRTI and PIs were generally high. With regard to NRTIs, 3TC resistance was seen in 81% of patients, with resistance to TDF in 35.5% and FTC in 75.2%, 137 (88.4%) patients had resistance to ABC and 59 (47.2%) resistance to AZT, which is noted in table 2.

Resistance in our cohort to NNRTIs showed an 81.3% resistance to EFV, with 80% of the cohort resistant to NVP. Etravarine resistance was noted in 79 (59%) of our cohort. The resistance to the protease inhibitors LPV/ATZ and DRV were collected for our study. It showed that 128 (83.1%) showed resistance to ATZ, with 126 (81.3%) showing resistance to LPV. DRV resistance was noted in 53 (34.2%) of our patients. DTG and DDI resistance profiles were not available for this study.

Univariate Analysis

A univariate logistic regression analysis assessing predictors of resistance to various ARV drugs was performed on the dataset, noted in table 3. Using this model, the data suggests a slight protective association between baseline CD_4 count and resistance to ABC with an odds ratio (95% CI, p-value) of 0.998 (0.996-0.999, 0.034) and EFV 0.998 (0.996-0.999, p= 0.015). This analysis also revealed a possible link between previous OI conferring a potential protective benefit with regard to resistance to LPV odds ratio 0.4 (CI: 0.17-0.95, p=0.038) and ATZ 0.4 (CI: 0.16-0.98, p=0.045). This relationship may benefit from a review in a further publication.

Multivariate Analysis

This analysis suggests a possible protective relationship between age and resistance to DRV medication odds ratio (95%CI, p-value) 1.04 (1.005-1.07,0.022), as noted in table 5 . Further categorical analysis of this relationship did not show a statistically significant relationship when age ranges were divided into decades.

Table 1: Patient Demographics and Baseline Data

Gender: Female n (%)	114 (61.3)

Age (years)	47.9 (11.4)	
Time since HIV diagnosis, years	14.1 [10.4-16.5]	
Time on third line therapy, years	4.3 (2.9)	
Time since TLART initiation from initial HIV diagnosis, years	9.1 (5.6-12.9)	
Previous Opportunistic infection n (%)	91 (54.2)	
Attendance at clinic 3 months post TLART initiation n (%)	163 (95.9)	
Evidence of prescription refill from pharmacy 1 month post TLART initiation, n (%)	162 (95.9)	
Baseline CD_4 count (Prior to TLART)	264.2 (216.8)	
Baseline VL (prior to TLART)	26500 [3750-127000]	
CD_4 count post (TLART) cells/ml	3 months 322.0 (211.9)	6 months 367.8 (222.6)
VL post TLART copies/ml	3 months 151 [27.5-1110]	6 months 59 [19-265]

N number, SD standard deviation (), CI confidence interval, IQR interquartile range [],

Table 2: Proportion of patients with resistance to specific ARV drugs

Previous ART Exposure	3TC	ATZ	ABC	EFV	TDF	LPV	Rit	NVP	AZT	FTC	D4T
n (%)	166 (96.5)	35 (24)	52 (30.6)	122 (71.4)	129 (75)	172 (100)		19 (11.1)	136 (79.5)	63 (36.8)	50 (29.2)
Current ART regime n (%)	110 (64.0)	NA	1 (0.6)	NA	169 (98.3)	1 (0.6)	163 (94.8)	NA	3 (1.7)	NA	NA
Evidence of drug resistance	127 (81.4)	128 (83.1)	137 (88.4)	126 (81.3)	55 (35.5)	126 (81.3)	3 (9.4)	124 (80.0)	59 (47.2)	106 (75.2)	NA

3TC(Lamivudine,ATZ(atazanavir),ABC(abacavir)EFV(efavirenz),TDF(tenofivir).LPV(lopinavir),Rit(ritinovir)NVP(nevirapine),AZT(zidovudine),FTC(emtricitabine),D4T(stavudine),DDI(didanosine),DTG(dolutegra vir),ETR(etravirine),DRV(darunavir),n(number),ART(anti-retroviral),NA(Not apllicable).

Table 3: Univariate logistic regression analysis assessing predictors of resistance to various ARV drugs

Variable	**3TC**	**P value**	**ATZ**	**P value**	**ABC**	**P value**	**EFV**	**P value**	**LP**
Univariate	*Odds ratio (95%CI)*		*Odds ratio (95%CI)*		*Odds ratio (95%CI)*		*Odds ratio (95%CI)*		*Od rat (95%*
Age	0.97(0.93-1.00)	0.088	0.99(0.99-1.00)	0.735	0.96 (0.92-1.00)	0.095	0.98 (0.94-1.02)	0.284	0.9 (0.9 1.0

Gender	1.95 (0.87-4.4)	0.107	0.98 (0.41-2.31)	0.954	1.3 (0.49-3.5)	0.596	1.62 (0.72-3.66)	0.243	0.9 (0.4 2.2
Baseline CD_4	1.00 (0.98-1.00)	0.108	0.99 (0.99-1.00)	0.068	**0.998 (0.996-0.999)**	**0.034**	**0.998 (0.996-0.999)**	**0.015**	0.9 (0.9 1.0
Baseline VL	1.00(0.99-1.00)	0.185	0.99(0.99-1.00)	0.735	1.00 (0.99-1.00)	0.432	0.99 (0.99-1.00)	0.266	0.9 (0.9 1.0
Previous Opportunistic infection	0.84 (0.37-1.89)	0.671	**0.40 (0.16-0.98)**	**0.045**	0.82 (0.31-2.21)	0.701	2.18 (0.93-5.1)	0.072	**0.4 (0.1 0.9**

3TC(lamivudine),ATZ(atazanavir),ABC(abacavir),EFV(efavirenz),LPV(lopinavir),NVP(nevirapine),VL(viral load),CI(confidence interval)|

Table 4: Univariate logistic regression analysis assessing predictors of resistance to various ARV drugs

Variable	***Odds ratio (95%CI)***	**P value**	***Odds ratio (95%CI)***	**P value**	***Odds ratio (95%CI)***	**P value**	***Od rat (95)***
Multivariate	*3TC*		*ATZ*		*ABC*		*EF*
Age	0.98 (0.94-1.01)	0.224	0.99 (0.95-1.04)	0.866	0.97 (0.92-1.01)	0.183	0.9 (0. 1.0
Gender	1.62 (0.69-3.78)	0.263	0.78(0.31-1.98)	0.598	1.00 (0.35-2.87)	0.997	1.8 (0. 4.5

Baseline_CD4	0.99 (0.99-1.00)	0.154	**0.99 (0.996-0.999)**	**0.032**	**0.99 (0.996-0.999)**	**0.048**	**0.9 (0. 0.9**
Previous Opportunistic infections	0.78 (0.34-1.86)	0.602	**0.33 (0.13-0.86)**	**0.023**	0.71 (0.25-2.01)	0.521	1.9 (0. 4.8

CI(confidence interval),3TC(lamivudine),ATZ(atazanavir),ABC(abacavir)EFV(efavirenz),LPV(lopinavir)

Table 5: Multivariate logistic regression analysis assessing predictors of resistance to various ARV drugs

Variable	**TDF**	**P value**	**DRV**	**P value**	**FTC**	**P value**	**ET**
Univariate	*Odds ratio (95%CI)*		*Odds ratio (95%CI)*		*Odds ratio (95%CI)*		*Od ra (9*
Age	0.99 (0.96-1.02)	0.469	**1.04 (1.005-1.07)**	**0.025**	**0.95 (0.91-0.98)**	**0.006**	1.0 (0 1.0
Gender	1.16 (0.59-2.30)	0.657	0.74 (0.38-1.46	0.388	1.97 (0.91-4.28)	0.085	1.1 (0. 3.4
Baseline CD_4	0.99 (0.99-1.00)	0.419	0.99 (0.99-1.00)	0.252	0.99 (0.99-1.00)	0.334	0.9 (0 1.0
Baseline VL	1.00 (0.99-1.00)	0.234	1.00 (0.99-1.00)	0.789	1.00 (0.99-1.00)	0.342	0.9 (0 1.0

Previous Opportunistic infection	0.87 (0.45-1.69)	0.683	1.26 (0.64-2.45)	0.501	1.31 (0.60-2.84)	0.496	1.5 2.3

TDF(tenofivir),DRV(darunavir),FTC(emtricitabine),ETR(etravirine),AZT(zidovudine),CI(confidence interval),VL(viral load)

Table 6: Multivariate logistic regression analysis assessing predictors of resistance to various ARV drugs

DRV(darunavir),FTC(emtricitabine)CI(confidence interval)

Variable	*Odds ratio (95%CI)*	P value	*Odds ratio (95%CI)*	P value
Multivariate	*DRV*		*FTC*	
Age	**1.04 (1.01-1.08)**	**0.022**	**0.95 (0.91-0.99)**	**0.011**
Gender	0.91 (0.44-1.86)	0.793	1.65 (0.72-3.78)	0.235
Baseline CD_4	0.998 (0.996-1.00)	0.171	0.99(0.9 98- 1.00)	0.676
Previous Opportunistic infections	1.34 (0.66-2.70)	0.418	1.30 (0.57-2.94)	0.537

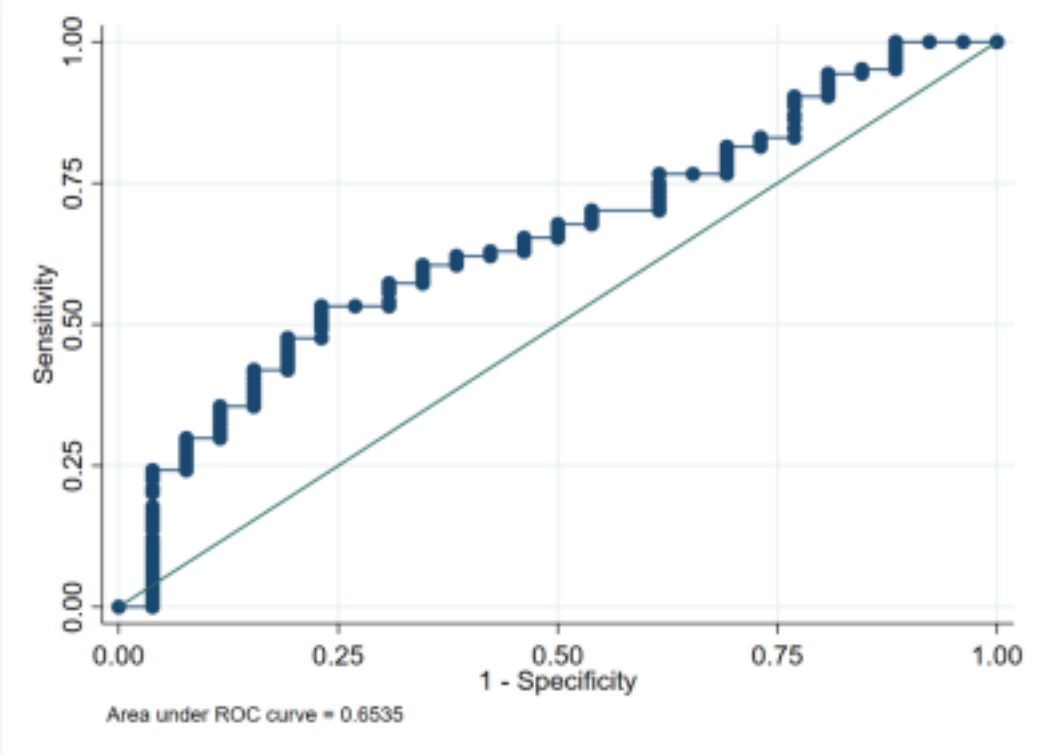

Figure 1: Receiver operating characteristic curve of the multivariate model for ATZ adjusting for age, gender, baseline CD4 and previous history of opportunistic infection

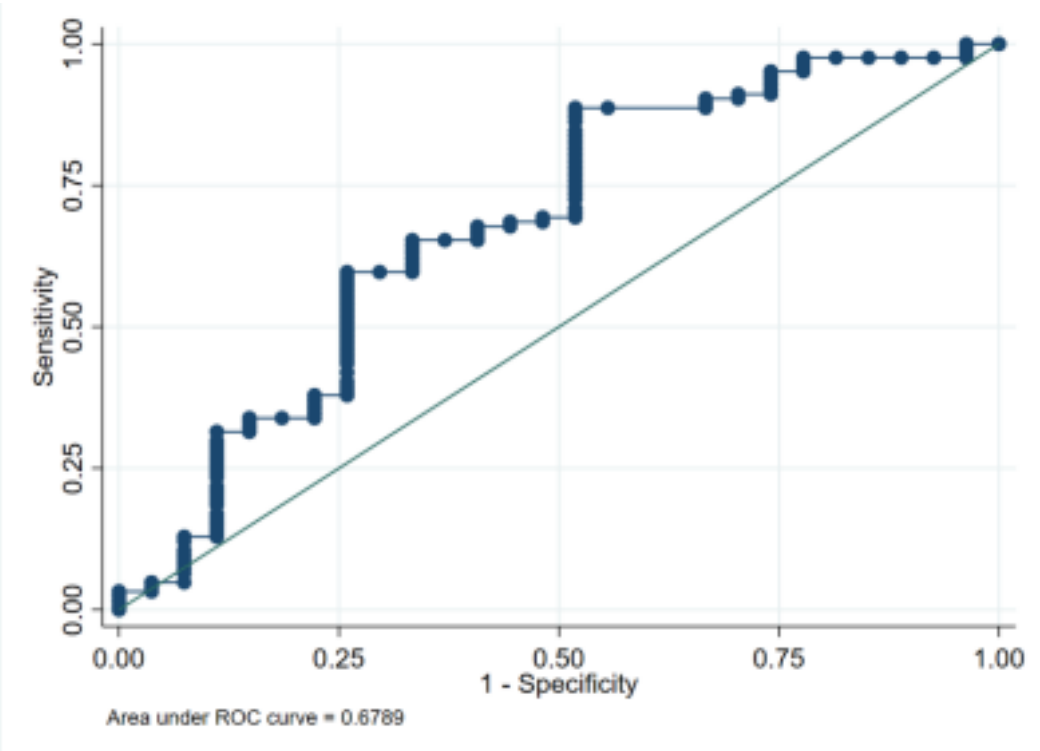

Figure 2: Receiver operating characteristic curve of the multivariate model for EFV adjusting for age, gender, baseline CD4 and previous history of opportunistic infection.

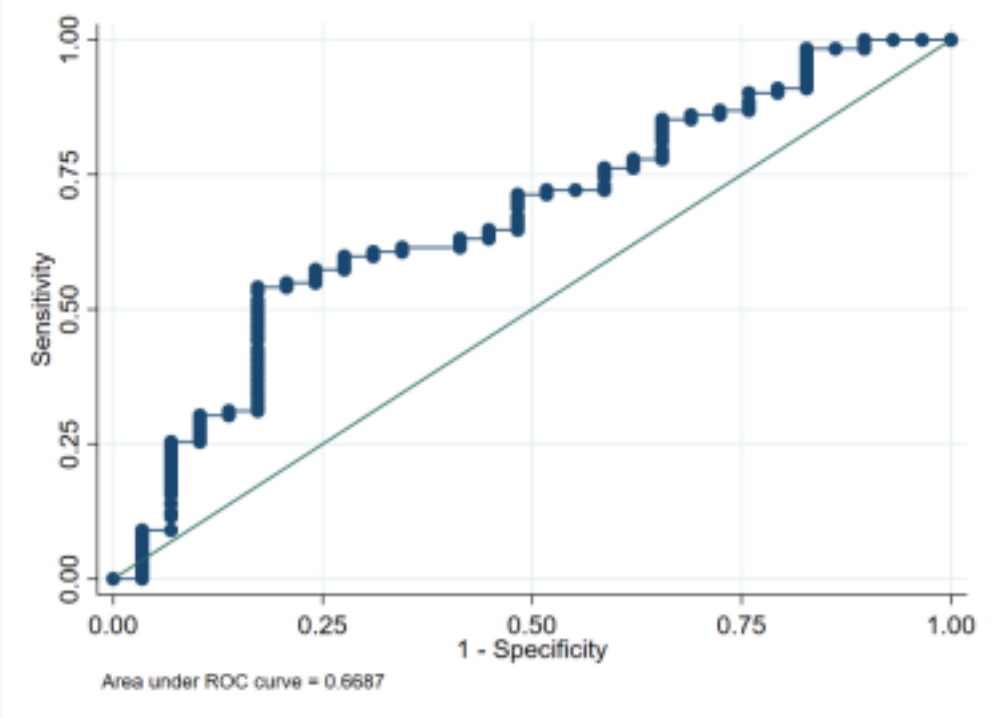

Figure 3: Receiver operating characteristic curve of the multivariate model for LPV adjusting for age, gender, baseline CD4 and previous history of opportunistic infection.

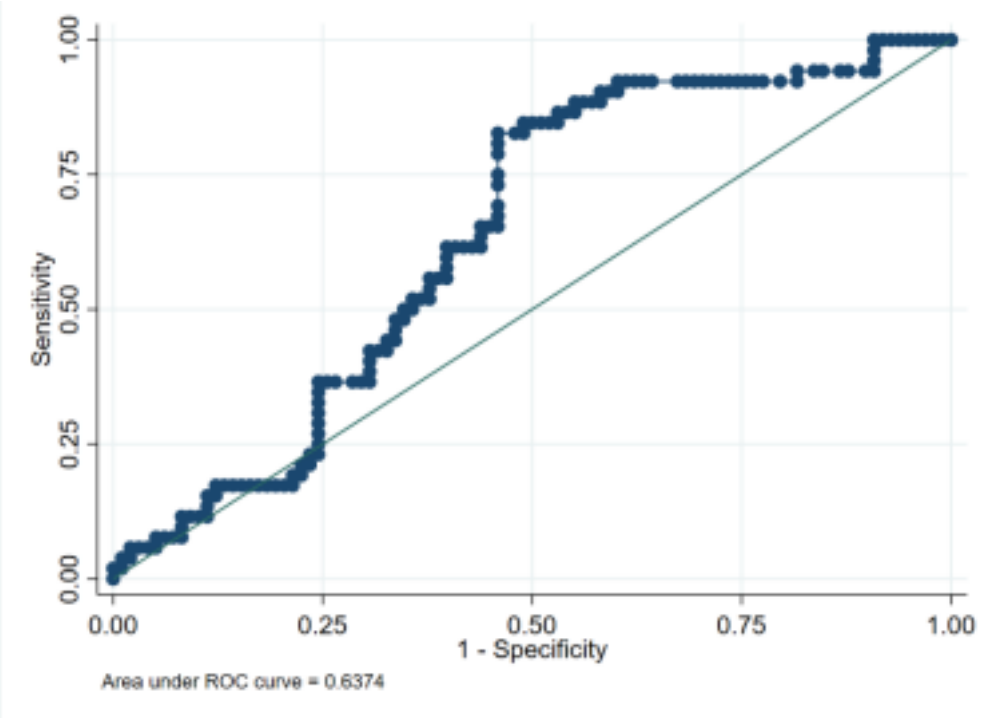

Figure 4: Receiver operating characteristic curve of the multivariate model for DRV adjusting for age, gender, baseline CD4 and previous history of opportunistic infection.

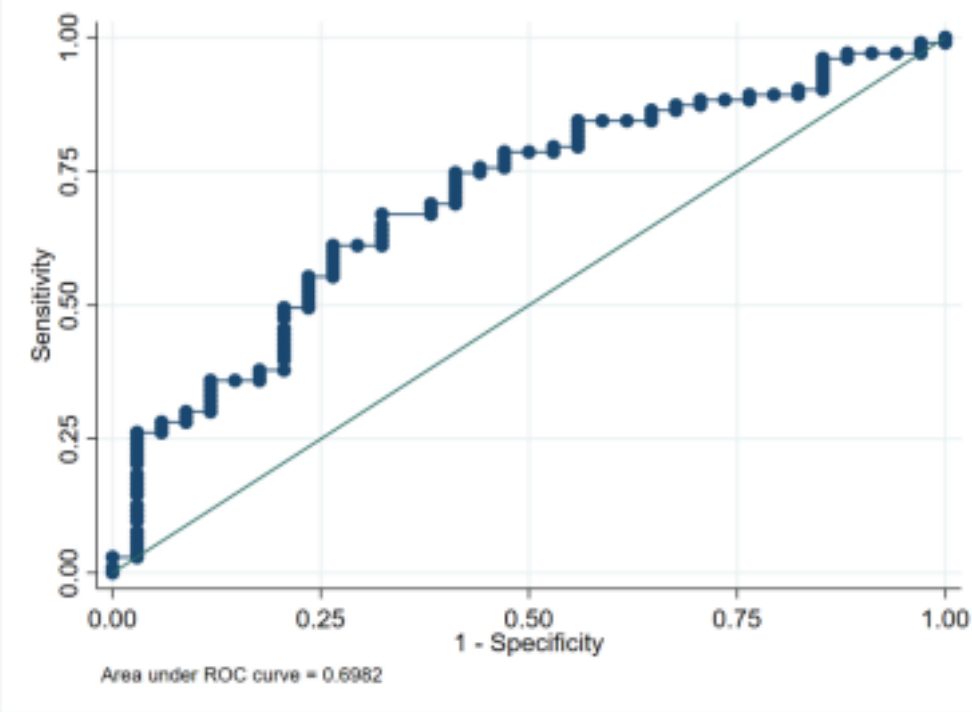

Figure 5: Receiver operating characteristic curve of the multivariate model for FTC adjusting for age, gender, baseline CD4 and previous history of opportunistic infection.

Table 7: Change in mean CD4 count and VL at 3 and 6 months post TLRT

	3 months	p-value	6 months	p-value
Change in mean CD_4 (95%CI) compared to baseline*	57.1 (21.6-92.5)	0.002	103.6 (69.0-138.2)	<0.001
Change in VL compared to baseline^{+}		<0.001		<0.001

CI(confidence interval),VL(viral load)

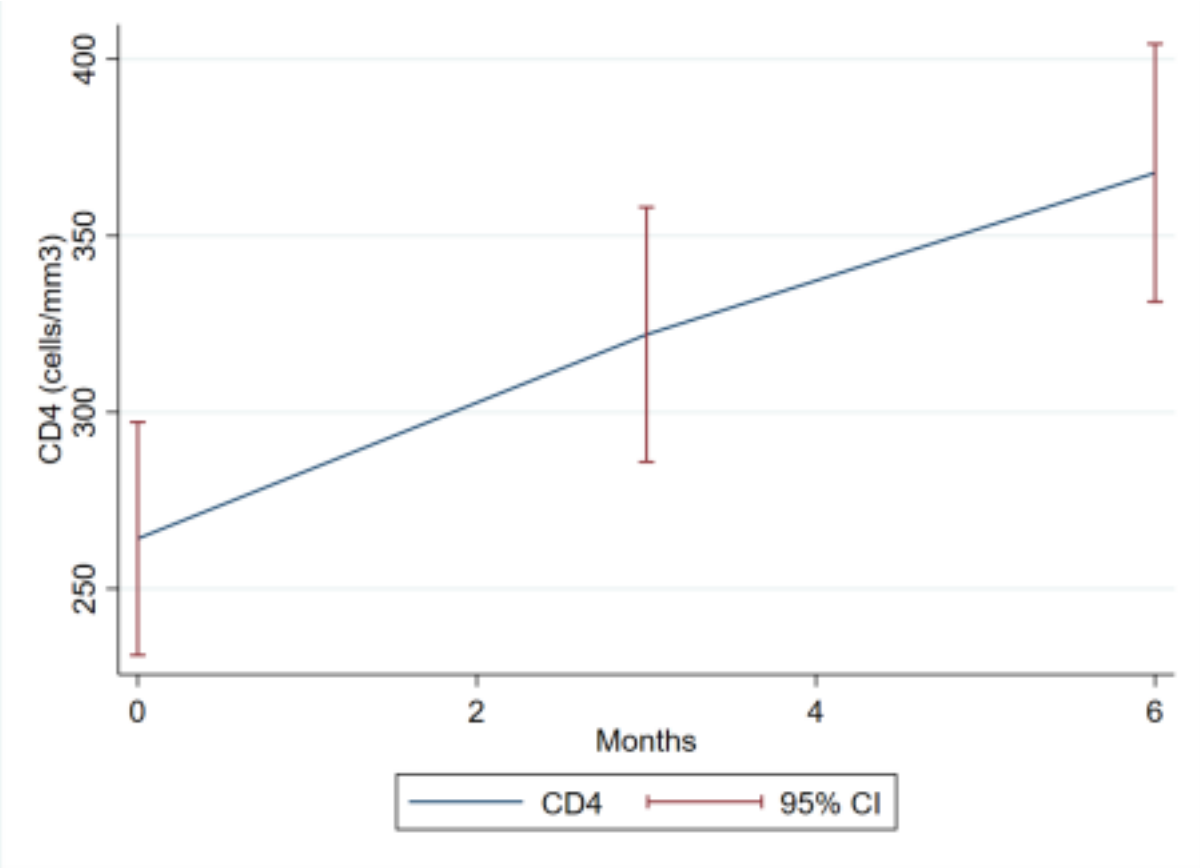

Figure 6: Response of CD4 count over time after initiation of third line therapy

Table 8: Multilevel mixed effects model for predictors of CD4 count over the 6 months adjusting for age, gender and previous opportunistic infection

Variable	Coefficient	95% CI	p-value
Age (Decade)	**19.66**	**1.53-37.6**	**0.03**
Gender	4.3	-37.8-46.5	0.842
Previous opportunistic infection	**-53.4**	**-94.7- - 12.1**	**0.01**

CI(confidence interval)

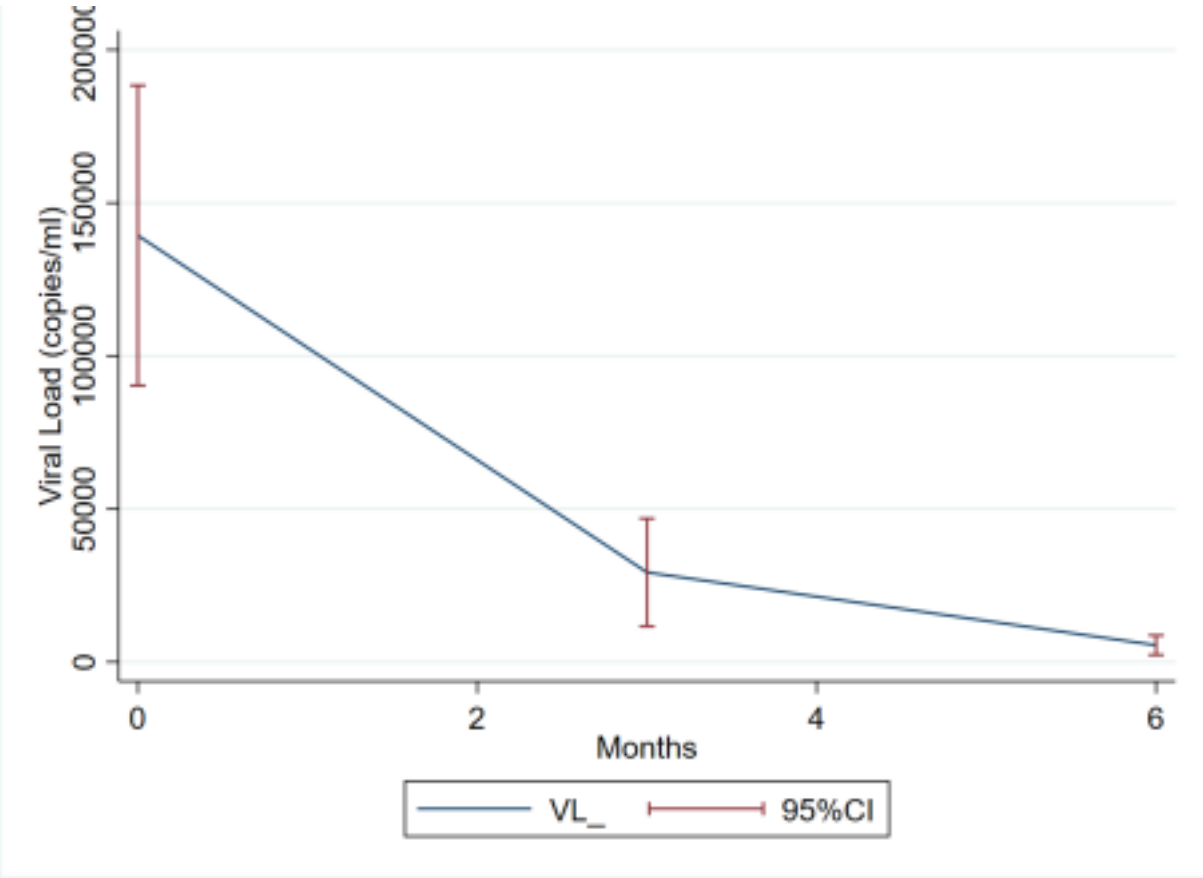

Figure 6: Response of viral load count over time after initiation of third line therapy).VL(viral load),CI(confidence interval)

Discussion

The main findings of our study of the cohort at CHBAH on TLART were a high level of

adherence to TLART, a robust immunological response and virological reduction of our patients, and a significant proportion of ART drug resistance in our cohort.

In South Africa's battle against the Human Immunodeficiency Virus/Acquired Immune Deficiency Virus **(**HIV/AIDS) epidemic, the ability of patients to readily access antiretroviral therapy (ART), has led to a significant reduction in mortality due to AIDS-associated diseases. Our study reviewed the cohort of patients at CHBAH on TLART, one of the largest TLART databases in SSA.

In our cohort for CHBAH, we noted a 95.9% rate of compliance to TLART, which correlates with the reduction in viremia and immune reconstitution as noted. Our study also showed that 163/176 (95.9%) patients attended clinic three months post initiation of TLART, signifying excellent patient retention in our cohort. As opposed to using patient-reported compliance, or the 'ever miss a dose' method as used in studies in Malawi (23), we used evidence of patients receiving and refilling their prescription at initiation and one month post initiation. Although we understand that there is no gold standard for assessing compliance to treatment, studies by Bessong *et al*. in South Africa (24) have shown that pharmacy refill records are a more accurate measure of assessing compliance than patient volunteered information (25). It is our postulate, that the degree of viral load reduction and immune reconstitution, may in part be due to the degree of compliance of our cohort. This level of compliance is achieved by having a dedicated TLART clinic, with more aggressive patient follow-up routine. Any patient enrolling in the TLART programme at CHBAH must have counselling by a trained ART counsellor and digital records kept with regard to their prescription refill from a devoted HIV pharmacy. The demographic makeup of our cohort was in line with other SSA studies done (12), with our mean age of 47 years old and a slightly higher female; male ratio. Univariate analysis on our dataset showed a possible relationship between a higher baseline CD_4 count and protection against resistance to ABC and EFV. This relationship will need to be investigated with further study and may prove fruitful with additional analysis.

With regard to the immunological and virological response to patients on TLART, we see a similar response to trials done in both the private and public sectors in RSA (3). Our cohort showed a median VL of 59 copies/million at six months post TLART initiation. This is slightly higher than those seen in the private sector, albeit our cohort having a higher baseline VL. The immunological response of our patients at CHBAH showing a change in mean CD_4 count of 103.6 cells/ml from baseline, is in concert with the data found in other

studies in SSA (12).

Multiple studies throughout the African continent investigating resistance profiles in patients failing ART regimes. Nanfack et al (26) investigated drug susceptibility in HIV-positive patients living in Cameroon. The outcome of this study gave further evidence to support the knowledge that, as the duration of PI exposure increases, drug resistance will subsequently increase. Thus, time on a PI-based regime correlated with the chance of failure on that regime.

Data from Uganda has revealed that in a population studied with virological failure, not only were the more common Nucleoside Reverse Transcriptase Inhibitor (NRTI) and non Nucleoside Reverse Transcriptase Inhibitor (NNRTI) mutations present, but as more patients were enrolled onto a PI based, second-line treatment regime, a greater prevalence of protease inhibitor mutational changes was noted (27).

When looking into resistance patterns of South African patients failing second-line ART, it is noted that PI resistance is not often the major obstacle in treatment failure. Rather it is driven by patient non-compliance. This issue will unfortunately lead to 'accumulation of mutations' (28) as more and more patients are exposed to second-line treatment. As noted in other SSA
countries, most patients are found to have minor PI mutations, which if left to accumulate, will only result in PI resistance a significant duration of time after initiation.

From our cohort of patients, a substantial proportion of them had baseline resistance to PIs, especially lopinavir and atazanvir, which are our commonly used at CHBAH. A significant majority of our cohort had been exposed to lopinavir as part of the Alluvia combination, which is often part of the second-line regime of choice. Resistance to other PIs such as darunavir was much less significant (34.2%). This is likely due to the fact that it is not used as part of the second-line armamentarium at CHBAH. The resistance patterns against NRTIs

and NNRTIs were similar to those seen in previous studies done in SSA (16), with the level being predictably high in our cohort, after failing two previous regimes.

Limitations

1. As this was a retrospective review, the main limitation to our study was the method of record keeping. No patient files were kept digitally. This inevitably led to data

failure with regard to certain parameters in our study, although, the data was able to remain statically significant. The fact that our study was done at a single center within a specialized clinic, was a limitation that we identified, and it may be difficult to extrapolate our data to more rural or even resource poorer settings than ours. The limitation of our data with regard to the resistance profile of our patients, is that we are looking at a subset of patients with a relatively similar exposure pattern to particular ARVs, as our patients are all from CHBAH. Variables preceding third-line initiation, including, but not limited to, non-compliance, adverse events to drugs in other regimens, and social circumstances which could prevented compliance were not documented and not collected. This would be an important consideration in future prospective studies. Furthermore, there was not information to be collected regarding the reasons for self-termination of treatment

Conclusion

Our results showed a reduced viraemia and a robust immunological response in the majority of our patients after initiating TLART, with evidence of high compliance. This may be due to the strict adherence of patient counselling and education as well as the retention of care that a committed ARV clinic for patients on TLART may provide as a service. The resistance profiles found in our cohort are in line with those that were noted in previous SSA studies. Future studies should review resistance profiles to newer drugs, such as INSTIs.

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CHAPTER 3: APPENDIX

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A: Abbreviations

Abacavir (ABC)
Antiretroviral (ARV)
Antiretroviral Therapy (ART)
Chris Hani Baragwaneth Academic Hospital (CHBAH)
Darunavir (DRV)
Dolutegravir (DTG)
Drug Resistance Test (DRT)

Efavirenz (EFV)
Emtricitibine (FTC)
Etravarine (ETR)
Human Immunodeficiency Virus / Acquired Immunodeficiency Syndrome
(HIV/AIDS) Integrase Strand Transfer Inhibitor (INSTI)
Lamivudine (3TC)
Lopinivir (LPV)
Non Nucleoside Reverse Transcriptase Inhibitor (NNRTI)
Nucleoside Reverse Transcriptase Inhibitor (NRTI)
Opportunistic Infection (OI)
Protease Inhibitor (PI)
Republic of South Africa (RSA)
Socio-economic status (SES)
Stavudine (D4T)
Sub Saharan Africa (SSA)
Third-line Antiretroviral Therapy (TLART)
Viral Load (VL)
Zidovudine (AZT)

B: Data collection sheet

Participant Number	
Gender	M=0/F=1
Date of birth	YYYY/MM/DD
Date of HIV diagnosis	YYYY/MM/DD

Previous Anti retro viral treatment (ART)	Exposed =1 Not exposed=o 3TC/ABC/EFV/TDF/LPV/rit/NVP/AZT/FTC/D4T/DDI/DTG/ATZ
Current ART regime	DRV/ETR/3TC/ABC/EFV/TDF/LPV/rit/NVP/AZT/FTC/D4T/DDI/DTG/ATZ
Date of third line ART initiation	DD/MM/YYYY
Previous adherence counselling	Yes=1 No=0
HIV viral load prior to third line regime	VL=
CD4 Prior to Third line	CD4=
Viral load post at 3/12 initiation	VL=
Viral load post at 6/12 initiation	VL=
CD4 count at 3/12 post initiation	CD4=
CD4 count at 6/12 post initiation	CD4=
Previous OI	Yes=1 No=0
Compliance to monthly script	Yes=1 No=0
Evidence of attendance at clinic 3/12 post initiation	Yes=1 No=0

Baseline resistance Profile	Resistance =1 Sensitive or not known =0 3TC/ABC/EFV/TDF/LPV/RIT/NVP/ATZ/DRV/FTC/ETR/AZT

C: Letter of permission Head of Unit Infectious Diseases

D: Permission letter from Chis Hani Baragwanath Academic Hospital Medical Advisory Committee

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

MEDICAL ADVISORY COMMITTEE

CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

PERMISSION TO CONDUCT RESEARCH

Date: 5th September 2023

TITLE OF PROJECT:

A Review of HIV-positive Patients at Chris Hani Baragwanath Academic Hospital on Third-line Antiretroviral Therapy

UNIVERSITY: Witwatersrand

PRINCIPAL INVESTIGATOR: Dr D Brozin

DEPARTMENT: INTERNAL MEDICINE

SUPERVISOR : Dr M Venter /Dr SA van Blydenstein

NHRD Number: GP_ 202308_092

Permission Head Department (where research conducted): Yes

The Medical Advisory Committee recommends that the said research be conducted at Chris Hani Baragwanath Academic Hospital. The CEO / management of Chris Hani Baragwanath Academic Hospital is accordingly informed and the study is subject to:-

- **Permission having been granted by the Committee for Research on Human Subjects of the University of the Witwatersrand.**
- The Hospital will not incur extra costs as a result of the research being conducted on its patients within the hospital
- The MAC will be informed of any serious adverse events as soon as they occur
- Permission is granted for the duration of the Ethics Committee Approval.

Recommended
(On behalf of the MAC)
Date: 05/09/2023

Approved/Not Approved
Hospital Management
Date: 06/09/2023

Appendix E : Human Ethics Committee Approval

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research and Innovation)

TO:	Dr D Brozin School of Clinical Medicine Department of Medicine Division of Internal Medicine Medical School University E-mail: Daniel.Brozin@gmail.com
CC:	Supervisor: Drs SA van Blydenstein and M Venter savanblydenstein@gmail.com and <HREC-Medical Research Office@wits.ac.za>
FROM:	Mr Iain Burns Human Research Ethics Committee (Medical) Tel: 011 717 1252 E-mail: Iain.Burns@wits.ac.za
DATE:	8 January 2024
REF:	R14/49
PROTOCOL NO:	**M23/09/88** (This is your ethics application reference number. Please quote it in all enquiries, oral or written, relating to this study.)
PROJECT TITLE:	*A review of HIV-positive patients at Chris Hani Baragwanath Hospital on third-line antiretroviral therapy*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to Government funding of the University.

MS Works2000/iain0007/Clearscan.wps

Appendix F : TurnItIn Report and Letter from Supervisor

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18/01/2024

Re: TurnItIn Similarity Report for MMED Student Daniel Brozin

MMED: A Review of HIV-positive Patients at Chris Hani Baragwanath Academic Hospital on Third-line Antiretroviral Therapy

To whom it may concern

The above student has completed his MMED and is submitting it for examination.

I have noted that she has run it through TurnItIn, and it has a 12% similarity index. The similarities identified are related to medical definitions found in multiple sources, and from standard layout. There is no evidence of plagiarism. This letter serves to confirm that I have reviewed his MMED with the TurnItIn report and approve for submission.

Sincerely

Dr Alex van Blydenstein
Supervisor
Specialist Physician, Pulmonologist
Chris Hani Baragwanath Hospital
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