MAKERERE  UNIVERSITY

HIV DRUG RESISTANT MUTATIONS ASSOCIATED WITH VIROLOGICAL FAILURE AMONG HIV-1 SEROPOSITIVE INFANTS IN UGANDA AFTER INTENSIFIED ADHERENCE COUNSELLING

BY

MUWABE FARYAD

BSc. Microbiology, Chemistry and Biotechnology

Registration No. 2018/HD17/3878U

Tel: +256706507807, Email: **faryadrenda@gmail.com**

A RESEARCH DISSERTATION SUBMITTED TO THE DIRECTORATE OF RESEARCH AND GRADUATE TRAINING IN PARTIAL FULFILMENT FOR THE AWARD OF A MASTER`S DEGREE IN MOLECULAR BIOLOGY AND BIOTECHNOLOGY OF MAKERERE UNIVERSITY

JANUARY, 2026

DECLARATION

I **Muwabe Faryad,** declare that this work is my original work and has never been submitted by any person (s) to any academic institution for any award

Signature: ..... Date....12 Jan 2026.....

This dissertation has been submitted for examination with the approval of the following supervisors.

Dr. Charles Drago Kato (BVM, MSc, MSc, PhD)
Lecturer,
Department of Biotechnical and Diagnostic sciences,
College of Veterinary medicine, Animal resources & Biosecurity,
P.O BOX 7062, Kampala, Uganda.

Signature: ..... Date....27/01/2026.....

Dr. Immaculate Nankya (MBChB, PhD),
Centre for AIDS Research Laboratory,
Joint Clinical Research Centre (JCRC),
P.O BOX 10005, Wakiso District, Uganda.

Signature: ..... Date....12th Jan 2026.

DEDICATION

This research thesis is dedicated to the omnipotent God who has served as a source of fortitude and celestial influence throughout the whole of this academic endeavour. To my supervisors, Dr. Immaculate Nankya and Dr. Charles Kato Drago, who have consistently served as my primary sources of inspiration and fortitude, providing unwavering support and encouragement which has motivated me to strive diligently in my endeavours.

ACKNOWLEDGMENTS

First and foremost, I extend my deepest gratitude to the Almighty for granting me the strength, resilience, good health, and intellectual capacity to pursue and complete this academic journey. Achieving this milestone has required immense dedication and sacrifice.

I am profoundly thankful to my supervisors, Dr. Immaculate Nankya and Dr. Charles Kato, for their exceptional mentorship, unwavering support, and insightful feedback throughout every phase of this work from the selection of the research topic to the development of the concept paper, research proposal, and final thesis. Their guidance has been instrumental to the successful completion of this dissertation.

I also wish to express my sincere appreciation to all those who have walked alongside me on this journey. Your encouragement and support have not gone unnoticed. May you be abundantly blessed and continue to thrive in all your endeavours.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND ACRONYMS

AIDS…….……Acquired Immune Deficiency Syndrome

ART…….…… Antiretroviral Therapy

CDC……. Centers for Disease Control

CPHL……..…… Central Public Health Laboratory

EID……..……. Early infant Diagnosis

EMTCT……...……. Early Mother to Child Transmission

HIV……..……. Human Immune virus

PLHIV……..……. People living with HIV

PCR……Polymerase Chain Reaction

NGS……..……. Next Generation Sequencing

IAC……...……. Intensified Adherence Counseling

WHO……..……World Health Organization

MoH…….……. Ministry of Health

PMTCT……. Prevention of mother to child transmission

GHOD……..……. Global Health Observatory Data

UNCST……. Uganda National Council of Science and Technology

UNAIDS……United Nations Program on HIV/AIDS

VL……...……..…… Viral Load

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Paediatric HIV treatment is setback by poor adherence and emerging drug resistance. This longitudinal cohort study assessed the impact of intensified adherence counselling (IAC) on viral suppression and the development of HIV drug resistance mutations in HIV-positive infants receiving antiretroviral therapy (ART).

100 HIV-positive infants (aged 4–12 months) with unsuppressed viral loads (>1000 copies/mL) were enrolled at the Joint Clinical Research Centre (JCRC), Uganda. Mother-infant pairs received IAC for a period of three months. After three months, re-evaluation was conducted, during which HIV viral load was measured using the Abbott assay and adherence was assessed using the pill-count method. Infants with persistent viremia were subjected to drug resistance testing using next-generation sequencing using a MiSeq from which the resulting FASTA files were downloaded from Hydra and submitted to the Stanford University HIV Drug Resistance Database (Version 9.8). The output, provided as a Comma-Separated Values (CSV) file, included classified mutations, mixtures, corresponding scores, susceptibility status, and other relevant parameters. A tabular summary of these findings was then generated to facilitate interpretation and further analysis.

Intensified Adherence Counselling (IAC) in mother–infant pairs prompted better results in viral suppression despite changes in adherence, with viral loads continually decreasing after IAC compared to before IAC. HIV drug resistance mutations showed changes in both NRTI and NNRTI profiles. The common NRTI mutation M184V/I reduced slightly post-IAC (43.5% to 40.2%), while increase in TAM-associated mutations (K219, K70, D67) suggested ongoing selective pressure from thymidine-based regimens. NNRTI resistance showed an overall decline, with major mutations such as K103N and Y181C decreasing significantly after IAC, with the disappearance of variants like E138Q and G190S, confirming improved viral suppression and reduced propagation of resistant quasispecies. Mixture mutation analysis showed reductions in NRTI (M184, K219) and NNRTI (V179, V108, Y181, V106) variants, further indicating suppression of resistant viral populations. Emergence and persistence of mutations such as E138A, K238, P225H reflects ongoing viral suppression under treatment pressure. Overall, IAC contributed to improved viral control and reductions in several resistance-associated variants, though there is a continued need for vigilant resistance monitoring and optimized ART regimens.

CHAPTER ONE: INTRODUCTION

1.1 Background

Since the discovery of the Human Immunodeficiency Virus (HIV) in the early 1980s, it remains a significant global public health concern. Regardless of the tremendous advances in medical research, approximate of 39 million individuals worldwide are living with HIV, of which 37.5 million are adults and 1.5 million being children under the age of 15 years as of 2022 (UNAIDS, 2024). The burden is worse in Africa with approximately 25.4 million people living with HIV (PLWHIV). There are over 1.5 million HIV diagnoses among children under the age of 15, which suggests both advancements and continued difficulties in stopping mother-to-child transmission (WHO, 2013). In East Africa, about 7.5 million people live with HIV even though, the region has made notable strides in reducing new infections and improving access to treatment, particularly through enhanced PMTCT programs. (Otieno *et al*.,2023)

As of 2023, 1.4 million PLWHIV in Uganda with roughly 80,000 of these being children (UAC, 2023). The number of children living with HIV has been greatly impacted by improvements in preventing mother-to-child transmission (PMTCT) over time (Valladolid-Acebes *et al.,* 2022). The combined efforts of routine HIV testing, lifelong antiretroviral medication (ART) for mothers, safe delivery practices and infant prophylaxis were responsible for the observed 77% decrease in new HIV diagnoses among infants between 2010 and 2022 (Mukose *et al*., 2024). Nonetheless, the UNAIDS 90-90-90 targets which aimed to end the global HIV epidemic by 2020, have not yet been fully actualised for children. These targets set goals for 90% of PLWHIV to know their status, 90% of those diagnosed to receive treatment, and 90% of those on treatment to attain viral suppression (UNAIDS, 2014). In comparison with 90% of adults in Uganda, only 76% of children with HIV are receiving ART and their viral suppression rate is only about 78%. This is could be due to the fact that children have unique challenges such as socioeconomic circumstances, adherence problems, and restricted access to age-appropriate ART formulations (Wakooko *et al*.,2020). It is therefore urgent to close these gaps as the world strives to achieve the new 95-95-95 targets for 2030 seeking to further improve treatment by aiming for 95% in each area to improve outcomes for everyone, particularly children and other vulnerable groups.

In Uganda, the government mandates viral load testing for everyone receiving ART for longer than six months and a patient with virologic failure is defined as one with a viral load greater than 1000 copies/ml. patients with virological failure are put on intensified adherence counselling (IAC) based on the national algorithm for HIV care and treatment. IAC consists of three-monthly sessions intended to enhance medication adherence, is given to those with high viral loads (Nyogea *et al*., 2015). Studies in Uganda, including the Uganda National Reference laboratories' data, indicate that many patients do not complete the full three counselling sessions as recommended, and even those who complete the sessions may not return for repeat viral load testing. Even patients who complete IAC and return for repeat viral load testing, may still have an unsuppressed viral load suggesting that IAC may not be sufficient to address all factors contributing to viral suppression. Several factors can hinder the effectiveness of IAC, including substance abuse, stigma, discrimination, motivation, deteriorating health and lack of incentives (Okot *et al*., 2024, Ndikabona *et al*., 2012 & Izudi *et al*., 2024). Even with widespread access to ART and IAC services, the risk of virologic failure persists due to the emergence of drug-resistant viruses, driven by HIV's high mutation rates during replication. (Silver *et al*., 2018). To complicate things further, drug-resistant mutations are often present at levels below the detection threshold of the routine sanger genotyping technology (Simen *et al*., 2009). During virologic failure, such mutations, often undetected by conventional genotyping, can increase the overall resistance burden. Next-generation sequencing offers valuable solution by generating bid data, that can detect low threshold mutation down to 5% mutant population in circulation compared to the conventional genotyping method with a detection limit of 20% with the former providing a more comprehensive data (Fokam *et al*., 2018).

1.2 Problem statement

Virological failure remains an urgent problem among infants with HIV, even with the major advancements in PMTCT of HIV, ART implementation and viral load monitoring in Uganda. An important strategy for increasing adherence and achieving viral suppression during virological failure (Viral load>1000 copies/ml) is IAC. However, many patients do not complete the full three counselling sessions as recommended, and even those who complete the sessions may not return for repeat viral load testing. Even after receiving IAC, a sizable percentage of kids may still experience virological failure. The existence of drug-resistant HIV strains, many of which are missed by traditional Sanger sequencing because of its low sensitivity to low frequency mutations may be the cause of this ongoing failure. These low

frequency mutations, which generally make up less than 20% of the virus population, can have a major impact on influencing treatment failure. Drug resistance mutations can be detected with higher sensitively with Next Generation Sequencing (NGS), which can detect alterations as low as 5%. The occurrence and evolution of HIV drug resistance mutations in infants undergoing IAC remain unknown in Uganda, a resource limited setting. Therefore, this study aims to study how these mutations appear and change throughout IAC in order to direct more efficient therapy plans and enhance virologic results for infants with HIV.

1.3 Objectives

1.3.1 General objective

To determine the prevalence of HIV drug-resistant mutations associated with virological failure among HIV-1 seropositive infants in Uganda after intensified adherence counselling

1.3.2 Specific objectives

i. To determine the viral load outcomes of infants following of Intensified Adherence Counselling (IAC) among mother-infant pairs with a viral load greater than 1000 copies/ml on a follow-up viral load test.
ii. To determine the presence HIV drug resistant mutations among infants experiencing virological failure before IAC and after IAC.
iii. To determine the evolution of HIV drug resistant mutations among infants experiencing virological failure during the course of IAC.

1.4 Research questions

i. What are the virological outcomes of Intensified Adherence Counselling (IAC) among mother-infant pairs with a follow-up viral load greater than 1000 copies/mL?
ii. What HIV drug resistance mutations are present in infants experiencing virological failure before and after undergoing IAC?
iii. How do HIV drug resistance mutations in infants change over the course of IAC?

1.5 Justification

In Uganda, virological failure among HIV positive infants remains a significant challenge even with the advancements in PMTCT, ART, and viral load monitoring. Although IAC is a key strategy to improve adherence, many infants do not complete it or fail to achieve viral suppression afterward. This may be due to undetected drug resistance mutations, particularly low frequency variants missed by traditional Sanger sequencing. However, Next NGS with its higher sensitivity offers a better tool for detecting these mutations. Therefore, this study sought to fill the knowledge gap on the prevalence and evolution of drug resistance in infants undergoing IAC, which is a crucial and timely initiative essential for improving treatment outcomes in HIV positive infants as Uganda and other similar resource limited settings strive to end HIV by 2030.

1.6 Significance

The findings from this study holds significant potential of informing and improving HIV treatment strategies for infants in Uganda and in other resource limited settings. This study evaluated the effectiveness of IAC in achieving viral suppression and provided key insights into the burden and role of drug resistance mutations including low-frequency variants in virological failure among HIV positive infants. The findings could be used by the ministry of health as a guide for revising treatment guidelines and integrating advanced resistance testing into the national programs. Healthcare providers may gain insights to tailor adherence support and manage treatment failure more effectively. National HIV programs use the findings to refine strategies for paediatric care and researchers may gain insights on resistance patterns and IAC in infants. The donors and development partners may be better positioned to fund impactful interventions based on this study findings with an ultimate goal of improving treatment outcomes for HIV positive infants and support Uganda's efforts in controlling paediatric HIV.

CHAPTER TWO: LITERATURE REVIEW

2.1 HIV/AIDS burden in Uganda among infants

Globally, an estimated 1.4 million children under the age of 15 are living with HIV, with approximately 120,000 newly acquiring the virus each year and about 76,000 dying annually from HIV related causes at the end of 2023. The sub-Saharan Africa region bears the weight of the burden with over 1.3 million children living with HIV (UNAIDS, 2024). Uganda being in the sub-Saharan Africa region is not exceptional, with approximately 80,000 children under 15 living with HIV as of 2022 (WHO, 2023). In spite of the advancements in treatment, including 66% of children globally receiving ART, mother-to-child transmission remains an issue (Namara-Lugolobi et al, 2022). It's therefore important to study children in the ART program in order to evaluate the outcomes and potential areas to improve IAC among all ART treated children with unsuppressed viral loads (Nassuna et al, 2018). In resource-limited settings, where genotypic drug resistance testing is rarely performed and poor adherence is the most common reason for treatment failure, programmatic approaches to handling treatment failure are essential (Okonji *et al*., 2022).

2.2 Virological failure

The use of antiretroviral therapy (ART) has led to significant reductions in morbidity and mortality(Bärnighausen et al, 2013; Broder et al, 2010; Mutevedzi et al, 2014). Despite the huge successes in increasing HIV treatment coverage, most patients who experience virologic failure on ART in low-middle-income countries fail due to poor adherence rather than resistance to a class of ART drugs (Leisegang *et al*., 2014) (McMahon et al, 2013). For patients on a protease inhibitor-based ART, high levels (≥80 %) of adherence are required for viral suppression, and poorer outcomes are observed when adherence drops (Viswanathan *et al*., 2015), (Chkhartishvili *et al*., 2014). The future success of ART programs depends on a good understanding of the behavioural determinants of acceptance and adherence to ART(Gill et al., 2005).. Studies have suggested several important factors influencing adherence, including forgetfulness, lack of understanding of treatment regimens or benefits, complexity of drug regimens, disclosure of status, stigma, and depression (Ammassari *et al*., 2002; E. J. Mills *et al*., 2006).

2.3 Intensified adherence counselling

In Uganda, the government mandates viral load testing for everyone receiving ART for longer than six months and a patient with virologic failure is defined as one with a viral load greater than 1000 copies/ml. patients with virological failure are put on intensified adherence counselling (IAC) based on the national algorithm for HIV care and treatment. IAC consists of three-monthly sessions intended to enhance medication adherence, is given to those with high viral loads. During IAC, adherence is evaluated to determine whether the caregiver understands the purpose of treatment and the importance of achieving virological suppression (Nyogea *et al*., 2015). Studies in Uganda, including the Uganda National Reference laboratories' data, indicate that many patients do not complete the full three counselling sessions as recommended, and even those who complete the sessions may not return for repeat viral load testing. Even patients who complete IAC and return for repeat viral load testing, may still have an unsuppressed viral load suggesting that IAC may not be sufficient to address all factors contributing to viral suppression. Numerous factors may hinder the effectiveness of IAC including substance abuse by clients, stigma, discrimination, deteriorating health and lack of incentives (Okot *et al*., 2024 & Izudi *et al*., 2024). However, when the viral load remains above 1000 copies per millilitre after IAC a drug resistance test is performed (Izudi,et al 2023).

2.4 HIV genome

The HIV virus has a complex structure consisting of 16 viral proteins that play a vital role during HIV life cycle. Three major genes, *gag*, *pol*, and *env*, code for structural proteins (matrix, capsid, nucleocapsid, and p6), viral enzymes (protease, reverse transcriptase [RT], and integrase), and envelope proteins (GP120 and GP41) respectively (Engelman & Cherepanov, 2012) . The *Pol* gene encodes the enzymes; protease, reverse transcriptase and integrase. Protease functions to cleave gag and gag/pol polyproteins. Reverse transcriptase functions to convert the viral RNA to DNA while integrase integrates the viral DNA into the host genome (Chan *et al*., 1999).

Although there are still problems, antiretroviral medications that target the protease, reverse transcriptase, and integrase enzymes are frequently used in paediatric HIV treatment. The development of drug resistance is a major obstacle, and it can be especially troublesome in children because of problems with medication adherence and the possibility of increased treatment failure rates (Peng et al, 2023).

Furthermore, while antiretroviral therapies can effectively suppress viral replication in paediatric patients, questions remain regarding the long-term effects of these treatments on children's growth, development, and overall health (Barlow-Mosha et al, 2017).

2.5 Antiretroviral regimen for children

Currently, there is no cure for HIV infection. For now, there are about 30 drugs in five main classes that have been approved for the treatment of HIV/AIDS. These drugs target different steps of the viral life cycle: (i) viral entry (e.g. coreceptor antagonists and fusion inhibitors); (ii) reverse transcription (reverse transcriptase (RT) inhibitors); (iii) integration (integrase (IN) inhibitors); and (iv) viral maturation (protease (PR) inhibitors)(Menéndez-Arias et al, 2013)

Nowadays, ART is used in combination as highly active antiretroviral therapy (HAART). The majority of these combination therapies include two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTI) or protease inhibitor (PI). Also, combinations of integrase or entry inhibitors with RT inhibitors and PIs are used as an alternative treatment strategy(Iyidogan et al, 2014) Uganda like most of the resource limited countries, has adapted the treatment guidelines set up by WHO for HIV/AIDS management, among these are the treatment options which include;

The preferred first-line regimen comprises two nucleoside reverse transcriptase inhibitors (NRTIs) and a non-nucleoside reverse transcriptase inhibitor (NNRTI). The most commonly recommended regimen is Tenofovir Disoproxil Fumarate (TDF) + Lamivudine (3TC) + Efavirenz (EFV). Alternatives include TDF + 3TC + Dolutegravir (DTG) and Abacavir (ABC) + 3TC + EFV, tailored to patient-specific factors and drug availability (MOH, 2024).

For pregnant and breastfeeding women, the first-line regimen is TDF + 3TC + EFV. Alternatives include TDF + 3TC + Atazanavir/Ritonavir (ATV/r) and ABC + 3TC + EFV, prioritizing both maternal and fetal health. The recommended regimen for children in this age group is ABC + 3TC + EFV, with alternatives such as ABC + 3TC + Nevirapine (NVP) based on individual health needs (MOH, 2024).

The preferred regimen for children under three years is ABC + 3TC + Lopinavir/Ritonavir (LPV/r) (MOH, 2024).

The recommended first-line regimen for infants 0-12 months is ABC + 3TC + LPV/r. Alternatives may include regimens such as AZT + 3TC + LPV/r, considering the infant's specific health conditions and resistance profiles (MOH, 2024)

For children who fail the first-line regimen, the second-line regimen consists of two NRTIs and ritonavir-boosted lopinavir (LPV/r). The recommended formulation is LPV/r 100/25mg tablets. The choice of NRTIs depends on the previous regimen: after failing an ABC + 3TC-based regimen, AZT + 3TC is recommended; after failing an AZT + 3TC-based regimen, ABC + 3TC is advised (MOH, 2024).

The second-line regimen for infants below 3 years includes two NRTIs and Raltegravir (RAL). The NRTI sequence is the same as for older children: after failing an ABC + 3TC-based regimen, AZT + 3TC is recommended; after failing an AZT + 3TC-based regimen, ABC + 3TC is advised (MOH, 2024).

For infants 0 to 12 months who fail the first-line regimen, the second-line regimen includes two NRTIs and RAL. The choice of NRTIs is based on the previous regimen, with a similar sequence as older children: after failing an ABC + 3TC-based regimen, AZT + 3TC is recommended; after failing an AZT + 3TC-based regimen, ABC + 3TC is advised (WHO, 2024).

2.6 Drug resistance

The use of combinational antiretroviral drugs has proved effective in controlling the progression of human immunodeficiency virus (HIV) disease and prolonging survival(Palella Jr *et al*., 1998) but these benefits can be compromised by the development of drug resistance.(DeGruttola *et al*., 2000; Ledergerber *et al*., 1999) This resistance is as a result of mutations that emerge in the viral proteins targeted by antiretroviral agents. The transmission of drug-resistant strains is also a growing concern(Grant *et al*., 2002; Little *et al*., 2002; Yerly *et al*., 1999) because drug-resistant HIV often exhibits resistance to several classes of antiretroviral drugs(Shafer et al, 1998) and also since cross-resistance between drugs within a class is frequent,(V. Miller et al, 2001; Richman et al, 1990; Shafer *et al*., 1998) the emergence of resistance always complicates further efforts to control viral replication. In Uganda most infected children have acquired HIV from their mothers, therefore they are at risk of HIV drug resistance in the context of prevention of mother-to-child transmission (PMTCT) or via transmission of resistant strains from their mothers.(Kuhn *et al*.,

2014)infants who become infected after PMTCT failure are at particular risk of HIV drug resistance (HIVDR), as a result of non–nucleoside reverse transcriptase inhibitors(NNRTIs) used in maternal or pediatric drug regimens.(Paredes *et al*., 2013; Ton Q et al, 2013). Therefore, the World Health Organization (WHO) currently recommends initiating ART with a protease inhibitor (PI)–based regimen in all children younger than 3 years (WHO, 2011).

2.6.1 Resistance to Antiretroviral Therapies

There are six different classes of drugs approved by US Food and Drug Administration (FDA) for HIV-1 treatment. The two drug classes that is NRTIs; and NNRTIs target the viral enzyme reverse transcriptase (RT). RT enzyme carries out RNA- and DNA-dependent DNA polymerase activities, as well as RNase H activity. All three activities are required for the synthesis of dsDNA from viral ssRNA genome (Chen et al, 2004).

The structure of non-B HIVRT has not been determined however, due to the high sequence conservation between HIV-1B and HIV-non-B RTs, the overall folding of HIV-non-B RT is expected to be similar. RT is an asymmetric heterodimer comprising two structurally distinct subunits (p66 and p51). The p66 subunit contains the active sites for the polymerase and RNase H activities of the enzyme (Jacobo-Molina *et al*., 1993; Kohlstaedt et al, 1992). The p51 subunit, which is derived from p66 by protease-mediated cleavage of the RNase H domain plays a structural role although some mutations in p51 have been reported to affect catalytic activity as well (Chung *et al*., 2013; Schuckmann *et al*., 2010). The first anti-HIV drugs were nucleoside analogues lacking the 3'-OH group, which is required for DNA synthesis(Singh, Marchand, Kirby, Michailidis, & Sarafianos, 2010). Currently, all approved NRTIs lack a 3'-OH, are phosphorylated in cells into dNTP analogues that bind at the dNTP-binding site and act as chain terminators after their incorporation into viral DNA by RT (De Clercq et al, 2004; Huang et al, 1998; Parniak et al, 2000; Tuske *et al*., 2004).

NNRTI are the second class of anti-HIV-1 RT drugs, bind in a hydrophobic pocket of HIV-1B RT at the base of the p66 thumb subdomain, ~10 Å away from the polymerase active site NNRTI binding has multiple effects, including restriction of the p66 thumb sub domain's flexibility, and repositioning of the nucleic acid (Smerdon *et al*., 1994; Tantillo *et al*., 1994).

2.6.2 Mechanism of NRTI Resistance in HIV-1

In HIV-1, resistance to nucleoside reverse transcriptase inhibitors (NRTIs) arises through two primary mechanisms: discrimination and excision. Discrimination mutations, such as K65R, K70E, L74V, V75I, Q151M, and M184V, reduce the affinity of the reverse transcriptase enzyme for NRTI triphosphates, thereby decreasing the incorporation of these drugs into the viral DNA chain. Excision mutations, including M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E, facilitate the ATP-mediated removal of incorporated NRTIs from the 3' end of the DNA primer, a process known as primer unblocking, which allows continued viral replication despite the presence of NRTIs (Das et al, 2013).

These resistance mechanisms are associated with specific mutations. The 69-insertion complex, comprising M41L, A62V, T69 insertions, K70R, L210W, T215Y/F, and K219Q/E, confers resistance to all approved NRTIs when present with one or more thymidine analogue mutations (TAMs) at codons 41, 210, or 215. The Q151M complex, formed by A62V, V75I, F77L, F116Y, and Q151M, results in reduced susceptibility to all NRTIs except tenofovir (TDF). TAMs themselves enhance resistance to all approved NRTIs (Boyer et al, 2022).

Regarding NNRTIs, resistance is predominantly conferred by mutations in the reverse transcriptase enzyme's non-nucleoside binding pocket (NBP). Major NNRTI resistance mutations include K103N, Y181C, G190A, and K103S, which significantly reduce the efficacy of NNRTIs such as efavirenz (EFV) and nevirapine (NVP). These mutations alter the enzyme's structure, decreasing the binding affinity of NNRTIs and thereby impairing their inhibitory effects (Iyidogan et al, 2014).

Understanding these resistance mechanisms is crucial for optimizing antiretroviral therapy (ART) regimens. The presence of specific mutations can guide clinicians in selecting appropriate drugs to overcome resistance and achieve effective viral suppression. For instance, the persistence of TAMs may necessitate the use of drugs like tenofovir, which retain activity against strains harbouring these mutations (Pennings et al, 2013).

2.7 Major, accessory, and minor mutations

Drug resistance mutations in HIV are typically classified into major, accessory and minor types. This is based on their role in confer drug resistance. The major mutations directly contribute to significant reductions in susceptibility to antiretroviral drugs and are frequently sufficient on their own to cause resistance. These mutations typically arise under strong

selective drug pressure and can rapidly dominate the viral population. Accessory mutations do not usually confer resistance autonomously but boosts the level of resistance when present alongside major mutations. They may also compensate for the fitness cost associated with major resistance mutations hence allowing the virus to replicate more efficiently regardless of drug presence (Tang et al, 2012). Minor mutations are frequently present at low frequencies within a person's viral populace. These mutations occur as a result of the high replication rate and error prone activity of HIV's reverse transcriptase enzyme which lacks proofreading capability. Consequently, the virus continuously generates a highly diverse population of variants through frequent mutation and recombination events. Although minority mutations are usually present at levels below the 20% detection threshold of conventional population based sequencing methods, they can gain a selective advantage under drug pressure. If a mutation provides decreased susceptibility to an antiretroviral agent, it can expand within the population following a Darwinian selection process, potentially becoming a major variant over time (Tang et al, 2012).

2.8 Next Generation Sequencing (NGS)

This is not the gold standard but has an advantage over the Sanger sequencing method in a way that any mutation present in the patient at a frequency as low as 1% will be detected by this technology. This method of sequencing is a usually done to obtain the exact order of occurrence of nucleotides in a DNA (Raza K et al, 2016). The principle behind NGS technology is similar to Sanger's capillary electrophoresis in which DNA polymerase catalyses the addition of fluorescently labelled deoxy ribonucleotide triphosphates (dNTPs) into a DNA template strand during sequential cycles of DNA synthesis. In every cycle, nucleotides will be uniquely identified by fluorophore excitation at the point of incorporation. The main difference is that instead of sequencing a single DNA fragment, NGS extends this process across millions of fragments in a massively parallel fashion (Katara et al, 2024).

CHAPTER THREE: METHODOLOGY

3.1 Study design

This longitudinal cohort study was nested within the larger DRIBS parent study, a four-year project aimed at evaluating the impact of low-frequency HIV drug-resistant polymorphisms in HIV-seropositive infants born to HIV-positive mothers following intensified adherence counselling. Infants who tested positive for HIV DNA via PCR and were receiving antiretroviral therapy (ART) were included, along with their mothers. During the initial visit, blood samples were obtained to assess viral load, and infants with unsuppressed viral loads (>1000 copies/mL) underwent intensified adherence counselling (IAC) for three months, with one session each month. Following IAC, another blood sample was collected from the infants for HIV viral load testing after 6 months from the date of initial viral load blood collection. Infants exhibiting persistent unsuppressed viral loads (>1000 copies/mL) underwent HIV drug resistance testing to detect drug resistance mutations present both at the initial visit and following intensified adherence counselling (IAC) if they remained unsuppressed.

3.2 Study site

The research was carried out at the Joint Clinical Research Centre (JCRC) located in Lubowa, Kampala. This facility offers HIV testing, treatment and counselling services to both infants and their mothers. The JCRC molecular biology laboratory is accredited by the College of American Pathologists (CAP). The lab is equipped with cutting-edge technology, including a high-throughput next-generation sequencer, Polymerase Chain Reaction (PCR) machines, and associated accessories, rendering it a contemporary molecular biology laboratory facility.

3.3 Study population

The study cohort comprised HIV-seropositive infants, both male and female, aged between 4 and 12 months at the time of study enrolment, along with their HIV-positive mothers.

3.4 Sample size determination

The sample size was determined using the formula outlined by Diggle *et al*., (2002) as follows;

$$n = \frac{2(Z_{\alpha/2} + Z_{\beta})^2)\sigma^2}{\Delta^2} * (1 + (m-1)\rho)$$

$$n = \frac{2(1.96 + 0.84^2) * 0.1^2}{0.05^2} * (1 + (2-1) * 0.05) = 66$$

The sample size (n) was determined using the following parameters; $Z_{\alpha/2}$ representing the Z-score corresponding to the selected significance level (α = 0.05) set at 1.96 for a two-tailed test, Z_{β} representing the Z-score corresponding to the desired statistical power (80%) set to 0.84, σ^2 representing the estimated standard deviation of adherence scores, set to σ=0.1, Δ representing the minimum clinically important difference in adherence scores, set to 0.05, m representing the number of repeated measurements per subject, set to 2 (before and after intervention), and ρ representing the intraclass correlation coefficient (ICC) depicting the correlation between repeated measurements within the same subject, set to 0.05. Through these parameters, the calculated minimum sample size required for the study was determined to be 66 participants. However, the study enrolled 100 participants further increasing the statistical power.

3.5 Inclusion, exclusion criteria and participant enrollment

3.5.1 Inclusion criteria

Infants between 4 and 12 months of age at the time of study enrolment, confirmed positive for HIV through early infant diagnosis (EID) DNA PCR testing, on ART and displaying unsuppressed viral loads (>1000 copies/mL, mother who consented for both herself and the infant to participate in the study were enrolled.

3.5.2 Exclusion criteria

Infants presenting with severe illnesses or documented medical conditions inadequately controlled, as well as mothers or primary caregivers unwilling to provide consent for both themselves and the infant, were excluded from the participant recruitment phase.

3.6 Intensified adherence counselling

Following the WHO and Ministry of Health guidelines, intensified adherence counselling (IAC) was initiated for the mother, adhering to standard procedures. This involved

identifying a primary dedicated parent or caregiver typically the infant's mother, who underwent counselling to assess the infant's adherence level. Given the mother's role in giving the baby medication, the reasoning behind this strategy was that a non-adherent mother would probably produce a non-adherent infant. Using the designated technique listed in Appendix 1 the counselling sessions were led by qualified experts at the Joint Clinical Research Centre (JCRC). Finding the root causes of poor adherence and providing mothers with individualised advice to improve adherence behaviours were the goals of the counselling sessions.

3.7 Laboratory procedures

3.7.1 HIV Viral load assay

EDTA purple tubes were used to collect venous blood samples, which were then processed to produce plasma. After processing, the samples were sent to the JCRC Viral Load Laboratory section's CAP-accredited processing unit for HIV viral load testing. The findings of the viral load were then obtained from an online database and sent to the appropriate practitioner. When infants had unsuppressed viral loads (≥1000 copies/mL), the referring clinician was notified immediately, and the mother's adherence counselling was enhanced. Following a 3month intensified counselling session, a repeat viral load assessment was conducted after the third month.

3.7.2 Adherence to Antiretroviral Therapy (ART)

The Pill count method was used as a practical and straightforward method to evaluate adherence to antiretroviral therapy (ART). At each clinic visit, healthcare provider (s) compared the number of pills dispensed to the number returned by the patient. Adherence was calculated using the formula:

$$Adherance\ (\%) = \frac{(Pills\ dispensed - Pills\ returned) * 100}{Pills\ prescribed\ for\ the\ period}$$

This method offered a relatively objective and low-cost way to monitor medication use, especially in resource-limited settings. However, it assumes that any pills not returned were taken as prescribed, which may not always be true. Patients might discard or hide pills before

their visit, potentially leading to overestimated adherence. Nonetheless, pill count remains a useful tool for identifying patients who may need additional support with adherence.

3.7.3 HIV Drug Resistance testing

A baseline drug resistance test was conducted for all study participants whose viral loads remained unsuppressed (≥1000 copies/mL) before initiation of intensified adherence counselling. Another drug resistance test was conducted for those infants who still had unsuppressed viral loads even after the

3.7.3.1 Sample preparation

Before extraction, plasma samples were retrieved from a storage at -80°C and allowed to thaw to room temperature. The samples were then processed in batches with uniform procedures applied to each batch.

3.7.3.2 Viral RNA extraction

HIV viral RNA isolation was conducted on thawed plasma samples with the spin column based QIAamp viral RNA mini kit (Qiagen, Germany) following the manufacturer's guidelines. The purified RNA was subsequently stored at -80°C in anticipation of PCR amplification.

3.7.3.3 Polymerase Chain Reaction

The purified viral RNA was then amplified through an external PCR process which was followed by nested PCR, using the SuperScript™ III One-Step RT-PCR System with Choice™ Taq DNA Polymerase enzyme (Invitrogen by Thermo Fisher Scientific, USA) and Choice-Taq™ DNA Polymerase (Thomas Scientific, USA) kits, respectively. Each reaction in the external PCR used a master mix comprising 4.5 µl, consisting of 0.75 µl of ultra-pure water (Invitrogen by Thermo Fisher Scientific, USA), 3.125 µl of 2X reaction buffer, 0.25 µl of MgSO4, 0.125 µl each of 25pM forward and reverse primers (Table 2), and 0.125 µl of SuperScript III enzyme mix. To this master mix, 1.75 µl of purified RNA was added resulting in a total reaction volume of 6.25 µl per sample. The reaction mixture was then amplified using a SimpliAmp™ Thermal Cycler (Thermo Fisher Scientific, USA) with cycling conditions as outlined in Table 1.

Table 1: Cycling Conditions for external PCR amplification

Step	Temp	Time	# cycles
Reverse Transcription	50°C	30 min	* 1
Denaturation	94°C	2 min	* 1
Denaturation	94°C	15 sec	* 2
Annealing	61°C	30 sec	
Elongation	68°C	1 min	
Denaturation	94°C	15 sec	* 14 (Touch down of -0.5 °C after cycle 1)
Annealing	60°C*	30 sec	
Elongation	68°C	1 min	
Denaturation	94°C	15 sec	* 34
Annealing	53°C	30 sec	
Elongation	68°C	1 min	
Final Extension	68°C	7 min	* 1
Hold	4°	Hold	* 1

The product obtained from the external PCR was further amplified via nested PCR using a set of primers positioned more internally compared to those used in the external primer set. Each reaction in the nested PCR utilized a master mix component totalling 11.5 µl, comprising 9.37 µl of ultra-pure water (Invitrogen by Thermo Fisher Scientific, USA), 1.25 µl of 10X reaction buffer, 0.25 µl of dNTP, 0.25 µl each of 25pM forward and reverse primers (Table 2), and 0.13 µl of Choice™ Taq DNA Polymerase enzyme. To this master mix, 1.0 µl of external PCR product was added, resulting in a total reaction volume of 12.5 µl per sample. The reaction mixture underwent amplification using a SimpliAmp™ Thermal Cycler (Thermo Fisher Scientific, USA), employing cycling conditions as follows: (1) An initial denaturation cycle of 2 min at 94°C; (2) 35 cycles consisting of 20 s at 94°C for DNA denaturation, 30 s at 55°C for annealing, and 2 minutes at 68°C for elongation; (3) a final extension step of 5 min at 68°C; and (4) holding at 4°C indefinitely.

Table 2: Primer sequences and characteristics for the external and nested PCR

Primer	Direction	PCR type	sequence	location
RTA8	Reverse	External	GCTATTAAGTCTTTTGATGGGTCAT	3505←3529
PS 3	Forward	External	GAAAGACTGCACTGAAAGACAGGC	2058→2081
RTA4	Revers	Nested	CTGTATATCATTGACAGTCCAGCT	3300←3323
PS2.5	Forward	Nested	ACAGCCCCACCAGCAGAG	2155→2172

3.7.3.4 PCR product visualization using gel illumination

Gel preparation began by mixing 1 gram of Agarose I (Thermo Fisher Scientific, USA) with 100 mL of 1X TAE buffer, which was prepared from 50X TAE Buffer (Thermo Fisher Scientific, USA). The mixture was heated to boiling until complete dissolution of the powder. Following this, the gel was allowed to cool under running water, and 5 µL of SYBR Safe DNA Gel Stain (Thermo Fisher Scientific, USA) was added. The prepared gel was then poured onto a loading tray assembled with combs and left to set. Subsequently, 1 µL of loading dye was combined with 2 µL of the DNA sample on a parafilm. The resulting mixture was loaded into wells on the agarose gel in the order corresponding to their respective runs for ease of identification. Furthermore, a GeneRuler 1 kb DNA Ladder (Thermo Fisher Scientific, USA) was loaded after every 8^{th} sample. Following the loading of all samples, the Bio-Rad PowerPac 3000 Electrophoresis Power Supply (Bio-Rad, USA) was activated and the gel was run for 30 minutes at 98 volts. Subsequently, the gel was transferred to the viewing block, and images were captured using the Samsung TL240 (Samsung, South Korea) lab camera. Gel images were then printed and attached to the laboratory notebook. For this report, gel pictures are associated with their respective PCR runs to facilitate result interpretation.

The gel electrophoresis image illustrates the results of a PCR assay targeting the protease and reverse transcriptase (PR-RT) region that was used to assess amplification success prior to sequencing. A 1 kb DNA ladder was loaded to serve as a molecular size marker permitting estimation of PCR product sizes. In the sample lanes the presence of a distinct DNA band at the expected size indicates successful amplification of the PR-RT region, while the absence of a band signifies amplification failure. The positive control shows a clear DNA band confirming that the PCR reagents and conditions were functional. Equally, the negative control displays no band verifying the absence of contamination and validating the specificity of the assay. This setup ensured a reliable interpretation of participant sample results.

3.7.3.5 PCR product clean up

The clean-up procedure was conducted using ExoSAP-IT™ Express PCR Product Clean-up (Thermo Fisher Scientific, USA) following the manufacturer's instructions. This method successfully removed any residual dNTPs and primers present in the PCR product thereby mitigating potential interference during sequencing process.

3.7.3.6 Library preparation and sequencing

Library preparation was carried out using the Illumina DNA Prep kit (Illumina, USA) in accordance with the manufacturer's instructions. The process involved several key steps to prepare cleaned DNA for sequencing. First, genomic DNA was fragmented during the tagmentation step which was followed by a clean-up and size selection. Sequencing primers, sample indices, and adapters were then added during amplification of the tagmented DNA, after which a second clean-up step was performed. The resulting libraries were quantified using the Qubit fluorometer (ThermoFisher, USA), normalized with nuclease-free water, and pooled to achieve equimolar concentrations. The pooled libraries were analyzed using the Agilent 2100 Bioanalyzer (Agilent, USA) with a High Sensitivity DNA kit (ThermoFisher, USA) using Qubit fluorometer 4.0 (ThermoFisher, USA) to determine average library fragment size and concentration respectively. The libraries were then diluted to 4 nM, denatured with 0.2 N sodium hydroxide, and further diluted to a final concentration of 20 pM with a 5% PhiX spike-in control. The prepared library mixture (600 µL) was loaded into the reagent cartridge. Prior to sequencing, the MiSeq instrument was cleaned and prepared using MiSeq Control Software, including flow cell cleaning, reagent loading, and system checks. Sequencing progress was monitored throughout the run, followed by a post-run wash to maintain instrument.

3.7.3.7 Genetic data Analysis and management

To guarantee that a sufficient number of clusters (>900,000) and a significant proportion (>70%) of sequences had Phred scores above 30, the obtained data was assessed using the run manager software after sequencing on the MiSeq sequencer was complete. This made sure that there were enough reads and that the bases were called correctly. The FASTQ files were then transferred from the MiSeq sequencer to an external hard drive. Then, using, these FASTQ files were uploaded to the web-based Hydra software (Version: v1.7.0) with the default parameters in order to perform quality controls, trimming, alignment, assembly, and consensus sequence generation for every sample in a FASTA file format. To predict drug resistance, the resultant FASTA files were downloaded from Hydra and then submitted to the Stanford University HIV drug resistance database (Version 9.8). Classified mutations, their corresponding scores, susceptibility status, and other parameters were obtained as an output in the form of a Comma-Separated Values (CSV) file. A tabular summary of these findings was provided to make interpretation and additional analysis simple.

3.8 Statistical analysis

To evaluate the outcomes of Intensified Adherence Counselling (IAC), viral load data before and after the intervention were analyzed alongside adherence levels, reported as percentages. The effects of IAC on both viral load and adherence over time were visualized by plotting these variables. On the y-axis, the average viral load before and after IAC, along with adherence percentages, are shown. The x-axis represents time in months.

To ascertain the presence of both NRTI, NNRTI, mixtures and absence of mutations in infants experiencing virological failure prior to and after intensified adherence counseling (IAC), the CSV data obtained from the Stanford University HIV drug resistance database was summarised using Microsoft Excel (2019) into a table form and later on represented as percentages on graphs.

3.9 Quality control

During the extraction phase, both positive and negative controls were incorporated to monitor for potential cross-contamination and amplification inhibitions. During library preparation, QC steps include assessing the integrity and quantity of input DNA using Agilent Bioanalyzer, which provided fragment size distribution, and Qubit for accurate quantification. For sequencing, the PhiX control library was included as an internal control, enabling real-time assessment of sequencing accuracy, instrument performance and evaluating the quality of the run, including metrics such as base calling accuracy and read misassignment. Moreover, statistical methods, particularly Phred score analysis, were employed to ensure sequencing accuracy and reliability during each sequencing run.

3.10 Ethical Consideration

The primary study obtained IRB approval from JCRC IRB, Uganda National Council for Science and Technology (UNCST) and consent from the babies' mothers for use of their samples for genetics and future studies.

3.11 Limitations of the study

The study was limited by the increased costs of enrolling mother to child pairs since they have to be facilitated to move from their home areas to come to the hospital for their scheduled counseling visits. The pill count method of comparing pills dispensed versus returned to identify patients needing additional support gave inconsistent results as patients might discard or hide pills before their visit, potentially leading to overestimated adherence.

CHAPTER FOUR: RESULTS

4.1 Patients Baseline Characteristics

In this study, a total of 100 HIV seropositive infants were enrolled, comprising 60% females (n = 60) and 40% males (n = 40). Only one new-born, whose mother had died, was breastfed by an HIV-negative caregiver while all the other infants were breastfed by their biological mothers. All infants were initiated on the same antiretroviral therapy regimen of abacavir/lamivudine/lopinavir-ritonavir (ABC/3TC/LPV/r), during the six months preceding the initiation of Intensified Adherence Counselling (IAC). At baseline, the mean age of the infants was 5 months, the mean weight was 6.0 kg, and the mean viral load was 5.6 $\log_{10}$ RNA copies/mL. Table 3 presents the baseline demographic characteristics of the infants and their mothers.

Table 3: Baseline Demographic and Clinical Characteristics of Study Participants (N = 100)

Variable	**Category/Statistic**	**Female (n=60)**	**Male (n=40)**	**Total (n=100)**
Age in months	Mean	8	8	8
Weight (kg)	Mean	6.1	5.9	-
Log10 viral load	Mean	5.6	5.5	-
Mother's education level	No formal/primary education	-	-	60 (60%)
	Secondary/tertiary education	-	-	40 (40%)
Mother's residence	Urban	-	-	55 (55%)
	Rural	-	-	45 (45%)
Nutrition assessment	Red	-	-	3 (3%)
	Yellow	-	-	8 (8%)
	Green	-	-	79 (79%)
Nevirapine exposure	Yes	-	-	64 (64%)
	No	-	-	36 (36%)

4.2 Viral Load Outcomes Following Intensified Adherence Counselling in Mother-Infant Pairs

The Figure 2 below, illustrates infants adherence trends and corresponding viral load levels before and after the implementation of Intensified Adherence Counselling (IAC). Adherence is represented by two bars with the blue indicating good adherence (95–100%), while orange represents poor adherence (<95%). The viral load levels before IAC are represented by the green dashed line, which exhibits a little rising trend in tandem with diminishing adherence. In contrast, the red solid line illustrates viral load levels following IAC, demonstrating a consistent decrease over time.

The visual data illustrates that prior to the intervention, decreasing adherence was associated with rising viral load levels. However, following the introduction of IAC, viral loads declined regardless of adherence fluctuations, implying a potential positive effect of IAC on viral load suppression.

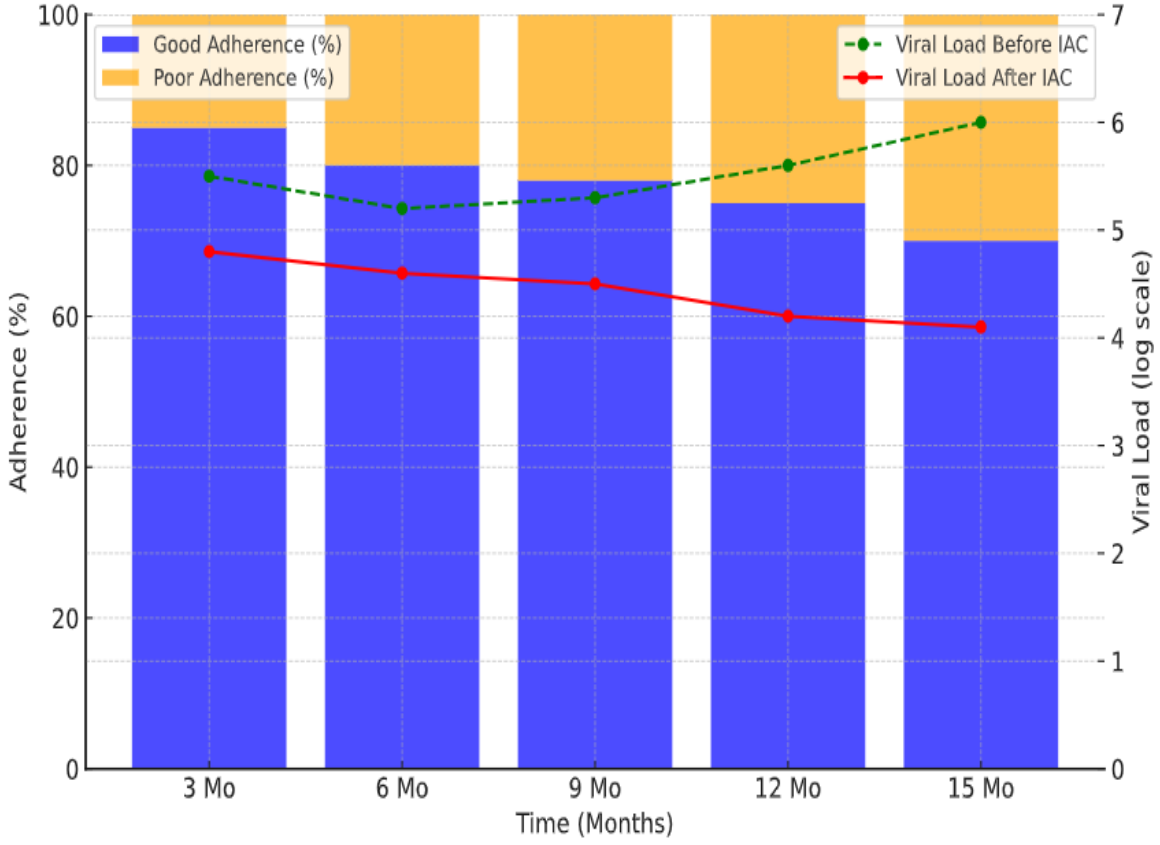

Figure 1: A bar and line graph, showing the trends in ART Adherence and Viral Load Response Over 15 Months Following IAC

4.3 HIV Drug Resistance Mutations in Infants with Virological Failure: Pre- and Post-IAC Findings

In this study we looked at nucleoside reverse transcriptase inhibitor (NRTI) mutations and non-nucleoside reverse transcriptase inhibitor (NNRTI) mutations before and after intensified adherence counselling (IAC) from 100 participants.

4.3.1 HIV drug resistance mutations before intensified adherence counselling

4.3.1.1 NRTI Mutations

A total of 115 samples were successfully amplified and sequenced for the HIV-1 reverse transcriptase (RT) region. Among the analysed samples, the most frequently observed mutation was M184V/M184I/M184MV, detected in 50 cases (43.5%). Additional noteworthy mutations were D67N/DN in 4 (3.5%), S68G in 6 (5.2%), L74V/LV/LI in 5 (4.3%), K70R/E/KE/KEGR in 7 (6.1%), and K219Q/E/KQ/KE in 10 samples (8.7%). The less common variants were T215Y/I/A/TA in four samples (3.5%), K65R/KR in three samples (2.6%), and V75M and E44D in one sample (0.9%) each. Notably, 65 samples (56.5%) showed no detectable NRTI resistance mutations. The figure below, illustrates the prevalence of NRTI resistance mutations in the study cohort prior to the initiation of IAC.

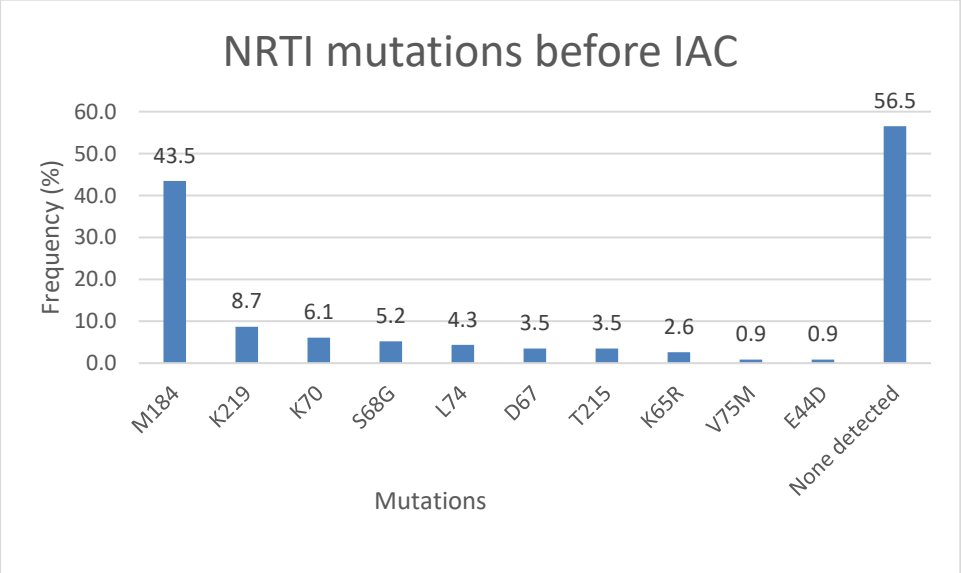

Figure 2: A bar graph showing the frequency of NRTI mutations before IAC

4.3.1.2 NNRTI Mutations

Among the 115 samples analysed for NNRTI resistance mutations, the most frequently detected mutation was K103N, present in 63.5% of participants. The Y181C (17.4%), G190A (11.3%), P225H and V108I (8.7% each) and E138A (7.8%) mutations followed. 15.7% of samples had mutations at position V179, whereas 9.6% had mutations at location V106. A98G and K101 mutations were found in 3.5% and 5.2% of samples, respectively. Additional mutations included E138Q and G190S (4.3% each), M230L (1.7%), F227L (0.9%), and H221Y (2.6%). Notably, 20.9% of participants had no detectable NNRTI resistance mutations.

The figure below illustrates the prevalence of NNRTI resistance mutations in the study cohort

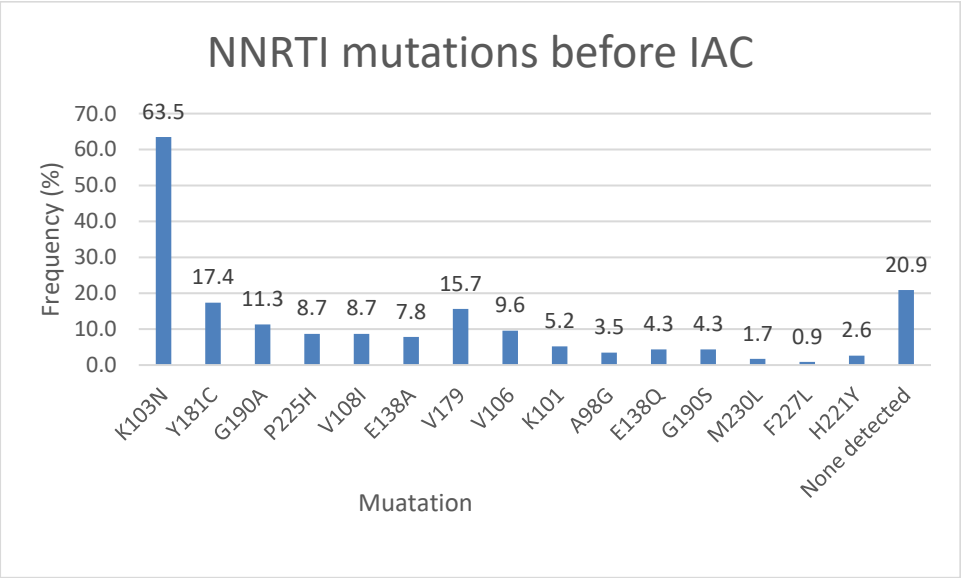

Figure 3: A bar graph showing the frequency of NNRTI mutations before IAC

4.3.2 HIV drug resistance mutation after intensified adherence counselling

4.3.2.1 NRTI Mutations

Among the 82 samples analysed after IAC, the most common NRTI mutation was M184 (including variants such as M184V and M184I), detected in 40.2% of participants. The D67N mutation was found in 6.1% of samples, whereas mutations at K70 and K219 were found in 9.8% of samples. T215, S68, and K65 variants were detected in 3.7% of the cohort, whereas L74 mutations were detected in 4.9%. V75I (1.2%) and M41 and E44D (2.4% each) were

less common alterations. Notably, 46.3% of participants exhibited no detectable NRTI mutations following IAC.

The figure below illustrates the prevalence of nucleoside reverse transcriptase inhibitor (NRTI) mutations in HIV patients following intensified adherence counselling (IAC).

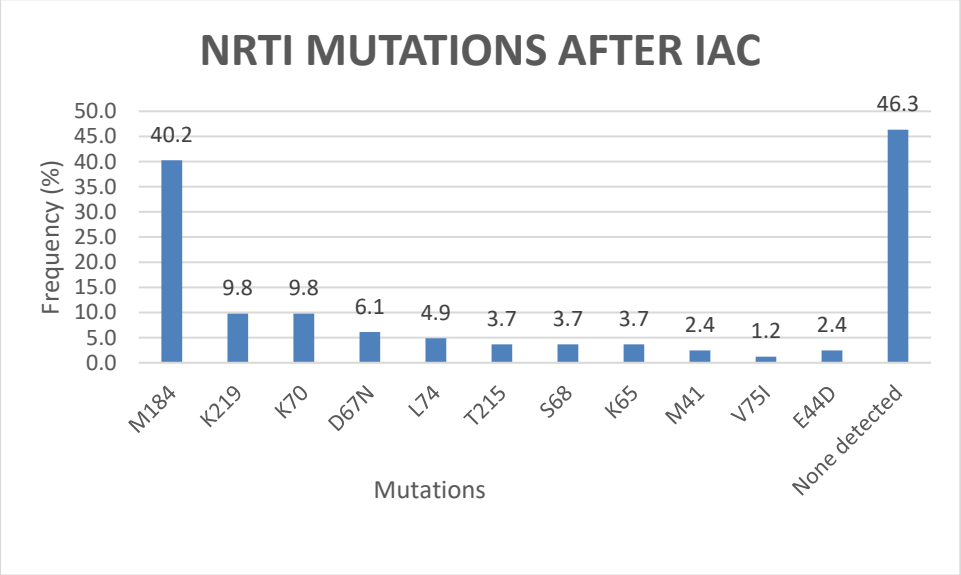

Figure 4: A bar graph showing the frequency of NRTI mutations after IAC

4.3.2.2 NNRTI Mutations

Among the 82 samples analysed, the most frequent NNRTI mutation was K103 (including variants K103N, K103KN, and K103S), detected in 56.1% of participants. This was followed by Y181 mutations (17.1%), G190 and V179 mutations (each at 15.9%), and E138 mutations (13.4%). Mutations at P225 and V108 were each observed in 9.8% of samples, while V106 mutations appeared in 6.1%. Mutations at K101 were seen in 4.9%, and M230 and A98G mutations were each detected in 3.7% of cases. Less frequent were H221 and K238 mutations, each at 2.4%. Notably, no NNRTI mutations were detected in 18.3% of the samples. The figure attached below illustrates the prevalence of non-nucleoside reverse transcriptase inhibitor (NNRTI) mutations in HIV patients following intensified adherence counselling (IAC).

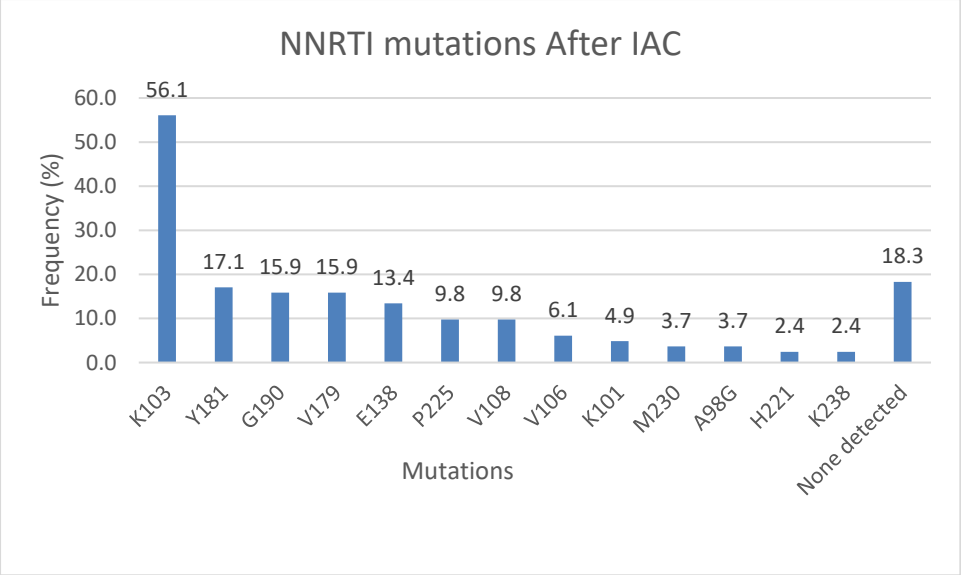

Figure 5: A bar graph showing the frequency of NNRTI mutations after IAC

4.3.3 Comparison of HIV Drug Resistance Mutations in Infants Pre- and Post-Intensified Adherence Counseling

4.3.3.1 NRTI Drug Resistance Mutations Before and After IAC

The most common mutation, M184, showed a modest decline from 43.5% before IAC to 40.2% after IAC. K219 and K70 mutations both increased post-IAC, rising from 8.7% to 9.8%, and 6.1% to 9.8%, respectively. The D67 mutation also increased, from 3.5% to 6.1%, while S68G decreased from 5.2% to 3.7%. Minor increases were noted for L74, T215, K65, V75, E44, and M41, all of which were either absent or less frequent before IAC. Overall, the proportion of participants with no NRTI mutations dropped from 56.5% before IAC to 46.3% after IAC, indicating a slight increase in NRTI resistance following IAC as illustrated in the figure below.

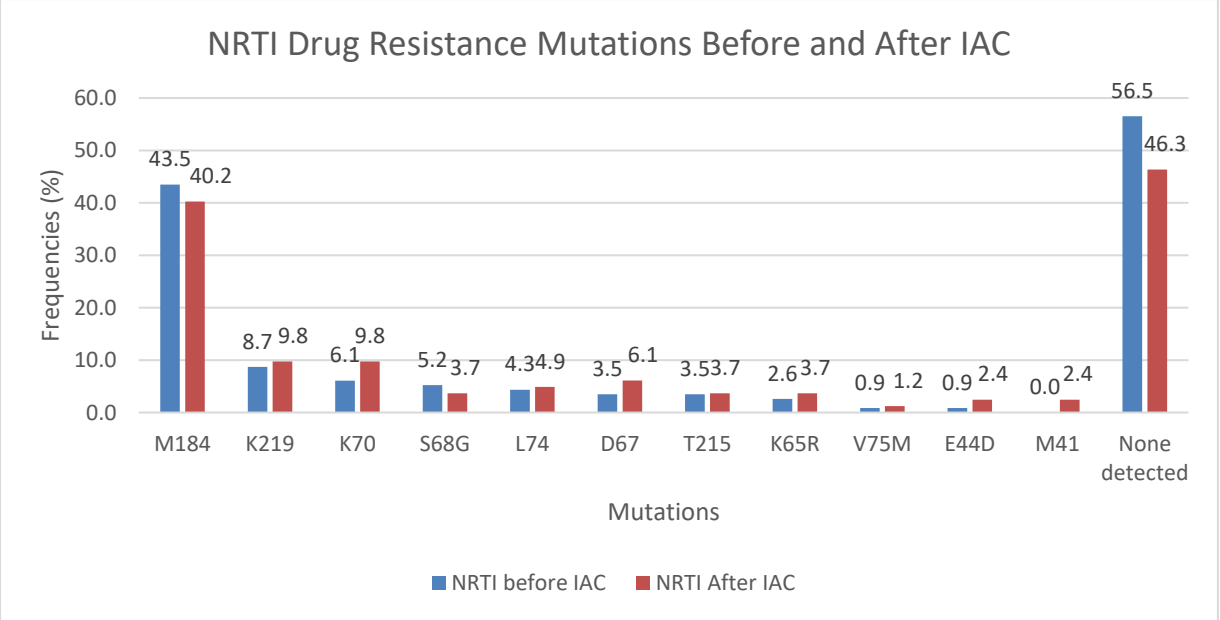

Figure 6: A bar graph showing the comparative Frequencies of NRTI Drug Resistance Mutations Before and After IAC

4.3.3.2 NNRTI Drug Resistance Mutations Before and After IAC

The Non-nucleoside reverse transcriptase inhibitor (NNRTI) mutation prevalence generally decreased following intensive adherence counseling (IAC) compared to previously. K103N, the most common mutation, dropped from 63.5% before to IAC to 40.0% following IAC. Similarly, P225H decreased from 8.7% to 7.0% while Y181C decreased from 17.4% to 12.2%. Some mutations including G190A, remained constant at 11.3%, E138A increased from 7.8% to 9.6% and K238 which wasn't there before IAC—appears at 1.7% after IAC. Interestingly, G190S and E138Q, which were each found in 4.3% of instances before to IAC, were no longer found following IAC. The percentage of those without NNRTI mutations increased from 20.9% to 13.0%, indicating a slight reduction in resistance profiles following IAC. However, important variants such as K103N and V179remain prevalent as illustrated in the figure 8 below.

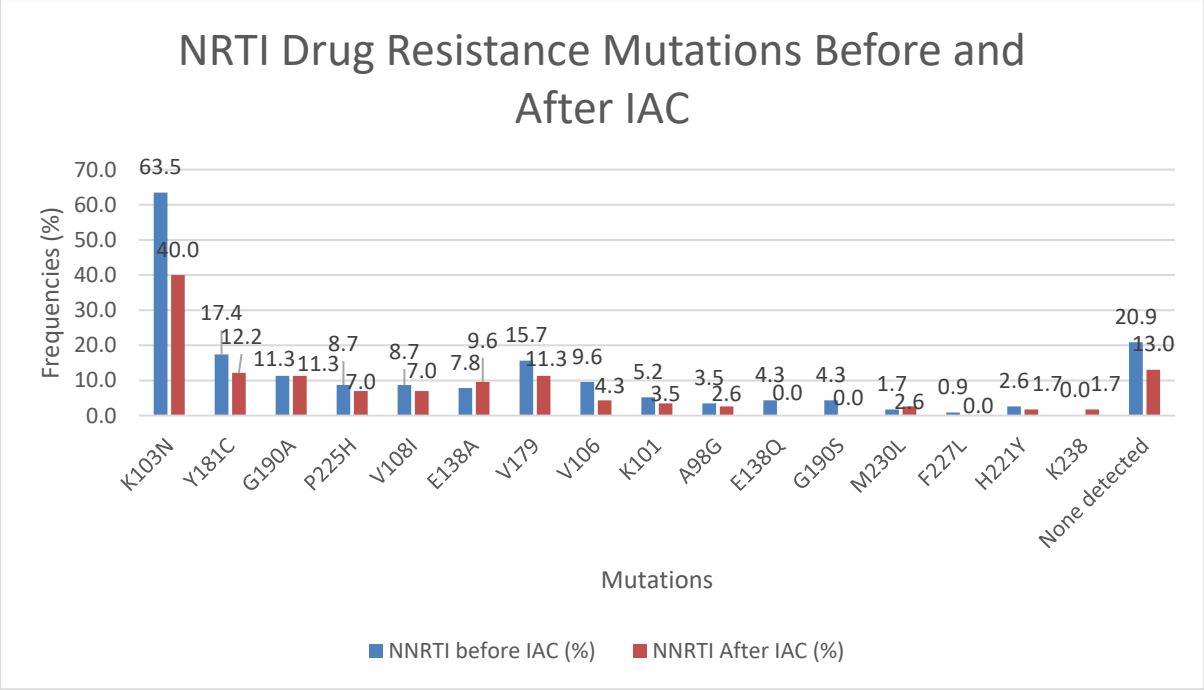

Figure 7: A bar graph showing the comparative Frequencies of NRTI Drug Resistance Mutations Before and After IAC

4.4 Evolution of the virus to pure sub type

4.4.1 NRTI Mixtures at DR positions

The changes in mixture mutation frequencies for different NRTI mutations before and after the implementation of IAC (Intervention-Affected Change) are summarized in figure 9 below. Note worth, the frequency of the M184 mutation dropped from 8% to 5%, from 9 (before) to 4 (after). In all cases, the K65 mutation maintained a 2% frequency and stayed constant at 1. The K70 mutation increased somewhat, from 3% to 5%, from 4 (before) to 5 (after). Frequencies for the K219 mutation changed from 7% to 4%, with a drop from 8 (before) to 3 (after). Mutations in S68, M41, L74, T215, and D67 either stayed the same or declined; the prevalence of S68 and M41 mutations was 0% before and after, whereas L74dropped from 3% to 1%, T215 stayed at 1%, and D67 went from 1% to 0%.

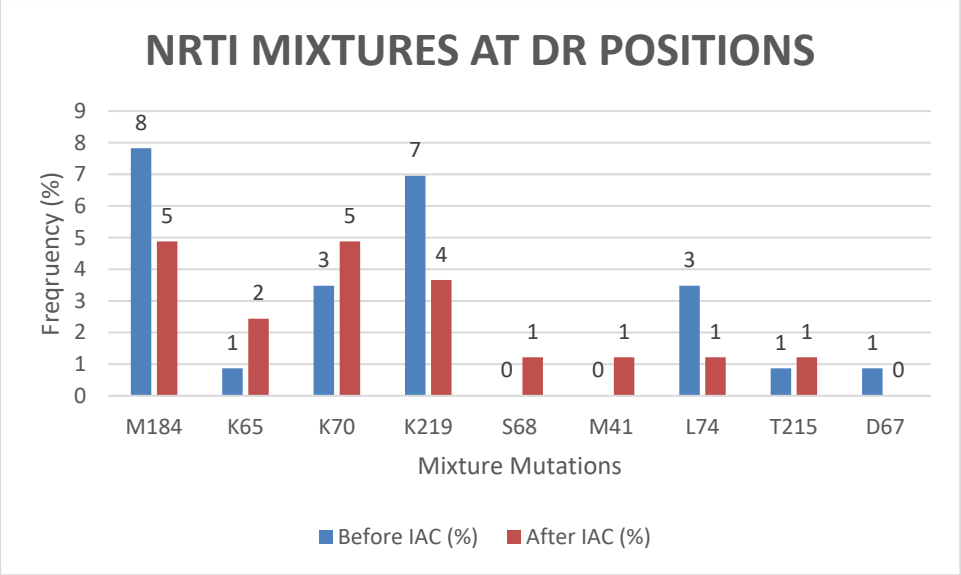

Figure 8: A bar graph showing the comparative frequencies of NRTI mixtures at DR positions before and after IAC.

4.4.2 NNRTI Mixtures at DR positions

The changes in mixture mutation frequencies for different NRTI mutations before and after the implementation of IAC (Intervention-Affected Change) are summarized in figure 10 below. The frequency of the K103 mutation dropped marginally from 8 (before) to 6 (after), while the proportion was constant at 7%. The percentages went from 3% to 5%, and the P225 mutation went from 3 to 4. Percentages decreased from 5% to 4% for the V108 mutation, which went from 6 to 3. With a constant frequency of 2 in both cases (2%), the G190 mutation stayed the same. With a drop-in frequency from 5% to 2%, the V179 mutation dropped from 6 to 2. The frequencies and percentages of the M230, K101, and H221 mutations stayed constant at 1. The Y181 and V106 and Y181 mutations saw complete elimination, with frequencies dropping to 0 after the intervention.

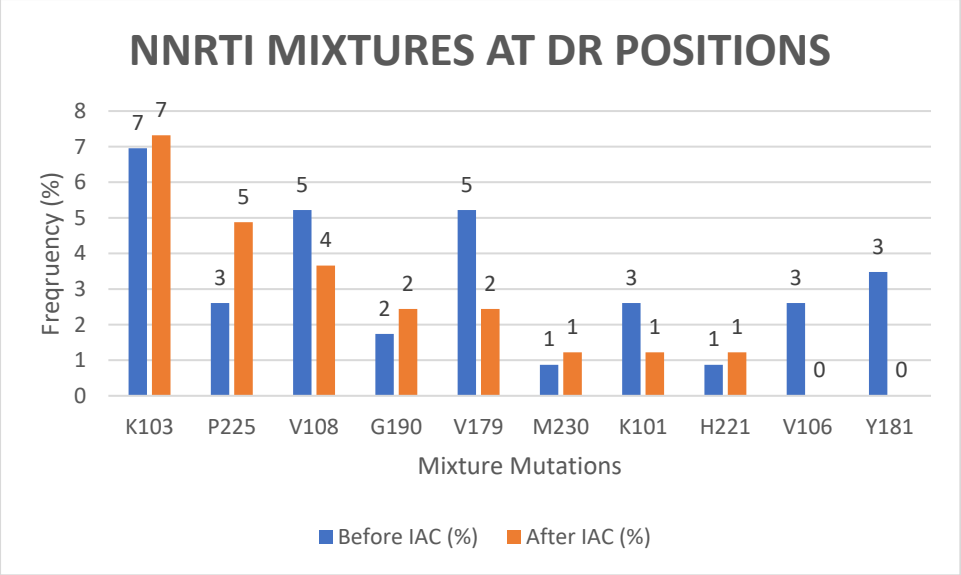

Figure 9: A bar graph showing the comparative frequencies of NNRTI mixtures at DR positions before and after IAC.

CHAPTER FIVE: DISCUSSION

Intensified Adherence Counselling (IAC) is one strategy used to improve ART adherence. It aims to improve viral suppression by informing patients on the value of adherence (Pius et al, 2021). The graphic data demonstrated that, before the intervention, rising viral load levels were linked to a decrease in adherence which is different from the findings of Izudi *et al*. (2023), who concluded that IAC was ineffective in achieving viral load suppression among people living with HIV (PLHIV) on first-line ART in Kampala, Uganda. However, our findings align with earlier research showing that treatment failure and increased viral loads were frequently associated with poor adherence to antiretroviral therapy (Phillips *et al*., 2019). However, even among those with adherence levels below 80%, viral load levels steadily decreased once IAC was introduced. This suggested that IAC had a positive impact on viral suppression, indicating that it could be effective even when adherence is poor. Relatedly, in a study by Manalel *et al*. (2024), individuals with at least 90% adherence did not significantly differ in viral suppression compared to those with 95% or greater adherence, supporting clinical guidelines and reinforcing the value of supportive interventions such as IAC. This is different from the findings of Izudi *et al.* (2023), who concluded that Intensive Adherence Counselling (IAC) was ineffective in achieving viral load suppression among people living with HIV (PLHIV) on first-line ART in Kampala, Uganda.

Interestingly, changes in nucleoside reverse transcriptase inhibitor (NRTI) resistance mutations before and after the introduction of IAC were notable in this study.

The M184V mutation that is associated with resistance to lamivudine (3TC) and emtricitabine (FTC) decreased from 43.5% before IAC to 40.2% after IAC. This supports the proposed explanation that, despite IAC's potential to improve adherence, M184V persists in a significant proportion of individuals. The data that M184V is quickly selected during non-suppressive antiretroviral therapy (ART) periods and can build up in the HIV reservoir, making it challenging to eradicate even with better adherence, is consistent with this persistence (Mimtsoudis *et al*., 2024).

The Thymidine analogue mutations (TAMs) may have developed in HIV as a result of the increased incidence of K219, K70 and D67 mutations following the deployment of Intensified Adherence Counselling (IAC), more so in patients receiving ZDV or d4T. It is well recognized that TAMs cause resistance to these medications. (Shafer et al., 2008). The observed changes in mutation frequencies (K219: 8.7% to 9.8%, K70: 6.1% to 9.8%, D67: 3.5% to 6.1%) indicate a potential accumulation of these resistance-conferring mutations.

According to Goodall *et al*. (2017), who discovered that prolonged viral failure on zidovudine-based ART was linked to rapidly increasing drug resistance, which could be mitigated with effective viral load monitoring, this increase after IAC may be a result of the selective pressure from continued use of ZDV or d4T in individuals.

After IAC, the frequency of the S68G mutation decreased from 5.2% to 3.7% which could indicate a change in the viral population as a result of better viral suppression. Previous research has indicated that the S68G mutation has a compensating function when combined with major resistance mutations even though it has not been as tin detail studied clinically as variants such as M184V. Li *et al*. (2020), for example discovered that S68G and the K65R mutation often co-occurred in patients who had received therapy for a diversity of HIV-1 subtypes and circulating recombinant forms. The S68G variant showed a competitive replication advantage over strains that only had the K65R mutation. In spite of this replication advantage, there were no observed genotypic resistance variations between K65R and K65R/S68G variants for zidovudine, tenofovir or lamivudine. As a result, the decrease in S68G following IAC may indicate less selection pressure for this compensatory mechanism under enhanced viral control, could be as a result of greater adherence or regimens that minimize the development of K65R-associated resistance. Nevertheless, because S68G is a polymorphic and compensating mutation, it's exact clinical effects remain unknown and warrant further investigation.

The modest post-IAC increases in L74, T215, K65, V75, E44, and M41 mutations suggest a gradual evolution of resistance, likely driven by cumulative antiretroviral therapy (ART) exposure and historical treatment pressures. Even with better adherence, these mutations—many of which have been connected to resistance to nucleoside reverse transcriptase inhibitors (NRTIs)—may represent the virus's adaptive response to sustained drug pressure. Notably, K65 and L74 decrease sensitivity to tenofovir and abacavir respectively, whilst T215 and M41 are thymidine analogue mutations (TAMs) linked to resistance to zidovudine (Shafer et al, 2008).

Importantly, the proportion of participants with no detectable NRTI mutations dropped from 56.5% to 46.3% after IAC, signalling a slight increase in overall NRTI resistance.

This result is consistent with research demonstrating that resistance may still arise even when adherence therapies such as IAC lead to better treatment results especially when the disease is advanced or there is a history of poor adherence (McCluskey *et al*., 2019). This is further supported by von Wyl et al (2013), who proposed that adherence rates below 89% increase

the likelihood of non-suppression and the formation of resistance mutations when compared to perfect adherence.

There was a decrease in prevalence of resistance mutations in non-nucleoside reverse transcriptase inhibitors (NNRTI) following the implementation of IAC from 20.9% to 13.0% and observed non detectable NNRTI mutations. This shows a possible desirable shift in resistance patterns among HIV-infected individuals (PLHIV). The major decrease was observed for the K103N mutation, decreasing from 63.5% to 40.0%. K103N is the most common NNRTI mutations and has been found to reduce susceptibility to first-generation NNRTIs like nevirapine and efavirenz (Wang *et al*., 2014). As the replication of the virus is being suppressed with improved adherence, there is minimal space for resistance mutations to emerge and establish themselves in the viral population. It's for this reason that the frequency of such mutations as K103N reduces after IAC (Lukyamuzi *et al*., 2021). Other mutations such as Y181C and P225H also reduced hence suggesting a general trend towards reduced NNRTI resistance following IAC.

These mutations are most commonly selected under the pressure of NNRTIs and cause cross-resistance among drugs within this class (Omooja *et al*., 2019). Loss of mutations like E138Q and G190S upon IAC also confirms the potential that increased adherence can suppress minor quasispecies with such mutations if they carry a fitness cost to the virus (Wang *et al*., 2006 & Murat *et al*., 2023). However, some mutations such as E138A did increase moderately from 7.8% to 9.6%, and K238 newly emerged (1.7%), indicating that while overall NNRTI resistance is declining, some still persist in developing or emerging under sustained drug pressure. The HIV virus is highly mutable, and drug resistance accumulates over time.

This dynamic nature is such that despite effective adherence interventions like IAC, new mutations or growth of existing ones can occur, ensuring the need for constant monitoring and re-tuning of treatment policies (Inzaule *et al*., 2025).

The decrease that was noted in M184 (8% to 5%) and K219 (7% to 4%) mutations reflects the fact that better compliance with antiretroviral therapy (ART) would have led to better viral suppression and the reduction in replication of drug-resistant viral mutants. Similarly, the observed prevalence reductions or stabilisation of mutations like S68G, D67, M41, and

T215 that are most commonly associated with nucleoside reverse transcriptase inhibitor (NRTI) resistance imply reduced viral capacity to maintain such mutations when replication is more effectively inhibited. The M184V mutation, in particular, is associated with high-level resistance to lamivudine (3TC) and emtricitabine (FTC) and typically arises under suboptimal adherence (HIVDB, 2023). Improved ART adherence leads to more viral suppression, lowering the selective pressure for resistant variants to multiply (Pouga *et al*., 2019). The drop in M184 and K219 mutations, as observed in this study, points towards the fact that improved adherence is inducing more viral suppression and thus reduced replication of drug-resistant variants (Miller *et al*., 2002). The modest increase in the K70 mutation (to 5%, up from 3%), thymidine analogue mutation (TAM), and stability of K65 signify that although resistant subpopulations are present, there is a tendency towards lower diversity in the resistant strains (Iyidogan et al., 2014). The observed changes in the mixture mutation frequencies of NNRTI resistance mutations following the implementation of IAC could suggest the dynamic balance between viral adaptation and change in therapeutic pressure. Some of the resistance mutations V179, V108, Y181 and V106 reduced in frequency, suggesting an improved viral suppression and reduced selection pressure against these mutants. Though there was a decrease in the percentage of the K103 mutation, it's overall prevalence did not change, as it continued to be prominent in the resistance profile. On the contrary, the increase in P225 and the fixed frequencies of mutations such as G190, M230, K101 and H221 show that while IAC might suppress some resistance patterns, others continue or emerge anew, as proof of the virus's ongoing evolution despite antiretroviral therapy (SeyedAlinaghi *et al*., 2023).

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study demonstrated the importance of IAC in improving viral suppression by showing that its use could result in an observable drop in viral load levels including those with less than ideal adherence. M184V slightly decreased but K219, K70 and D67 mutations increased in the analysis of NRTI mutations before and after IAC, suggesting that zidovudine use continues to exert selection pressure even in the face of improved adherence. The slight increase in K65 and L74 mutations points to cumulative resistance brought on by prolonged medication use. NNRTI resistance mutations steadily decreased after IAC; significant drops were seen in K103N, V179, V108, Y181 and V106, suggesting better viral suppression. There were more newborns with no detectable NNRTI mutations, indicating less resistance. Ongoing viral adaptation to ART pressure was reflected in the persistence of mutations such as P225 and K238. Numerous NRTI and NNRTI mixture mutations have decreased in HIV resistance evolution following IAC indicating an improved viral suppression brought about by better adherence. However, the persistence of certain mutations such as K70 and P225 suggests that resistance may still emerge even in the presence of ongoing medication pressure.

6.2 Recommendations

1. This study recommends that IAC should be reinforced to improve viral suppression in babies. This can be achieved by routinely checking viral loads both before and after IAC in an effort to reduce viral load to less than 1000 copies/ml.
2. Other methods of measuring adherence could be adopted like drug level monitoring in the plasma since the pill count method has potential limitations.
3. It is recommended to perform routine resistance testing for new-borns with detectable viremia in order to identify significant drug-resistant mutations.
4. Tracking the evolution of drug-resistant mutations, with special attention to K70, K219, and K103N mutations, as well as P225 and K238, is recommended. This is because these mutations have persisted despite improved adherence, which indicates ongoing viral adaptability under ART pressure.

REFERENCES

1. Ammassari, A., Trotta, M. P., Murri, R., Castelli, F., Narciso, P., Noto, P., . . . Antinori, A. (2002). Correlates and predictors of adherence to highly active antiretroviral therapy: overview of published literature. *Journal of acquired immune deficiency syndromes (1999), 31*, S123-127.
2. Bor, J., Herbst, A. J., Newell, M. L., & Bärnighausen, T. (2013). Increases in adult life expectancy in rural South Africa: valuing the scale-up of HIV treatment. *Science (New York, N.Y.), 339*(6122), 961–965. https://doi.org/10.1126/science.1230413
3. Boyer, P. L., Rehm, C. A., Sneller, M. C., Mican, J., Caplan, M. R., Dewar, R., Ferris, A. L., Clark, P., Johnson, A., Maldarelli, F., & Hughes, S. H. (2022). A Combination of Amino Acid Mutations Leads to Resistance to Multiple Nucleoside Analogs in Reverse Transcriptases from HIV-1 Subtypes B and C. *Antimicrobial agents and chemotherapy*, *66*(1), e0150021. https://doi.org/10.1128/AAC.01500-21
4. Broder, S. (2010). The development of antiretroviral therapy and its impact on the HIV-1/AIDS pandemic. *Antiviral research, 85*(1), 1-18.
5. Chan, D. C., & Kim, P. S. (1998). HIV entry and its inhibition. *Cell*, *93*(5), 681-684.
6. Chen, R., Quinones-Mateu, M. E., & Mansky, L. M. (2004). Drug resistance, virus fitness and HIV-1 mutagenesis. *Curr Pharm Des, 10*(32), 4065-4070.
7. Chkhartishvili, N., Sharvadze, L., Dvali, N., Karchava, M., Rukhadze, N., Lomtadze, M., . . . Tsertsvadze, T. (2014). Virologic outcomes of second-line antiretroviral therapy in Eastern European country of Georgia. *AIDS research and therapy, 11*(1), 18.
8. Chung, S., Miller, J. T., Lapkouski, M., Tian, L., Yang, W., & Le Grice, S. F. (2013). Examining the role of the HIV-1 reverse transcriptase p51 subunit in positioning and hydrolysis of RNA/DNA hybrids. *J Biol Chem, 288*(22), 16177-16184. doi:10.1074/jbc.M113.465641
9. Das, K., & Arnold, E. (2013). HIV-1 reverse transcriptase and antiviral drug resistance. Part 2. *Current opinion in virology*, *3*(2), 119–128. https://doi.org/10.1016/j.coviro.2013.03.014
10. De Clercq, E. (2004). Antiviral drugs in current clinical use. *J Clin Virol, 30*(2), 115-133. doi:10.1016/j.jcv.2004.02.009
11. DeGruttola, V., Dix, L., D'Aquila, R., Holder, D., Phillips, A., Ait-Khaled, M., . . . Harrigan, R. (2000). The relation between baseline HIV drug resistance and response to antiretroviral therapy: re-analysis of retrospective and prospective studies using a standardized data analysis plan. *Antiviral therapy, 5*(1), 41-48.

12. Diggle, P. (2002). *Analysis of longitudinal data*. Oxford university press. Dynamics of Rilpivirine Resistance-Associated Mutation: E138 in Reverse Transcriptase among Antiretroviral-Naive HIV-1-Infected Individuals in Turkey. Murat Sayan, Nazife Sultanoglu, and Tamer Sanlidag. AIDS Research and Human Retroviruses 2023 39:2, 84-90
13. Engelman, A., & Cherepanov, P. (2012). The structural biology of HIV-1: mechanistic and therapeutic insights. *Nat Rev Microbiol, 10*(4), 279-290. doi:10.1038/nrmicro2747
14. Fokam, J., Bellocchi, M. C., Armenia, D., Nanfack, A. J., Carioti, L., Continenza, F., . . . Torimiro, J. N. (2018). Next-generation sequencing provides an added value in determining drug resistance and viral tropism in Cameroonian HIV-1 vertically infected children. *Medicine, 97*(13).
15. Gill, C. J., Hamer, D. H., Simon, J. L., Thea, D. M., & Sabin, L. L. (2005). No room for complacency about adherence to antiretroviral therapy in sub-Saharan Africa. *Aids, 19*(12), 1243-1249.
16. Goodall, R. L., Dunn, D. T., Nkurunziza, P., Mugarura, L., Pattery, T., Munderi, P., Kityo, C., Gilks, C., Kaleebu, P., Pillay, D., Gupta, R. K., & DART Virology Group (2017). Rapid accumulation of HIV-1 thymidine analogue mutations and phenotypic impact following prolonged viral failure on zidovudine-based first-line ART in sub-Saharan Africa. *The Journal of antimicrobial chemotherapy*, *72*(5), 1450–1455. https://doi.org/10.1093/jac/dkw583
17. Grant, R. M., Hecht, F. M., Warmerdam, M., Liu, L., Liegler, T., Petropoulos, C. J., . . . Kahn, J. O. (2002). Time trends in primary HIV-1 drug resistance among recently infected persons. *Jama, 288*(2), 181-188.
18. Huang, H., Chopra, R., Verdine, G. L., & Harrison, S. C. (1998). Structure of a covalently trapped catalytic complex of HIV-1 reverse transcriptase: implications for drug resistance. *Science, 282*(5394), 1669-1675.
19. Inzaule, S., Jordan, M. R., Fokam, J., Hamers, R. L., Paredes, R., Crowell, T. A., Shafer, R. W., Schapiro, J., Wit, T. F. R., Charurat, M., Kuritzkes, D. R., & Ndembi, N. (2025). Non-canonical HIV drug resistance mutations: need to close existing gaps. *AIDS (London, England)*, 10.1097/QAD.0000000000004170. Advance online publication. https://doi.org/10.1097/QAD.0000000000004170
20. Iyidogan, P., & Anderson, K. S. (2014). Current Perspectives on HIV-1 Antiretroviral Drug Resistance. *Viruses, 6*(10), 4095-4139. https://doi.org/10.3390/v6104095

21. Iyidogan, P., & Anderson, K. S. (2014). Current perspectives on HIV-1 antiretroviral drug resistance. *Viruses*, *6*(10), 4095-4139.
22. Izudi, J., Castelnuovo, B., King, R., & Cattamanchi, A. (2023). Impact of intensive adherence counseling on viral load suppression and mortality among people living with HIV in Kampala, Uganda: A regression discontinuity design. PLOS global public health, 3(8), e0002240. https://doi.org/10.1371/journal.pgph.0002240
23. Izudi, J., Castelnuovo, B., King, R., & Cattamanchi, A. (2023). Impact of intensive adherence counseling on viral load suppression and mortality among people living with HIV in Kampala, Uganda: A regression discontinuity design. *PLOS global public health*, *3*(8), e0002240. https://doi.org/10.1371/journal.pgph.0002240
24. Izudi, J., Cattamanchi, A., Castelnuovo, B., & King, R. (2024). Barriers and facilitators to viral load suppression among people living with HIV following intensive adherence counseling in Kampala, Uganda: A qualitative study. *Social science & medicine (1982)*, *343*, 116595. https://doi.org/10.1016/j.socscimed.2024.116595
25. Jacobo-Molina, A., Ding, J., Nanni, R. G., Clark, A. D., Jr., Lu, X., Tantillo, C., . . . *et al*. (1993). Crystal structure of human immunodeficiency virus type 1 reverse transcriptase complexed with double-stranded DNA at 3.0 A resolution shows bent DNA. *Proc Natl Acad Sci U S A*, *90*(13), 6320-6324.
26. Joint United Nations Programme on HIV/AIDS, & Joint United Nations Programme on HIV/Aids. (2014). 90-90-90: an ambitious treatment target to help end the AIDS epidemic. *Geneva: Unaids*, 9-23.
27. Jonah Omooja, Maria Nannyonjo, Grace Sanyu, Stella E Nabirye, Faridah Nassolo, Sandra Lunkuse, Anne Kapaata, Farouk Segujja, David Patrick Kateete, Eric Ssebaggala, Nicholas Bbosa, Emmanuel Aling, Rebecca N Nsubuga, Pontiano Kaleebu, Deogratius Ssemwanga, Rates of HIV-1 virological suppression and patterns of acquired drug resistance among fisherfolk on first-line antiretroviral therapy in Uganda, *Journal of Antimicrobial Chemotherapy*, Volume 74, Issue 10, October 2019, Pages 3021–3029, Karin J. Metzner, Stefano G. Giulieri, Stefanie A. Knoepfel, Pia Rauch, Philippe Burgisser, Sabine Yerly, Huldrych F. Gunthard, Matthias Cavassini, Minority Quasispecies of Drug-Resistant HIV-1 That Lead to Early Therapy Failure in Treatment-Naive and -Adherent Patients, *Clinical Infectious Diseases*, Volume 48, Issue 2, 15 January 2009, Pages 239–247, https://doi.org/10.1086/595703 https://doi.org/10.1093/jac/dkz261

28. Katara, A., Chand, S., Chaudhary, H., Chaudhry, V., Chandra, H., & Dubey, R. C. (2024). Evolution and applications of Next Generation Sequencing and its intricate relations with chromatographic and spectrometric techniques in modern day sciences. *Journal of Chromatography Open*, *5*, 100121. https://doi.org/10.1016/j.jcoa.2024.100121
29. Kityo, C., Sigaloff, K. C., Sonia Boender, T., Kaudha, E., Kayiwa, J., Musiime, V., Mukuye, A., Kiconco, M., Nankya, I., Nakatudde-Katumba, L., Calis, J. C., Rinke de Wit, T. F., & Mugyenyi, P. N. (2016). HIV Drug Resistance Among Children Initiating First-Line Antiretroviral Treatment in Uganda. *AIDS research and human retroviruses*, *32*(7), 628–635. https://doi.org/10.1089/AID.2015.0215
30. Kohlstaedt, L. A., Wang, J., Friedman, J. M., Rice, P. A., & Steitz, T. A. (1992). Crystal structure at 3.5 A resolution of HIV-1 reverse transcriptase complexed with an inhibitor. *Science*, *256*(5065), 1783-1790.
31. Kuhn, L., Hunt, G., Technau, K.-G., Coovadia, A., Ledwaba, J., Pickerill, S., . . . Black, V. (2014). Drug resistance among newly-diagnosed HIV-infected children in the era of more efficacious antiretroviral prophylaxis. *AIDS (London, England)*, *28*(11), 1673.
32. Ledergerber, B., Egger, M., Erard, V., Weber, R., Hirschel, B., Furrer, H., . . . Opravil, M. (1999). AIDS-related opportunistic illnesses occurring after initiation of potent antiretroviral therapy: the Swiss HIV Cohort Study. *Jama*, *282*(23), 2220-2226.
33. Leisegang, R., Stewart, A., Sunpath, H., Murphy, R., Winternheimer, P., Ally, M., & Maartens, G. (2014). Short term adherence tool predicts failure on second line protease inhibitor-based antiretroviral therapy: an observational cohort study. *BMC infectious diseases*, *14*(1), 664.
34. Li, S., Ouyang, J., Zhao, B., An, M., Wang, L., Ding, H., Zhang, M., & Han, X. (2020). The S68G polymorphism is a compensatory mutation associated with the drug resistance mutation K65R in CRF01_AE strains. *BMC infectious diseases*, *20*(1), 123. https://doi.org/10.1186/s12879-020-4836-z
35. Little, S. J., Holte, S., Routy, J.-P., Daar, E. S., Markowitz, M., Collier, A. C., . . . Conway, B. (2002). Antiretroviral-drug resistance among patients recently infected with HIV. *New England Journal of Medicine*, *347*(6), 385-394.
36. Lukyamuzi, Z., Etajak, S., Katairo, T. *et al.* Effect and implementation experience of intensive adherence counseling in a public HIV care center in Uganda: a mixed-methods study. *BMC Infect Dis* **21**, 1168 (2021). https://doi.org/10.1186/s12879-021-06862-6

37. Manalel, J.A., Kaufman, J.E., Wu, Y. *et al.* Association of ART regimen and adherence to viral suppression: an observational study of a clinical population of people with HIV. *AIDS Res Ther* **21**, 68 (2024). https://doi.org/10.1186/s12981-024-00657-w
38. McCluskey, S. M., Siedner, M. J., & Marconi, V. C. (2019). Management of Virologic Failure and HIV Drug Resistance. *Infectious disease clinics of North America*, *33*(3), 707–742. https://doi.org/10.1016/j.idc.2019.05.004
39. McMahon, J. H., Elliott, J. H., Bertagnolio, S., Kubiak, R., & Jordan, M. R. (2013). Viral suppression after 12 months of antiretroviral therapy in low-and middle-income countries: a systematic review. *Bulletin of the World Health Organization*, *91*, 377-385.
40. Miller, V., Stark, T., Loeliger, A. E., & Lange, J. M. (2002). The impact of the M184V substitution in HIV-1 reverse transcriptase on treatment response. *HIV medicine*, *3*(2), 135–145. https://doi.org/10.1046/j.1468-1293.2002.00101.x
41. Mills, E. J., Nachega, J. B., Buchan, I., Orbinski, J., Attaran, A., Singh, S., . . . Thabane, L. (2006). Adherence to antiretroviral therapy in sub-Saharan Africa and North America: a meta-analysis. *Jama*, *296*(6), 679-690.
42. Mills, E., Nachega, J., Bangsberg, D., Singh, S., Rachlis, B., & Wu, P. (2006). Adherence to antiretroviral therapy: A systematic review of developed and developing nation patient-reported barriers and facilitators. *PLoS Medicine 2006a*, *3*(11), 0001-0025.
43. Mimtsoudis, I., Tsachouridou, O., Akinosoglou, K., & Metallidis, S. (2024). Treatment Management Challenges in Naïve and Experienced HIV-1-Infected Individuals Carrying the M184V Mutation. *Viruses*, *16*(9), 1392. https://doi.org/10.3390/v16091392
44. Mukose, A. D. (2024). Implementation of lifelong ART (Option B+) for Prevention of Mother-to-Child Transmission (PMTCT) of HIV in Uganda (Doctoral dissertation, University of Antwerp).
45. Mutevedzi, P. C., & Newell, M. L. (2014). The changing face of the HIV epidemic in sub-S aharan A frica. *Tropical Medicine & International Health*, *19*(9), 1015-1028.
46. Namara-Lugolobi, E., Namukwaya, Z., Owor, M., Ouma, J., Namale-Matovu, J., Nakabiito, C., Ndugwa, C., Fowler, M. G., & Musoke, P. (2022). Twenty years of Prevention of Mother to Child HIV Transmission: research to implementation at a national referral hospital in Uganda. *African health sciences*, *22*(Spec Issue), 22–33. https://doi.org/10.4314/ahs.v22i2.5S
47. Nasuuna, E., Kigozi, J., Babirye, L. *et al.* Low HIV viral suppression rates following the intensive adherence counseling (IAC) program for children and adolescents with viral

failure in public health facilities in Uganda. *BMC Public Health* **18**, 1048 (2018). https://doi.org/10.1186/s12889-018-5964-x

48. Ndikabona, G., Alege, J.B., Kirirabwa, N.S. *et al.* Unsuppressed viral load after intensive adherence counselling in rural eastern Uganda; a case of Kamuli district, Uganda. *BMC Public Health* **21**, 2294 (2021). https://doi.org/10.1186/s12889-021-12366-4
49. Nyogea, D., Mtenga, S., Henning, L., Franzeck, F. C., Glass, T. R., Letang, E., . . . Geubbels, E. (2015). Determinants of antiretroviral adherence among HIV positive children and teenagers in rural Tanzania: a mixed methods study. *BMC infectious diseases*, *15*(1), 28.
50. Okonji, E. F., Wyk, B. V., Hughes, G. D., & Mukumbang, F. C. (2022). Implementation of a Psychosocial Support Intervention for Adolescents on Antiretroviral Treatment: Challenges and Experiences from Ehlanzeni District, South Africa. *Journal of the International Association of Providers of AIDS Care*, *21*, 23259582221121094. https://doi.org/10.1177/23259582221121094
51. Okot, S.P., Izudi, J., Ssenyonga, R., & Babirye N.J. (2024). Factors associated with completion of intensive adherence counseling among people living with HIV at a large referral hospital in Uganda: a retrospective analysis. *BMC Health Serv Res* **24**, 1093 (2024). https://doi.org/10.1186/s12913-024-11528-5
52. Otieno, P. (2023). Determinants Of Hiv Serostatus Disclosure Among People Living With Hiv/Aids In Msambweni County Referral Hospital, Kwale County, Kenya (Doctoral dissertation, The Catholic University of Eastern Africa).
53. Paredes R, M. V., Lockman S, Abrams EJ, Kuhn L:, & 2:S93–S100., J. I. D. S. (2013). Impact of antiretroviral drugs in pregnant women and their children in Africa: HIV resistance and treatment outcomes.
54. Parniak, M. A., & Sluis-Cremer, N. (2000). Inhibitors of HIV-I reverse transcriptase *Advances in pharmacology* (Vol. 49, pp. 67-109): Elsevier.
55. Peng, Y., Zong, Y., Wang, D., Chen, J., Chen, Z., Peng, F., & Liu, Z. (2023). Current drugs for HIV-1: from challenges to potential in HIV/AIDS. *Frontiers in Pharmacology*, *14*. https://doi.org/10.3389/fphar.2023.1294966
56. Pennings P. S. (2013). HIV Drug Resistance: Problems and Perspectives. *Infectious disease reports*, *5*(Suppl 1), e5. https://doi.org/10.4081/idr.2013.s1.e5
57. Phillips, T. K., Wilson, I. B., Brittain, K., Zerbe, A., Mellins, C. A., Remien, R. H., Orrell, C., Abrams, E. J., & Myer, L. (2019). Decreases in Self-Reported ART Adherence Predict HIV Viremia Among Pregnant and Postpartum South African Women. *Journal of*

acquired immune deficiency syndromes (1999), *80*(3), 247–254. https://doi.org/10.1097/QAI.0000000000001909

58. Pius, A., Josephine, N. N., Erick, S., Winifred, A., Rita, M., Silverjoseph, O., Lucy, E., & Novatus, N. (2021). Influence of intensified adherence counselling on viral load suppression of people receiving antiretroviral therapy at a health centre IV in southwestern Uganda: a qualitative study. *AIDS research and therapy*, *18*(1), 45. https://doi.org/10.1186/s12981-021-00372-w
59. Pouga L, Santoro MM, Charpentier C, Di Carlo D, Romeo I, Artese A, Alcaro S, Antinori A, Wirden M, Perno CF, Ambrosio FA, Calvez V, Descamps D, Marcelin AG, Ceccherini-Silberstein F and Lambert-Niclot S. New resistance mutations to nucleoside reverse transcriptase inhibitors at codon 184 of HIV-1 reverse transcriptase (M184L and M184T). Chemical Biology & Drug Design 2019.
60. Raza K, A. S. (2016). Principle, analysis, application and challenges of next-generation sequencing: a review. arXiv preprint arXiv:160605254.
61. Richman, D. D. (1990). Susceptibility to nucleoside analogues of zidovudine-resistant isolates of human immunodeficiency virus. *The American journal of medicine*, *88*(5), S8-S10.
62. Schuckmann, M. M., Marchand, B., Hachiya, A., Kodama, E. N., Kirby, K. A., Singh, K., & Sarafianos, S. G. (2010). The N348I mutation at the connection subdomain of HIV-1 reverse transcriptase decreases binding to nevirapine. *J Biol Chem*, *285*(49), 38700-38709. doi:10.1074/jbc.M110.153783
63. SeyedAlinaghi, S., Afsahi, A.M., Moradi, A. *et al.* Current ART, determinants for virologic failure and implications for HIV drug resistance: an umbrella review. *AIDS Res Ther* **20**, 74 (2023). https://doi.org/10.1186/s12981-023-00572-6
64. Shafer, R. W., & Schapiro, J. M. (2008). HIV-1 drug resistance mutations: an updated framework for the second decade of HAART. *AIDS reviews*, *10*(2), 67–84.
65. Shafer, R. W., & Schapiro, J. M. (2008). HIV-1 drug resistance mutations: an updated framework for the second decade of HAART. *AIDS reviews*, *10*(2), 67–84.
66. Shafer, R. W., Winters, M. A., Palmer, S., & Merigan, T. C. (1998). Multiple concurrent reverse transcriptase and protease mutations and multidrug resistance of HIV-1 isolates from heavily treated patients. *Annals of internal medicine*, *128*(11), 906-911.
67. Silver, N., Paynter, M., McAllister, G., Atchley, M., Sayir, C., Short, J., . . . Bergefall, K. (2018). Characterization of minority HIV-1 drug resistant variants in the United Kingdom

following the verification of a deep sequencing-based HIV-1 genotyping and tropism assay. *AIDS research and therapy, 15*(1), 18.

68. Simen, B. B., Simons, J. F., Hullsiek, K. H., Novak, R. M., MacArthur, R. D., Baxter, J. D., . . . Braverman, M. S. (2009). Low-abundance drug-resistant viral variants in chronically HIV-infected, antiretroviral treatment–naive patients significantly impact treatment outcomes. *The Journal of infectious diseases, 199*(5), 693-701.
69. Singh, K., Marchand, B., Kirby, K. A., Michailidis, E., & Sarafianos, S. G. (2010). Structural Aspects of Drug Resistance and Inhibition of HIV-1 Reverse Transcriptase. *Viruses, 2*(2), 606-638. doi:10.3390/v2020606
70. Smerdon, S., Jäger, J., Wang, J., Kohlstaedt, L., Chirino, A., Friedman, J., . . . Steitz, T. (1994). Structure of the binding site for nonnucleoside inhibitors of the reverse transcriptase of human immunodeficiency virus type 1. *Proceedings of the National Academy of Sciences, 91*(9), 3911-3915.
71. Stanford HIV Drug Resistance Database (HIVDB). 2023, Version 9.8. https://hivdb.stanford.edu/ [Accessed: May 5, 2025
72. Tang, M. W., & Shafer, R. W. (2012). HIV-1 antiretroviral resistance: scientific principles and clinical applications. *Drugs, 72*(9), e1–e25. https://doi.org/10.2165/11633630-000000000-00000
73. Tantillo, C., Ding, J., Jacobo-Molina, A., Nanni, R. G., Boyer, P. L., Hughes, S. H., . . . Arnold, E. (1994). Locations of anti-AIDS drug binding sites and resistance mutations in the three-dimensional structure of HIV-1 reverse transcriptase: implications for mechanisms of drug inhibition and resistance. *J Mol Biol, 243*(3), 369-387.
74. THE REPUBLIC OF UGANDA MINISTRY OF HEALTH (MOH). (2024). *THE REPUBLIC OF UGANDA CONSOLIDATED GUIDELINES FOR THE PREVENTION AND TREATMENT OF HIV AND AIDS IN UGANDA*. Retrieved from https://dsduganda.com/wp-content/uploads/2023/05/Consolidated-HIV-and-AIDS-Guidelines-20230516.pdf
75. Ton Q, F. L. C. H. R. (2013). HIV drug resistance in mothers and infants following use of antiretrovirals to prevent mother to child transmission
76. Tuske, S., Sarafianos, S. G., Clark Jr, A. D., Ding, J., Naeger, L. K., White, K. L., . . . Clark, P. (2004). Structures of HIV-1 RT–DNA complexes before and after incorporation of the anti-AIDS drug tenofovir. *Nature Structural and Molecular Biology, 11*(5), 469.
77. Uganda Aids Commission (UAC0. (2023). ANNUAL JOINT AIDS REVIEW REPORT. https://uac.go.ug/images/2024/jar-2024/jar-2022-2023-annual-report.pdf

78. UNAIDS. (2024). *Global HIV & AIDS statistics — Fact sheet | UNAIDS*. https://www.unaids.org/en/resources/fact-sheet
79. Valladolid-Acebes, I., & Steven, A. W. Prevalence of HIV and Antiretroviral Therapy (ART) Coverage in Uganda: A Comprehensive Study of 2022 Trends.
80. von Wyl V, Klimkait T, Yerly S, Nicca D, Furrer H, Cavassini M, *et al*. Adherence as a predictor of the development of class-specific resistance mutations: the swiss HIV cohort study. PLoS One 2013;8:1–11. doi:10.1371/journal.pone.0077691.
81. Wakooko, P., Gavamukulya, Y., & Wandabwa, J. N. (2020). Viral load suppression and associated factors among HIV patients on antiretroviral treatment in Bulambuli district, eastern Uganda: a retrospective cohort study. *Infectious Diseases: Research and Treatment*, *13*, 1178633720970632.
82. Wang, J., Dykes, C., Domaoal, R. A., Koval, C. E., Bambara, R. A., & Demeter, L. M. (2006). The HIV-1 reverse transcriptase mutants G190S and G190A, which confer resistance to non-nucleoside reverse transcriptase inhibitors, demonstrate reductions in RNase H activity and DNA synthesis from tRNA(Lys, 3) that correlate with reductions in replication efficiency. *Virology*, *348*(2), 462–474. https://doi.org/10.1016/j.virol.2006.01.014
83. Wang, Y., Xing, H., Liao, L. *et al*. The development of drug resistance mutations K103N Y181C and G190A in long term Nevirapine-containing antiviral therapy. *AIDS Res Ther* **11**, 36 (2014). https://doi.org/10.1186/1742-6405-11-36
84. WHO | Regional Office for Africa. (2023, December 2). Retrieved from Uganda records significant reduction in new HIV infections among newborns website: https://www.afro.who.int/photo-story/uganda-records-significant-reduction-new-hiv-infections-among-newborns
85. Yerly, S., Kaiser, L., Race, E. e., Bru, J., Clavel, F., & Perrin, L. (1999). Transmission of antiretroviral-drug-resistant HIV-1 variants. *The Lancet*, *354*(9180), 729-733.

APPENDIX

Appendix I: Intensified Adherence Questionnaire

Key area of investigation	***Rationale***	**Themes**	**Example questions** These are not to be followed as a script. The flow of the interview can change depending on the discussion
Back ground information/Household dynamics	Introductory questions to encourage them to talk about themselves, for the interviewer to demonstrate interest in them individually and contextualise the household in which they live with infant X This will give help to give an idea of the carer's priorities. *This stage is intended to: Demonstrate interest in their lives* *Encourage participants to start talking freely (warm up)* Caring for specific child	• Explore background circumstances • Household structure (who they live with exploring relationships) • Marital status and relationship status • Occupation and education • Sources of income and support • Explain to what extent you feel your life to be meaningful. • Relationship with child. • Status of others within the household. • Attitude towards this responsibility of caring for the child, particularly in	➢ Can you tell me a bit about yourself? (probe for age, marital status, education level, sources of income, religion, place of residence, number of children)**Nsabakumbuulirakokubikukwatakoebitonotono (olinaemyakaemeka? olimufumbo?,wasomako? Bikiebikuyingirizasenteezikubeezawo?oliwaddinikki?, obeerawwa?,olinaabaanabameka?)** ➢ Can you tell me about your family and the household that you live in with the infant you care for?(probe for how many people live there, who?) **Nsabakumbuulirakokumaakamwobeeran'omwana X. (Bantu bameekaababeera mu maaka go? Bebaani?)** ➢ Please can you tell the story of how infant X came to live with you? (if not biological parent). **Nsabakumbuulirakoomwana X bweyajjaokubeeranawe** ➢ Is infant X the only one taking ART in the household? **Omwana X yeyeekaalikuddagalaeriweweezaakawuka?** ➢ How do you feel about life? would you say that you have a meaningful life? **Obulamubwoobulowoozakokki? Waligambyenti**

	Exploring how this care differs from the care given to others in the household Attitudes of others in the household towards the child	comparison with other children in the household. • Other adults or older children within the house and their role and contribution. • Specific care given to X? Including clinic attendance, nursing, treatment support, cooking and feeding.	➢ What care do you give to X?**Omwana X omulabiriramungerikki?** ➢ What it is like for you caring for X?**okulabiriraomwana X okisanzeotya?** ➢ Does this differ in any way from the care that you give the others?**Endabiriragyowaomwana X erinaengeriyonnagyeyawukanaune yabalala?** ➢ Do you have any support?**Olinaobuyambibwonna mu kulabiriraowana X?** ➢ Is there anyone that can you really count on to care about you, regardless of what is happening to you? Who? Give me an example when they were there for you **Olinaomuntuyennagwoyinzaokwesigamakookubeereerawo mu mbeerayonna?**
trial experience	This is an opportunity to understand their transition from their child's former standard health facility into the trial and to understand how carers perceive the trial.	• Transition from standard clinic to DRIBS clinic.(transfer in from other health facilities) • Initiation onto ART • Reflections on the experience of coming to the DRIBS clinic.	➢ Can you please tell me how your child came to be involved in the trial**?Nsabakumbuulirakoomwanawobweyajjaokubeera mu kunonyerezakuno?** ➢ Whose decision was it to have X join the trial? (probe for motivation to join study) **Ani yasalawontiomwanayetabe mu kunoonyereza?Lwaki?** ➢ How do you feel about participating in the study? Do you think your child has benefited in any way from participating in this study? **Owuliraotyaokubangaoli mu kunoonyereza?Olowoozantiomwanawoalinakyafunyemukyonnamukwetaba mu kunoonyerezakuno?** ➢ Is coming here different from the care you were receiving before

			coming here?**Okujjawanookisangangakyanjawulookuvakubujjanjabibwewaliofunangatonajjawano?** ➢ Do you have any questions about the study?**Olinaebibuuzobyonnangabikwatakukunoonyerezakuno?** ➢ Do you think the healthcare workers at this clinic have been helpful? In what way, can you give me examples?**Olowoozantiabasawokuddwalirobalinaengeriyonnagyebakuyambyemu?Bakuyambyemu ngerikki? ngerezako** ➢ Is there anything at the clinic that you would like to change?**Waliwokyonnakyewaliyagaddeokukyukaku clinic?**
Adherence, support and challenges	Exploring carers' understanding of what is "good adherence"	• Treatment support – what it looks like • Challenges to treatment support (e.g. side effects and other obstacles) • Examples of adherence problems- how find out about them, how manage them and whether the clinic was involved. • The role of others in the household in supporting or hindering adherence	➢ What do you understand by the adherence to ART?**Okumiraobulungieddagalaolowoozakitegeezakki?** ➢ What does good adherence mean to you? ➢ What would you say is bad adherence?**Okumiraobubieddagalakyekki?** ➢ What sorts sort of things can be done to improve adherence? **Bikiebiyinzaokolebwaokulabantiomuntuamirabulungieddagala?** ➢ Who is primarily responsible for giving the child their treatment? **Ani alinaobuvunanyizibwaokulabantiomwanaaweebwaeddagala lye?** ➢ What has this been like? Easy or difficult**? Kino okisanzeotya? Kibaddekyanguobakizibu? Lwaki?** ➢ What would make treatment taking for X easier?**Bikiebiyinzaokwanguyizao**

			mwanaokumiraeddagala? ➢ Do you think that taking treatment has had any effect on infant X in anyway? How?**Olowoozantieddagalalirina engeriyonnagyelikyusizamuomwana? Litya?** ➢ Do you think that people would blame you if adherence for X was poor? What would help in those circumstances?**Olowoozantiabantubayinzaokuvunaanasingaomwanatamirabulungiddagala?Bikiebiyinzaokuyambasingaomwanatamirabulungi?** ➢ Have you ever talked to the clinic about any adherence problems? Would you talk to them if you had any issues?**walioyogeddeko ne clinic kubuzibumukumiraeddagala?** ➢ What support do you think people caring for infants on ART need in order to support adherence as recommended?**Olowoozantiabalabiriraabaanabetaagabuyambikkiokulabantiabaanabamirabulungieddagalangabwekiragibwa?** ➢ What support do you need as a carer to ensure that X adheres to medication as recommended?**Gwengaalabiriraomwanawetaagabuyambikkiokulabantiomwanaamirabulungieddagalangabwekiragibwa?** ➢ Do you feel that you have this support now? What do you think you might need or benefit from?**Owulirantiolinaobuyambiobukumalaokulabantiomwanawoamirabulungieddagala?Olowoozawetaagakki?** ➢ What advice can you give to other carers taking care of infants on ART/ on how they can ensure optimum adherence?**Oyinzakuwamagezikkieriabalabiriraabaanaabalinaaka**

			wukaokulabantiabaanababwebamirabulungieddagala?
Disclosure	Extent and impact of disclosure, experiences and perceptions Are there plans to disseminate to other close relatives who sometimes look after children such as grandparents, aunts and uncles Views on risks or benefits of disclosure of infant's status to both infant and carer. Any negative feelings towards your own life or your baby and how often?	• Disclosure plans • Disclosing to other relatives • Feelings about disclosure Explore any blame games	➢ Have you told anyone about your own status?**Olinaomuntuyennagwewaliobuliddekokubwoyimiriddekubyakawukakamukenenya?** ➢ What was that like for you? How did you feel at the time? Do you still feel the same?**Wakisangaotya? Wawuliraotya? Okyawulirabwotyo?** ➢ Who else knows about X's status? **Ani omulalaakimanyintiomwanawoalinaakawuka?** ➢ Are there other people whom you think should know who doesn't know? Who are they?**Olowoozantiwaliyoabantuabasaanideokumanyaomwanantialinaakawukaabatakimanyi? Ngaani?** ➢ Do you intend to tell them? **Osuubiraokubagamba?** ➢ Do you think it is helpful to disclosure to other family members/relatives? **Olowoozakirinaengerigyekikuyambamuokubuuliraabengandangaolinaakawuka aka mukenenya?** ➢ What do you think will happen to you, your baby or your partner if you disclosed to others?**Olowoozakkiekinakutuukakoobamwanawo? Omwagalwawo singaobuliraabalalakubwemuyimiriddekubyakawukakamukenenya?** ➢ Have you experienced any

			changes with your body as a result of HIV? How did that make you feel?**Waliwoebikyusebyonnakumubirigwokubangaolinaakawukakamukenenya? Wawulira otyangakibaddewo?**

Digitisation and Self-Archiving Consent Agreement: Theses

Agreement between Makerere University & Students (Authors of Theses / Dissertations / Reports)

1. The author is a student of Makerere University and author of the thesis / dissertation entitled:

HIV drug resistant mutations associated with virological failure among HIV-1 seropositive infants in uganda after intensified adherence counselling

2. The author grants to the University:
   a. The right to deposit the electronic version of the Thesis / Dissertation into Makerere University Institutional Repositories (Mak IR) or (Mak UD); and
   b. The right to store the thesis / dissertation in Mak IR / Mak UD and make it permanently available to the general public via the Internet at no cost to the general public after a grace period (if any is specified).
   Choose one of the three options below (c, d, or e):
   c. The Author may opt for immediate open access to the public ✓
   d. Or Restrict access indefinitely: .....
   e. Or Restrict access for the specified number of years: .....
   f. Reason for restriction: .....

3. The author warrants that to the best of the authors knowledge and belief:
   a. The thesis / dissertation is an original work;
   b. The author is the owner of all the intellectual property in the thesis / dissertation;
   or
   c. The Author is entitled to deal with the intellectual property in the thesis / dissertation by publishing it on the Internet
   d. The Author has the right, power and authority to enter into this Agreement and to grant the University the rights contained in this Agreement; and
   e. The University's use of the thesis / dissertation pursuant to this Agreement will not infringe the intellectual property rights of any third party.

4. The Author acknowledges and agrees that the University is not responsible or liable for any breach of the intellectual property rights in the thesis / dissertation, in particular any breach of copyright, as a result of the use of the thesis / dissertation pursuant to this Agreement.

5. The University acknowledges that the rights granted by the Creator in clause 2 of this Agreement, do not cause any transfer or assignment of any proprietary rights in the intellectual property in the article to the University.

Signed by the Author as confirmation that the Author has read and accepted the terms of this Agreement:

Name: Muwabe Faryad

College: COVAB School: _____

(Tick) Type of Degree: (Undergraduate / PGD / Masters ✓ / PhD), Reg. No.: 2018/HD17/3878U

Tel No.: 0706507807 E-Mail: faryadvenda@gmail.com

Signature: _____ Date: 12 Jan 2026

Supervisor's endorsement: _____

MakIRs Policy