PREVALENCE AND RISK FACTORS OF VIROLOGIC FAILURE AND HIV-1 DRUG RESISTANCE AMONG CHILDREN AND ADOLESCENTS IN NAIROBI, KENYA

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Prevalence and risk factors of virologic failure and HIV-1 drug resistance among children and adolescents in Nairobi, Kenya

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DECLARATION

This Thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

I dedicate this Thesis to my beloved parents Paul Kabogo (R.I.P) and Beatrice Kabogo.

Your love, wisdom, support and encouragement over the years have made me a person

of integrity, discipline and resoluteness.

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LIST OF ACRONYMS AND ABBREVIATIONS

3TC	Lamivudine
AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral therapy
AZT	Azidothymidine/Zidovudine
C	Cysteine
CD4	Cluster of differentiation type 4
CVR	Centre for Viral Research
D	Aspartic Acid
DBS	Dried blood spot
DDI	Didanosine
DOT	Directly observed therapy
DTG	Dolutegravir
E	Glutamic Acid
EFV	Efavirenz
F	Phenylalanine
FDA	Food and Drug Administration
F.U.T.	The total follow-up time in person-years.
G	Glycine
H	Histidine
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
HIV-1	Human Immunodeficiency Virus type-1
HIV-DR	Human Immunodeficiency Virus drug resistance

IgG	Immunoglobulin G
IgM	Immunoglobulin M
INI	Integrase Inhibitor
ITROMID	Institute of Tropical Medicine and Infectious Diseases
JKUAT	Jomo Kenyatta University of Agriculture and Technology
K	Lysine
K103N	Lysine to Asparagine mutation at position 103 of the gene
KEMRI	Kenya Medical Research Institute
KMTC	Kenya Medical Training College
KNH	Kenyatta National Hospital
L	Leucine
LMICs	Low- and middle-income countries
M	Methionine
MEMS	Medication event monitoring system
N	Asparagine
NASCOP	National AIDS and STI Control Programme
NGO	Non-Governmental Organization
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
Ns	The number of study participants switched from first-line ART to second-line ART.
NVP	Nevirapine
PCR	Polymerase Chain Reaction
PEPFAR	President's Emergency Plan for AIDS relief

PI	Protease Inhibitor
PMTCT	Prevention of mother-to-child transmission
PR	Protease
Q	Glutamine
QALY	Quality-adjusted life year
R	Arginine
RAL	Raltegravir
RIP	Rest in peace
RNA	Ribonucleic Acid
RT	Reverse transcriptase
RT-PCR	Reverse transcriptase polymerase chain reaction
S	Serine
SAM	Severe acute malnutrition
SSA	Sub-Saharan Africa
SQV	Saquinavir
TAM	Thymidine Analogue Mutation
TDF	Tenofovir
USA	United States of America
USD	United States Dollars
V	Valine
VF	Virological Failure
W	Tryptophan
WHO	World Health Organization
Y	Tyrosine

ABSTRACT

Highly active antiretroviral therapy (HAART) in people infected with the Human Immunodeficiency Virus type-1 (HIV-1) is effective when backed up with adequate clinical, immunological, and virologic monitoring. However, a lack of viral suppression after at least 6 months of ART - known as virologic failure (VF) - may result in the emergence of HIV-1 drug resistance (HIV-DR). The twin phenomena of VF and HIV-DR can then lead to an increased incidence of opportunistic infections (OIs), the need for costlier second-line HAART drugs and a higher likelihood of death. To evaluate the prevalence and risk factors of VF and HIV-DR among children and adolescents in Kenya, a study was conducted in the Lea Toto Programme (LTP). The LTP is a multi-Centre community outreach Programme in Nairobi, Kenya providing care to 3,500 HIV-infected children and adolescents who live in resource-limited settings. This prospective cohort study involved 438 HIV-infected children and adolescents aged 2-17 years, who were recruited between December 2013 and April 2014: 210 (47.9%) females and 228 (52.1%) males. Medical and demographic data including, HIV-1 viral loads, CD4+ T-cell counts, information on adherence to HAART, HIV-1 drug resistance mutations (DRMs), malnutrition and OIs were collected over a period of four years. A threshold of 1,000 HIV-1 RNA copies/ml was used to determine treatment outcome. Treatment regimens with the Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) drugs Nevirapine (NVP) and Efavirenz (EFV), were prescribed to 284 (64.8%) and 141 (32.2%) of the children, respectively. Treatment regimens with the protease inhibitor drugs Ritonavir-boosted Lopinavir (LPV/r) were prescribed to 13 (3%) individuals in the cohort. The median $\log_{10}$ viral load (VL) for the cohort at baseline was 4.82 (Interquartile range [(IQR]: 4.37-5.39). After 24 months of first-line ART, the $\log_{10}$ VL fell to 2.43 (IQR, 2.28-2.59). The prevalence of VF during first-line antiretroviral therapy (ART) was 32.2%, 23.9%, 24.2%, and 26.1% after 12, 24, 36, and 48 months of treatment, respectively. For the 438 individuals initiated on first-line ART, the outcomes were as follows: nine (2.1%) died; 49 (11.2%) transferred out to other Comprehensive Care Centres (CCCs); 20 (4.6%) were lost to follow-up; 114 (26.0%) failed first-line ART and were switched to second-line ART. Sanger sequencing determined that among these 114 individuals, there were a total of 16 HIV-1 subtypes: four (25%) pure HIV-1 subtypes, namely A1, B, C and D; and 12 (75%) possibly recombinant HIV-1 subtypes, including A1B, DG, A1J, CRF_01AE and CRF_35AD. The VF rate among the 114 second-line ART individuals was 19.3% (n=22) after a median of 17 months of treatment. Of these 22 individuals, 15 (68.2%) were switched to Salvage ART; two (1.8%) were lost to follow-up; two (1.8%) transferred out of the LTP; and three (13.6%) died. The median $\log_{10}$ VL for the second-line ART cohort fell from 4.86 (IQR, 4.42-5.20) at baseline to 2.06 (IQR, 1.81-2.31) HIV RNA copies/ml at the end of the study. At second-line ART baseline, 95.6% (n=109) of the cohort had at least 1 Nucleoside Reverse Transcriptase Inhibitor (NRTI) DRM and 92.1% (n=105) had at least 1 NNRTI DRM. Fifty percent (n=57) had 1 or more Thymidine Analogue mutations (TAMs), 50% (n=57) had 1 or more Type-I TAMs, while 45.7% (n=52) had 1 or more Type-II TAMs. No DRMs to the protease inhibitor drugs LPV/r were found. Among the 15 patients switched from second-line ART to salvage ART, 13 succeeded on treatment, one (6.7%) transferred out of the LTP and one (6.7%) died. The risk factors for VF and HIV-DR were

determined using Cox Proportional Hazards Ratio (CPHR) analysis. Firstly, this analysis found that children and adolescents with suboptimal adherence to first-line ART were 37 times more likely to experience VF than those with optimal adherence (Hazard ratio [HR] = 36.99, 95% Confidence Interval [CI]=8.21-166.66, $P<0.001$). Secondly, those with severe acute malnutrition (SAM) were three times more likely to experience VF than the well-nourished (HR= 2.93, 95% CI=1.54-5.99, $P<0.01$). Thirdly, teenagers aged 14 to 17 years were three times more likely to have suboptimal adherence to first-line ART than children aged two to five years old (HR = 2.67, 95% CI=1.36-5.24, $P<0.01$). Fourthly, children and adolescents with suboptimal adherence to second-line ART were seven times more likely to experience VF than those with optimal adherence. Analysis of the salvage ART cohort data revealed a statistically significant increase in the number of NRTI DRMs (from 35 to 69) and OIs (from 22 to 34) among the 15 patients transitioned from second-line ART to salvage ART ($P=6.12\times10^{-10}$ and $P=2.14\times10^{-6}$, respectively). In light of these data, measures to reduce the rates of VF and HIV-1 among children and adolescents should be ramped up. These measures include increased child and adolescent HIV testing, counselling, linkage to care, and better adherence to ART. Moreover, teenagers should be encouraged to form peer support groups so that they can receive moral support and reduce self-stigma. On the HIV-1 treatment Programmatic level, the Government should revise their guidelines to recommend the use of protease inhibitors more frequently, as they have a higher barrier to HIV-DR. Given that HIV-1 infection is lifelong, reducing the rates of VF and HIV-DR among children and adolescents is crucial to ensuring that they will live long, healthy and productive lives.

CHAPTER ONE
INTRODUCTION

1.1 Background of the study

The HIV and AIDS pandemic has ravaged Sub-Saharan Africa for many years primarily because of a lack of adequate funds to purchase highly active antiretroviral therapy (HAART) for those infected (The Joint United Nations Programme on HIV/AIDS, 2017). Children and adolescents have been especially hard hit. The WHO defines children as individuals aged 1 to 9 years old, while adolescents are those aged 10 to 19 years of age (World Health Organization, 2016a). Sub-Saharan Africa accounts for 71% of all Human Immunodeficiency Virus (HIV) - infected individuals and 91% of all HIV-infected children below the age of 15 years (World Health Organization, 2015). In Kenya in 2016, the HIV-1 prevalence among adults was 5.4%, amounting to 1.6 million people living with HIV; 64% of these people were on ART (The Joint United Nations Programme on HIV/AIDS, 2017). There were 62,000 new infections and 36,000 AIDS-related deaths that year. There are approximately 190,000 HIV-infected children, and 65% of these were on ART (The Joint United Nations Programme on HIV/AIDS, 2017).

Over the last 10 years, the President's Emergency Fund for AIDS Relief (PEPFAR), the Global Fund and the World Bank multi-country AIDS Programme have invested heavily in antiretroviral therapy (ART) provision to resource-limited countries. Consequently, the number of HIV-infected persons on ART has drastically increased; for example, more than 4 million HIV-infected people in Africa now receive HAART, a 100-fold increase compared to 10 years ago (The Joint United Nations Programme on HIV/AIDS, 2010; World Health Organization, 2012; Ssemwanga *et al.*, 2015). This widespread availability of ART to HIV-infected persons in resource-limited countries has greatly reduced morbidity and mortality rates (The Joint United Nations Programme on HIV/AIDS, 2017). Unfortunately, there are HIV-infected individuals who are unaware of their status, while others know they are HIV-infected but continue to engage in high-risk sexual behaviour. Consequently, the horizontal spread of HIV within the population continues at a high rate (Ssemwanga *et al.*, 2015). Vertical HIV transmission still occurs too, because some pregnant women fail to receive Prevention

of mother to child transmission (PMTCT) drugs while pregnant, leading to the birth of HIV-infected babies (National AIDS and STI Control Programme, 2017).

In 2014, the Joint United Nations Programme on HIV and AIDS launched the three 90-90-90 targets for 2020 as a major step towards eliminating the AIDS epidemic (The Joint United Nations Programme on HIV/AIDS, 2014). Related to this, the World Health Organization (WHO) directed that from the 1st of September 2016, every HIV-positive person should be initiated on ART immediately, regardless of their CD4+ T-cell count (World Health Organization, 2016a); this is commonly called the "Universal Test and Treat" (UTT) strategy. By availing ART to HIV-infected people and expanding prevention choices to the uninfected, 21 million AIDS-related deaths and 28 million new infections can be prevented by 2030 (Levi *et al.*, 2016). The 90-90-90 targets recognize and utilize antiretroviral therapy (ART) as a life-saving treatment (Lundgren *et al.*, 2015 and Levi *et al.*, 2016), a transmission prevention measure (Cohen *et al.,* 2011; Levi *et al.*, 2016) and a human right (Gruskin *et al.*, 2008; Levi *et al.*, 2016). Target one is successfully diagnosing 90% of all HIV positive people. In target two, 90% of those diagnosed will be started on ART while target three entails achieving viral suppression for 90% of those on ART (Levi *et al.*, 2016). While allowing for serial 10% losses at each subsequent step, the implementation of these targets should result in 73% of all HIV-infected individuals achieving viral suppression, thereby improving their health and reducing their ability to infect others. These measures - in addition to vaccination of the uninfected - would eliminate HIV/AIDS as a public health problem (Levi *et al.*, 2016; World Health Organization, 2016b).

The ART protocols used in Kenya are based on the World Health Organization (WHO) guidelines (World Health Organization, 2016a). Treatment involves the use of various classes of drugs: Nucleoside Reverse Transcriptase Inhibitors (NRTIs), Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) and Protease Inhibitors (PIs). A cocktail of three or more drugs is given in order to reduce the risk of virologic failure (VF). The phenomenon of VF is often accompanied by HIV-1 drug resistance (HIV-DR). The Kenyan ART guideline for children is as follows: the NRTIs combination chosen is either Zidovudine and Lamivudine (AZT/3TC) or Abacavir and Lamivudine (ABC/3TC); Tenofovir and Lamivudine(TDF/3TC) could also be used, especially for

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older children or adolescents; the NNTRI selected depends on the child's exposure to Nevirapine (NVP) during the mother's pregnancy; those exposed are to be put on a PI such as Ritonavir-boosted Lopinavir (LPV/r); for those not NVP-exposed, either NVP or Efavirenz (EFV) is recommended according to the age and/or weight of the child (National AIDS and STI Control Programme, 2017). Those with VF are switched to second-line therapy, which involves the use of two new NRTIs (Abacavir and Didanosine) and a boosted PI (Lopinavir + Ritonavir or Saquinavir + Ritonavir). The next option of treatment is salvage therapy, where active drugs (drugs to which the patient's HIV-1 is not resistant) are prescribed. There is no specific Salvage Therapy drug regimen; it is a mix-and-match exercise that eliminates the drugs to which the patient is resistant - as per a drug resistance test - and adds first- and/or second-line ART drugs that may work. (World Health Organization, 2016a). The last treatment option is third-line therapy, where integrase inhibitors and attachment inhibitor ARVs - such as Raltegravir, Etravirine and Darunavir - are used in conjunction with first- and/or second-line ART drugs to which the patient's virus is still susceptible (World Health Organization, 2016a). In this study, the WHO definition of treatment success was used: a viral load below 1,000 HIV RNA copies/ml after 6 months of ART. Conversely, we will define treatment failure / virological failure (VF) as a viral load of 1,000 or more HIV RNA copies/ml after 6 months of ART (World Health Organization, 2016a).

The Lea Toto Programme is a community outreach initiative whose story begins in 1992. That year, Father Angelo D'Agostino, an American medical doctor and Jesuit Priest, in partnership with Sister Mary Owens an Irish nurse, established the Nyumbani Children's Home (NCH) located in Karen, Nairobi (Nyumbani Children's Home, 2019). The mission of the NCH was to provide medical care, food, shelter and clothing to orphaned HIV-infected children and adolescents who had no extended family willing or able to care for them. The capacity of the NCH was only 120, yet Father D'Agostino and Sister Owens wanted to help more of Kenya's HIV-infected children and adolescents. Therefore in 1998, they established the Lea Toto Programme (LTP), to provide care to thousands of HIV-infected children and adolescents living in resource-limited settings (Nyumbani Lea Toto Community Outreach, 2019). Since its inception, the LTP has grown tremendously, and now cares for 3,700 children and

adolescents through a network of eight Clinics in the following low-income suburbs of Nairobi: Dagoretti, Dandora, Kangemi, Kariobangi, Kawangware, Kibera, Mukuru, and Zimmerman. An important distinction between the NCH and the LTP is that while the NCH cares for 120 children and adolescents in a directly observed therapy (DOT) setting, the LTP children and adolescents live with their parents or guardians and visit their local LTP Clinic as needed. There, they receive ART, food assistance, treatment for opportunistic infections, counselling, and school fees assistance. Additionally, the parents and guardians of these children and adolescents receive caregiver counselling as well as micro-loans to start small businesses. Each LTP Clinic is staffed by a Centre Administrator, Clinical Officer, Nurse, Counsellor, Social worker(s), Community Health Volunteers, and a Data Officer. These specialists work in tandem to ensure that the medical, emotional, physical and financial needs of the child or adolescent are met (Nyumbani Lea Toto Community Outreach, 2019).

1.2 Statement of the problem

In sub-Saharan Africa, VF and HIV-DR are responsible for an ever-increasing percentage of AIDS-related deaths and AIDS-related costs. Statistical models show that between 2015 and 2030, 16% (n = 890,000) of HIV- and AIDS-related deaths, 9% of new infections (n=450,000) and 8% (USD 6.5 billion) of ART-programme costs in sub-Saharan Africa will be attributable to VF and HIV-DR (Phillips *et al.,* 2017). Therefore, VF and HIV-DR pose a major challenge to the elimination of HIV and AIDS as a public health problem. Moreover, the lack of adequate data about the extent of the problems of virologic failure and HIV-DR only serves to hinder the fight against HIV and AIDS. Indeed, as of 2016 the WHO had reliable HIV-DR data from only 59 countries in the world, indicating a major data gap in this field (World Health Organization, 2016b).

The lack of sufficient resources to monitor VF and HIV-DR affects virtually all of sub-Saharan Africa (SSA). Consequently, HIV-infected people are routinely initiated on ART without being tested for HIV-DR mutations (World Health Organization, 2016a). Such HIV patients are subjected to potentially ineffective treatment from the very start. It is only after the patients are suspected to be failing first-line ART that tests for VF and HIV-DR are conducted. By that point, they may be too far gone. It is therefore

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important to determine the prevalence of both VF and HIV-DR in the Lea Toto cohort, so as to paint a more accurate picture of the prevalence of paediatric VF and HIV-DR in Kenya (World Health Organization, 2016b). The outcome of second-line ART regimens depends in large part on the presence or absence of drug resistance mutations (DRMs), especially Thymidine analog mutations (TAMs) (Dow *et al.*, 2014). The amount of time on a failing first-line ART regimen is thought to be a predictor of accumulation of TAMs, which in turn impede the proper working of second-line ART regimens (Sigaloff *et al.*, 2012a; Hosseinipour *et al.,* 2013). It is therefore important to determine the prevalence of pre-treatment HIV-1 DR in Kenya, so as to inform future treatment policy.

1.3 Justification of the study

Antiretroviral therapy (ART) is now widely available in Kenya and other other sub-Saharan African (SSA) countries, ensuring that HIV-infected people can live long healthy lives should they adhere to treatment (Sawe *et al.*, 2009; Kantor *et al.*, 2009). Poor adherence to ART - and the resulting virologic failure (VF) and HIV-1 drug resistance HIV-DR - are the biggest challenges to curbing the spread, morbidiy and mortality of HIV-1. This is especially true in resource-limited settings such as Kenya and other SSA countries: the ART drugs are widely available, but there are insufficient funds to monitor patients' viral loads. Lack of HIV virologic monitoring is accompanied by the risk of VF going undetected (Calmy *et al.*, 2007; Mugyenyi *et al.*, 2010), causing the emergence of HIV-1 drug resistance mutations (DRMs) (Calmy *et al.*, 2007; Sawe *et al.*, 2009). The presence of DRMs in the patient increases the incidence of opportunistic infections (OIs), leading to deteriorating health and possibly death (Kantor *et al.*, 2009; Mugyenyi *et al.*, 2010). Moreover, VF and HIV-DR in the patient necessitate the switch from cheaper first-line ART drugs to more expensive second-line and third-line ART drugs (Sawe *et al.*, 2009; Kantor *et al.*, 2009). On the population level, the transmission of drug-resistant HIV strains within the population makes the fight against HIV and AIDS even more challenging and expensive (Sawe *et al.*, 2009; Mugyenyi *et al.*, 2010).

1.4 Null Hypothesis

There is no virologic failure or HIV-1 drug resistance among the children and adolescents of the Lea Toto Programme in Nairobi.

1.5 Research questions

1) What is the prevalence of virologic failure in children within the Lea Toto Programme in Nairobi?
2) What is the prevalence of HIV-1 drug resistance in children and adolescents within the Lea Toto Programme in Nairobi?
3) What are the medical and demographic risk factors for virologic failure and HIV-1 drug resistance within the Lea Toto Programme in Nairobi?

1.6 General objective

To determine the prevalence and risk factors of virologic failure and HIV-1 drug resistance among children and adolescents within the Lea Toto Programme in Nairobi.

1.7 Specific objectives

1) To determine the prevalence of virologic failure among the children and adolescents within the Lea Toto Programme in Nairobi.
2) To determine the prevalence of HIV-1 drug resistance among the children and adolescents within the Lea Toto Programme in Nairobi.
3) To determine the medical and demographic risk factors for virologic failure and HIV-1 drug resistance within the Lea Toto Programme in Nairobi.

CHAPTER TWO
LITERATURE REVIEW

2.1 Biology of HIV

2.1.1 The origin of the Human Immunodeficiency Virus

Genetic evidence supports the Hypothesis that the Human Immunodeficiency Virus (HIV) is a zoonotic infection: our species has acquired HIV at least thrice from the Chimpanzee sub-species *Pan troglodytes schweinfurthii* and *Pan troglodytes troglodytes* (HIV-1); once from the Gorilla (HIV-1); and once from the Sooty Mangabey (HIV-2) (Sharp *et al.,* 2011; Peeters *et al.,* 2014) (Figure 2.1). From the view-point of the virus, infecting our species is a host range expansion. The Phylogenetic tree of various HIV, Simian Immunodeficiency (SIV), and other Lentivirus strains demonstrates that HIV is closely related to SIV, and supports the Zoonosis hypothesis of HIV (Hemelaar, 2012; Foley *et al*., 2018).

In order to determine the evolutionary relationships among the various Lentiviruses, Scientists obtained SIV *gag, pol,* and *env* gene sequences from a variety of primate species (Sharp *et al.,* 2011; Foley *et al*, 2018). These SIV sequences were then compared to their HIV homologues. The most similar DNA sequences share a more recent common ancestor (Figure 2.1). It was determined that the most recent common SIV ancestor is the source of HIV. Moreover, HIV-1 has a different origin than HIV-2: HIV-1 is most closely related to the SIV from the Chimpanzee, while HIV-2 is most closely related to the SIV from the Sooty Mangabee (Sharp *et al.,* 2011; Foley *et al*, 2018) (Figure 2.1).
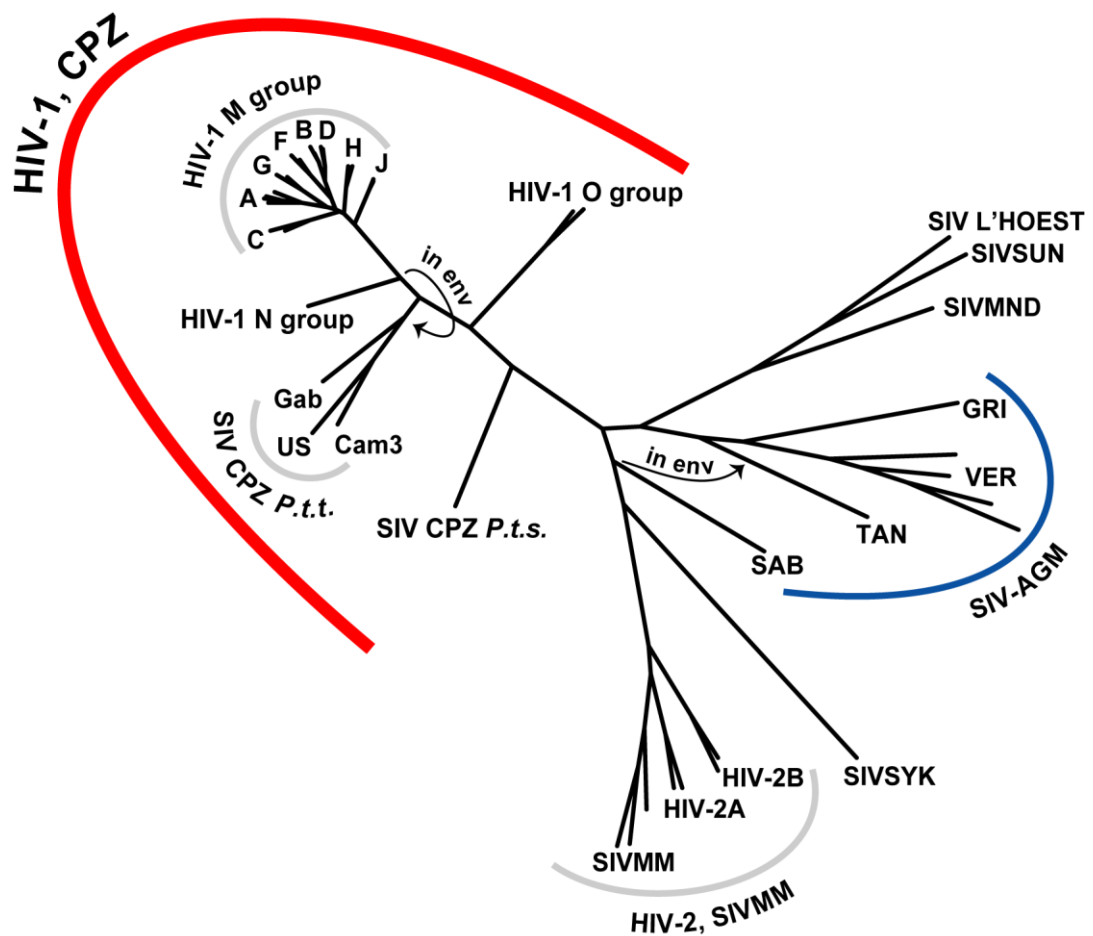
Figure 2.1: The evolutionary relationships of SIV, HIV-1 and HIV-2 strains as well as the HIV-1 groups and subtypes. The phylogenetic tree is based on maximum-likelihood phylogenetic analyses of full-length envelope protein sequences. (Foley *et al*., 2018)

2.1.2 Classification of HIV by types, groups and subtypes

Ninety percent of all HIV infections worldwide are caused by HIV-1 (World Health Organization, 2016a). The HIV-1 type can be further classified into 4 viral groups: M, N, O, and P (Sharp *et al.,* 2011; Hemelaar, 2012) (Figure 2.2). The 'M' stands for "major", and is by far the most common type of HIV, with more than 90% of HIV/AIDS cases deriving from infection with HIV-1 group M (Peeters *et al.,* 2014). The M group is subdivided further into subtypes, which are also assigned letter designations: namely A, B, C, D, F, G, H, J, and K (Figure 2.2). The 'N' in N group stands for "non-M, non-O". This group was discovered in 1998 and has only been seen

in Cameroon, Gabon, and Equatorial Guinea. As of 2006, only 10 Group N infections had been identified. The O ("Outlier") group is not usually seen outside of West-Central Africa (Sharp *et al.,* 2011; Hemelaar, 2012). In 2009, a study was published reporting the discovery of a divergent HIV sequence isolated from a Cameroonian woman residing in France. The scientists reporting this sequence placed it in a proposed Group P "pending the identification of further human cases" (Sharp *et al.,* 2011; Hemelaar, 2012).

The Human immunodeficiency virus type 2(HIV-2) less pathogenic than HIV-1; consequently, the HIV-2 transmission rate is much lower than that of HIV-1 (Santos *et al.*, 2010). However, both can lead to AIDS in affected individuals and both can mutate to develop drug resistance. Also, HIV-2 is not common outside of Africa (Sharp *et al.,* 2011; Hemelaar, 2012). The mechanism of HIV-2 infection is not yet fully understood. However, it has been established that there are 8 known HIV-2 subtypes (A to H).
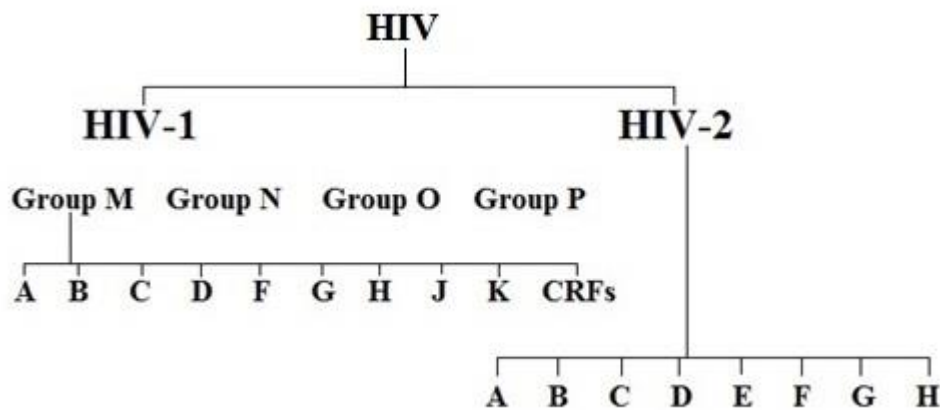
Figure 2.2: The HIV types, groups and subtypes.

2.1.3 The Genetics and Structure of HIV

The Human Immunodeficiency virus (HIV) is a type of RNA virus known as a Retrovirus, which can affect only human beings (Coffin *et al.*, 1997; NIH, 2018). A Retrovirus utilizes the Enzyme Reverse Transcriptase to convert its viral RNA genome to DNA, which is then integrated into the host DNA genome (Sharp *et al.,* 2013). HIV belongs to the RNA virus family *Retroviridae*, and the Genus *Lentiviridae* (Coffin *et*

al., 1997; Sharp *et al.,* 2013). The virus has 3 main genes: *gag*, *pol* and *env*, which encode various proteins (Sharp *et al.,* 2013; Bracq *et al.*, 2018) (Figure 2.3). The *gag* gene encodes the proteins p17 (Matrix protein), p24 (capsid protein), and p7 (nucleocapside protein). The *pol* gene encodes p9 (Protease protein), p66 (Reverse transcriptase protein), and p32 (Integrase protein). The *env* gene encodes gp120 (Surface glycoprotein) and gp41 (Transmembrane protein) (Sharp *et al.,* 2013; Bracq *et al.*, 2018).
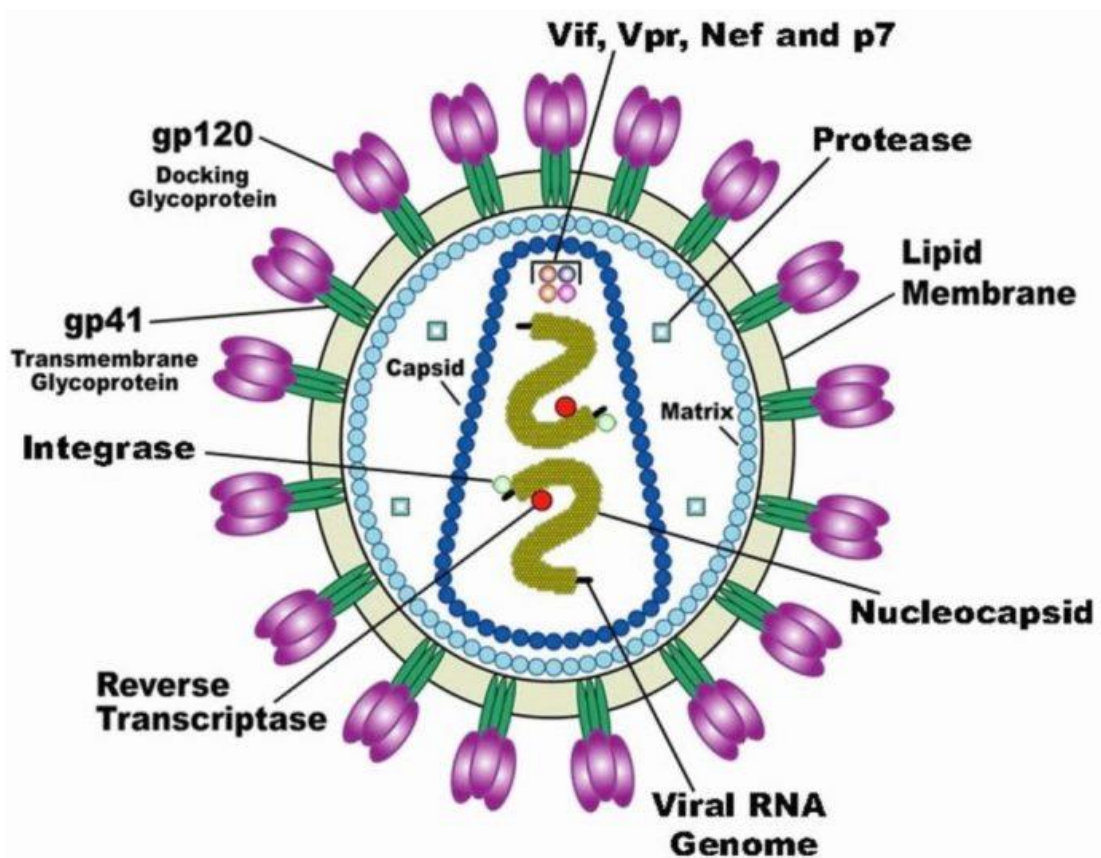
Figure 2.3: The Structure of HIV, showing the important proteins involved in its life cycle and functions (National Institutes of Health, 2018).

2.2 Transmission and pathogenesis of HIV
2.2.1 Transmission of HIV
Transmission of HIV from person to person occurs in several ways: sexual contact, pregnancy, childbirth, breastfeeding, injecting drug use, blood transfusion, organ donation, or occupational exposure among those handling infected materials through

mucous membrane or skin and travels to dendritic phagocytes beneath the epithelium, multiplies and is shed (Bracq *et al.*, 2018; NIH, 2018). The virus is then taken up into, and multiplies within macrophages in the skin, lymph organs, bone marrow, and blood. To effect infection at the cellular level, the virus's gp120 protein - which faces outward on the viral membrane - attaches to the CD4 protein on the surface of the host's T-cells (Alkhatib, 2009). The binding of the virus's gp120 protein to the host's CD4 protein happens with the help of the host's CCR5 co-receptor (for macrophage-tropic HIV strains) or the CXCR4 co-receptor (for T-cell-tropic HIV strains) (NIH, 2018) (Figure 2.4). Upon binding to the host CD4 protein, HIV's gp120 protein undergoes a conformational change that exposes a hidden co-receptor-binding site (Alkhatib, 2009). The binding of gp120 to the CCR5 or CXCR4 co-receptor then brings the viral envelope into close proximity to the host cell surface and induces gp120 to undergo a second conformational change. This second conformational change allows the gp41 transmembrane viral protein to penetrate the host cell membrane and form a six helices bundle. Through processes that are still not understood, fusion occurs between the cell and viral membranes allowing entry of the viral capsid and proteins into the host cell (Alkhatib, 2009).

Inside the host cell, the HIV Reverse transcriptase protein then makes a DNA copy of the HIV RNA, which makes its way to the host cell nucleus, where it integrates into the host cell DNA randomly via the virus's Integrase protein (NIH, 2018). Gene expression for the HIV genes relies on the host cell's transcription machinery; thus, the integrated HIV DNA is transcribed along with the host's DNA. A new DNA strand called the leading strand and its new complementary strand - known as the lagging strand - is synthesized in the opposite direction and in short fragments known as Okazaki fragments, which require multiple RNA primers. The protein DNA polymerase I removes the RNA primers from the newly produced DNA strands, while DNA ligase fills in the gaps left by the removal of the RNA primers (Coffin *et al.,* 1997; Bracq *et al.,* 2018). The penultimate step in viral replication is the assembly of both the new viral DNA and the new viral proteins into new virus particles (Coffin *et al.,* 1997; Bracq *et al.,* 2018). These new active viruses then float through the blood stream and attach to new host T-cells (NIH, 2018) (Figure 2.4). The life cycle continues *ad infinitum* until all the T-cells are destroyed, or until the viral life cycle is

stopped by Antiretroviral Therapy (ART) (Coffin *et al.,* 1997, Bracq *et al.*, 2018; NIH, 2018).
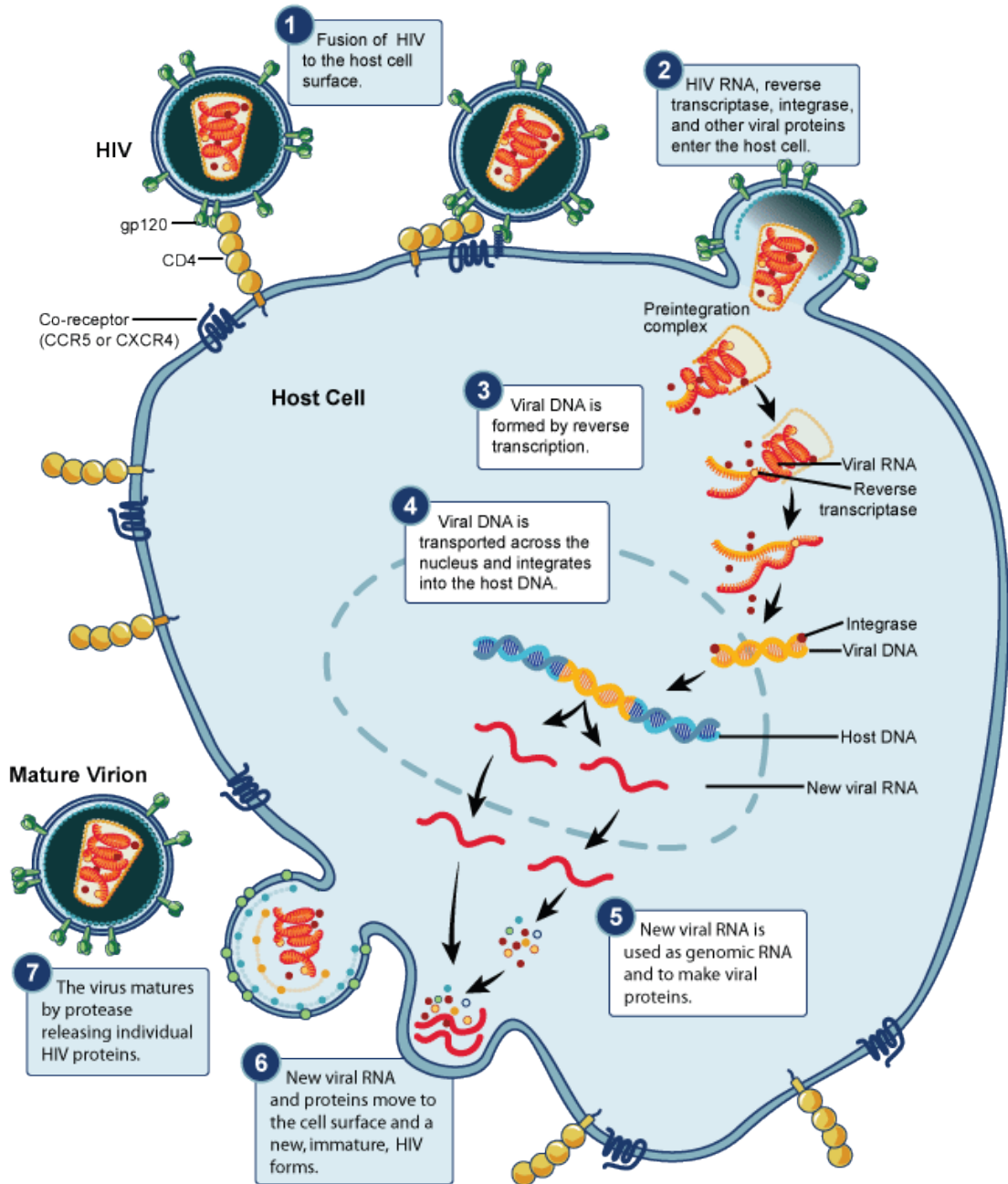
Figure 2.4: The life cycle of HIV within and outside the host's CD4+ T-cells (National Institutes of Health, 2018).

2.2.2 Pathogenesis of HIV

Infection with HIV has several phases: acute infection, clinical latency, and AIDS (Sharp *et al.*, 2013; NIH, 2018) (Figure 2.5). Within 3 to 12 weeks of infection, there is a spike in the number of virus particles and the seeding of macrophage cells in the lymphoid organs. This phase may be characterized by flu-like symptoms. Antibodies to HIV are detectable during this period of seroconversion. The acute phase of HIV infection is followed by a prolonged latency period that can last 1 to 10 years (Sharp *et al.*, 2013; NIH, 2018). During the latency period, the patient is often asymptomatic, or may display minor symptoms. Untreated HIV infection is an unrelenting war between the virus and the host immune system, with billions of host cells infected and even more virus particles produced on a daily basis. The entire process from initial infection to the development of AIDS can take as long as 12 years (Sharp *et al.*, 2013; NIH, 2018).
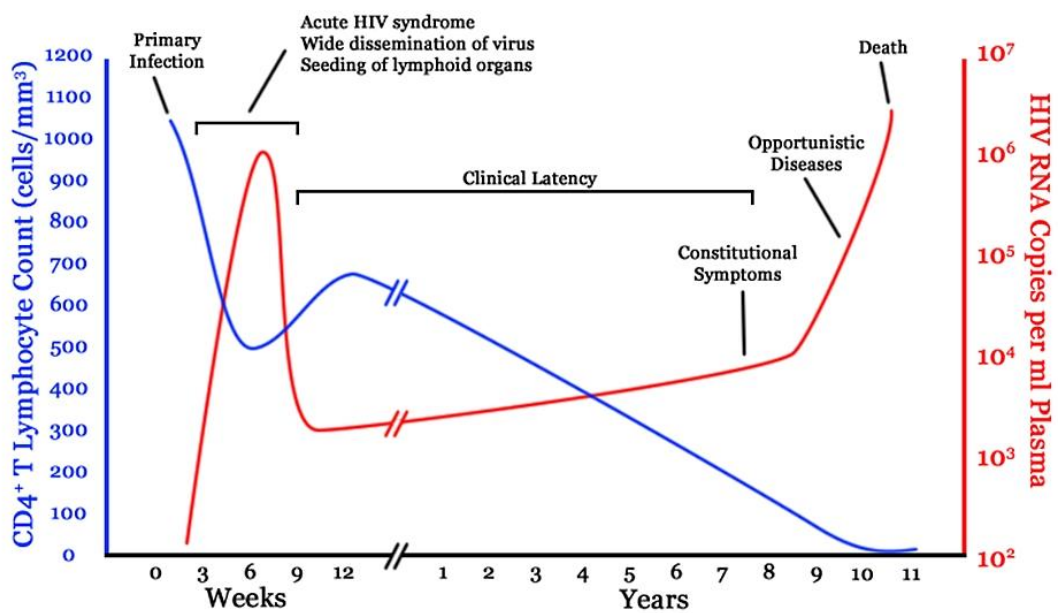
Figure 2.5: The time course of HIV infection and its progression to AIDS (National Institutes of Health, 2018).

2.3 Epidemiology of HIV

The global epidemic of HIV and AIDS has been a scourge for amost 40 years, claiming 35 million lives since 1981 (The Joint United Nations Programme on HIV/AIDS, 2017). There are 36.7 million people currently living with HIV and AIDS worldwide (0.8% global prevalence); of these, 34.5 million are adults, and 2.1 million are children below the age of 15 years (The Joint United Nations Programme on HIV/AIDS, 2017). The prevalence of HIV varies widely by region: on the low end, Bhutan has only 246 cases (0.0003%) of HIV out of a total population of 800,000 in 2016 (The Joint United Nations Programme on HIV/AIDS, 2017) (Figure 2.6). On the high end, three countries in Southern Africa have the highest HIV prevalences worldwide: Swaziland (27.4%), Lesotho (22.9%) and Botswana (21.9%) (World Health Organization, 2015; The Joint United Nations Programme on HIV/AIDS, 2017).
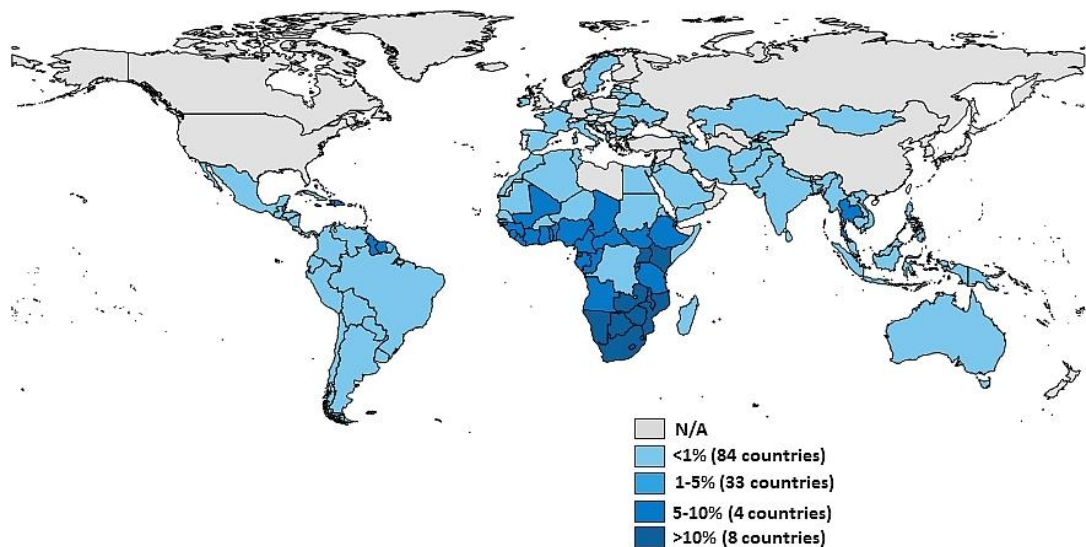
Figure 2.6: The global adult HIV prevalence in 2017 (The Joint United Nations Programme on HIV/AIDS, 2017).

2.3.1 Epidemiology of HIV outside sub-Saharan Africa

Western Europe, Central Europe, and North America show an adult HIV-1 prevalence of 0.3%, or 2.1 million people. The Eastern Europe and Central Asia region has an adult HIV prevalence of 0.9%, or 1.6 million infected people. Meanwhile in Asia and the Pacific region, the adult HIV-1 prevalence is 0.2%, or 5.1 million people. The

Middle East and North African region shows an adult HIV prevalence of 0.1%, or 230,000 people, which is the lowest prevalence of any region worldwide. Finally, the region of Latin America and the Caribbean has an HIV-1 prevalence of 0.5% or 2.1 million people. Only 56% of infected adults and 37% of infected children are on ART. Men who have sex with men (MSMs), commercial sex workers (CSWs), injecting drug users (IDUs) and transgender women show the highest infection rates (The Joint United Nations Programme on HIV/AIDS, 2017).

2.3.2 Epidemiology of HIV in West, Central and Southern Africa

Sub-Saharan Africa (SSA) has historically been the poorest region in the world in economic terms. It also bears the greatest HIV burden: Although SSA has 15% of the world's population, the region accounts for 71% of all HIV-infected individuals, and 91% of all HIV-infected children below the age of 15 years (World Health Organization, 2015; The Joint United Nations Programme on HIV/AIDS, 2017). Unfortunately, the ones can least afford to bear its burden have been the hardest hit.

The West and Central Africa region shows a 2.2% adult HIV prevalence, or 6.1 million people. Southern Africa is the region of the world with the greatest HIV and AIDS burden in the world (The Joint United Nations Programme on HIV/AIDS, 2017). Swaziland has the highest HIV prevalence worldwide (27.4%), followed by Lesotho (22.9%). Botswana is third with 21.9%. South Africa has the highest number of infected people worldwide (6.3 million people), and a prevalence of 11.3% (The Joint United Nations Programme on HIV/AIDS, 2017). Countries in this region have had to contend with cultural and religious practices that have exacerbated the spread of HIV over the years. Additionally, systemic poverty has hampered HIV prevention and treatment efforts. Over the last 10 years, Governments in this region have committed increased resources to this fight, and they have benefitted from great financial assistance from Donors (World Health Organization, 2015; The Joint United Nations Programme on HIV/AIDS, 2017).

2.3.3 Epidemiology of HIV in Eastern Africa

Our regional neighbours have had to contend with the tremendous burden of HIV as well. For example, Tanzania has an adult HIV prevalence of 4.7%, Uganda has

reported a prevalence of 6.5% and Ethiopia is at 2.4% (The Joint United Nations Programme on HIV/AIDS, 2017). Unprotected heterosexual sex remains the main mode of HIV transmission in Eastern Africa (The Joint United Nations Programme on HIV/AIDS, 2017). Truck drivers and female commercial sex workers along the transport corridors have played a large role in the spread of the virus (Shoepf 2001; Velayati *et al.*, 2007). This problem is exacerbated by the fact that many of these truck drivers are married or have multiple sexual partners, leading to the spread of HIV from a key population (CSWs) to the general population (Velayati *et al.*, 2007). Moreover, breastfeeding - a cultural norm in Eastern Africa - has contributed to the vertical transmission of HIV, due to the attached stigma: refusal to breastfeed is seen as a sign that a mother is HIV-positive (Velayati *et al.*, 2007).

2.3.4 Prevalence of HIV in Kenya

Kenya has an average HIV prevalence of 6%, and there are 1.6 million people living with HIV infection (The Joint United Nations Programme on HIV/AIDS, 2017). The predominant HIV-1 subtypes in Kenya are subtypes A, followed by D, followed by C, then CRFs (Lihana *et al.,* 2009; Kiptoo, *et al.,* 2009; Nyagaka *et al.,* 2012; Wambui *et al.,* 2012; Onywera *et al.*, 2017). The Nyanza region, including Homa Bay, Siaya and Kisumu are the most affected by HIV with rates of 25.7%, 23.7% and 19.3%, respectively. The least affected counties are Wajir, Tana River and Marsabit with rates of 0.2%, 1% and 1.2%, respectively (The Joint United Nations Programme on HIV/AIDS, 2017). There were 62,000 new infections and 36,000 AIDS-related deaths in Kenya in 2017. There are approximately 190,000 HIV-infected children, 65% were on ART (The Joint United Nations Programme on HIV/AIDS, 2017). By 2016, the Voluntary Male Medical Circumcision Programme had circumcised 860,000 males (aged 15-49) and met its universal coverage target of 80% (The Joint United Nations Programme on HIV/AIDS, 2016). The prevalence of HIV among key populations in Kenya is as follows: MSMs (18.2%); IDUs (18.3%); and 29.3% among CSWs (International Organization for Migration, 2010; The Joint United Nations Programme on HIV/AIDS, 2016). In 2014, 59,000 Kenyan women received PMTCT services, out of an estimated 79,000 who were eligible (74% coverage) (National AIDS Control Council of Kenya, 2014). That year, only 6,600 children below the age of 15 years

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were newly infected with HIV, due in large part to PMTCT services (National AIDS Control Council of Kenya, 2014; The Joint United Nations Programme on HIV/AIDS, 2016).

2.4 The Management of HIV and AIDS

During the beginning of the HIV and AIDS Epidemic in the early 1980s, infected persons would invariably progress from being HIV-positive, to developing opportunistic infections, to having full-blown AIDS, and finally death (Palmisano *et al.*, 2011; Vella *et al.*, 2012). The only exception are the small minority of patients known as "elite controllers", who are able to naturally maintain low viraemia without ART (Palmisano *et al.*, 2011). For the majority of HIV-infected people in the 1980s and early 1990s, HIV was a death sentence waiting to happen (Vella *et al.*, 2012).

2.4.1 Antiretroviral Therapy in the Management of HIV and AIDS

The deployment of donor-funded Antiretroviral Therapy (ART) on a large scale in Sub-Saharan Africa has greatly reduced HIV-related morbidity and mortality (Brady *et al.*, 2010; Vella *et al.*, 2012). HIV virologic suppression is usually achieved within the first year of ART initiation in 40% to 81% of HIV-infected children from Sub-Saharan Africa (Sutcliffe *et al.*, 2008; Ciaranello *et al.*, 2009; Wamalwa *et al.*, 2013). The success of ART is reliant on effective clinical, immunological, and especially virologic monitoring (Paintsil *et al.,* 2011, Barth *et al.,* 2011; Ruel *et al.,* 2011).

The Kenya National AIDS and Sexually Transmitted Infections Programme (National AIDS and STI Control Programme) continually updates its recommendations for ART regimens. During the period of study, the Kenyan ART guideline for children was: the Nucleoside Reverse Transcriptase Inhibitors (NRTIs) combination chosen was either: Zidovudine and Lamivudine (AZT/3TC) or Abacavir and Lamivudine (ABC/3TC) (National AIDS and STI Control Programme, 2017). Tenofovir and Lamivudine (TDF/3TC) could also be used, especially for older children or adolescents; the Non-Nucleoside Reverse Transcriptase Inhibitors (NNTRI) selected depended on the child's exposure to Nevirapine (NVP) during the mother's pregnancy; those exposed were to be put on a Protease inhibitor (PI) namely Lopinavir boosted with Ritonavir (LPV/r); for those not NVP-exposed, either NVP or Efavirenz (EFV) was to be used

according to the age and/or weight of the child (National AIDS and STI Control Programme, 2017).

2.4.2 Antiretroviral Therapy and the United Nations 90-90-90 Targets

In 2014, the UNAIDS Programme launched the three 90-90-90 targets as a major step towards eliminating the AIDS epidemic (The Joint United Nations Programme on HIV/AIDS, 2014). Target one is successfully diagnosing 90% of all HIV-positive people. In target two, 90% of those diagnosed will be started on ART, and target three entails achieving viral suppression for 90% of those on ART. While allowing for serial 10% losses at each subsequent step, the implementation of these targets should result in 73% of all HIV-infected individuals achieving viral suppression (The Joint United Nations Programme on HIV/AIDS, 2014; Levi *et al.*, 2016).

Additionally, the World Health Organization (WHO) directed that from the 1st of September 2016, every HIV-positive person should be initiated on ART immediately, regardless of their CD4+ count (World Health Organization, 2016a); this is commonly called the "Universal Test and Treat" (UTT) strategy. Previously, ART was reserved only for those with a CD4+ T-cell count below 500 cells/µl and for those below the age of 10. By availing ART to HIV-infected people and expanding prevention choices to the uninfected, it is estimated that 21 million AIDS-related deaths and 28 million new infections can be prevented by 2030 (Levi *et al.*, 2016). These efforts have begun to bear fruit: 64% of adults and 65% of children in Kenya are now receiving ART (The Joint United Nations Programme on HIV/AIDS, 2017).

2.5 Challenges and failures in the Management of HIV and AIDS

Given the wide availability of ART worldwide, the main challenge that patients, Clinicians, and Scientists have to contend with is poor adherence to ART, which in turn causes virologic failure (VF) and HIV-1 drug resistance HIV-DR). The WHO defines treatment success as a viral load of below 1,000 HIV-1 RNA copies/ml, while virologic failure is defined as a viral load of above 1,000 HIV-1 RNA copies/ml after 6 or more months of ART (World Health Organization, 2013). Lack of HIV virologic monitoring is accompanied by the risk of virologic failure going undetected (Calmy *et al.*, 2007; Mugyenyi *et al.*, 2010), potentiating the emergence of HIV-1 drug resistance

(HIV-DR) (Calmy *et al.*, 2007; Sawe *et al.*, 2009), increasing morbidity and mortality (Kantor *et al.*, 2009; Mugyenyi *et al.*, 2010), enhancing the need for costly second-line or third-line ART (Sawe *et al.*, 2009; Kantor *et al.*, 2009), as well as the transmission of drug-resistant HIV strains within the population (Sawe *et al.*, 2009; Mugyenyi *et al.*, 2010).

2.5.1 Types and causes of virologic failure and HIV-1 drug resistance
Antiretroviral therapy (ART), while effective in suppressing the patient's viral load, can fail, a condition known as virologic failure (VF), which many or may not be accompanied by HIV Drug Resistance (HIV-DR) (Ssemwanga *et al.*, 2015). Virologic Failure is a condition where the wild-type HIV strain has been out-populated by a mutant strain that is resistant to the ART regimen being administered (Ssemwanga *et al.*, 2015; Onywera *et al.*, 2017). The patient's viral load therefore, continues to increase unabated, leading to the development of opportunistic infections. If a new, effective ART regimen is not administered, the patient develops full-blown AIDS and eventually dies (Gupta *et al.*, 2009; Haberer *et al.*, 2010; Onywera *et al.*, 2017).

The development of HIV-DR happens in one of two ways: when drug resistance mutations (DRMs) arise within the patient, during the HIV replication cycle; this is called acquired drug resistance (ADR) (Onywera *et al.*, 2017). Alternatively, the HIV-DR mutations could be transmitted to the patient either perinatally (*in utero*, during birth or via breastfeeding), or horizontally (via sexual contact or injecting drug use); this is called primary or transmitted drug resistance (TDR) (Chan *et al.*, 2009).

Low- and middle-income countries (LMICs) are more susceptible to HIV-DR because of their structural and economic deficiencies such as: inadequate laboratories and insufficient trained lab and medical personnel (Rasschaert *et al.*, 2011; Ssemwanga *et al.*, 2015); irregular lab monitoring of HIV patients (Gupta *et al.*, 2009); counterfeit ART drugs; and drug stock-outs (Oyugi *et al.*, 2007; Bateman *et al.*, 2013; Ssemwanga *et al.*, 2015). Research has shown that 38% of countries reporting had at least one drug stock-out, despite the fact that most of these Programmes were donor-funded. The culprit for these stock-outs was determined to be inefficient procurement and supply chain management systems (Schouten *et al.*, 2011).

On the patient level, suboptimal adherence to ART dramatically increases the likelihood of VF and HIV-DR (Bansberg *et al.*, 2000; Haberer *et al.*, 2010). Suboptimal adherence to ART may be caused by depression, anxiety, stigma from others, self-stigma, high pill burden, a lack of a syrup formulation for young children, adverse drug reactions, and poor nutrition (Birungi *et al.*, 2011; Wolf *et al.*, 2014; Adejumo *et al.*, 2015; Madiba *et al.*, 2015; Denison *et al.*, 2015).

2.5.2 The emergence of HIV-1 Drug Resistance during ART

The emergence of drug resistance mutations (DRMs) to Nucleoside Reverse Transcriptase Inhibitor (NRTI) drugs occurs primarily because NRTIs have a low genetic barrier to resistance (Luber, 2005). In the absence of resistance, the HIV-1 is susceptible to NRTI drugs, and these drugs are incorporated by the wild-type HIV-1 reverse transcriptase enzyme into the elongating HIV-1 DNA strand (Clavel *et al.*, 2004; Schauer *et al.*, 2013). By design, NRTIs lack a 3' hydroxyl group, thereby making them chain terminators during DNA replication (Singh *et al.*, 2010; Schauer *et al.*, 2013). Due to the high mutability of HIV-1, DRMs arise randomly during the reverse transcription phase of its life cycle.Some benefit the virus, some are neutral while others are detrimental to the virus (Spach and Kinney, 2019). Mutations beneficial to HIV-1 are in two groups: firstly, discriminatory DRMs - such as M184V/I, L74V, K70E, and K65R - cause the virus to preferentially use naturally occurring deoxynucleotides for DNA elongation, while discriminating against the NRTI drug (Clavel *et al.*, 2004; Schauer *et al.*, 2013; Spach and Kinney, 2019). The second group are known as excision/primer unblocking DRMs; they include M41L, D67N, K70R, L210W, T215Y/F and K219Q/E. Excision DRMs enhance the phosphorylitic excision of the NRTI-triphosphate already added to the elongation HIV-1 RNA-DNA complex, leading to the unblocking of the primer by the NRTI drug and preventing chain termination. This allows the viral to replicate unhindered (Singh *et al.*, 2010; Tu *et al.*, 2010; Tang *et al.*, 2012).

Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) drugs - such as Nevirapine (NVP) and Efavirenz (EFV) - have a low genetic barrier to HIV-1 resistance as well (Spach and Kinney, 2019). Resistance in HIV-1 to NNRTI drugs occurs when these amino acid residues that line the hydrophobic binding pocket of the HIV-1 reverse

transcriptase enzyme are mutated. These binding pocket amino acids include L100, K101, K103, V106, T107, V108, V179, Y181, Y188, V189, G190, F227, W229, L234 and Y318. Mutation of these residues leads to one or both of the following outcomes: an altering of the NNRTI binding site itself or reduced access to the binding site for the NNRTI drug (Clavel *et al.*, 2004; Schauer *et al.*, 2013). Consequently, the NNRTI is unable to limit viral replication, and the patient's viral load increases. The most common NNRTI DRMs that emerge in a patient on NVP are are K103N, Y181C/I, V106A/M and Y188C/L/H. Patients on EFV predominantly develop the K103N, Y188L, and G190S/A DRMs (Rhee *et al.*, 2003; Shafer, 2006; Spach and Kinney, 2019).

2.5.3 Virologic Failure and HIV-1 Drug Resistance worldwide

The WHO periodically updates and publishes a list of TDR for surveillance worldwide (Bennett *et al.*, 2009). The TDR rate is highest in North America at 12.9%, followed by Europe at 10.9%, then Latin America at 6.3%, Africa at 4.7%, and Asia at 4.2% (Frentz *et al.*, 2012; Ssemwanga *et al.*, 2015). Due to the scale-up of ART deployment in LMICs in recent years, the greatest increase in TDR prevalence has occurred primarily in Africa and Asia (Frentz *et al.*, 2012). One caveat is that natural sequence polymorphisms can lead to overestimation of TDR rates (Frentz *et al.*, 2011).

2.5.4 Virologic Failure and HIV-1 Drug Resistance in Africa

Several HIV-1 studies among children and adolescents have been conducted in SSA and reported varying rates of virologic failure and HIV-DR (Reddi *et al.,* 2007; Emmet *et al.*, 2010; Lihana *et al.*, 2011; Mutwa *et al.*, 2014). These studies have - among other things - compared the accuracy of clinical and immunological markers such as adherence level, CD4+ T-cell count, and complete blood cell count, in identifying children with VF. Virologic failure rates have been found to be as much as 10 times higher than immunological and clinical failure rates, with the former being accurate and the latter two being underestimations (Chaix *et al.*, 2005; Kamya *et al.*, 2007; Kekitiinwa *et al.,* 2012; Wamalwa *et al.*, 2013).

The advent of PMTCT has greatly reduced the rate of TDR to below 5%, demonstrating the importance of early ART intervention for HIV-infected pregnant

women and their babies (Nyombi *et al.*, 2008). Virologic suppression rates have also increased drastically in recent years, as demonstrated by the high treatment success rates in SSA countries among patients with good adherence to ART (Johannessen *et al.*, 2009; Bratholm *et al.*, 2010).

2.5.5 Virologic Failure and Drug Resistance in Kenya

Here in Kenya, several studies have demonstrated that VF and HIV-DR are major problems that threaten to undo the progress made in fighting HIV/AIDS. A survey of prevalence done in the North-Rift region of Kenya found that 28% of mothers attending ante-natal clinics had HIV-DR mutations. The presence of DRMs in pregnant women confirms the importance of integrating HIV-DR monitoring as a key component in PMTCT Programmes (Kiptoo *et al.*, 2008). This is important because vertically transmitted HIV-DR DRMs can persist for up to 7 years, even in the absence of ART drug pressure (Lwembe *et al.*, 2007).

Long-term ART often produces virologic success, but also an increasing number of DRMs. Therefore, HIV-DR monitoring must go hand in hand with virologic monitoring (Hamers *et al.*, 2011; Price *et al.*, 2011). Ideally, testing for DRMs should occur before initiating patients on ART, in order to avoid putting patients on ART drugs they are already resistant to (Steegen *et al.*, 2009; Lihana *et al.*, 2009). Among HIV-infected infants, it has been shown that DRMs emerge between 2 weeks and 6 months post-partum, probably due to exposure to maternal ART drugs found in breast milk.

2.6 Solutions to the problems of Virologic Failure and HIV-1 Drug Resistance

Several studies have shown that good adherence to ART is the most important way to ensure viral suppression, lower the risk of opportunistic infections, and minimize the risk of HIV-DR. (World Health Organization, 2003; Chesney *et al.*, 2006; Ajose *et al.*, 2012). Another important solution to VF and HIV-DR is the use of drugs with a high genetic barrier to HIV-DR, such as protease inhibitor drugs (Luber, 2005). Thirdly, good nutrition must be provided in conjunction with the ARV drugs (Bukusuba *et al.*, 2007).

2.6.1 Adherence to Antiretroviral Therapy in Children and Adolescents

Adherence to ART in children or adolescents is influenced by their 'care' environment (Ene *et al.*, 2007; Polisset *et al.*, 2009), the level of vigilance of their primary caregiver (Ware *et al.*, 2009; Sutcliffe and Moss, 2011), peer influence (Polisset *et al.*, 2009), ARV pill burden, and ARV formulation: pill or syrup (Ene *et al.*, 2007; Polisset *et al.*, 2009; Sutcliffe and Moss, 2011). Adolescents are especially prone to the fear of stigmatization, and have an increased desire to 'fit in' with their HIV-negative peers (Ware *et al.*, 2009). Moreover, treatment fatigue is quite common in children and adolescents with prolonged ART usage (Haberer and Mellins, 2009). The reduction in morbidity during prolonged ART usage creates a feeling of cure. This may lead to declines or stoppage of ART use, accompanied with viral load rebound, recurrence of opportunistic infections and development of HIV-DR (Haberer and Mellins, 2009).

2.6.2 Adherence Measurement Methods

There are several different methods used to measure adherence to ART: direct and indirect (Adejumo *et al.*, 2015). There are 2 direct measurement methods: plasma drug assays and directly observed therapy (DOT) (Adejumo *et al.*, 2015). Plasma drug assays are not commonly used in SSA due to their high cost and the limited number of well-equipped laboratories in the region. The second direct adherence measurement method is directly observed therapy (DOT), where the patient ingests their ART drugs in the presence of a Clinician or Nurse. The benefits of DOT are that drug ingestion is ensured; however the downside is that is each patient consumes a lot more Clinician time and energy, thus raising total Healthcare costs (Kaai *et al.*, 2010; Munyao *et al.*, 2010).

Indirect methods of adherence measurement include: self-report and electronic monitoring methods and devices. Self-report is the most widely used method of adherence measurement in SSA (Vreeman *et al.*, 2010; Mghamba *et al.*, 2013). The second indirect method of adherence measurement is electronic monitoring methods and devices, such as phone or text reminders, and Medication Event Monitors (MEMs). The MEMs devices electronic detect and transmit data every time a pill is removed from the medication bottle, thus ensuring an accurate pill count (Muller *et al.*, 2008; Haberer *et al.*, 2012).

Pharmacy-based methods of adherence measurement include pill counts and Pharmacy visit/refill records. Pill counts are quickly and easily obtained when the patient visits the Clinic (Adejumo *et al.*, 2015). The Clinician or Pharmacist counts the number of pills the patient returns in order to determine the number of missed doses (Ndiaye *et al.*, 2013). One modification to this method is the unannounced home visit and pill count, which improves the likelihood of patients adhering to their ART (Bansberg *et al.*, 2001; Kalichman *et al.*, 2007). The second Pharmacy-based method of adherence measurement is Pharmacy visit records and drug refill records. In this method, the exact number of pills is dispensed to last a particular period, e.g. a month; there may be additional drugs given to last 1 or 2 extra days. Any delay by the patient in returning to the Clinic on the scheduled day is taken as an indication of a missed dose (Chi *et al.*, 2009).

2.6.3 The rates of Adherence to Antiretroviral Therapy outside of Kenya

Worldwide, reported adherence rates among Paediatric patients on ART vary widely depending on the region and the adherence measurement method used (Kim *et al.*, 2014; Adejumo *et al.*, 2015). In North America, self-report, pill counts, Plasma assays, and MEMs are the predominant methods used. In Europe, pill counts are the measurement method of choice, while in Asia, self-reports are the metric of choice. Self-reports are preferred in South America too (Kim *et al.*, 2014; Adejumo *et al.*, 2015). Adherence rates worldwide vary widely - from 62% to 84% - depending on the method used (Kim *et al.*, 2014; Adejumo *et al.*, 2015).

Studies conducted in Cote d'Ivoire, Nigeria, Togo, and South Africa have shown that Clinic attendance records, self-reports, caregiver reports are the most common metrics for measuring adherence to ART in Africa. The reported adherence rates in these countries range from 67% to 86% (Elise *et al.*, 2005; Mukhtar-Yola M *et al.*, 2006; Nachega *et al.,* 2009; Iroha *et al.*, 2010; Ugwu *et al.*, 2013).

Research cohorts in Ethiopia, Rwanda, Tanzania and Uganda have revealed that there are large discrepancies in adherence rates, depending on the measurement method used, with plasma drug assays being the most accurate, and caregiver reports/patient self-report being the least accurate (Biadgilign *et al.*, 2008; Biressaw *et al.*, 2013; Mghamba *et al.*, 2013).

2.6.4 The rates of Adherence to Antiretroviral Therapy in Kenya

Children and adolescents in Kenya have widely varying adherence scores – from 46% to 95% - depending on the measurement method used. The adherence measurement methods of choice are drug-refill data, Clinical appointments records, pill-counts, caregiver reports and self-reports (Wamalwa *et al.*, 2007, Vreeman *et al.*, 2008; Langat *et al.*, 2012). Moreover, Researchers have reported that the likelihood of suboptimal adherence increases with the death of both parents (Vreeman *et al.*, 2008).

2.7 HIV and AIDS in Children and Adolescents

Worldwide, 90% of HIV-infected children and adolescents below the age of 15 years were infected perinatally: *in utero*, during birth, or through breastfeeding (World Health Organization, 2007a). Fortunately, the rate of perinatal infections has declined sharply in recent years due to increased awareness and greater funding towards Prevention of Mother to Child Transmission (PMTCT) (The Joint United Nations Programme on HIV/AIDS, 2013). The remaining 10% of young people who are not infected perinatally were infected mainly through sexual contact or through injecting drug use (Cowan *et al.*, 2009; Adejumo *et al.*, 2015). Unfortunately, many of these youth are unaware that they are HIV-infected, thus they become unwitting carriers, spreading the virus through the population (UNICEF, 2010; The Joint United Nations Programme on HIV/AIDS, 2013).

Adolescents are especially vulnerable to HIV and AIDS; this period of life is characterized by significant physical, emotional and mental changes, including an increased desired to assert one's independence from parents, the need to be affirmed by peers, and the desire to engage in risky behaviour. Therefore, they are very likely to engage in unprotected sex with multiple partners or experiment with injectable drugs (Adejumo *et al.*, 2015). African adolescents aged 10 to 19 years accounted for 80% of all worldwide infections (UNICEF, 2014).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study site

This study focused on the children and adolescents served by the Lea Toto Programme (LTP). The LTP is a multi-Centre community outreach program in Nairobi, Kenya providing care to 3,500 HIV-infected children and adolescents. The LTP has Clinics in each of the following eight low-income suburbs of Nairobi: Dagoretti, Dandora, Kangemi, Kariobangi, Kawangware, Kibera, Mukuru, and Zimmerman. Blood samples were transported from these eight Clinics to the Nyumbani Diagnostic Lab in Karen for analysis. Additional laboratory work was done at the Kenya Medical Research Institute (KEMRI) Centre for Virus Research (CVR) Annex Laboratory located in the Kenyatta National Hospital (KNH) Campus (Figure 3.1).

Figure 3.1: The study sites and their locations in Nairobi County, Kenya. The map of Kenya is shown on the left, with Nairobi County blown up and shown on the right. The study sites are numbered: (1) Dagoretti Lea Toto Clinic (2) Dandora Lea Toto Clinic (3) Kangemi Lea Toto Clinic (4) Kariobangi Lea Toto Clinic (5) Kawangware Lea Toto Clinic (6) Kibera Lea Toto Clinic (7) Mukuru Lea Toto Clinic (8) Zimmerman Lea Toto Clinic (9) Nyumbani Diagnostic Lab (Karen) (10) KEMRI Centre for Virus Research Annex Laboratory (KNH Campus) (United Nations Geospatial Information Section, 2019).

3.2 Study design

This was a prospective cohort study.

3.3 Study population

This study involved Lea Toto children aged two to nine years and adolescents aged 10 to 17 years of age.

3.4 Sample size

To calculate the minimum sample size for the study, the formula for cohort studies was used (Charan & Biswas, 2013).

Cohort sample size =

$\{[Z\alpha\sqrt{(1 +1/w)} * P_\Omega(1 - P_\Omega) + Z_\beta\sqrt{P1}] / [1 - P1)/w + P2(1-P2)]\}^2 / [P1 - P2]^2$

Where: $P_\Omega = (P2 + wP1) / (w + 1)$

Where:

Zα: 1.96, the standard normal variate for 0.05 level of significance.

w: 1, Number of control subjects per experimental subject.

Z$_\beta$: 0.84, Standard normal variate for power.

P1: 0.28: Probability of virologic failure in control group; taken from an average published virologic failure rate of 29% in Sub-Saharan Africa (Lihana *et al.*, 2011).

P2: 0.14: Probability of virologic failure in experimental group:

P$_\Omega$: $(0.14 + 1*0.29) / (1 + 1) = 0.22$

The sample size that results from the above calculation is 58.8, rounded up to **59** persons. When 10% is added to account for loss-to-follow-up, the minimum sample size was 65.

3.5 Sampling design

Stratified consecutive sampling was used to calculate the number of study participants from each LTP to be included in the sample (Table 3.1). The total sample population of children and adolescents in the LTP at the start of the study was 2,466, stratified amongst the eight regions served by the LTP Clinic. The number of children and adolescents from each LTP Clinic was calculated based on the percentage that Clinic

contributed to the total LTP population. Children and adolescents were recruited consecutively at each LTP Clinic until the required minimum number was reached.

Table 3.1: Sampling design for the Lea Toto Programme (LTP) cohort in Nairobi. Children and adolescents at the 8 LTP Centres were recruited consecutively until the minimum number per Centre was reached.

LEA TOTO PROGRAMME CLINIC LOCATION	TOTAL NUMBER OF CHILDREN & ADOLESCENTS	% OF SAMPLE	MINIMUM SAMPLE SIZE (n)
Dagoretti	217	8.8%	6
Dandora	349	14.2%	9
Kangemi	288	11.7%	8
Kariobangi	423	17.2%	11
Kawangware	354	14.4%	9
Kibera	282	11.4%	7
Mukuru	243	9.9%	6
Zimmerman	310	12.6%	8
TOTAL	**2,466**	**100.0%**	**65**

3.6 Inclusion Criteria

1) Children in the LTP aged two to nine years of age.
2) Adolescents in the LTP aged 10 to 17 years of age.
3) Children in the LTP aged two to nine years old whose parents consented for their children to be included in the study.
4) Adolescents aged 10 to 17 years old whose parents consented for them to be included in the study and who assented to be included in the study.

3.7 Exclusion Criteria

1) Infant children who could not be bled due to difficulty in finding their arm veins.

2) Children and adolescents whose parents or guardians refuse to give consent for their children to participate in the study.

3) Adolescents who refused to assent to participate in the study, or who refuse to sign the LTP treatment contract promising to adhere to ART.

3.8 Clinical Records Review

The following primary Clinical data were collected by the principal investigator from the patients' Clinical records using a data abstraction form (Appendix VIII): ART regimen the study participant was initiated on, ART first-line start date, all the viral loads on file, all the CD4+ T cell counts on file, CD4+ T cell percentages (only available for children aged 2 to 5 years), information from clinical evaluation for opportunistic infections, duration on first-line ART, the second-line ART start date where applicable, the second-line ART regimen, ART monthly pill counts, punctuality in attending Clinic visits, punctuality in undergoing blood tests, as well as HIV-DR data where applicable. Percentages, not counts were used for the 2 to 5 year-olds; the former are considered more accurate than the latter, as the immune systems of 2 to 5 year-olds are not fully formed yet (World Health Organization, 2016a).

The following secondary data were determined from the primary data; adherence to ART, the WHO immunological stage, weight-for-height Z score and height-for-age Z score at baseline.

3.8.1 Adherence scores

To calculate adherence rates, four parameters were used (Focà *et al.*, 2014): firstly, the pill counts done by the Clinicians during the monthly Clinic visits; secondly, the pill counts done by the LTP Community Health Workers (CHWs) during unannounced home visits, with the latter serving as verification of the former; thirdly punctuality in attending Clinic sessions, with a maximum allowance of 2 days late; fourthly, undergoing the required blood tests on schedule. Each parameter contributed 25% to the overall score. Within each parameter, full compliance was scored as 25%, partial compliance was scored as 12.5%, and zero compliance was scored as 0%. Any overall

score between 95% and 100% was defined as optimal, while a score below 95% was defined as suboptimal (Ware *et al.*, 2009; Haberer *et al.*, 2011).

3.8.2 Length of follow-up on ART and the rate of switching from first- to second-line ART

The length of follow-up on ART was measured in person-years, which was the sum of time that all persons were followed-up while in the study

The rate of switching from first-line to second-line ART was given by the formula:

{Ns / F.U.T}

Where:

Ns: The number of study participants switched from first-line ART to second-line ART.

F.U.T.: The total follow-up time in person-years.

3.8.3 Cox Proportional Hazards Regression

Univariate and Multivariate Cox proportional hazards regression analysis was also performed to determine the risk factors that lead to virologic failure, slow response to ART as well as sub-optimal adherence to ART. For this analysis, various variables were simultaneously compared between the treatment success group and virologic failure group. The risk factors associated with virologic failure were considered to be predictive and statistically significant when 3 conditions were met: firstly, the Cox proportional hazard ratio (HR) had to be greater than 1.96; secondly, the lower limit of the 95% confidence interval (CI) had to be greater than 1; and thirdly, the *P*-value had to be less than 0.05 (Walters, 2009).

3.9 Laboratory methods

3.9.1 Blood collection

One vacutainer tube (Becton Dickinson Biosciences, Franklin Lakes, New Jersey) containing the anticoagulant Ethylenediaminetetraacetic acid (EDTA) was labelled with the name and Lea Toto number of each study subject. A tourniquet was then applied to the upper arm of the child or adolescent to enable the veins to be seen and felt. The venipuncture site was sterilized with a cotton swab dampened with 70%

alcohol, after which the venipuncture site was allowed to dry for 30 seconds. The needle of the syringe was then inserted into the venipuncture site, after which the vacutainer tube was inserted into the tube holder, allowing blood to flow from the vein, through the syringe and into the vacutainer. The syringe was kept in place until five millilitres of blood had flowed into the vacutainer, after which the syringe was removed from the vein. The venepuncture site was swabbed with a cotton swab dampened with 70% alcohol and covered with an adhesive bandage /Elastoplast. The vacutainer was inverted six times to ensure mixing of the blood with the EDTA. The vacutainer was then stored at 4°C and transported to the Nyumbani Laboratory within 4 hours of blood draw.

3.9.2 CD4+ T cell Count and percentage determination

The BD FACSCalibur flow cytometry system (Becton Dickinson Biosciences, Franklin Lakes, New Jersey) was used to CD4+ T cell counts and percentages, according to the manufacturer's instructions and previously published protocols (Böhler *et al.,* 2007; Sitoe *et al.*, 2007; Novitsky *et al.*, 2009). Briefly, 50 µl of whole blood was added to 20µl BD Tritest reagent in a BD Trucount tube (Becton Dickinson Biosciences, Franklin Lakes, New Jersey). After a 15-minute incubation in the dark, 450µl of FACSlyse buffer was added to the BD Trucount tube to lyse the red blood cells. CD3+, CD4+, and CD8+ T cell absolute counts and percentages were then acquired and analyzed by the BD FACSCalibur machine and software (Böhler *et al.,* 2007; Novitsky *et al.*, 2009).

3.9.3 Viral Load determination

The remaining 4.95 ml of blood was centrifuged at 1,500 rpm in a table top centrifuge. One millilitre of plasma was then pipetted into a sterile 2ml tube. The plasma was loaded into the Cobas AmpliPrep-Cobas TaqMan (CAP/CTM) HIV-1 test, v.2.0 (Roche Molecular Systems, Branchburg, New Jersey). The assay was done according to the manufacturer's instructions (Zeh *et al.*, 2017). Briefly, the CAP/CTM assay was used for automated extraction, amplification and quantification on plasma in two phases. Firstly, one milliliter of plasma of each sample was aliquoted into the respective sample tube after a brief vortexing to obtain a uniform mix. The samples

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were then transferred to the COBAS AmpliPrep machine for processing using the HI2CAP96 method (Zeh *et al.*, 2017). Secondly, the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) was performed on the Cobas TaqMan 96 analyzer. The quantification range of the CAP/CTM v2.0 was from 20 copies/mL to 10 million copies/ml.

3.9.4 Sequencing of DNA to identify HIV-1 drug resistance mutations

The HIV-1 *pol* gene - specifically, the reverse transcriptase (RT) gene amino acids 1 to 299 and the protease gene (PR) amino acids 1 to 99 - were sequenced by the Sanger method to check for the presence of HIV-DR mutations. The protocol was taken from the Manufacturer's instructions (Roche Inc., Carlsbad CA) and a previously paper (Ndembi *et al.*, 2008). Briefly, HIV-1 RNA was extracted from 200 µl of blood plasma using the QIAamp viral RNA mini kit (Qiagen Inc., Chatsworth, CA). All sequencing reactions were performed using an in-house assay already optimized/validated at the Nyumbani Diagnostic Laboratory using the ABI 3100 automated capillary DNA Sequencer (Thermo Fisher Scientific Inc., Carlsbad CA). The Polymerase gene-specific primers RT18 and KS104 were used for the first-round PCR, with the parameters shown below (Lwembe *et al.*, 2009; Bittker *et al.*, 2012) (Table 3.2 and Appendix IX).

Table 3.2: The sequences of the Primers used and the PCR parameters for the First-round PCR to determine what HIV-1 drug resistance mutations were present in the Lea Toto study participants.

First-round PCR	Primer Sequence			Source of Primers
Forward Primer (RT18)	5'- GGAAACCAAAAATGATAGGG GGAATTGGAGG-3'			Songok *et al.*, 2004
Reverse Primer (KS104)	5'-TGACTTGCCCAATTTAGTTTTCC CACTAA-3'			Songok *et al.*, 2004
Reverse Transcription Step	$50{}^{o}C$ 30 min			
Denature DNA & Activate Taq	$94{}^{o}C$ 2 min			
PCR Cycles	**Melt**	**Anneal**	**Extend**	**# Cycles**
	$94{}^{o}C$	$50{}^{o}C$	$68{}^{o}C$	$40{}^{o}C$
	20 sec	30 sec	1 min 30 sec	
Final Extension	$68{}^{o}C$ 5 min			
Hold	$4{}^{o}C$ Forever			

PCR:- Polymerase Chain Reaction.

Thereafter, nested PCR were done using a protocol taken from the Manufacturer's instructions (Thermo Fisher Scientific Inc., Carlsbad CA) and a previously paper (Ndembi *et al.*, 2008), using with primers KS101 and KS102, with the parameters shown below (Hass *et al.*, 2008) (Table 3.3 and Appendix X).

Table 3.3: The sequences of the Primers used and the PCR parameters for the Nested PCR to determine what HIV-1 drug resistance mutations were present in the Lea Toto study participants.

Nested PCR	Primer Sequence			Source of Primers
Forward Primer (KS101)	5'-GTAGGACCTACACCTGTTCAACATA ATTGGAAG-3'			Songok *et al.*, 2004
Reverse Primer (KS102)	5'-CCCATCCAAAGAAATGGAGGAGGT TCTTTCTGATG-3'			Songok *et al.*, 2004
Denature DNA & Activate Taq	98°C 30 sec			
PCR Cycles	**Melt**	**Anneal**	**Extend**	**# Cycles**
	98 °C	62 °C	72 °C	35
	10 sec	20 sec	40 sec	
Final Extension	72 °C 10 min			
Hold	4 °C Forever			

PCR:- Polymerase Chain Reaction.

The presence of a PCR amplicon was confirmed using a Nanodrop Spectrophotometer (Thermo Fisher Scientific Inc., Carlsbad CA), and the presence of a PCR amplicon of the expected size was determined by agarose gel electrophoresis with ethidium bromide staining. The PCR products were then purified with a QIAquick PCR purification kit (Qiagen Inc., Valencia, CA) and sequenced in the Sense and Antisense direction with a set of nested primers (Ndembi *et al.*, 2008).

3.9.5 Data analysis

The data analysis was done using SPSS software version 22 (IBM Corporation, Armonk, New York) and MedCalc software version 15.2.2 (MedCalc Software BVBA, Ostend, Belgium). *P*-values were determined using the Fisher's exact test - a parametric test - used for normally distributed data. The Mann Whitney U test – a non-

parametric test − was used for skewed data. Univariate and Multivariate Cox proportional hazards regression analysis were used to establish the effect of different variables on virologic failure. A threshold of $P \leq 0.05$ for statistical significance was set.

3.9.6 Bioinformatics Analysis

The chromatogram files from the DNA sequencing reactions were read using the Sequencher 4.7 program (GeneCodes, Ann Arbor, MI) and edited with the BioEdit program version 7.0 (T. Hail, Carlsbad, CA). These sequences were confirmed to be HIV-1 sequences by comparing against a reference database using the Basic Alignment Search Tool (BLAST) (Boratyn *et al.*, 2012). The HIV-1 RT nucleotide sequences were analyzed for drug resistance mutations previously reported in the Stanford University HIV database (Rhee *et al.*, 2003; Shafer, 2006).

The REGA HIV-1 subtyping tool was used to determine the HIV-1 subtype (Rhee *et al.*, 2003; Shafer, 2006). The sample sequences were aligned with subtype reference sequences from the European Bioinformatics Institute (EBI) CLUSTAL Omega service (Madeira *et al.*, 2019). The SIV reference sequence was also included in the alignment. A phylogenetic tree was constructed by the neighbor-joining method, and its reliability was estimated by 1000 bootstrap replications (Ndembi *et al.*, 2004).

3.10 Ethical considerations

Informed consent and assent forms were prepared (Appendices I, II, III and IV). This study - designated SSC 2500 - was granted scientific and ethical approval from the KEMRI Scientific and Ethics Review Unit (SERU) (Appendices V and VI). Additionally, the Nyumbani Medical Board (NMB) - which oversees all Research and Treatment operations in the LTP - granted approval for this study (Appendix VII).

Confidentiality of data was maintained at all times. In order to protect the privacy of the study participants, serial numbers were assigned during data entry. Names, Lea Toto identification numbers, and any other personal identifiers were then deleted. All subsequent presentations and publications used these serial numbers.

CHAPTER FOUR
RESULTS

4.1 Description of the cohort

A total of 438 Lea Toto Programme (LTP) children and adolescents met the inclusion criteria and were enrolled in the study. The study participants were recruited into the study at the same time that they were enrolled into the LTP. The study participants were recruited from all eight LTP Centres: Dagoretti, Dandora, Kangemi, Kariobangi, Kawangware, Kibera, Mukuru, and Zimmerman.

Of the study participants, 210 (47.9%) were females, while 228 (52.1%) were males (Table 4.1). Eighty study participants (18.3%) were aged between 2 and 5 years old; 136 (30.8%) were between 6 and 9 years old; 130 (29.8%) were aged 10 to 13 years old; and 92 (21.1%) were aged 14 to 17 years old. Sixty children (13.7%) were being raised by a guardian, 153 (34.9%) were being raised by one parent while 225 (51.4%) were being raised by two parents. The median $\log_{10}$ viral load for the cohort at baseline was 4.82 (Interquartile range [(IQR]: 4.37 to 5.39). After 24 months of first-line ART, it fell to 2.43 (IQR, 2.28 to 2.59). The median CD4+ T-cell count at baseline was 423 (IQR, 266 to 715); after 24 months of first-line ART, it rose to 486 (IQR, 273 to 741). In this cohort, 2 Nucleoside Reverse Transcriptase Inhibitors (NRTIs) were prescribed to each child. Abacavir and Lamivudine (ABC/3TC) were prescribed to 151 (34.5%) children, while and Zidovudine and Lamivudine (AZT/3TC) were prescribed for 277 (63.2%) of the children. The Nucleotide Analogue Reverse Transcriptase Inhibitor (NtRTI) Tenofovir was prescribed to 10 (2.3%) of the children. Moreover, 1 Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) or 1 Protease inhibitor (PI) was prescribed to each child. The NNRTIs Nevirapine (NVP) and Efavirenz (EFV), were prescribed to 284 (64.8%) and 141 (32.2%) of the children respectively. These drug permutations resulted in seven unique ART regimens being prescribed to children in this cohort. Forty-nine point one percent (n=215) of the children were on AZT/3TC/NVP, 13.5% (n=59) were on AZT/3TC/EFV, 15.7% (n=69) were on ABC/3TC/NVP, 16.4% (n=72) on ABC/3TC/EFV; 2.1% (n=10) were on TDF/3TC/EFV; 2.1% (n=10) were on ABC/3TC/LPV/r; and 0.7% (n=3) were on AZT/3TC/LPV/r (Table 4.1).

Table 4.1: Baseline medical and demographic characteristics of the Lea Toto cohort initiated on first-line ART.

Characteristics	n (%)	Characteristics	n (%)
Age group		WHO Clinical stage	
2 to 5 years	80 (18.3%)	1	110 (25.1%)
6 to 9 years	136 (30.8%)	2	100 (22.8%)
10 to 13 years	130 (29.8%)	3	140 (31.9%)
14 to 17 years	92 (21.1%)	4	88 (20.2%)
Gender		**Adherence to ART Regimen**	
Male	228 (52.1%)	**Optimal**	279 (63.7%)
Female	210 (47.9%)	**Suboptimal**	159 (36.3%)
Primary caregiver		**Weight-for-height Z score$^{\Omega}$**	-0.8 (-2.7 to -0.2) [Ψ]
Both parents	225 (51.4%)	**Height-for-age Z score$^{\theta}$**	-0.8 (-2.3 to -0.3) [Ψ]
One parent	153 (34.9%)	**First-line ART regimen**	
Guardian	60 (13.7%)	AZT/3TC/NVP	215 (49.1%)
Baseline HIV-1 RNA, $\log_{10}$ copies/ml	4.82 (2.28 to 2.59) [Ψ]	AZT/3TC/EFV	59 (13.5%)
Post-treatment HIV-1 RNA, $\log_{10}$ copies/ml	2.43 (4.12 to 5.49) [Ψ]	ABC/3TC/NVP	69 (15.7%)
		ABC/3TC/EFV	72 (16.4%)
Post-treatment CD4 T-cell count, cells/µl	486 (273 to 741) [Ψ]	TDF/3TC/EFV	10 (2.3%)
Baseline CD4 T-cell count, cells/µl	423 (266 to 715) [Ψ]	ABC/3TC/LPV/r	10 (2.3%)
		AZT/3TC/LPV/r	3 (0.7)

$^{\Psi}$ Medians and interquartile ranges (IQRs); $^{\Omega}$ The Weight-for-height Z score measures malnutrition, and was calculated for the entire cohort, from ages 2 to 17 years; $^{\theta}$ The Height-for-age Z score measures stunting, and was calculated for the entire cohort, from ages 2 to 17 years;   WHO:- World Health Organization; ART:- Antiretroviral therapy; NRTI:- Nucleoside Reverse Transcriptase Inhibitor; NtRTI:- Nucleotide Analogue Reverse Transcriptase Inhibitor; NNRTI:- Non-Nucleoside Reverse Transcriptase Inhibitor; Pi:- Protease inhibitor; ABC:- Abacavir; 3TC:- Lamivudine; AZT:- Zidovudine; TDF:- Tenofovir; NVP:- Nevirapine; EFV: Efavirenz.

Out of the 438 study participants, 9 died during the course of the 48 month follow-up, 20 were lost to follow-up, and 49 Transfer out to other Paediatric HIV Care Programmes. Of the remaining 360 study participants, 246 remained on first-line ART and 114 were switched to second-line ART. Of these (114), 92 remained on second-line ART and 22 were switched to salvage ART (Figure 4.1).
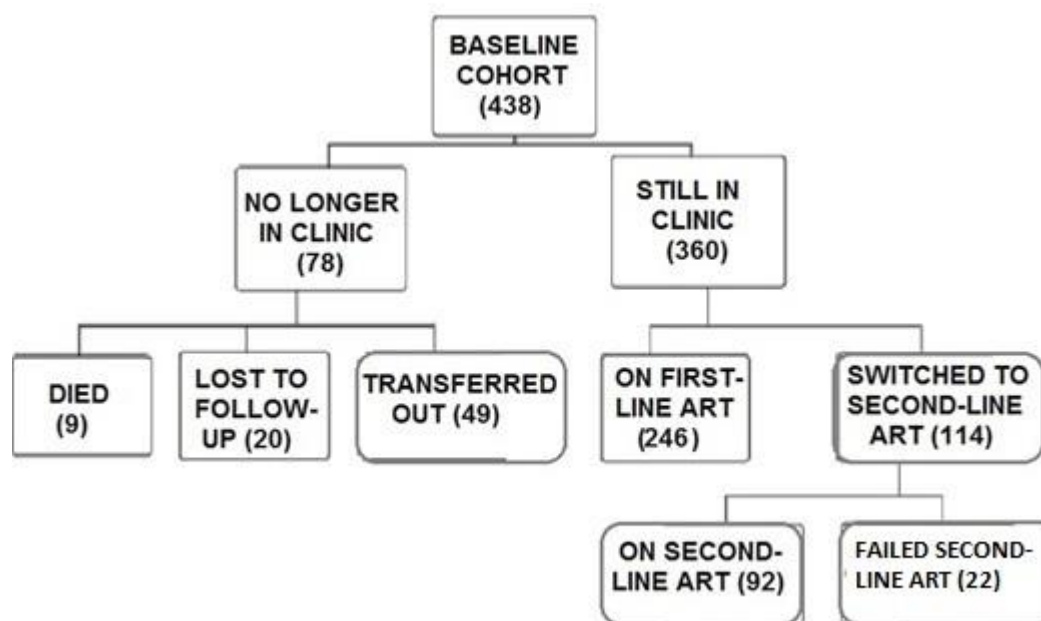
Figure 4.1: Study outcomes for the 438 Lea Toto children and adolescents in the study.

4.1.1 Length of follow-up and survival during follow-up

The total length of follow-up for all study participants was 1,545.3 person-years (Figure 4.2 and Table 4.3). The median time of follow-up on first-line ART before switching from first-line ART to second-line ART was 31 months (IQR, 29 to 37 months).
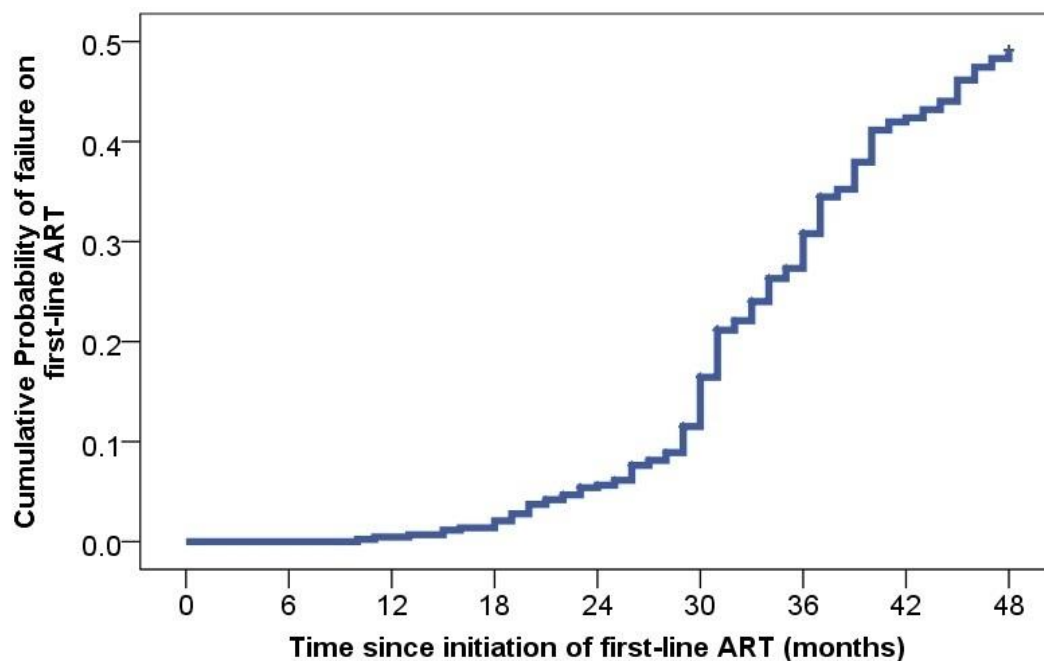
Figure 4.2: The cumulative probability of virologic failure for the Lea Toto cohort during 48-month study.

Table 4.2: Length of follow-up in person-years for the Lea Toto cohort.

Group, n	Sub-group, n		Person-years of follow-up	Person-years of follow-up	Total person-years of follow-up
Still in Clinic: n = 360	First-line ART: n= 282		1,128 person-years	1,334 person-years	
	Second-line ART: n = 78		206.3 person-years		
No longer in LTP Clinic: n = 78	Died: n = 9	First-line ART: n = 2	6 person-years	27.6 person-years	
		Second-line ART: n = 7	21.3 person-years		1,545.3 person-years
	LTFU: n = 20	First-line ART: n = 14	32.5 person-years	48 person-years	
		Second-line ART: n = 6	15.5 person-years		
	Transfer out of LTP: n = 49	First-line ART: n = 26	75 person-years	135.7 person-years	
		Second-line ART: n = 23	60.7 person-years		

4.1.2 Adherence to ART

All the adherence rates were below the mandated WHO target of 95% of the cohort achieving 95%, except the two to five year old age group. Children and adolescents raised in two parent homes (n = 225) had an adherence rate of 93.5%; those in one parent homes (n = 152) had a rate of 91.8%; those in homes headed by guardians (n = 61) had a rate of 87.2% (Table 4.4). The *P*-values were as follows: two parent homes vs. one parent homes *P*=0.06; two parent homes vs. guardian homes *P*<0.001; one parent homes vs. guardian homes *P*<0.001. Age group comparisons were also done vis-à-vis adherence rates: the 2 to 5 years old group (n = 80) had a adherence rate of 95.9%; the 6 to 9 years old group (n = 135) had a rate of 93.6%; the 10 to 13 years old group (n = 130) had a rate of 88.7%; and the 14 to 17 years old group (n = 93) had a rate of 90.8%. The *P*-values were as follows: 2 to 5 years old group vs. the 6 to 9 years old group, $P = 0.03$; 2 to 5 years old group vs. the 10 to 13 years old group, $P < 0.001$; 2 to 5 years old group vs. the 14 to 17 years old group, $P < 0.001$. The adherence rates according to gender were as follows: males (n = 229) was 91.6% and females (n = 209) was 91.2% ($P = 0.35$).

Table 4.3: Adherence rates in the Lea Toto cohort after 24 months of first-line ART, compared across caregiver status, age group and gender demographic groups.

	n	Adherence rate, %	*P*
Caregiver status			
Two parent home	225	93.5%	2-PH$^{\Psi}$ vs 1-PH: *P* = 0.06
One parent home	152	91.8%	2-PH vs GH$^{\Omega}$: *P* < 0.001
Guardian home	61	87.2%	1-PH vs GH: *P* < 0.001
Age group			
2 to 5 years old	80	95.9%	2 to 5 vs 6 to 9 yrs old: *P* = 0.03
6 to 9 years old	135	93.6%	2 to 5 vs 10 to 13 yrs old: *P* < 0.001
10 to 13 years old	130	88.7%	2 to 5 vs 14 to 17 yrs old: *P* < 0.001
14 to 17 years old	93	90.8%	
Gender			
Male	229	91.6%	*P* = 0.35
Female	209	91.2%	

$^{\Psi}$ PH: Parent-home; $^{\Omega}$ GH: Guardian Home.

4.2 Prevalence and patterns of virologic failure among children and adolescents in the Lea Toto Programme

4.2.1 Viral Suppression and virologic failure rates among demographic groups

Children and adolescents raised in two parent homes (n = 185) had a viral suppression rate of 83.2%; those in one parent homes (n = 125) had a rate of 77.0%; those in guardian homes (n = 50) had a rate of 73.2%. The virologic failure rates were 16.8%, 23%, and 26.8%, respectively. The *P*-values were as follows: two parent homes vs. one parent homes *P* < 0.001; two parent homes vs. guardian homes *P* < 0.001; one parent homes vs. guardian homes *P* < 0.01 (Table 4.5).

Age group comparisons were also done vis-à-vis viral suppression rates: the 2 to 5 years old group (n = 66) had a viral suppression rate of 81.3%; the 6 to 9 years old group (n = 111) had a rate of 77.4%; the 10 to 13 years old group (n = 107) had a rate of 75.4%; and the 14 to 17 years old group (n = 76) had a rate of 73.9%. The virologic

failure rates were 18.7%, 22.6%, 24.6%, and 26.1%, respectively. The *P*-values were as follows: 2 to 5 years old group vs. the 6 to 9 years old group, $P < 0.01$; 2 to 5 years old group vs. the 10 to 13 years old group, $P < 0.001$; 2 to 5 years old group vs. the 14 to 17 years old group, $P < 0.001$. The viral suppression rates according to gender were as follows: males (n = 188) was 75.6% and females (n = 172) was 77.6%; while virologic failure rates were 24.4% and 22.4%, respectively ($P = 0.04$).

Table 4.4: Viral Suppression failure rates in the Lea Toto cohort after 24 months of first-line ART.

	n	Viral suppression rate, %	Virologic failure rate, %	*P*
Caregiver status				
Two parent home	225	83.2%	16.8%	2-PH$^\Psi$ vs 1-PH: $P < 0.001$
One parent home	152	77.0%	23%	2-PH vs GH$^\Omega$: $P < 0.001$
Guardian home	61	73.2%	26.8%	1-PH vs GH: $P < 0.01$
Age group				
2 to 5 years old	80	81.3%	18.7%	2-5 vs 6-9 yrs; $P < 0.001$
6 to 9 years old	135	77.4%	22.6%	2-5 vs 10-13 yrs; $P < 0.01$
10 to 13 years old	130	75.4%	24.6%	2-5 vs 14-17 yrs; $P < 0.001$
14 to 17 years old	93	73.9%	26.1%	
Gender				
Male	229	75.6%	24.4%	$P = 0.04$
Female	209	77.6%	22.4%	

$^\Psi$ PH: Parent-home; $^\Omega$ GH: Guardian Home.

4.2.2 First-line ART Treatment Regimens and their success rates

The AZT/3TC/LPV/r and TDF/3TC/EFV regimens both showed a 100% treatment success rate throughout 24 month follow-up period (Table 4.6). The other 5 ART regimens showed an increasing rate of treatment success over time The ABC/3TC/LPV/r regimen showed a 70% treatment success rate after 6 and 12 months of ART, and a 100% treatment success rate after 18 and 24 months of ART. The

difference in treatment outcome amongst the 7 ART regimens was not statistically significant at any time point: according to the Pearson χ^2 test, the *P* values were 0.45, 0.91, 0.40, and 0.74 after six, 12, 18, and 24 months of ART, respectively.

Table 4.5: Treatment success rates for the Lea Toto cohort while on various first-line ART regimens measured at six, 12, 18, and 24 months after initiation of first-line ART.

		Treatment success (n, %)			
	n (%)	6 months	12 months	18 months	24 months
AZT/3TC/ NVP	215 (100%)	101 (47%)	129 (60%)	129 (60%)	159 (74%)
AZT/3TC/ EFV	59 (100%)	41 (69%)	45 (76%)	51 (86%)	51 (86%)
ABC/3TC/ NVP	69 (100%)	34 (49%)	46 (67%)	46 (67%)	50 (73%)
ABC/3TC/ EFV	72 (100%)	37 (51%)	50 (69%)	50 (69%)	58 (81%)
TDF/3TC/ EFV	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
ABC/3TC/ LPV/r	10 (100%)	7 (70%)	7 (70%)	10 (100%)	10 (100%)
AZT/3TC/ LPV/r	3 (100%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)
P-value		0.45	0.91	0.40	0.74

ART:- Antiretroviral Therapy; ABC:- Abacavir; 3TC:- Lamivudine; AZT: Zidovudine; TDF:- Tenofovir; NVP:- Nevirapine; EFV: Efavirenz; LPV/r:- Lopinavir/ritonavir; *P*-value: Measure of Statistical Significance.

4.2.3 First-line ART treatment outcomes

In this study, treatment success for the cohort increased over time. The treatment success rate was 56.2% after 6 months of ART, 67.8% after 12 months, and 71.2%

after 18 months, 76.1% after 24 months, 75.8% after 36 months and 73.9% after 48 months of ART (Table 4.7).

Table 4.6: Treatment success and failure in the Lea Toto cohort during 48 months of first-line antiretroviral therapy (ART).

Duration on First-line ART	n (%)	Treatment Success, n (%)	Virologic Failure, n (%)
6 months	438 (100%)	246 (56.2%)	192 (43.8%)
12 months	434 (100%)	294 (67.8%)	140 (32.2%)
18 months	418 (100%)	298 (71.2%)	120 (28.8%)
24 months	392 (100%)	298 (76.1%)	94 (23.9%)
30 months	340 (100%)	263 (77.3%)	77 (22.7%)
36 months	302 (100%)	229 (75.8%)	73 (24.2%)
42 months	287 (100%)	213 (74.2%)	74 (25.8%)
48 months	282 (100%)	208 (73.9%)	74 (26.1%)

Those with virologic rebound had initial treatment success after 12 months of ART, followed by a viral load of 1,000 RNA copies/ml after 18 and /or 24 months of ART. After 18 months of ART, 37 (10.3%) children had experienced virologic rebound, while 35 (9.7%) had experienced virologic rebound after 24 months of ART (Table 4.8). Seventeen children (4.7%) had virologic failure after both 18 and 24 months of ART. Also, 30 (8.3%) of the 360 children were slow responders: they initially experienced virologic failure after 12 months of ART, but had treatment success after 18 to 24 months of ART.

Table 4.7: **Slow responders to ART and Virologic rebounders in the Lea Toto cohort during the first 24 months of first-line antiretroviral therapy (ART).**

ART Treatment Outcome	n (%)	Proportion with ART treatment outcome, n (%)
Slow responders	438 (100%)	30 (6.8%)
Virologic rebound at 18 months	418 (100%)	37 (8.9%)
Virologic rebound at 24 months	392 (100%)	35 (8.9%)
Virologic rebound at 18 and 24 months	392 (100%)	17 (4.3%)

4.2.4 Dynamics of response to first-line ART

The Log_{10} viral loads over 24 months of the 30 individuals with slow response to first-line ART were compared to the median Log_{10} viral loads of the success group (n = 297) (Figure 4.3). There was a statistically significant difference between the Log_{10} viral load of the success group, and the Log_{10} viral loads of the 12 slow responders after 0, 6, and 12 months of ART. This difference grew more significant over this period: the Mann-Whitney U-test P values were $P = 0.03$ at baseline, $P < 0.001$ after 6 months and $P < 0.001$ after 12 months of ART. Additionally, the median Log_{10} viral loads of the success group were compared to the median Log_{10} viral loads of the failure group (N = 111). The failure group were those with a viral load of 1,000 HIV-1 RNA copies/ml or more, even after 24 months of ART. The differences between the success group and the failure group were statistically significant after 6, 12, 18, and 24 months of ART. The time-point with the most statistically significant difference was 24 months (Mann-Whitney U-test $P < 0.001$).
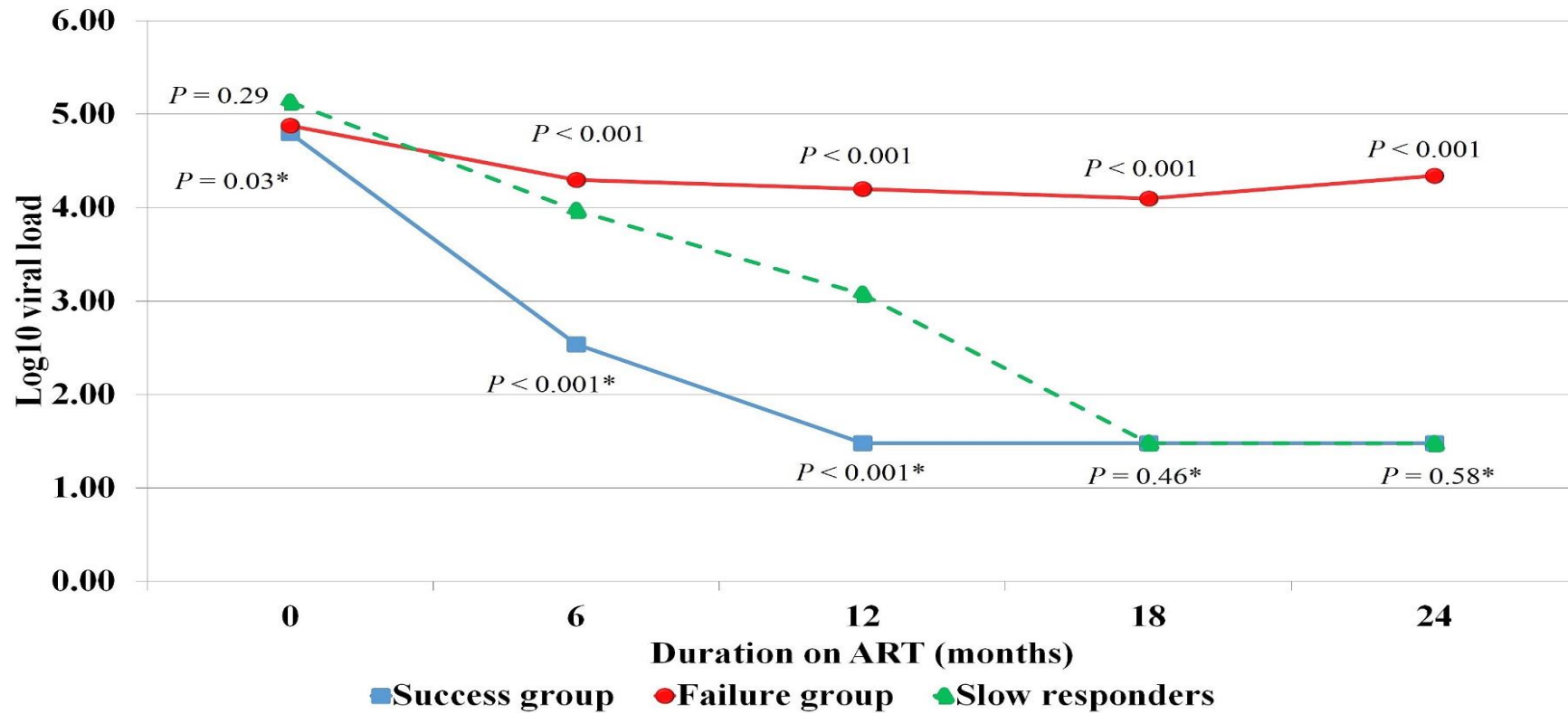
Figure 4.3: The HIV-1 viral loads over 24 months of first-line ART in the Lea Toto cohort for the treatment success group (n=297), slow responders group (n=30), and the virologic failure group (n=111).

4.2.5 Immune System recovery among children and adolescents aged six to 17 years

To determine the rate of recovery of the immune systems of patients aged six to 17 years, the CD4+ T cell counts for 358 children and adolescents six years of age and older were compared in terms of treatment outcome (Figure 4.4). The median CD4 T cell count for the treatment success group (n = 271) was 402 cells/µl at baseline. It increased over time, and the value at 24 months was 721 cells/µl. The median CD4 T cell count for the virologic failure group (n = 87) at baseline was 466 cells/µl. It declined over time, with the value at 24 months being 353 cells/µl. The difference in the median CD4 T cell count between the treatment success and virologic failure groups was statistically significant at 18 months ($P = 0.03$) and 24 months ($P = 0.03$).
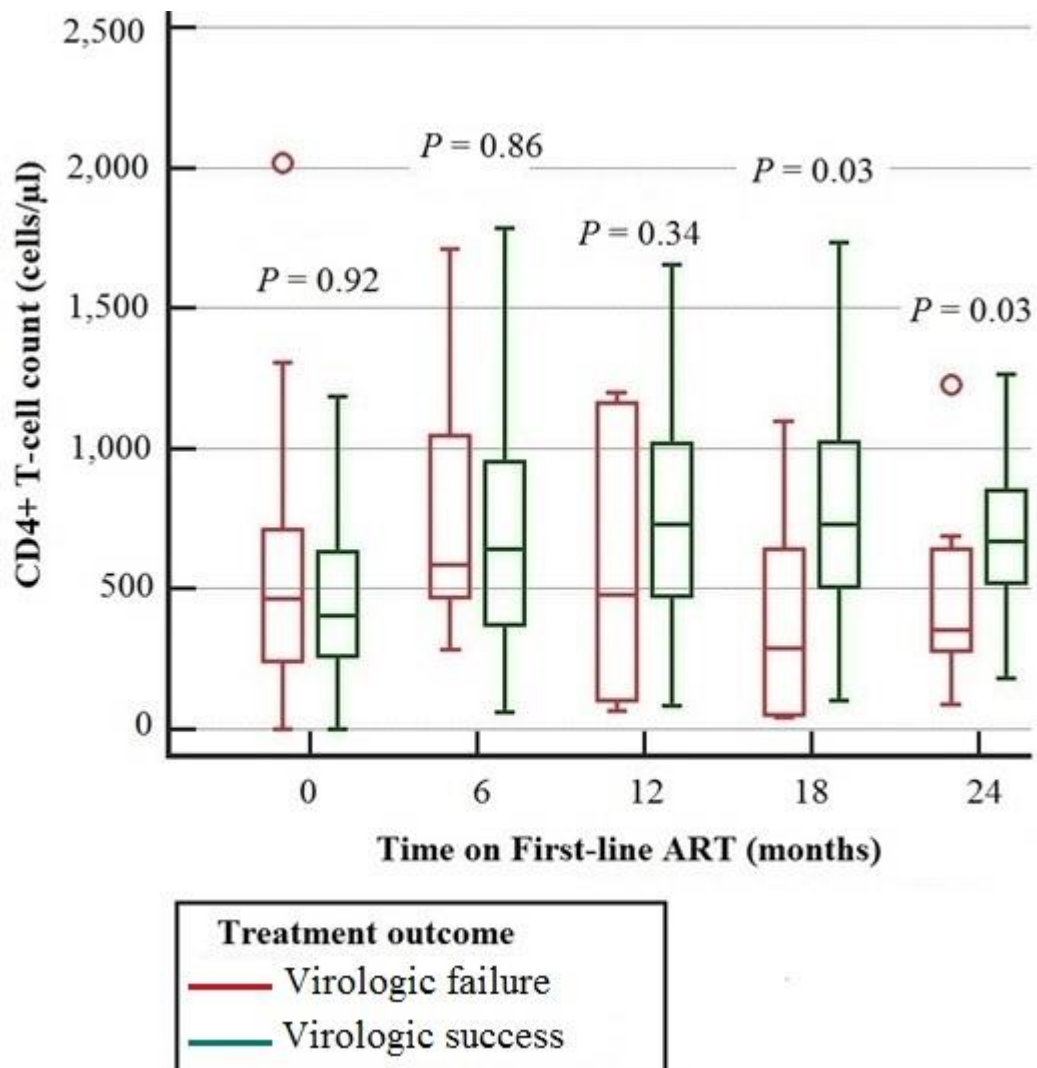
Figure 4.4: The CD4+ T cell counts of the Lea Toto treatment success and failure groups among children and adolescents aged six to 17 years during 24 months of first-line ART. The upper and lower borders of the flying boxes represent the interquartile range, while the central line indicates the median value. The whiskers indicate the minimum and maximum values excluding outliers.

4.2.6 Immune System recovery among children aged two to five years.

To determine the rate of recovery of the immune systems of patients aged 2 to 5 years the CD4+ T cell percentage (%) values for 80 children vis-à-vis treatment outcome, were compared (Figure 4.5). Percentages, not counts were used because in this age range, the former is considered accurate than the latter. Data CD4+ T cell % for children aged 6 years or older was not measured. The median CD4+ T cell % for the treatment success group (n = 61) was 18% at baseline. It improved over time, and was 36% at 24 months. For the virologic failure group (n = 19), the median CD4+ T cell % was 22% at baseline. It also improved over time, and was 28% at 24 months. The difference in the median CD4+ T cell % between the treatment success and virologic failure groups was not statistically significant at any time-point.
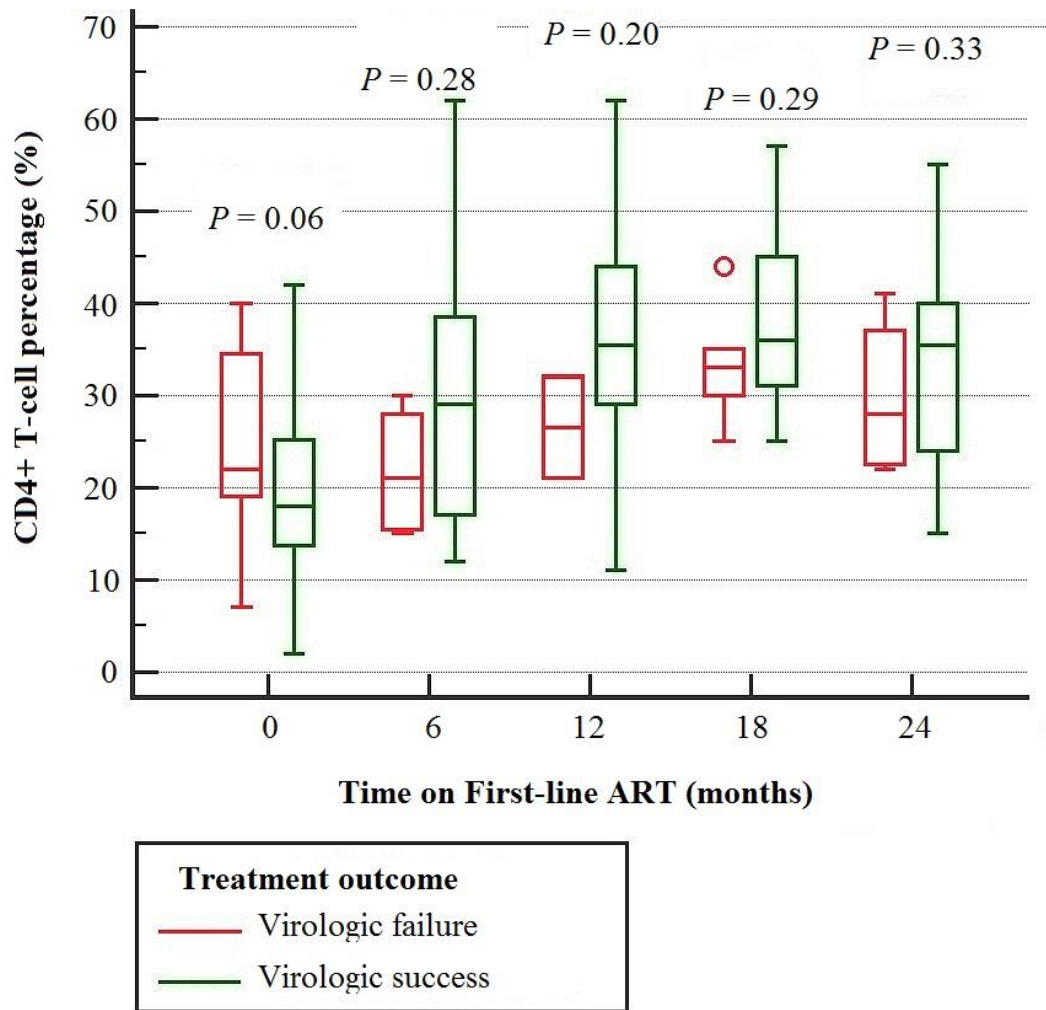
Figure 4.5: The CD4+ T cell percentages of the Lea Toto treatment success and failure groups among children aged two to five years old (n = 80) during 24 months of first-line ART. The upper and lower borders of the flying boxes represent the interquartile range, while the central line indicates the median value. The whiskers indicate the minimum and maximum values excluding outliers.

4.2.7 Immune System dynamics across age groups over time

The CD4+ T-cell counts for the different age groups were compared over the first 24 months of first-line ART (Figure 4.6). The median baseline CD4+ T-cell counts were: 2-5 years age group (n=80), 873 cells/µl; 6-9 years age group (n=136), 437 cells/µl; 10-13 years age group (n=130), 486 cells/µl; 14-17 years age group (n=92), 360 cells/µl. The median CD4+ T-cell counts after 12 months of first-line ART were 862, 724, 889, and 571 cells, respectively. After 24 months of first-line ART, the median CD4+ T-cell counts were 1,384, 849, 692, and 1,173 cells/µl, respectively. The *P*-values of the difference between the CD4+ T-cell counts for the 2-5 years age group vs the average for the other 3 age groups, were all statistically significant ($P < 0.001$).
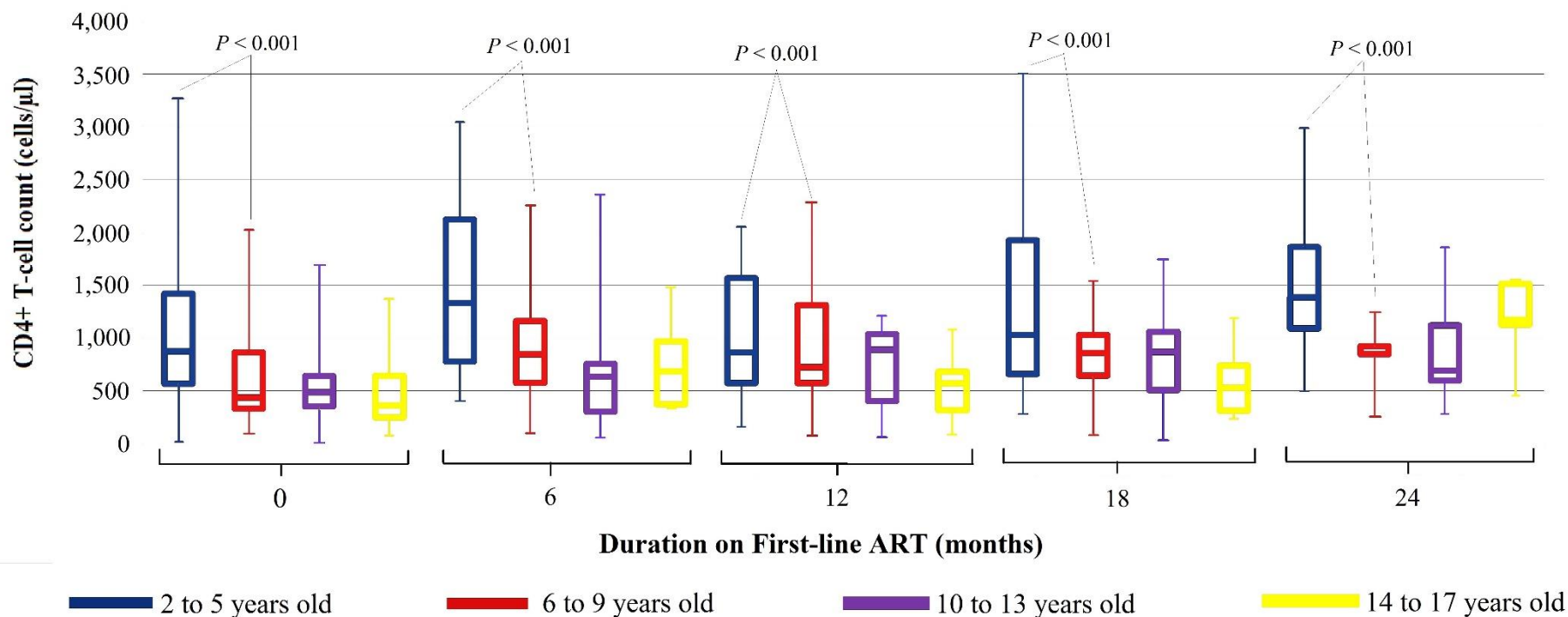
Figure 4.6: The CD4+ T-cell counts for the different Lea Toto cohort age groups during the first 24 months of first-line ART. The upper and lower borders of the flying boxes represent the interquartile range, while the central line indicates the median value. The whiskers indicate the minimum and maximum values excluding outliers.

4.3 Prevalence and patterns of HIV-1 drug resistance among the children and adolescents of the Lea Toto Programme

4.3.1 Clinical and demographic characteristics for those in the second-line ART cohort

Among the 114 study participants who failed first-line ART, the most common HIV-1 subtypes were subtype A (54.4%), and subtype D (15.8%) (Table 4.9). The median time of follow-up on first-line ART before switching to second-line ART was 31 months (IQR, 29 to 37 months). The median CD4+ T-cell count cells/µl was 192 (IQR, 73 to 432) cells/µl. The median Baseline HIV-1 RNA was 4.86 (IQR, 4.42 to 5.20) $\log_{10}$ copies/ml. After second-line ART, the median HIV-1 RNA dropped to 2.06 (IQR, 1.81 to 2.31) $\log_{10}$ copies/ml. Optimal adherence to ART was found in 70 (61.4%) study participants, while 44 (38.6%) had suboptimal adherence to ART.

The first-line ART regimens initially prescribed to these 114 study participants that failed first-line ART were: AZT/3TC/NVP, 73 (64.0%); AZT/3TC/EFV, 19 (n = 16.7%); ABC/3TC/NVP, 13 (11.4%); ABC/3TC/EFV, 7 (6.1%); and TDF/3TC/EFV, (n = 2, 1.8%). Nine different second-line ART regimens were prescribed to these patients, with the most common being TDF/3TC/LPV/r, given to 40 (35.1%) children; the least common was ABC/3TC/TDF prescribed to 1 child (0.9%) (Table 4.9).

Table 4.8: Clinical characteristics of those in the Lea Toto cohort who failed first-line ART and were switched to second-line ART.

Characteristics	n (%)
Time of follow-up on First-line ART (months)	31 (29 to 37) [ψ]
Baseline HIV-1 RNA $\log_{10}$ copies/ml	4.86 (4.42 to 5.20)[ψ]
Post-treatment HIV-1 RNA $\log_{10}$ copies/ml	2.06 (1.81 to 2.31) [ψ]
CD4+ T-cell count cells/µl	192 (73 to 432) [ψ]
Adherence to ART Regimen [φ]	
Optimal	70 (61.4%)
Suboptimal	44 (38.6%)
Second-line ART regimen	
TDF/3TC/LPV/r	40 (35.1%)
ABC/3TC/LPV/r	17 (14.9%)
ABC/DDI/LPV/r	21 (18.4%)
AZT/3TC/LPV/r	16 (14.0%)
ABC/TDF/LPV/r	10 (8.8%)
TDF/3TC/EFV	5 (4.4%)
TDF/3TC/NVP	2 (1.8%)
TDF/DDI/LPV/r	2 (1.8%)
ABC/3TC/TDF	1 (0.9%)

[ψ] Medians and IQRs; IQR:- Interquartile Range; ART:- Antiretroviral therapy; NRTI:- Nucleoside Reverse Transcriptase Inhibitor; NtRTI:- Nucleotide Analogue Reverse Transcriptase Inhibitor; NNRTI:- Non-Nucleoside Reverse Transcriptase Inhibitor; Pi:- Protease inhibitor; ABC:- Abacavir; 3TC:- Lamivudine; AZT:- Zidovudine; TDF:- Tenofovir; NVP:- Nevirapine; EFV: Efavirenz; LPV/r:- Lopinavir/ritonavir.

Among the 114 study participants who failed first-line ART, the median time of follow-up on first-line ART before switching to second-line ART was 31 months (IQR, 29 to 37 months) (Table 4.10). Of the study participants, males were 63 (55.3%), while 51 (44.7%) were females. The median age was 13.5 years (IQR, 10.1 to 18.1). Eighteen study participants (15.8%) were aged between 2 and 5 years old; 28 (24.6%)

were between 6 and 9 years old; 31 (27.2%) were aged 10 to 13 years old; 34 (29.8%) were aged 14 to 17 years old; and 3 (2.6%) were aged 18 to 20 years old. Twenty-eight children (24.6%) were being raised by guardian, 39 (34.2%) were being raised by one parent while 47 (41.2%) were being raised by two parents.

Table 4.9: Demographic characteristics of the 114 Lea Toto cohort members who failed first-line ART and were switched to second-line ART.

Characteristics	n (%)
Age at start of Second-line ART (years)	13.5 (10.1 to 18.1)$^{\psi}$
Age group	
2 to 5 years	18 (15.8%)
6 to 9 years	28 (24.6%)
10 to 13 years	31 (27.2%)
14 to 17 years	34 (29.8%)
18 to 20 years	3 (2.6%)
Gender	
Male	63 (55.3%)
Female	51 (44.7%)
Primary caregiver	
Two parents	47 (41.2%)
One parent	39 (34.2%)
Guardian	28 (24.6%)

ψ Medians and IQRs; IQR:- Interquartile Range; ART:- Antiretroviral therapy.

4.3.2 Agarose gel of the DNA amplicons that were sequenced

Results of the PCR were visualized by gel electrophoresis. Plate 4.1 show positive bands for amplicons of HIV-1 *pol*, which includes the protease (PR) gene amino acids 1-99 and the reverse transcriptase (RT) gene, amino acids 1-299. The amplicons then underwent automated sequencing.

Plate 4.1: PCR gel picture showing successfully amplified products with a size of 1.332 kilobases (kb). Seven of the 114 amplicons for members of the Lea Toto second-line ART cohort are shown. L: Ladder; Lanes 1 to 7: study participants A100, A102, A107, A111, A114, A116 and A119, respectively.

4.3.3 HIV-1 subtypes and Phylogenetic tree for those in the second-line ART cohort

Subtyping analysis determined that the 114 DNA sequences of the second-line patients were clustered into multiple HIV-1 subtypes for the Protease gene (Pr) and the reverse transcriptase (RT) gene. The only pure subtypes were subtype A1 (n=41, 35.9%), recombinant subtype A1B (n=19, 16.6%), subtype B (n=9, 7.9%), subtype C (n=8, 7.0%) and subtype D (n=9, 7.9%). There were a total of 16 subtypes represented: 4 pure (25%) and 12 possibly recombinant (75%) subtypes. (Table 4.11).

Table 4.10: The HIV-1 subtypes of the 114 sequences of the Lea Toto cohort that were sequenced before they were switched from first-line ART to second-line ART.

HIV-1 Subtype (Pr, RT)	n (%)
A1	40 (35.1%)
B	9 (7.9%)
D	8 (7.0%)
C	8 (7.0%)
Possibly recombinant A1B	19 (16.6%)
Possibly recombinant A1D	7 (6.1%)
Possibly recombinant A1C	6 (5.3%)
Possibly recombinant DG	4 (3.6%)
Possibly recombinant BC	3 (2.5%)
Possibly recombinant A1H	2 (1.8%)
Possibly recombinant CRF_35AD	2 (1.8%)
Possibly recombinant BD	2 (1.8%)
Possibly recombinant A1J	1 (0.9%)
Possibly recombinant A1K	1 (0.9%)
Possibly recombinant BJ	1 (0.9%)
CRF_01AE	1 (0.9%)
Total	**114 (100%)**

Pr: Protease; RT: Reverse Transcriptase

The alignment of the protease amplicon sequences to a reference HIV-1 protease sequence is shown. (Appendix XI). The resulting phylogenetic tree is also shown (Figure 4.7). The alignment of the reverse transcriptase amplicon sequences to a reference HIV-1 reverse transcriptase sequence is shown. (Appendix XII). The resulting phylogenetic tree is also shown (Figure 4.8).

Figure 4.7: Phylogenetic tree of the HIV-1 protease gene for the 114 Lea Toto cohort members who were switched from first-line ART to second-line ART. The phylogenetic relationships were rooted with a SIV chimpanzee reference sequence. Bootstrap values of > 70% of 1000 replicates are indicated next to the nodes. Brackets on the right indicate the subtype clusters. The scale in the bottom right corner indicates the number of nucleotide substitutions.
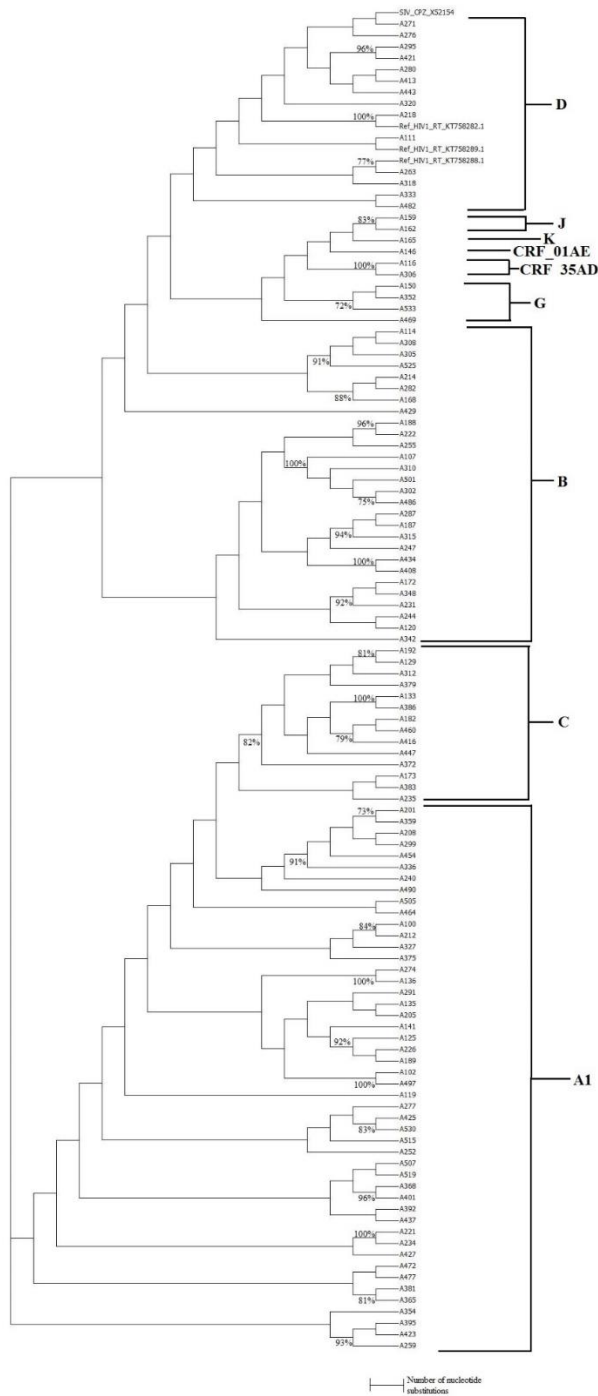
Figure 4.8: Phylogenetic tree of the HIV-1 reverse transcriptase gene for the 114 Lea Toto cohort members switched from first-line ART to second-line ART. The phylogenetic relationships were rooted with a SIV chimpanzee reference sequence. Bootstrap values of > 70% of 1000 replicates are indicated next to the nodes. Brackets on the right indicate the subtype clusters. The scale in the bottom right corner indicates the number of nucleotide substitutions.

4.3.4 The drug resistance mutation profiles

The DRM profiles of the 114 study participants who failed first-line ART and were initiated on second-line ART showed the presence of NRTI and NNRTI mutations. Among the NRTI mutations, M184V was the most common, occurring in 90 out of 114 study participants (78.9%); the L74V mutation occurred in 19 persons (16.7%) (Table 4.12).

Mutations of the NRTI class, especially Thymidine Analog Mutations (TAMs), are significant in terms of their influence on the success of second-line regimens in resource-limited settings. Time on a failing ART regimen is thought to be a predictor of accumulation of TAMs, which then compromise second-line therapy (Dow *et al.*, 2014). In this cohort: among type-I pattern TAMs, the T215Y mutation was present in 61 (53.5%) of the children and adolescents; the M41L mutation was found in 38 (33.3%) individuals, while the L210W mutation occurred in 28 (24.6%) persons. The following type-II pattern TAMs were found: T215F in 64 (56.1%) persons; K70R occurred in 40 (35.1%) persons; D67N was present in 37 (32.5%) individuals, K219Q mutation occurred in 6 (5.3%) individuals, while the K219E mutation occurred in 3 (2.6%) persons (Table 4.12).

Table 4.11: Drug resistance mutations of the 114 Lea Toto cohort members who failed first-line ART and were switched to second-line ART.

NRTI mutations	
At least one NRTI Mutation	**109 (95.6%)**
M184V	**109 (95.6%)**
L74V	**24 (21.1%)**
K65R	**2 (1.8%)**
Q151M	**0.9 (1.8%)**
TAMs	
At least one TAM	**57 (50.0%)**
Type-I pattern TAMs	
M41L	**25 (21.9%)**
L210W	**18 (15.8%)**
T215Y	**14 (12.3%)**
Type-II pattern TAMs	
D67N	**18 (15.8%)**
K70R	**15 (13.2%)**
T215F	**10 (8.8%)**
K219Q	**6 (5.3%)**
K219E	**3 (2.6%)**
NNRTI mutations	
At least one NNRTI Mutation	105 (92.1%)
K103N	31 (27.2%)
K103S	15 (13.2%)
Y181C	22 (19.3%)
Y181I	13 (11.4%)
Y181V	8 (7.1%)
V106A	4 (3.5%)
V106M	2 (1.8%)
G190A	18 (15.8%)
G190S	10 (8.8%)
G190E	5 (4.4%)
Y188L	6 (5.3%)
Y188C	2 (1.8%)
Y188H	1 (0.9%)

NRTI:- Nucleoside Reverse Transcriptase Inhibitor; NtRTI:- Nucleotide Analogue Reverse Transcriptase Inhibitor; NNRTI:- Non-Nucleoside Reverse Transcriptase Inhibitor; TAM:- Thymidine Analog Mutation; C:- Cysteine; D:- Aspartic Acid; E:- Glutamic acid; F:- Phenylalanine; G:- Glycine; H:- Histidine; L: Leucine; K:- Lysine; M:- Methionine; N:- Asparagine; Q:- Glutamine; R:- Arginine; S:- Serine; V:- Valine; W:- Tryptophan; Y: Tyrosine.

4.3.5 Treatment outcomes for the second-line ART cohort

Of the 114 persons switched from first-line ART to second-line ART, 92 (80.7%) experienced treatment success by suppressed their viral load to below 1,000 HIV-1 RNA copies per ml by the end of the 48 month study (Table 4.13). The median follow-up time on second-line ART was 17 months (IQR, 12 to 19). Twenty-two persons (19.3%) failed second-line ART, meaning they did not suppress their viral load to below 1,000 HIV-1 RNA copies per ml. Fifteen (68.2%) of these 22 individuals were switched to Salvage ART; two (9.1%) were lost to follow-up; two (9.1%) transferred out of the LTP and into other comprehensive care centres (CCCs); and three (13.6%) died (Table 4.13).

Table 4.12: Treatment outcomes for the 114 children and adolescents in the Lea Toto cohort initiated on second-line ART.

Second-line Outcome	n (%)
Treatment Success	92 (80.7%)
Treatment Failure	22 (19.3%)
Total	**114 (100%)**
Outcome for the 22 patients who had second-line ART treatment failure	
Salvage ART	15 (68.2%)
Lost to follow-up	2 (9.1%)
Transferred out	2 (9.1%)
Died	3 (13.6%)
Total	**22 (100%)**

4.3.6 Opportunistic infections among the second-line ART cohort

Data abstracted from the Lea Toto patients' records showed that several opportunistic infections (OIs) occurred in the children within 2 months of the time of switching to second-line ART (Figure 4.9). The most common OIs were respiratory tract infections (RTIs), including Pneumonia, Bronchitis, Coryza, and Rhinitis (n = 42, 36.8%). Pneumonia on its own was found in 12 persons (10.5%). The Skin infections Eczema, Pruritic Papular Eruption (PPE), Tinea Capitis, and Dermatitis were found in 29 persons (25.4%). Gastrointestinal tract (GIT) infections occurred in 14 individuals (12.3%). Other OIs found were Ear, Nose and Throat (ENT) infections (n=8, 7.0%), Pulmonary Tuberculosis (PTB) (n=6, 5.3%), Lymphadenopathy (n=3, 2.6%), Extra-pulmonary TB (EPTB) (n=2, 1.8%), and Malaria (n=1, 0.9%).
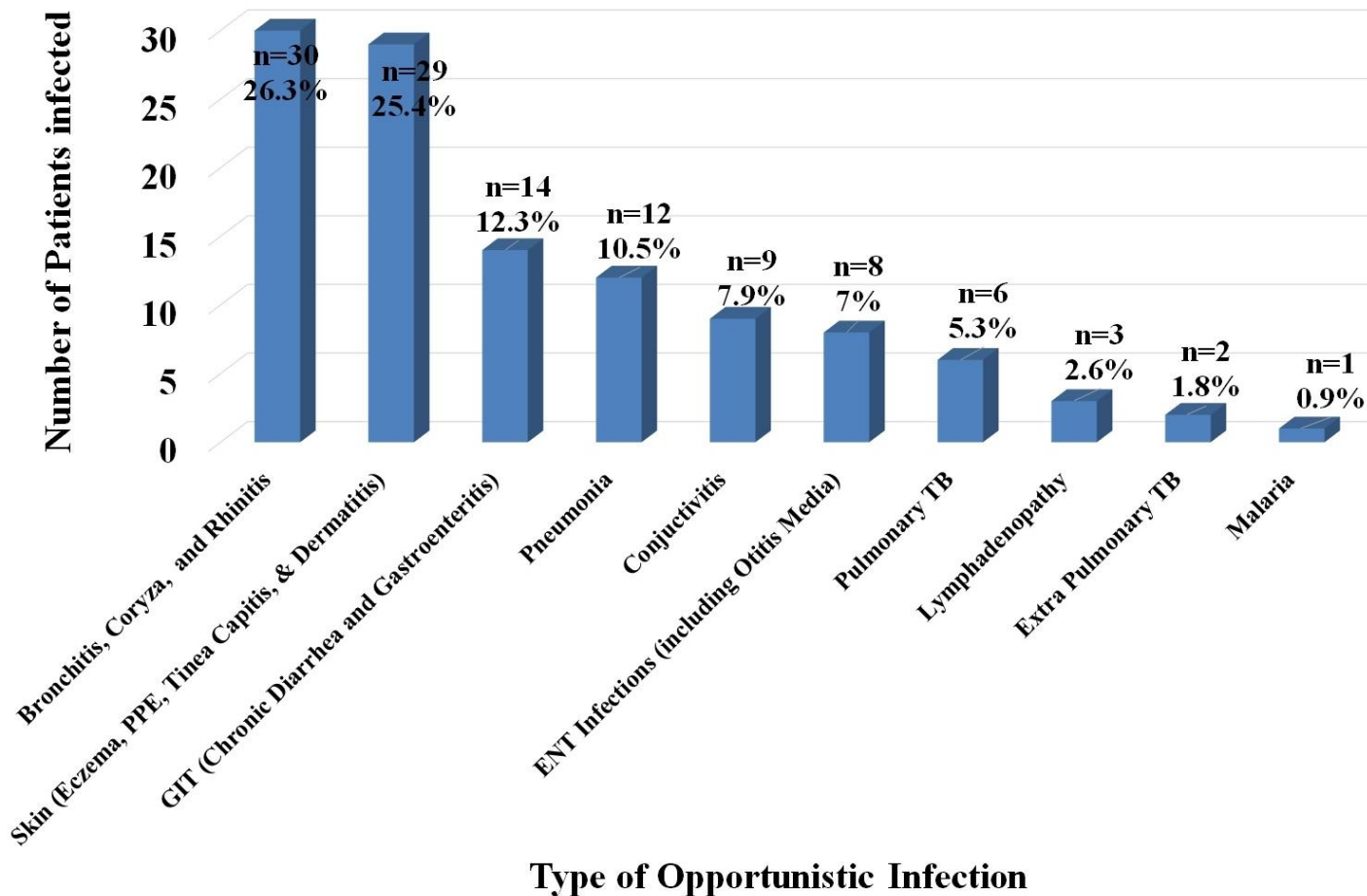
Figure 4.9: Prevalence of opportunistic infections in the Lea Toto cohort for the 114 members who were switched from first-line ART to second-line ART.

4.3.7 The Thymidine Analog Mutations in the second-line ART cohort

The frequencies of patients with 0 TAMs was 57 (50.0%); those with 1 TAM were 18 (15.8%); those with 2 TAMs were 16 (14.0%), and those with 3 or more TAMs were 23 (20.0%) (Figure 4.10). The highest number of TAMs in a single patient was 6. Of the 57 patients with 0 TAMs, 47 (82.5%) succeeded on second-line ART, suppressing their viral load to below 1,000 HIV-1 RNA copies/ml; 10 (17.5%) failed to suppress their viral load while on second-line ART. Among the 18 patients with 1 TAM, 15 (83.3%) succeeded on second-line ART, while 3 of them (16.7%) failed. Of the 16 patients with 2 TAMs, 12 (75.0%) succeeded on second-line ART, while 4 of them (25.0%) failed. Among the 23 patients with 3 or more TAMs, 18 (78.3%) succeeded on second-line ART, while 5 of them (21.7%) failed on second-line ART. Those with any TAMs at all were 57 in total, with 45 (78.9%) succeeding on second-line ART and 12 (21.1%) failing.

The rates of second-line ART treatment success were compared among the TAM groups, using the Fisher's exact test. Firstly, the difference between the treatment success rates of the 0 TAMs group and the "any TAMs" group was statistically significant: 82.5% vs. 78.9%, $P = 0.004$. The difference between the treatment success rates of the 0 TAMs group and the 1 TAM group however, was not statistically significant: 82.5% vs. 83.3%, $P = 0.22$. The difference between the treatment success rates of the 0 TAMs group and the 2 TAMs group was statistically significant: 82.5% vs. 75.0%, $P < 0.001$. The difference between the treatment success rates of the 0 TAMs group and the 3 or more TAMs group was also statistically significant: 82.5% vs. 78.3%, $P < 0.01$. Regarding the follow-up on first-line ART per TAM group, the average follow-up times were: the 0 TAMs group was followed up on first-line ART for an average of 2.7 years; the any TAMs group was followed up on first-line ART for an average of 2.5 years; the 1 TAM group was followed up on first-line ART for an average of 2.5 years; the 2 TAMs group was followed up on first-line ART for an average of 2.5 years; finally, the 3 or more TAMs group was followed up on first-line ART for an average of 2.4 years. These follow-up times were compared using the Fisher's exact test: the 0 TAMs group vs. the any TAMs group, 2.7 years vs. 2.5 years, $P = 0.04$; the 0 TAMs group vs. the 1 TAM group, 2.7 years vs. 2.5 years, $P = 0.04$; the 0 TAMs group vs. the 2 TAMs group, 2.7 years vs. 2.5 years, $P = 0.04$; and the 0

TAMs group vs. the 3 or more TAMs group, 2.7 years vs. 2.4 years, $P < 0.01$ (Figure 4.10).
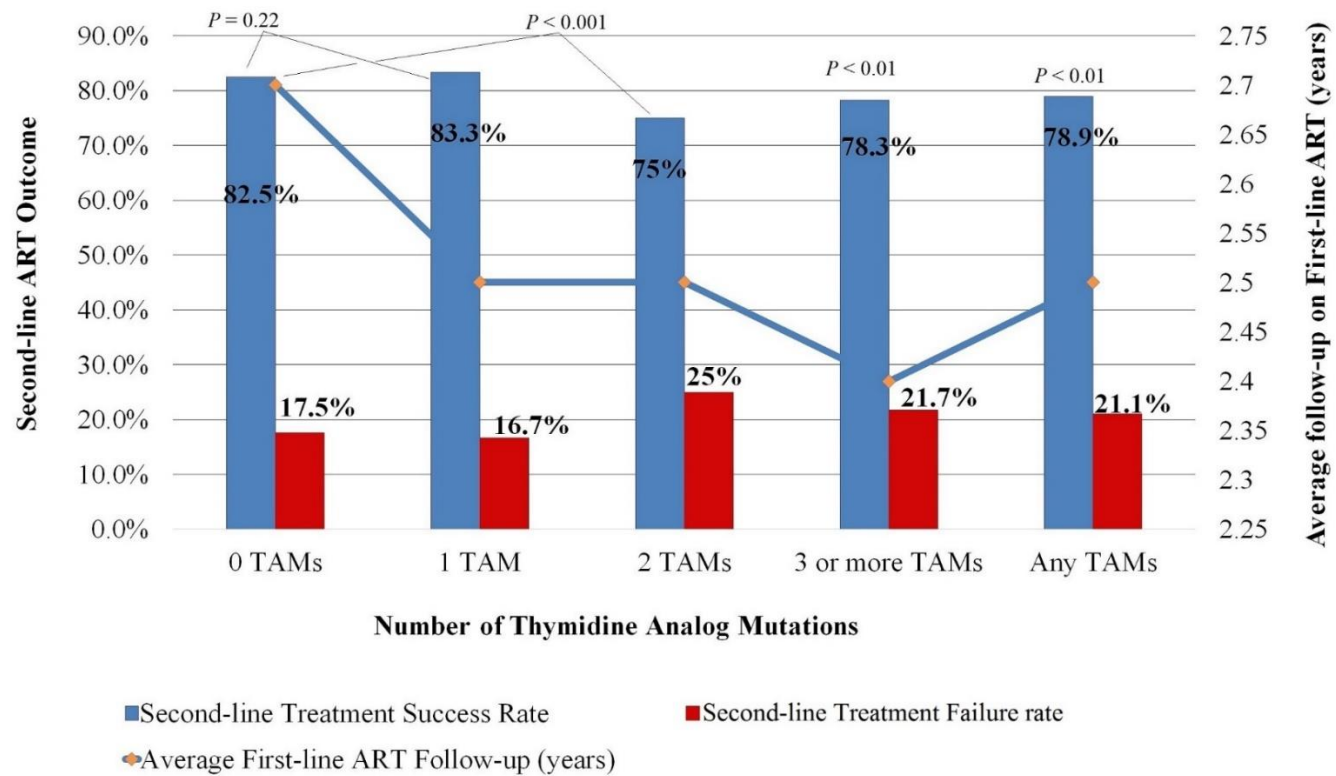
Figure 4.10: Prevalence of Thymidine Analog Mutations (TAMs) and average follow-up time in the Lea Toto cohort at the onset of for those initiated on second-line ART.

4.3.8 Treatment outcomes for the salvage ART cohort

A drug resistance test (DRT) was conducted to determine what DRMs existed in the 15 patients who failed second-line ART and were switched to salvage ART. These patients were then initiated on a salvage ART regimen to which their virus was still susceptible. Five of the 15 patients (33.3%) were female, while 10 (66.7%) were male (Table 4.14). The median first-line baseline age was 7.1 years (IQR, 5.1-9.9). The three salvage ART regimens used were ABC/DDI/LPV/r (n=8, 53.3%); AZT/DDI/LPV/r (n=3, 20%); and TDF/3TC/LPV/r (n=4, 26.7%). The median follow-up time on first-line ART was 1.8 years (IQR, 1.4-2.0). The median follow-up time on second-line ART was 1.7 years (IQR, 1.6-2.3). By the end of the study, 13 (86.6%) patients were still succeeding on salvage ART; one (6.7%) had died of severe acute malnutrition and severe Pneumonia; and one (6.7%) had transferred out to another Comprehensive Care Centre (CCC).

Table 4.13: Clinical history for the 15 individuals in the Lea Toto salvage ART cohort.

Study ID #	Gender	Age at 1st line ART Start (years)	1st line ART Regimen	1st Line Follow-up (years)	2nd Line ART Regimen	2nd Line Follow-up (years)	Salvage ART Regimen	Salvage ART outcome
A100	Male	5.2	AZT/3TC/NVP	1.8	ABC/3TC/LPV/r	1.5	TDF/3TC/LPV/r	On salvage ART
A114	Male	8.7	AZT/3TC/NVP	1.5	TDF/3TC/LPV/r	1.7	ABC/DDI/LPV/r	On salvage ART
A125	Male	11.0	ABC/3TC/NVP	2.2	ABC/DDI/LPV/r	1.4	ABC/DDI/LPV/r	On salvage ART
A129	Female	7.6	TDF/3TC/EFV	1.4	ABC/3TC/LPV/r	2.3	ABC/DDI/LPV/r	On salvage ART
A136	Female	4.6	AZT/3TC/NVP	2.3	ABC/3TC/LPV/r	1.2	ABC/DDI/LPV/r	Transferred-out
A159	Male	2.9	ABC/3TC/NVP	1.8	AZT/3TC/LPV/r	2.1	AZT/DDI/LPV/r	On salvage ART
A165	Female	11.3	AZT/3TC/NVP	2.0	ABC/3TC/LPV/r	1.9	ABC/DDI/LPV/r	Died (SAM, SP)
A173	Female	5.5	AZT/3TC/NVP	1.2	ABC/3TC/LPV/r	2.3	TDF/3TC/LPV/r	On salvage ART
A192	Male	9.3	AZT/3TC/NVP	2.4	TDF/3TC/LPV/r	1.4	ABC/DDI/LPV/r	On salvage ART
A205	Male	13.5	AZT/3TC/NVP	1.9	TDF/3TC/LPV/r	1.5	ABC/DDI/LPV/r	On salvage ART
A310	Male	10.5	AZT/3TC/EFV	0.8	ABC/3TC/LPV/r	2.9	TDF/3TC/LPV/r	On salvage ART
A333	Male	3.6	ABC/3TC/EFV	1.9	ABC/DDI/LPV/r	1.7	AZT/DDI/LPV/r	On salvage ART
A379	Female	4.0	ABC/3TC/NVP	1.4	TDF/3TC/LPV/r	1.8	ABC/DDI/LPV/r	On salvage ART
A383	Male	7.1	AZT/3TC/EFV	1.3	ABC/3TC/LPV/r	1.7	TDF/3TC/LPV/r	On salvage ART
A401	Male	6.4	AZT/3TC/NVP	1.6	ABC/TDF/LPV/r	2.2	AZT/DDI/LPV/r	On salvage ART

ART: Antiretroviral therapy; ABC: Abacavir; 3TC: Lamivudine; AZT: Zidovudine; DDI: Didanosine; TDF: Tenofovir; NVP: Nevirapine; EFV: Efavirenz; LPV/r: Lopinavir/ritonavir; SAM: Severe acute malnutrition; SP: Severe Pneumonia.

The DRM profiles of the 15 study participants who failed second-line ART and were initiated on salvage ART showed the presence of NRTI and NNRTI mutations, but no PI mutations. The DRT conducted at second-line ART baseline revealed that among the NRTI mutations, M184V was the most common, occurring in 15 (100%) patients (Table 4.15). The E44D, Y115F, K70R, L74V, F77L and T69N mutations occurred in 2 patients each. Also, all 15 patients had 2-3 major NRTI DRMs. The DRT conducted at salvage ART baseline showed an increase in the number of DRMs in each of the 15 patients: each of the 15 patients had 3-5 major NRTI DRMs. In this salvage ART cohort, several TAMs were detected: type-1 pattern TAMs found were M41L (n=3, 20%); L210W (n=6, 40%); and T215Y (n=1, 6.7%). The type-2 TAMs found were K70R (n=4, 26.7%) and T215F (n=4, 26.7%). All 15 patients had NNRTI mutations at pre-second-line ART baseline, with the K103N DRM being found in 13 (86.7%) patients; other DRMs present were G190A (53.3%); V179L/F (n=7, 46.7%); and A98G (n=6, 40%). The pre-salvage ART DRT revealed no changes from the pre-second-line NNRTI profiles.

The clinical history of the 15 salvage ART patients showed the presence of several opportunistic infections (OIs) during second-line ART and salvage ART. The most common OIs seen during second-line ART were lower respiratory tracts infections (RTIs) (n=7, 46.7%); upper RTIs (n=4, 26.7%); skin infections (SIs) (n=4, 26.7%); and gastro-intestinal tract (GIT) infections (n=2, 13.3%) (Table 4.15). During salvage ART, the most common OIs were lower RTIs (n=8, 53.3%); GIT infections (n=6, 40.0%); SIs (n=5, 33.3%); and upper RTIs (n=4, 26.7%). Moreover, some OIs occurred during salvage ART that had not occurred during second-line ART: Kaposi's sarcoma (KS) (n=1, 6.7%) and Pneumocyctis carinii pneumonia (PCP) (n=1, 6.7%).

Table 4.14: Drug resistance mutations and opportunistic infections for the 15 individuals in the Lea Toto salvage ART cohort.

Study ID #	Pre-2nd-line ART NRTI DRMs	Pre-2nd-line ART NNRTI DRMs	Pre-2nd-line ART PI DRMs	Opportu-nistic infections	Pre-salvage ART NRTI DRMs	Pre-salvage ART NNRTI DRMs	Pre-salvage ART PI DRMs	Opportu-nistic infections
A100	M184V, E44D	K103N, A98G, Y181C	None	RTI	*M41L, L210W,* Y115F, M184V, E44D	K103N, A98G, Y181C	None	RTI, SI, URTI
A114	M184V, Y115F	K103N, G190A, A98G	None	RTI, URTI, SI	*T215F*, Y115F, T69N, M184V, Y115F	K103N, G190A	None	RTI, URTI, SI
A125	M184V, E44D	K101H,G190S, K103N	None	RTI	T69N, M184V, E44D	K101H,G190S, K103N	None	GIT
A129	M184V, *K70R*	A98G, V179L, G190A	None	GIT, SI	*M41L, L210W,* Y115F, M184V, *K70R*	A98G, V179L, G190A	None	RTI, LA
A136	M184V, T69N	K101E, V179F, Y181C, G190A.	None	PTB	V75M , M184V, T69N	K101E, V179F, Y181C, G190A.	None	GIT
A159	M184V, L74V	K103N, V90I, Y181C, V179L, G190A	None	URTI, TB, EPTB	*T215F*, Y115F, E44D, M184V	K103N, V90I, Y181C, V179L, G190A	None	LA, PCP

A165	M184V, V75M	K103N, Y188L, G190A	None	PTB	*L210W, K70R*, Y115F, M184V, V75M	K103N, Y188L, G190A	None	EPTB
A173	*L210W*, T69N, *M184V*	A98G, K101E, V179L, G190A	None	SI	*M41L*, T215A, *L210W*, T69N, *M184V*	A98G, K101E, V179L, G190A	None	RTI, SI, PTB
A192	Y115F, M184V	K103N, Y181C, G190S, V108I	None	GIT, RTI	*T215F*, K70Q, L74V, Y115F, M184V	K103N, Y181C, G190S, V108I	None	RTI, ENT
A205	L74V, Y115F, M184V	A98G, K103N, Y318F	None	URTI	*L210W*, V75M, L74V, Y115F, M184V	A98G, K103N, Y318F	None	RTI, GIT, URTI
A310	M184V, F77L	K101Q, K103N, Y188L, Y181C	None	RTI, SI	*K70R*, T215A, M184V, F77L	K101Q, K103N, Y188L, Y181C	None	RTI, SI, LA
A333	L74V, Y115F, M184V	K103N, V179T, G190S	None	RTI	T69N, K70Q, L74V, Y115F, M184V	K103N, V179T, G190S	None	GIT
A379	*L210W,* F77L, M184V	K103N, Y188L, G190A	None	URTI	*M41L*, T69N, *L210W,* F77L, M184V	K103N, Y188L, G190A	None	GIT, SI, URTI
A383	T69N, M184V	V179T, G190S, K103N, A98G	None	ENT	*T215Y, L210W*, V75M, T69N, M184V	V179T, G190S, K103N, A98G	None	KS, ENT
A401	*K70R*, M184V, T215N	K103H, V108I, V179L, G190A	None	RTI	*T215F*, T69D, *K70R*, M184V, T215N	K103H, V108I, V179L, G190A	None	PTB, RTI, GIT, ENT

Thymidine Analog Mutations (TAMs) are shown in boldface and italicized. DRM:- Drug resistance mutation; ART:- Antiretroviral therapy; NRTI:- Nucleoside Reverse Transcriptase Inhibitor; NtRTI:- Nucleotide Analogue Reverse Transcriptase Inhibitor; NNRTI:- Non-Nucleoside Reverse Transcriptase Inhibitor; TAM:- Thymidine Analog Mutation; C:- Cysteine; D:- Aspartic Acid; E:- Glutamic acid; F:- Phenylalanine; G:- Glycine; H:- Histidine; L: Leucine; K:- Lysine; M:- Methionine; N:- Asparagine; Q:- Glutamine; R:- Arginine; S:- Serine; V:- Valine; W:- Tryptophan; Y: Tyrosine; ENT:- Ear, nose and throat; EPTB:- Extra-Pulmonary Tuberculosis; GIT:- Gastrointestinal tract; KS:- Kaposi's Sarcoma; LN:- Lymphadenopathy; PCP:- Pneumocystis carinii pneumonia; PTB:- Pulmonary Tuberculosis.

4.4 Risk factors for virologic failure and HIV-1 drug resistance among the children and adolescents of the Lea Toto Programme

4.4.1 Risk factors for virologic failure to first-line ART

A multivariate Cox proportional hazards regression analysis comparing the 297 children and adolescents with virologic success after 12 months of ART with the 111 who had virologic failure. The Univariate analysis revealed that children and adolescents with suboptimal adherence to ART, were 15.68 times more likely to develop treatment failure when compared to individuals with optimal adherence (HR= 15.68, CI 4.79 to 51.29, *P*<0.001) (Table 4.15).

Multivariate analysis showed even more statistically significant values: HR= 36.99, CI 8.21 to 166.66, *P*<0.001. Severe malnutrition was also predictive of virologic failure, both by Univariate analysis (HR= 2.01, CI 1.15 to 3.79, *P*-value of 0.02) and Multivariate analysis (HR= 2.93, CI 1.54 to 5.59, *P*<0.001). Those with severe malnutrition were two to three times more likely to experience virologic failure than the well-nourished: Univariate HR: 2.01 CI 1.15 to 3.79 *P* = 0.01; Multivariate HR: 2.93 CI 1.54 to 5.59 *P* = 0.001. None of the other variables tested met the 3 conditions for being a risk factor. Three of them: baseline CD4 T cell count of 199 or less, baseline CD4% of 14% or less, and being raised by one parent, had HRs > 1.96, but the lower limits of their 95% CIs were less than 1.

Table 4.15: Risk factors of virologic failure in the Lea Toto cohort during the first 24 months of first-line ART.

	Univariate HR	95% CI	*P*	Multivariate HR	95% CI	*P*
Optimal adherence to ART	1.0			1.0		
Suboptimal adherence to ART	*15.68*	*4.79 to 51.29*	*P<0.001*	*36.99*	*8.21 to 166.66*	*P<0.001*
Malnutrition (Weight for Height Z-score)	1.0			1.0		
Healthy (+1 to < -1)	1.54	0.76 to 3.13	0.23	1.67	0.79 to 3.55	0.18
Mild (-1 to < -2)	1.15	0.66 to 2.03	0.62	1.24	0.69 to 2.22	0.48
Moderate (-2 to < -3)	*2.01*	*1.15 to 3.79*	*0.01*	*2.93*	*1.54 to 5.59*	*0.001*
Severe (≤ -3)						
NRTI drug combination: AZT/3TC	1.0			1.0		
NRTI drug combination: ABC/3TC	0.97	0.23 to 2.15	0.63	1.45	0.39 to 5.51	0.71
CD4 T cell count of ≥ 500 at baseline	1.0			1.0	1.0	1.0
CD4 T cell count of ≤199 at baseline	1.20	0.69 to 2.09	0.51	3.38	0.77 to 14.89	0.11

	HR	CI	P-value	HR	CI	P-value
CD4% of $\geq 25\%$ at baseline	1.0			1.0		
CD4% $\leq 14\%$ or less at baseline	1.59	0.56 to 4.53	0.39	2.24	0.28 to 17.86	0.046
Primary Caregiver: Two parents	1.0			1.0		
Primary Caregiver: One parent	0.73	0.33 to 1.60	0.43	2.08	0.44 to 9.90	0.36
Primary Caregiver: Guardian	1.23	0.72 to 2.11	0.45	1.27	0.34 to 4.72	0.72
Age of > 35 months baseline	1.0			1.0		
Age of 35 months or less at baseline	1.03	0.70 to 1.50	0.89	0.85	0.17 to 4.31	0.85
Male Gender	1.0			1.0		
Female gender	0.69	0.48 to 1.00	0.05	0.73	0.26 to 2.07	0.56
HIV RNA, $\log_{10} < 5.0$ at baseline	1.0			1.0		
HIV RNA, $\log_{10} \geq 5.0$ at baseline	0.76	0.56 to 1.07	0.11	0.46	0.17 to 1.21	0.12

HR: Hazard Ratio; CI: Confidence Interval; ART: Antiretroviral Therapy; CD4: Cluster of Differentiation 4**;** ABC:- Abacavir; 3TC: Lamivudine; AZT: Zidovudine; *P*-value: Measure of Statistical Significance.

4.4.2 Risk factors for slow response to first-line ART

Multivariate Cox proportional hazards regression analysis was also performed to determine the risk factors that lead to a slow response to ART. For this analysis, various variables were simultaneously compared between the group of 297 children who had treatment success after 12 months of ART initiation, and the group of 30 who attained treatment success only after 18 to 24 months of ART. The Univariate analysis revealed that children and adolescents with suboptimal adherence to ART, were 3.67 times more likely to have a slow response to ART when compared to individuals with optimal adherence (HR= 3.67, CI 1.37 to 9.80, *P*=0.01) (Table 4.16). Multivariate analysis showed even more statistically significant values: HR= 8.91, CI 2.08 to 38.12, *P*=0.003. None of the other variables tested met the 3 conditions to be a risk factor. Children with suboptimal adherence to ART, were 3.2 times more likely to develop have a slow response to ART, when compared to individuals with optimal adherence (HR= 3.20, CI 0.55 to 18.72 *P*=0.20). Although the HR was greater than 1.96, the lower limit of the 95% CI was less than 1, and the *P*-value was greater than 0.05.

Table 4.16: Risk factors of slow response to ART in the Lea Toto cohort during the first 24 months of first-line ART.

	Univariate HR	95% CI	P	Multivariate HR	95% CI	P
Optimal adherence to ART	1.0			1.0		
Suboptimal adherence to ART	*3.67*	*1.37 to 9.80*	*0.01*	*8.91*	*2.08 to 38.12*	*0.003*
Malnutrition (Weight for Height Z-score)						
Healthy (+1 to < -1)	1.0			1.0		
Mild (-1 to < -2)	2.38	0.25 to 22.38	0.45	1.13	0.15 to 2.96	0.11
Moderate (-2 to < -3)	2.20	0.55 to 8.86	0.27	0.96	0.08 to 2.88	0.15
Severe (≤ -3)	1.88	0.21 to 17.18	0.58	0.83	0.10 to 2.01	0.20
NRTI drug combination: AZT/3TC	1.0			1.0		
NRTI drug combination: ABC/3TC	0.93	0.05 to 2.32	0.35	1.53	0.31 to 7.50	0.61
CD4 T cell count of ≥ 500 at baseline	1.0			1.0		
CD4 T cell count of ≤199 at baseline	2.79	0.29 to 27.26	0.26	1.51	0.31 to 7.50	0.61
CD4% of ≥ 25% at baseline	1.0			1.0		
CD4% ≤ 14% or less at baseline	0.69	0.07 to 7.17	0.76	0.20	0.01 to 3.59	0.27
Primary Caregiver: Two parents	1.0			1.0		
Primary Caregiver: One parent	0.71	0.16 to 3.25	0.66	0.22	0.02 to 3.22	0.27
Primary Caregiver: Guardian	0.22	0.02 to 2.19	0.20	0.13	0.01 to 2.80	0.19

Age of > 35 months baseline	1.0			1.0		
Age of 35 months or less at baseline	3.18	0.86 to 11.75	0.08	1.02	0.07 to 14.47	0.20
Male Gender	1.0			1.0		
Female gender	0.42	0.12 to 1.47	0.18	1.03	0.09 to 11.15	0.98
HIV RNA, $\log_{10} < 5.0$ at baseline	1.0			1.0		
HIV RNA, $\log_{10} \geq 5.0$ at baseline	0.97	0.30 to 3.13	0.96	1.11	0.22 to 5.64	0.90

HR: Hazard Ratio; CI: Confidence Interval; ART: Antiretroviral Therapy; CD4: Cluster of Differentiation 4; ABC: Abacavir; 3TC: Lamivudine; AZT: Zidovudine; *P*-value: Measure of Statistical Significance.

4.4.3 Risk factors for sub-optimal adherence to first-line ART

Given that suboptimal adherence to ART was a risk factor for both virologic failure and a slow response to ART, the risk factors for suboptimal adherence itself were investigated. Univariate analysis revealed that adolescents aged 14 to 17 years, were 2.44 times more likely to have suboptimal adherence to ART than children aged 2 to 5 years (HR= 2.44, CI 1.25 to 4.78, *P*=0.009) (Table 4.17). When multivariate analysis was conducted, it was confirmed that this age group was indeed most vulnerable: adolescents aged 14 to 17 years, were 2.67 times more likely to have suboptimal adherence to ART than children aged 2 to 5 years (HR= 2.67, CI 1.36 to 5.24, *P*=0.004). Neither the caregiver nor the gender variables met the 3 conditions to be a risk factor.

Table 4.17: Risk factors for suboptimal adherence to ART in the Lea Toto cohort during the first 24 months of first-line ART.

Characteristic	Univariate Analysis			Multivariate Analysis		
	HR	*P*	95% CI	HR	*P*	95% CI
Two Parent Homes	1.0			1.0		
One Parent Homes	1.44	0.08	0.96 to 2.16	1.31	0.22	0.85 to 2.01
Guardian Homes	0.68	0.18	0.39 to 1.19	0.74	0.29	0.42 to 1.29
2 to 5 years old	1.0			1.0		
6 to 9 years old	1.01	0.96	0.60 to 1.71	1.17	0.56	0.69 to 1.98
10 to 13 years old	0.99	0.98	0.58 to 1.69	1.05	0.86	0.61 to 1.81
14 to 17 years old	*2.44*	*0.009*	*1.25 to 4.78*	*2.67*	*0.004*	*1.36 to 5.24*
Female Gender	1.0			1.0		
Male Gender	0.59	0.005	0.41 to 0.85	0.64	0.025	0.44 to 0.95

HR: Hazard Ratio; CI: Confidence Interval; *P*-value: Measure of Statistical Significance.

4.4.4 Risk factors for virologic failure on second-line ART

A multivariate Cox proportional hazards regression analysis was conducted to determine the risk factors associated with second-line ART treatment failure. For this analysis, various variables were simultaneously compared between the treatment success group (n=92) and virologic failure group (n = 22) (Table 4.18). The Univariate analysis revealed that children and adolescents with suboptimal adherence to ART, were 5.3 times more likely to develop treatment failure when compared to individuals with optimal adherence (HR=5.3, CI 1.93-14.62, *P*=0.001); multivariate analysis showed even more statistically significant values: HR= 6.56, CI 1.56 to 27.47, *P*-value of 0.01. None of the other variables tested met the 3 conditions for being a risk factor. Some of them – age groups 6 to 9 years, 10 to 13 years, 14 to 17 years, and 18 to 20 years; also CD4+ T-cell count of 199 cells/µl or less; as well as HIV-1 subtypes B, C, G, K, CRF_01AE and CRF02_AG - had HRs > 1.96, but the lower limits if their 95% CIs were less than 1.

Table 4.18: Risk factors of virologic failure in the Lea Toto cohort during second-line ART.

	Univariate HR	95% CI	*P*	Multivariate HR	95% CI	*P*
Adherence to second-line ART						
Optimal	1.0			1.0		
Suboptimal	*5.31*	*1.93 to 14.62*	*0.001*	*6.56*	*1.56 to 27.47*	*0.01*
Malnutrition						
(Weight for Height *Z*-score)						
Healthy (+1 to < -1)	1.0			1.0		
Mild (-1 to < -2)	0.83	0.01 to 2.16	0.98	0.22	0.01 to 2.61	0.98
Moderate (-2 to < -3)	1.10	0.52 to 2.33	0.86	3.15	1.06 to 9.35	0.04
Severe (≤ -3)	1.22	0.60 to 2.51	0.70	0.48	0.12 to 2.02	0.32
Second-line ART regimen						
ABC/3TC/LPV/r	1.0			1.0		
TDF/3TC-based	0.72	0.23 to 2.21	0.56	1.75	0.83 to 3.67	0.82
AZT/3TC/LPV/r	0.13	0.04 to 1.65	0.93	1.43	0.54 to 4.32	0.55
ABC/DDI/LPV/r	1.17	0.35 to 3.87	0.80	0.87	0.16 to 2.18	0.34
Primary Caregiver						
Two parents	1.0			1.0		
One parent	0.73	0.33 to 1.60	0.43	0.89		0.88

Guardian	1.23	0.72 to 2.11	0.45	0.06	0.18 to 4.38	0.07
					0.01 to 1.23	
Age at start of second-line ART						
2 to 5 years old	1.0			1.0		
6 to 9 years old	1.58	0.16 to 15.59	0.70	2.16	0.11 to 41.46	0.61
10 to 13 years old	2.62	0.33 to 21.16	0.37	2.01	0.12 to 37.37	0.61
14 to 17 years old	4.05	0.48 to 33.88	0.20	3.32	0.14 to 76.89	0.46
18 to 20 years old	6.58	0.57 to 75.93	0.13	9.24	0.10 to 817.9	0.33
Gender						
Male	1.0			1.0		
Female	0.69	0.48 to 1.00	0.05	1.44	0.20 to 10.35	0.72
HIV RNA at start of second-line ART						
1,000 to 9,999 copies/ml				1.0		
10,000 to 99,999 copies/ml	1.0			0.25	0.02 to 2.51	0.24
100,000 or more copies/ml	0.76	0.56 to 1.07	0.11	0.03	0.001 to 0.64	0.03
CD4+ count at second-line ART start						
500 or more cells/µl	1.0			1.0		
350 to 499 or less cells/µl	1.11	0.20 to 6.07	0.91	0.25	0.03 to 2.43	0.23
200 to 349 cells/µl	1.33	0.36 to 4.96	0.67	0.43	0.05 to 3.86	0.45

	HR	CI	p	HR	CI	p
199 or less cells/µl	0.88	0.27 to 2.90	0.83	3.45	0.32 to 37.56	0.31
HIV Subtype						
A	1.0			1.0		
B, C, G or K	1.77	0.39 to 8.11	0.46	9.62	0.39 to 234.68	0.17
D	0.57	0.13 to 2.53	0.46	0.25	0.02 to 3.69	0.31
Recombinant (A/D, A/B, D/B…)	0.82	0.10 to 6.46	0.85	0.72	0.06 to 9.31	0.80
CRF (CRF01_AE and CRF02_AG	1.88	0.40 to 8.78	0.42	6.32	0.55 to 72.15	0.14
TAMs at second-line ART start						
0	1.0			1.0		
1	0.47	0.11 to 1.98	0.30	0.27	0.02 to 3.93	0.34
2	0.91	0.27 to 3.03	0.87	0.48	0.06 to 3.97	0.50
3 or more	0.47	0.15 to 1.49	0.20	0.38	0.04 to 3.90	0.42

CI: Confidence Interval; HR: Hazard Ratio; ART: Antiretroviral Therapy; ABC: Abacavir; 3TC: Lamivudine; AZT: Zidovudine; TDF: Tenofovir; DDI: Didanosine; NVP: Nevirapine; EFV: Efavirenz; LPV/r: Lopinavir/ritonavir; NRTI: Nucleoside Reverse Transcriptase Inhibitor; NtRTI: Nucleotide Analogue Reverse Transcriptase Inhibitor; NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitor; TAM: Thymidine Analog Mutation.

4.4.5 The link between drug resistance mutations and opportunistic infections in the salvage ART cohort

Given that no DRT was performed at first-line ART baseline, it was not possible to study the evolution of DRMs during first-line ART. However, the DRTs done prior to the start of second-line ART and salvage ART made it possible to study the evolution of DRMs for the 15 individuals who received all 3 stages of treatment. The number of DRMs that had a direct impact on NRTI drugs was 35 during second-line ART and 69 during salvage ART; this difference was statistically significant by the Fisher's exact test ($P=6.12 \times 10^{-10}$) (Figure 4.11). The number of opportunistic infections (OIs) that occurred among these 15 patients was 22 during second-line ART and 34 during salvage ART; this difference was also statistically significant ($P=2.14 \times 10^{-6}$).
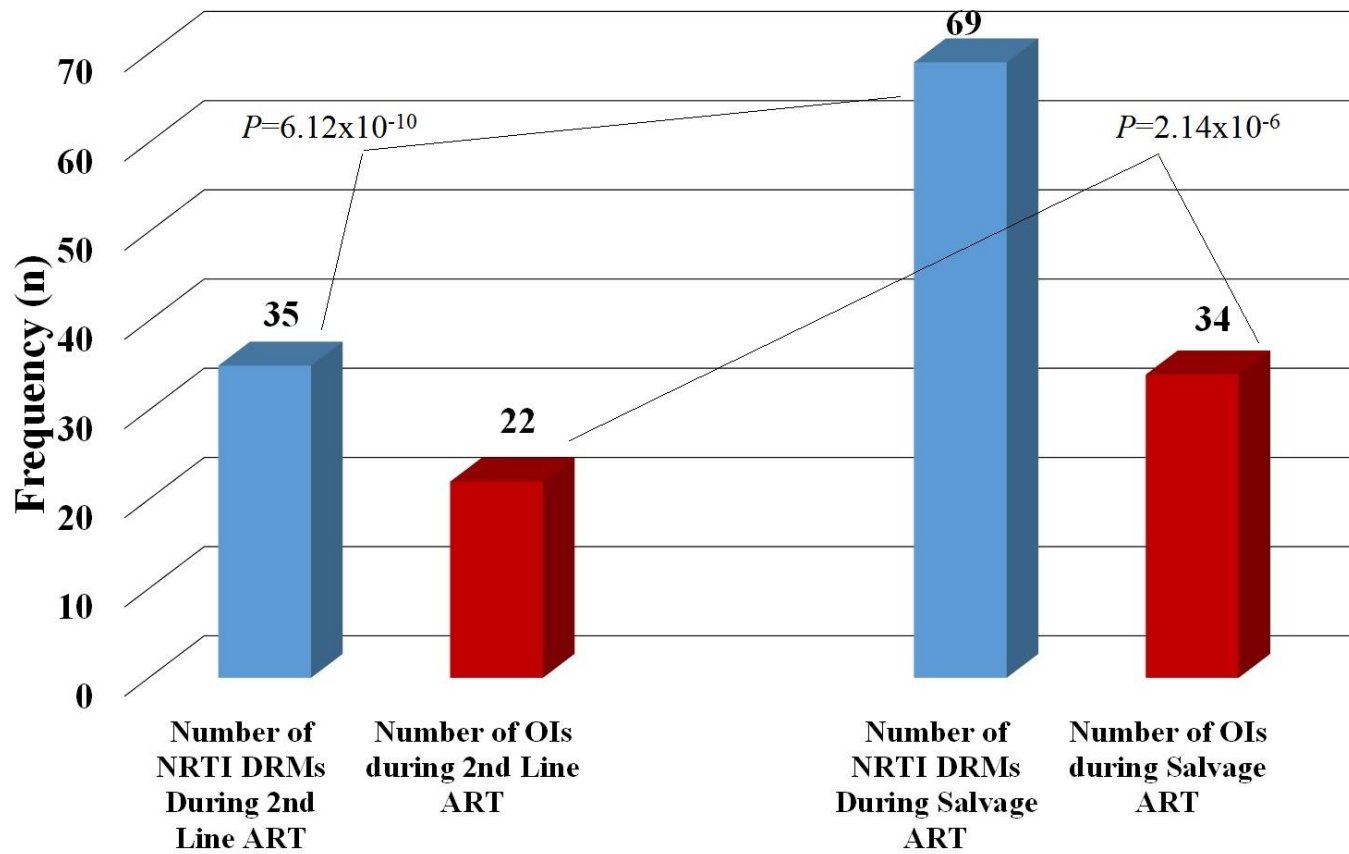
Figure 4.11: The number of Nucleoside Reverse Transcriptase Inhibitor (NRTI) drug resistance mutations (DRMs) and Opportunistic infections (OIs) that occurred during second-line ART and salvage ART among the 15 patients who failed second-line ART and were switched to salvage ART.

4.5 Model and summary of results

A model summarizing the findings of this study is shown below, including the risk factors for virologic failure and suboptimal adherence to ART, as well as the Hazard ratios for the same (Figure 4.12). In the Lea Toto cohort, suboptimal adherence carried a 37-fold increased risk for first-line ART virologic failure; it also carried a 9-fold risk for slow response to ART. Moreover, severe malnutrition carried a 3-fold increased risk for first-line ART virologic failure. Teenagers aged 14 to 17 years old were 2.7 times more likely to have suboptimal adherence to first-line ART than children aged 2 to 5 years old.

The emergence of the NRTI DRMs M184V, E44D, K70R, L74V, F77L and T69N occured during first-line ART and they contributed to virologic failure (VF) and the emergence of opportunistic infections (OIs). Although NNRTI DRMs also arose in this cohort, their number and composition did not change during second-line ART. This is because all the NNRTIs were subsituted with the protease inhibitor ritonavir-boosted Lopinavir during second-line ART. No Protease inhibitor DRMs arose in any of the 15 patients during first-line and second-line ART. Suboptimal adherence to second-line ART carried a 6.7-fold increased risk for second-line ART virologic failure. Moreover, OIs emerged in patients with sub-optimal ART adherence, and these included respiratory tract infections (RTIs), upper respiratory tract infections (URTIs), gastro-intestinal tract (GIT) infections, skin infections (SIs) pulmonary Tuberculosis (PTB), extra-Pulmonary Tuberculosis (EPTB) as well as ear, nose and throat (ENT) infections. With continued sub-optimal adherence to second-line ART, the patients developed additional NRTI DRMs: L210W, T215F, T215Y and M41L; these DRMs further increased the likelihood of VF and the emergence of OIs. Although it is beyond the scope of this study, it is predicted that upon switching from second-line ART to salvage ART, patients with continued sub-optimal adherence to ART will develop additional NRTI DRMs, which will eventually lead to VF on salvage ART and the emergence of additional OIs. In the model, these predicted data are indicated with question marks and dotted lines (Figure 4.12).

First-line ART

Severe malnutrition increases risk of virologic failure 2.9-fold

Optimal adherence to first-line ART

Teens aged 14-17 years have a 2.7-fold greater risk of sub-optimal adherence to ART

Stably on first-line ART

Sub-optimal adherence to first-line ART increases the risk of virologic failure 37-fold

Sub-optimal adherence to ART increases the risk of slow response to first-line ART 9-fold

The emergence of the NRTI DRMs M184V, E44D, K70R, L74V, F77L and T69N

Sub-optimal adherence to second-line ART increases the risk of virologic failure 6.7-fold

Second-line ART

Optimal adherence to second-line ART

The emergence of OIs, namely: RTIs, URTIs, GIT infections, SIs, PTB, EPTB and ENT infections

Staby on second-line ART

The emergence of additional NRTI DRMs: L210W, T215F and M41L

Salvage ART

Optimal adherence to salvage ART

Sub-optimal adherence to salvage ART leads to virologic failure. Hazard ratio?

The emergence of additional OIs, namely: LA, PCP and KS

Stably on salvage ART

Virologic failure on salvage ART

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Figure 4.12: Model summarizing the findings of the Lea Toto cohort study. Statements with question marks and dotted lines indicate predicted data that are beyond the scope of this study. DRM:- Drug resistance mutation; ART:- Antiretroviral therapy; NRTI:- Nucleoside Reverse Transcriptase Inhibitor; C:- Cysteine; D:- Aspartic Acid; E:- Glutamic acid; F:- Phenylalanine; G:- Glycine; H:- Histidine; L: Leucine; K:- Lysine; M:- Methionine; N:- Asparagine; Q:- Glutamine; R:- Arginine; S:- Serine; V:- Valine; W:- Tryptophan; Y: Tyrosine; ENT:- Ear, nose and throat; EPTB:- Extra-Pulmonary Tuberculosis; GIT:- Gastrointestinal tract; KS:- Kaposi's Sarcoma; LN:- Lymphadenopathy; PCP:- Pneumocystis carinii pneumonia; PTB:- Pulmonary Tuberculosis.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

This study evaluated the prevalence, patterns and risk factors associated with virologic failure (VF) and HIV-1 drug resistance (HIV-DR) among children and adolescents in the LTP cohort of Nairobi, Kenya. Our findings show that the prevalence of VF in this cohort declined steadily from 32% after 12 months, to 29% after 18 months, to 23.9% after 24 months, and to 24.2% after 36 months of first-line ART. The VF rate then increased slightly to 26.1% after 48 months of first-line ART. Additionally, the VF rate among those switched to second-line ART was 19.3% after a median time of 17 months of follow-up. These findings are comparable to VF rates ranging from 20% to 34% found in other paediatric cohort studies done in Sub-Saharan Africa (Chaix *et al.*, 2005, Reddi *et al.,* 2007, Kamya *et al.*, 2007, Emmet *et al.*, 2010, Lihana *et al.*, 2011, Kekitiinwa *et al.,* 2012,Wamalwa *et al.*, 2013 and Mutwa *et al.*, 2014). One notable exception is an Ethiopian study that found a VF rate of 13% in 100 children after 24 months of ART (Mulu *et al.*, 2014). Perhaps the lower VF rate in the Ethiopian study can be explained by the fact that their cohort included only children who had been on ART successfully for 12 or more months. Moreover, that study excluded children who had experienced interruptions in ART as well as those with chronic illnesses. Our study sought to follow children from the beginning of ART, including children with ART interruptions and those with chronic illnesses. Since opportunistic infections and treatment disruptions are realities for HIV patients, the findings of our study may be more relevant to the general population.

This study did not find a higher likelihood of VF for children with a baseline CD4% below 15%, or a baseline CD4 T-cell count below 200 cells/µl. This agrees with previous studies in low- and middle-income countries (LMICs) which show that clinical and immunological parameters are poor predictors of VF (Emmett *et al.*, 2010, Davies *et al.*, 2011, Barth *et al.*, 2011, Paintsil *et al.*, 2011, Ruel *et al.*, 2011; Mutwa *et al.,* 2014). The findings of our study confirm that viral load - not CD4+ T-cell count or CD4+ T-cell % - is and should continue to be the gold standard in measuring treatment outcome for HIV-infected persons. Indeed, in many cases faithful adherence

to ART reduced viral loads from above 100,000 HIV-1 RNA copies per ml to below 1,000 copies/ml in a matter of 6 months. There were some curious cases however, where the viral load was undetectable, yet the CD4+ T-cell count was still below the 350 cells/µl threshold, even after 12 to 18 months of ART. Such immunologic non-reponders have been found in previous studies at a rate of 15-30% (Autran *et al.*, 1999). Fortunately, for the majority of patients, a falling viral load led to a rising CD4+ T-cell count.

The presence of at least 1 drug resistance mutation (DRM) among 92% of patients in the Lea Toto cohort agrees with the findings of previous studies: a Tanzanian study found a DRM prevalence of 89% (Dow *et al.*, 2014) while a Ugandan study found a prevalence of 90% (Musiime *et al.*, 2013). Given that testing for DRMs in this cohort was not done at first-line ART baseline, we do not know what percentage of DRMs were transmitted from the patients' mothers and how many were acquired during the child's treatment. Consequently, it is possible that some children and adolescents were placed on failing ART regimens from the start, thereby accelerating the pace of first-line ART failure. Furthermore, the outcome of second-line ART regimens depends in large part on the presence or absence of NRTI DRMs, especially TAMs (Dow *et al.*, 2014). The amount of time on a failing first-line ART regimen is thought to be a predictor of accumulation of TAMs, which then impede the proper working of second-line ART regimens (Sigaloff *et al.*, 2012b; Hosseinipour *et al.,* 2013). Musiime *et al.,* showed that the M184V DRM occurs as early as 1.5 months during a failing first-line ART viraemia, while TAMs begin to accumulate 12 months post-viraemia (Musiime *et al.*, 2013). Therefore, if virologic failure is detected early on, the patient can be promptly switched to second-line ART, and that second-line ART regimen is more likely to result in treatment success (Ruel *et al.*, 2011, Musiime *et al.*, 2013 and Dow *et al.*, 2014). There was a statistically significant difference in the second-line ART treatment success rate between patients with 0 TAMs and those with any number of TAMs (P=0.004). Unfortunately, the prohibitive cost of testing for DRMs at treatment baseline is a major hindrance to treatment success in Kenya and other LMICs. Hopefully, cheaper DNA sequencing methods will be developed and deployed in the future.

The emergence of the NRTI DRMs M184V, E44D, K70R, L74V, F77L and T69N during first-line ART is indicative of the low genetic barrier for NRTI drugs (Luber, 2005). This low genetic barrier to drug resistance contributed to the doubling of the number of NRTI DRMs during second-line ART through the emergence of additional NRTI DRMs such as L210W, T215F and M41L. The presence of these NRTI DRMs led directly to a 55% increase in the incidence of opportunistic infections (OIs), as patients were transitioned from second-line ART to salvage ART. While these DRMs did not directly cause the OIs, they significantly reduced the efficacy of the NRTI drugs and paved the way for pathogens to easily cause disease in the immunocompromised patients. The pathway for the development of NRTI resistance is as follows: NRTI drugs are incorporated by the wild-type HIV-1 reverse transcriptase enzyme into the elongating HIV-1 DNA strand (Clavel *et al.*, 2004; Schauer *et al.*, 2013). Due to a lack of a 3' hydroxyl group, the NRTI drug acts as a chain terminator. The emergence of NRTI DRMs in the HIV-1 during ART gives the virus an advantage in two ways: firstly, discriminatory DRMs - such as M184V/I, L74V, K70E, and K65R - cause the virus to preferentially use naturally occurring deoxynucleotides for DNA elongation, while discriminating against the NRTI drug (Clavel *et al.*, 2004; Schauer *et al.*, 2013; Spach and Kinney, 2019). Secondly, excision/primer unblocking DRMs - such as M41L, D67N, K70R, L210W, T215Y/F and K219Q/E - enhance the phosphorylitic excision of the NRTI-triphosphate already added to the elongation HIV-1 RNA-DNA complex, leading to the unblocking of the primer by the NRTI drug (Singh *et al.*, 2010; Tu *et al.*, 2010; Tang *et al.*, 2012). These two pathways significantly increase viral fitness resulting in a higher viral load. Eventually, the HIV-1 patient develops VF, which further weakens their immune system and leads to a higher incidence of OIs (Spach and Kinney, 2019).

Antiretroviral drug efficacy is reduced significantly in the presence of HIV-1 DRMs. For example, the M184V DRM - which develops via the discriminatory resistance pathway - causes a 100-fold reduction in the efficacy of the NRTI drug 3TC. (Spach and Kinney, 2019). This DRM emerges early during treatment: an early 3TC monotherapy study showed that the M184V DRM arises within 4 weeks of treatment initiation (Eron *et al.*, 1995). The M184V + L74V DRM pair is the most common pattern that emerges in HIV-infected patients receiving an ABC/3TC ART regimen.

This DRM pair increases viral replication fitness and reduces the efficacy of 3TC fivefold (Moyle *et al.*, 2005). The L210W TAM often occurs in combination with M41L and T215Y. This triad of DRMs confers a high level of resistance to the NRTI drugs AZT, ABC, d4T, ddI, TDF (Rhee *et al.*, 2003; Shafer, 2006).

Just like NRTIs, Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) drugs - such as NVP and EFV - have a low genetic barrier to HIV-1 resistance (Rhee *et al.*, 2003; Shafer, 2006; Spach and Kinney, 2019). The NNRTIs inhibit HIV-1 replication by non-competitive inhibition of the HIV-1 reverse transcriptase enzyme (Clavel *et al.*, 2004; Schauer *et al.*, 2013; Spach and Kinney, 2019). Resistance in HIV-1 to NNRTI drugs occurs by mutation of one of the amino acid residues that line the hydrophobic binding pocket of the HIV-1 reverse transcriptase enzyme. These binding pocket amino acids include L100, K101, K103, V106, T107, V108, V179, Y181, Y188, V189, G190, F227, W229, L234 and Y318. Mutation of one or more of these residues leads to one or both of the following outcomes: an altering of the NNRTI binding site itself or reduced access to the binding site for the NNRTI drug (Clavel *et al.*, 2004; Schauer *et al.*, 2013). Consequently, the NNRTI is unable to limit viral replication, and the patient's viral load increases. Alarmingly, sub-optimal levels of NNRTI drugs lead to the emergence of NNRTI DRMs within only 1-4 weeks of treatment (Spach and Kinney, 2019). The most common NNRTI DRMs that emerge in a patient on NVP are are K103N, Y181C/I, V106A/M and Y188C/L/H. Patients on EFV predominantly develop the K103N, Y188L, and G190S/A DRMs (Rhee *et al.*, 2003; Shafer, 2006; Spach and Kinney, 2019). In this cohort, all the patients on first-line NVP- and EFV-based regimens were switched to second-line ART regimens that replaced the NNRTI drug with the protease inhibitor ritonavir-boosted Lopinavir. Therefore, the emergence of NNRTI DRMs was put to a stop after first-line ART, and the adverse effect of the existing NNRTI DRMs was not as big as that of the NRTI DRMs. It is important to note that no NNRTI drugs were used during second-line ART and salvage ART; it was therefore not possible to compare the NNRTI DRMs found at the pre-second-line ART baseline with those found at the pre-salvage ART baseline. Three out of four HIV-1 subtypes in this second-line ART cohort were recombinants; this suggests that HIV-1 subtypes from various parts of Kenya and the world are mixing together among Nairobi's residents. Previous studies have shown that the pure

HIV-1 subtypes A, C and D are the most dominant HIV-1 subtypes in circulation in Kenya, comprising 9 out of 10 of all Kenyan HIV-1 subtypes (Lihana *et al.*, 2006; Kageha *et al.*, 2012; Nyagaka *et al.*, 2012). Our study found that only 6 out of 10 HIV-1 subtypes were pure, suggesting an elevated rate of infection and re-infection with HIV-1 subtypes from all over Kenya and the world among Nairobi residents (Lihana *et al.*, 2009). Given that people continue to migrate to Nairobi from all over Kenya and the world, the rate of recombination among HIV-1 subtypes will likely increase in the future.

Malnutrition is an important factor for ART outcome, as has been shown in previous studies (Barth *et al.*, 2011; Bacha *et al.*, 2012; Mutwa *et al.*, 2014). It increases the incidence of opportunistic infections, while also making ART drug adsorption difficult. The findings of this study show that severe malnutrition in children and adolescents on first-line ART increases the risk of virologic failure 3-fold. The analysis of second-line ART data did not reveal malnutrition as a risk factor for virologic failure, possibly because as ART progresses, the patient begins to understand his or her nutritional needs vis-à-vis ART treatment, and ensures that he or she gets proper nutrition. The children and adolescents in the LTP cohort were regularly checked for signs of malnutrition. In addition, nutritionists provided regular nutritional counselling and food aid to needy families. This ensured that nutritional health was maximized and that the patients' bodies could tolerate the ART medication.

Suboptimal adherence to ART is the greatest risk factor for virologic failure, as evidenced by the finding that children and adolescents with suboptimal adherence during first-line ART in this study were 37 times more likely to experience virologic failure than those with optimal adherence. During second-line ART, children with suboptimal adherence were 7 times more likely to experience virologic failure than those with optimal adherence. Moreover, persons with suboptimal adherence were 9 times more likely to experibachaence a slow response to ART. This is in line with several other studies that have shown the paramount importance of adherence to ART in treatment outcome for HIV-infected children and adolescents (Nachega *et al.*, 2009; Ajose *et al.*, 2012; Dow *et al.*, 2014; Adejumo *et al.*, 2015).

The presence of individuals with virologic rebound from month 13 to 24 of first-line ART after initial treatment success suggests that there was decline in the adherence

levels during this period. Perhaps in the initial 12 months of ART, patients were likely to adhere strictly to ART because they were eager to attain an undetectable viral load and freedom from opportunistic infections. It is possible that once they achieved one or both of these goals, they became complacent, even careless in their adherence to ART. This is supported by 2 findings from the current study: firstly, 9.7% (n = 35) experienced virologic rebound at 24 months after initial treatment success after 12 months of ART. Secondly, the difference in adherence levels between the first-line treatment success and virologic failure groups was large, as reflected in the large Hazard ratio (HR) and the level of statistical significance: (HR 36.99, $P = 0.000003$). The decline in adherence levels after the first year of ART may reflect the onset of treatment fatigue among the children and adolescents. Given that HIV is a lifelong condition that requires a sustained level of near-perfect ART adherence, treatment fatigue after only one year of treatment does not bode well for these non-adherent individuals.

In contrast to previous studies, our study did not find a higher likelihood of virologic failure for children below 3 years of age at baseline (Frange *et al.,* 2011; Wamalwa *et al.*, 2013). This is probably because in this cohort, body weight and height were measured monthly to ensure that the correct ART dosage was prescribed. This in turn ensured that children did not have sub-therapeutic ART levels in their blood (Wamalwa *et al.*, 2013). Moreover, caregivers were counselled on the proper administration of ART drugs to their infants, as well as on the importance of adherence.

The Null Hypothesis set forth at the beginning of the study which stated that "There is no virologic failure or HIV-1 drug resistance among the children and adolescents of the Lea Toto Programme in Nairobi", was rejected.

5.2 Conclusions

1) One in four children and adolescents in the Lea Toto Programme experienced virologic failure during first-line ART. One in five children and adolescents experienced virologic failure during second-line ART.

2) Nine out of ten children and adolescents in the Lea Toto Programme who were switched from first- to second-line ART had at least 1 NRTI and at least 1 NNRTI drug resistance mutation.

3) The medical and demographic risk factors for VF and HIV-DR:

    i) Suboptimal adherence to ART.

    ii) Severe malnutrition.

    iii) Being a teenager aged 14 to 17 years old.

    iv) The doubling in the number of NRTI DRMs.

5.3 Recommendations

1) The Kenya Government's should change its treatment guidelines for first-line ART regimens to utilize protease inhibitor drugs more frequently, as they have a higher barrier to HIV-1 drug mutations than NRTIs and NNRTIs.

2) The Government and Non-Governmental organizations (NGOs) should boost their Advocacy efforts and public awareness campaigns towards increased child and adolescent HIV testing, counselling, linkage to care, and adherence to ART.

3) Given that teenagers aged 14 to 17 years old are the most vulnerable to suboptimal adherence to ART, virologic failure and HIV-DR, they require special attention. For example, teenagers should be encouraged to form peer support groups and enroll in a "buddy system", so that they can receive moral support and reduce self-stigma.

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APPENDICES

Appendix I: English Informed Parental Consent Form for children 0-12 years old.

Title of Research Study: "Prevalence and Risk Factors of Virologic Failure and HIV-1 Drug Resistance among Children and Adolescents in the Lea Toto Programme in Nairobi, Kenya".

Name of principal investigator..............................Joseph Mbugua Kabogo

Name of organization……………………………JKUAT / KEMRI

Introduction

We are requesting your consent for your child to participate in a research study titled: "Prevalence and Patterns of HIV-1 Drug Resistance among children in the Lea Toto Program in Nairobi, Kenya." Since your child is below the age of 12 years, we need your informed consent as his / her parent for your child to participate in our research study.

Aim of the study

This study aims to determine the percentage of those children in the Lea Toto Program who stop responding to antiretroviral therapy (ART) due to treatment failure and HIV-1 drug resistance. The results of this study will help us to find out the causes of the problems of treatment failure and drug resistance. Then we will be able to come up with solutions that can help save children's lives and improve children's health.

Your role and your child's role in the study

Your role and your child's role in this study is very easy because all the work has already been done. The blood to be tested has already been collected and stored every 6 months that your child visits the Lea Toto Program clinic for a CD4+ test and viral load test. The other data needed for the study has already been collected and is on file.

All we need is your permission to allow your child's blood and data to be used for our study.

Benefits to your child

By your child participating in this study, your child is helping all HIV-1 positive children in the Lea Toto Program and beyond, to improve their quality of life by reducing ART treatment failure and HIV-1 drug resistance.

Risks and discomforts to your child being in the study

We will NOT collect any blood from your child for this study. We will use your child's CD4+ count, viral load results, and other relevant medical and social data that have already been collected at the Lea Toto clinic. Therefore, your child will NOT experience any risk or discomfort.

Permission to access your child's Lea Toto medical records

We are requesting your consent to access and use your child's Lea Toto Program medical records to obtain the following information: age, gender, weight, when they joined the Lea Toto Program, ART regimen, what percentage of their ART drugs they have taken, CD4+ T-cell count, CD4%, and viral load.

Analysis and discarding of blood if your child develops treatment failure

In case your child stops responding to antiretroviral therapy (ART), we will need to test your child's blood which has remained after your child is tested every 6 months at the Lea Toto Clinic. This remaining blood is already stored in a freezer at the Nyumbani Diagnostic Laboratory in Karen, Nairobi. We will NOT collect any blood from your child.

In case your child stops responding to ART, we are requesting your consent to use your child's blood samples, collected ever since your child joined the Lea Toto Program. We will use your child's blood to perform tests to find out the exact cause of the treatment failure and drug resistance. After that, the tested blood will be burned in an incinerator.

Privacy of your child's information

We will protect your child's privacy by keeping this signed consent form and your child's medical information under lock and key. Only the principal investigator of this study and the Laboratory Directory of the Nyumbani Diagnostic Laboratory will have the key. Moreover, after you have signed the consent form, we shall assign your child a serial number to identify your child. We will NOT use your child's name during this study or in the publication of our results. Also, any information entered into our computer databases will be password-protected, and only the principal investigator of the study and his data entry assistant will have that password.

At the end of our study, we shall publish our results in a scientific journal for other scientists to read. Also, we shall write a report to the Clinical Officers at the Lea Toto program. Thus, you can find out the results of our research by asking the Clinical Officer at the Lea Toto Program clinic. In all our reports, we shall use serial numbers in our publications, so that no one will be able to identify your child personally.

Your right to refuse your child participating in the study, and your right to withdraw your child from the study

Your child does NOT have to take part in this research study if you do not wish him / her to do so. This will NOT affect your child's participation in the Lea Toto Program. Also, if you give consent for your child to participate in this study, you still have the right to withdraw your child from this study any time, without penalty.

If you agree to give consent for your child to participate in this research study, and for the results to be published, please read and sign the following consent form.

Signed, informed Consent

I, the undersigned, confirm that as I give consent for my child to participate in this research study, it is with a clear understanding of the objectives and conditions of the study, and with the recognition of my right to withdraw my child from the study at any time, without penalty.

I…………………………………………………………., do hereby give consent to
………………………………………………….….... for my child to participate in
the proposed research study. I have been given all the necessary information to
understand the purpose and nature of this research study. I have also been assured that
I can withdraw my child from the study at any time, without penalty or loss of benefits
that my child enjoys as a participant in the Lea Toto Program. The research proposal
has been explained to me in the language I understand.

Name of parent / guardian……………………………………………..

Parent's / guardian's signature…………………………………………..

Date…………………………   Place……………………..…

Name of principal investigator…………………………………………..

Principal investigator's signature…………………………………………..

Date…………………………   Place……………………..………

Name of independent witness…………………………………………..

Independent witness' signature……………………………………….…..

Date…………………………   Place…………………..…………………

In case of any questions regarding this research study, please contact the principal
investigator Joseph Kabogo, by calling 0714-108786. You may also contact the
chairman of the KEMRI Ethical Review Commission using the following address and
telephone number:

Chairman, KEMRI/ Ethical Review Committee (ERC)

P.O Box 54840 Nairobi. ; Tel 020-2722541 ; Email : ercsecretariat@kemri.org

Appendix II: English Informed Parental Consent Form for children aged 13-17 years old.

Title of Research Study: "Prevalence and Risk Factors of Virologic Failure and HIV-1 Drug Resistance among Children and Adolescents in the Lea Toto Programme in Nairobi, Kenya".

Name of principal investigator..............................Joseph Mbugua Kabogo

Name of organization…………………………JKUAT / KEMRI

Introduction

We are requesting your permission to include your child in our research study titled: "Prevalence and Patterns of HIV-1 Drug Resistance among children in the Lea Toto Program in Nairobi, Kenya." Since your child is a minor aged between 13 and 17 years old, we are seeking both your permission as their parent, and in a separate form, we are seeking his or her assent / agreement to participate in the study.

Aim of the study

This study aims to determine the percentage of those children in the Lea Toto Program who stop responding to antiretroviral therapy (ART) due to treatment failure and HIV-1 drug resistance. The results of this study will help us to find out the causes of the problems of treatment failure and drug resistance. Then we will be able to come up with solutions that can help save children's lives and improve children's health.

Your role and your child's role in the study

Your role and your child's role in this study is very easy because all the work has already been done. The blood to be tested has already been collected and stored every 6 months that your child visits the Lea Toto Program clinic for a CD4+ test and viral load test. The other data needed for the study has already been collected and is on file. All we need is your permission to allow your child's blood and data to be used for our study.

Benefits to your child

By your child participating in this study, your child is helping all HIV-1 positive children in the Lea Toto Program and beyond, to improve their quality of life by reducing ART treatment failure and HIV-1 drug resistance.

Risks and discomforts to your child being in the study

We will NOT collect any blood from your child for this study. We will use your child's CD4+ count, viral load results, and other relevant medical and social data that have already been collected at the Lea Toto clinic. Therefore, your child will NOT experience any risk or discomfort.

Permission to access your child's Lea Toto medical records

We are requesting your informed parental permission and your child's informed assent to access and use your child's Lea Toto Program medical records to obtain the following information: age, gender, weight, when they joined the Lea Toto Program, ART regimen, what percentage of their ART drugs they have taken, CD4+ T-cell count, CD4%, and viral load.

Analysis and discarding of blood if your child develops treatment failure

In case your child stops responding to antiretroviral therapy (ART), we will need to test your child's blood which has remained after your child is tested every 6 months at the Lea Toto Clinic. This remaining blood is already stored in a freezer at the Nyumbani Diagnostic Laboratory in Karen, Nairobi. We will NOT collect any blood from your child.

In case your child stops responding to ART, we are requesting your informed parental permission and your child's informed assent to use your child's blood samples, collected ever since your child joined the Lea Toto Program. We will use your child's blood to perform tests to find out the exact cause of the treatment failure and drug resistance. After that, the tested blood will be burned in an incinerator.

Privacy of your child's information

We will protect your child's privacy by keeping your informed parental permission form, your child's signed informed assent form, and your child's medical information

under lock and key. Only the principal investigator of this study and the Laboratory Directory of the Nyumbani Diagnostic Laboratory will have the key. Moreover, after you have signed this informed parental permission form, we shall assign your child a serial number to identify your child. We will NOT use your child's name during this study or in the publication of our results. Also, any information entered into our computer databases will be password-protected, and only the principal investigator of the study and his data entry assistant will have that password.

At the end of our study, we shall publish our results in a scientific journal for other scientists to read. Also, we shall write a report to the Clinical Officers at the Lea Toto program. Thus, you can find out the results of our research by asking the Clinical Officer at the Lea Toto Program clinic. In all our reports, we shall use serial numbers in our publications, so that no one will be able to identify your child personally.

Your right to refuse your child participating in the study, and your right to withdraw your child from the study
Your child does NOT have to take part in this research study if you do not wish him / her to do so. This will NOT affect your child's participation in the Lea Toto Program. Also, if you give permission for your child to participate in this study, you still have the right to withdraw your child from this study any time, without penalty.
If you agree to give permission for your child to participate in this research study, and for the results to be published, please read and sign the following informed parental permission form.

Signed, informed Parental permission
I, the undersigned, confirm that as I give permission for my child to participate in this research study, it is with a clear understanding of the objectives and conditions of the study, and with the recognition of my right to withdraw my child from the study at any time, without penalty.

I……………………………………………………………., do hereby give permission to …………………………………………………….…..... for my child to participate

in the proposed research study. I have been given all the necessary information to understand the purpose and nature of this research study. I have also been assured that I can withdraw my child from the study at any time, without penalty or loss of benefits that my child enjoys as a participant in the Lea Toto Program. The research proposal has been explained to me in the language I understand.

Name of parent / guardian……………………………………………..

Parent's / guardian's signature…………………………………………..

Date……………………….   Place…………………..……………

Name of principal investigator…………………………………………..

Principal investigator's signature…………………………………………..

Date……………………….   Place…………………..……………

Name of independent witness…………………………………………..

Independent witness' signature…………………………………….…..

Date……………………….   Place…………………..……………

In case of any questions regarding this research study, please contact the principal investigator Joseph Kabogo, by calling 0714-108786. You may also contact the chairman of the KEMRI Ethical Review Commission using the following address and telephone number:

Chairman, KEMRI/ Ethical Review Committee (ERC)

P.O Box 54840 Nairobi. ; Tel 020-2722541; Email : ercsecretariat@kemri.org

Appendix III: English Informed Assent Form for minors aged 13-17 years old

Title of Research Study: "Prevalence and Risk Factors of Virologic Failure and HIV-1 Drug Resistance among Children and Adolescents in the Lea Toto Programme in Nairobi, Kenya".

Name of principal investigator..............................Joseph Mbugua Kabogo

Name of organization…………………………JKUAT / KEMRI

Introduction

We are requesting for your assent / agreement to participate in our research study titled: "Prevalence and Patterns of HIV-1 Drug Resistance among children in the Lea Toto Program in Nairobi, Kenya." Since you are a minor between 13 years old and 17 years old, we are seeking both your assent to participate in our study, and in a separate form, we are also seeking your parent's or guardian's permission for you to participate in our study.

Aim of the study

This study aims to determine the percentage of those children in the Lea Toto Program who stop responding to antiretroviral therapy (ART) due to treatment failure and HIV-1 drug resistance. The results of this study will help us to find out the causes of the problems of treatment failure and drug resistance. Then we will be able to come up with solutions that can help save children's lives and improve children's health.

Your role in the study

Your role in this study is very easy because all the work has already been done. The blood to be tested has already been collected and stored every 6 months that you visit the Lea Toto Program clinic for a CD4+ test and viral load test. The other data needed for the study has already been collected and is on file. All we need is your assent / agreement to allow your blood and data to be used for our study.

Benefits to you

By you participating in this study, you are helping all HIV-1 positive children in the Lea Toto Program and beyond, to improve their quality of life by reducing ART treatment failure and HIV-1 drug resistance.

Risks and discomforts to you while in the study

We will NOT collect any blood from you for this study. We will use your CD4+ count, viral load results, and other relevant medical and social data that have already been collected at the Lea Toto clinic. Therefore, you will NOT experience any risk or discomfort.

Permission to access your Lea Toto medical records

We are requesting your assent / agreement to access and use your Lea Toto Program medical records to obtain the following information: age, gender, weight, when your joined the Lea Toto Program, ART regimen, what percentage of your ART drugs you have taken, CD4+ T-cell count, CD4%, and viral load.

Analysis and discarding of blood if you develop treatment failure

In case you stop responding to antiretroviral therapy (ART), we will need to test your blood which has remained after you are tested every 6 months at the Lea Toto Clinic. This remaining blood is already stored in a freezer at the Nyumbani Diagnostic Laboratory in Karen, Nairobi. We will NOT collect any blood from you.

In case you stop responding to ART, we are requesting your assent / agreement to use your blood samples that have been collected ever since you joined the Lea Toto Program. We will use your blood to perform tests to find out the exact cause of the treatment failure and drug resistance. After that, the tested blood will be burned in an incinerator.

Privacy of your information

We will protect your privacy by keeping your signed informed assent form and your medical information under lock and key. Only the principal investigator of this study and the Laboratory Directory of the Nyumbani Diagnostic Laboratory will have the key. Moreover, after you have signed this informed assent form, we shall assign you a

serial number to identify you. We will NOT use your name during this study or in the publication of our results. Also, any information entered into our computer databases will be password-protected, and only the principal investigator of the study and his data entry assistant will have that password.

At the end of our study, we shall publish our results in a scientific journal for other scientists to read. Also, we shall write a report to the Clinical Officers at the Lea Toto program. Thus, you can find out the results of our research by asking the Clinical Officer at the Lea Toto Program clinic. In all our reports, we shall use serial numbers in our publications, so that no one will be able to identify you personally.

Your right to refuse to participate in the study

You do NOT have to take part in this research study if you do not wish to do so. This will NOT affect your participation in the Lea Toto Program. Also, if you agree to participate in this study, you still have the right to withdraw from this study any time, without penalty.

If you agree to participate in this research study, and for the results to be published, please read and sign the following informed assent form.

Signed, informed assent form for minors aged 13-17 years old

I, the undersigned, confirm that as I agree to participate in this research study, it is with a clear understanding of the objectives and conditions of the study, and with the recognition of my right to withdraw from the study at any time, without penalty.

I……………………………………………………………., do hereby agree to participate in the proposed research study led by…………………………………………………... I have been given all the necessary information to understand the purpose and nature of this research study. I have also been assured that I can withdraw from the study at any time, without penalty or loss of benefits that I enjoy as a participant in the Lea Toto Program. The research proposal has been explained to me in the language I understand.

Name of minor …………………………………

Minor's signature…………………………….

Name of parent/guardian………………………………………..

Date………………………. Place………………………..………………

Name of principal investigator…………………………………………..

Principal investigator's signature…………………………………………..

Date………………………. Place………………………..………………

Name of independent witness…………………………………………..

Independent witness' signature……………………………………..…..

Date………………………. Place………………………..………………

In case of any questions regarding this research study, please contact the principal investigator Joseph Kabogo, by calling 0714-108786. You may also contact the chairman of the KEMRI Ethical Review Commission using the following address and telephone number:

Chairman, KEMRI/ Ethical Review Committee (ERC)

P.O Box 54840 Nairobi. ; Tel 020-2722541; Email : ercsecretariat@kemri.org

Appendix IV: Verification of guardianship form

Title of Research Study: "Prevalence and Risk Factors of Virologic Failure and HIV-1 Drug Resistance among Children and Adolescents in the Lea Toto Programme in Nairobi, Kenya".

Name of principal investigator..............................Joseph Mbugua Kabogo

Name of organization…………………………JKUAT / KEMRI

Introduction

This form is to verify that you are the legal guardian of the child or minor being recruited to participate in our research study titled: "Prevalence and Patterns of HIV-1 Drug Resistance among children in the Lea Toto Program in Nairobi, Kenya."

Aim of the study

This study aims to determine the percentage of those children in the Lea Toto Program who stop responding to antiretroviral therapy (ART) due to treatment failure and HIV-1 drug resistance.

Name of legal guardian …………………………………………………..

Legal guardian's signature…………………………………………………..

National ID Number………………………………………………………….

Name of child………………………………………………………………...

Child's date of birth………………………………………………………….

Date that guardianship began…………………………………………………

Date……………………….   Place……………………..………………

Name of Lea Toto Program Clinical Officer…………………………………

Signature of Lea Toto Program Clinical Officer……………………………

Date……………………….   Place……………………..………………

Name of principal investigator…………………………………………………..

Principal investigator's signature…………………………………………………..

Date……………………….   Place……………………..………………

In case of any questions regarding this research study, please contact the principal investigator Joseph Kabogo, by calling 0714-108786. You may also contact the chairman of the KEMRI Ethical Review Committee (ERC) using the following address and telephone number:

Chairman, KEMRI/ Ethical Review Committee (ERC)

P.O Box 54840 Nairobi. ; Tel 020-2722541 ; Email : ercsecretariat@kemri.org

Appendix V: KEMRI Scientific Steering Committee Approval (2013)

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org   info@kemri.org   Website:www.kemri.org

ESACIPAC/SSC/101599   25th April, 2013

Joseph Kabogo

Thro'
    Director, CVR
    NAIROBI

REF: SSC No. 2500 (Revised) – Prevalence and patterns of HIV-1
      drug resistance among children in Nairobi, Kenya

Thank you for your letter dated 24th April, 2013 responding to the comments raised by the KEMRI SSC.

I am pleased to inform you that your amendment now has formal scientific approval from SSC.

The SSC however, advises that work on the proposed study can only start after ERC approval.

Sammy Njenga, PhD
SECRETARY, SSC

Appendix VI: KEMRI Ethics Review Committee Approval (2013)

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1   November 27, 2013

TO: **JOSEPH MBUGUA KABOGO**
             PRINCIPAL INVESTIGATOR

THROUGH: **DR. GEORGE NAKITARE,**
             ACTING DIRECTOR, CVR,
             NAIROBI

Dear Sir,

RE: **SSC PROTOCOL NO. 2500 (*RE-SUBMISSION 2*): PREVALENCE AND PATTERNS
        OF VIROLOGICAL FAILURE AND HIV-1 DRUG RESISTANCE AMONG CHILDREN
        IN THE LEA TOTO PROGRAM IN NAIROBI, KENYA.**

Reference is made to your letter dated November 25, 2013. The ERC Secretariat acknowledges
receipt of the revised proposal on November 26, 2013.

This is to inform you that the Ethics Review Committee (ERC) reviewed the documents submitted
and is satisfied that the issues raised at the 215th meeting have been adequately addressed.

The study is granted approval for implementation effective this **November 27, 2013**. Please
note that authorization to conduct this study will automatically expire on **November 26, 2014.**
If you plan to continue with data collection or analysis beyond this date, please submit an
application for continuing approval to the ERC Secretariat by **October 15, 2014**.

Any unanticipated problems resulting from the implementation of this protocol should be brought
to the attention of the ERC. You are also required to submit any proposed changes to this
protocol to the ERC prior to initiation and advise the ERC when the study is completed or
discontinued.

You may embark on the study.

Yours faithfully,

DR. ELIZABETH BUKUSI,
ACTING SECRETARY,
KEMRI/ETHICS REVIEW COMMITTEE

In Search of Better Health

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Appendix VII: Nyumbani Medical Board Approval (2013).

NYUMBANI®

THE CHILDREN OF GOD RELIEF INSTITUTE
A Registered Charity in Kenya, USA, UK, Italy, Ireland & Spain
P.O. Box 24970-00502, Nairobi, Kenya - Office Tel: 254-20-3882429, Fax: 3882430, e-mail: mary@nyumbani.org
Hospice: Tel: 2015573 / 3883731, Fax: 3882371, Mobile: 0722-201163, 0733-661418, e-mail: admin@nyumbani.org
Homepage:http://www.nyumbani.org - Dagoretti, Karen

18 November 2013

Mr. Joseph Mbugua Kabogo
Ph.D. student, Molecular Medicine Program,
Institute of Tropical Medicine & Infectious Diseases (ITROMID),
Jomo Kenyatta University of Agriculture & Technology (JKUAT).

Dear Joseph,

This is to confirm that Nyumbani Children's Home Lea Toto Program have no objection in allowing you access to archived blood samples from the Children in our program. However, we will require a letter confirming approval of your study from the KEMRI Ethical Review Board before we can consider your request further.

Kindly make the necessary arrangements to provide us with the said ethical clearance letter.

Yours sincerely,

Sr. Mary Owens, IBVM
Executive Director

Appendix VIII: Data abstraction form

Title of Research Study: "Prevalence and Risk Factors of Virologic Failure and HIV-1 Drug Resistance among Children and Adolescents in the Lea Toto Programme in Nairobi, Kenya".

Name_____ Age _____ Gender_____

Lea Toto Admission Number_____ Lea Toto Admission Date_____

First line ART regimen_____First line ART start date_____

Second line ART regimen (If applicable)_____

Second line ART start date (If applicable)_____

Viral Loads test dates and results from December 2013 to December 2016.

1)_____
2)_____
3)_____
4)_____
5)_____
6)_____
7)_____
8)_____
9)_____
10)_____
11)_____
12)_____

CD4+ T-cell count test dates and results from December 2013 to December 2016.

1)_____
2)_____
3)_____
4)_____
5)_____
6)_____
7)_____
8)_____

Appendix IX: The reagents and amounts used in the Master-mix for the First-round PCR.

First round PCR Master-mix	Volume / reaction (µl)	Master-mix volume (µl)	# Tests	Final Concentration
RNase free H$_2$O	4	40	10	
SuperScript III 2x Reaction Buffer	25	250		1x
5mM MgSO$_4$	4	40		1.6 mM
Forward Primer RT18 (5µM)	2	20		200 nM
Reverse Primer KS104 (5µM)	2	20		200 nM
SuperScript III Enzyme Mix	1	10		N/A
Template (RNA)	12			
Total Volume	50	380		Master-mix volume
		38		Volume / tube

PCR:- Polymerase Chain Reaction; RNase:- Ribonuclease-free water; MgSO4:- Magnesium Suphate.

Appendix X: The reagents and amounts used to prepare the Master-mix for the Nested PCR.

Nested PCR Master-mix	Volume / reaction (µl)	Master-mix volume (µl)	# Tests	Final Concentration
RNase free H₂O	24.5	245	10	
Phusion Taq 5x Reaction Buffer	10	100		1x
dNTP mix (10 mM each)	1	10		200 µM
Forward Primer KS101 (5µM)	5	50		500 nM
Reverse Primer KS102 (5µM)	5	20		500 nM
Phusion Taq	0.5	5		1 Unit
Template DNA (First-round PCR Amplicon	4			
Total Volume	50	460		Master-mix volume
		46		Volume / tube

PCR:- Polymerase Chain Reaction; RNase:- Ribonuclease-free water; dNTP: deoxyribonucleotide phosphate

Appendix XI: Alignment of HIV-1 protease gene (amino acids 1-99) for the 114 Lea Toto cohort study participants switched from first-line ART to second-line ART.

| | 1650 | 1660 | 1670 | 1680 | 1690 | 1700 | 1710 | 1720 | 1730 | 1740 | 1750 |

Conservation %

Consensus sequence
NFPQI.TLWQRDPLVTKDRVTKIGGQLKEALLIYSI.L..KKHQ....FK.MICDTGADDTVLEDINLCPSK.DSMNRY..GKWKPKMIGGIGG.T.RNLFIKKNSV

A189	SSIEL-GIDYT-RHKV---T-V--D.------------
A271	---------VE-AQCS---D-.-N.VQLKK.I------------------------RS....QY-KR-IC-Q-----------ENGNQK--.ELEV------LS.---
A359	S....-....-..V.----.G.....L-----------------------------------.E.-.----------.....K.......------...---
A413	S....-....-.LV.----.G.....L-----------------------------------.E.-.----------.....K.......------...---
A443	S....-....-.LV.---.R.G.....L-----------------------------------.....-.----------.....K.......------...---
A188	V....-....-.LV.---.-.....IR...L-------------------------------.....G.Q.-E------.....K.......------...---
A222	ASLKS-LFGND-.LLP-----R-GDS-RKLY----------------------IQEQMIQY-KT-IC-Q-----------.NGNQK--.ELEV------LS.---
A280	.IKTK-WTFG--VFT---L-PPG.S.YL.V--
A172	-L.SN-HSLAT-TPC.----.-.VG.....L-----------------------------------.E.-.----------.....K.......------...---
A182	-L.S-----KD-TPC------.-G..VR...L--.....K.......------...---
A146	--
A192	LISLK-S.FGN-DPLS--Q--KR.TCR...L-------------------------------.....D.-.----------.....K.......------...---
A114	--
A497	....-....-.LV.---.-.XG...R...L-----------------------------------.E.-.----------.....K.......------...---
A218	-L.SN-HSLAA-TLV.----.-R.G...I...L-----------------------------------.E.-.----------.....K.......------...---
A212	....-....-.LXX---.-.....R...L--.....K.......------...---
A327	--
A318	--
A333	--
A205	--
A135	--
A291	--
A274	--
A263	--SIRRHRF-A----------R.METKNDR.N..----...---
A421	S....-.....-.IVA---.-.G..I...L-------------------------.....D.-.----------.....K.......------...---
A173	.....-L--E.-.LSQ------.-KDS-.KLY-IQEQ--------MIQY-KI-ICQENGNQK--.ELEV-LSK-DSMNRYYRNL..KGYRYS.S..------T-----
A187	.....-.--W-.LV.---I-.G.....L-----------------------------------.EMS.-.----------.R...K.......------...---
A287	--
A277	S....-....-.LV.---I-.....L---------------------------------------S-.----------.....K.......------...---
A464	ASLN.-....-..V.---.G.....L-----------------------------------.E.-.----------.....K.......------...---
A525	.....-....-.LV.---I-.G.....L-----------------------------------.EMS.-.----------.R...K.......------...---

Appendix XII: Alignment of HIV-1 reverse transcriptase gene (amino acids 1-299) for the 114 Lea Toto cohort members switched from first-line ART to second-line ART.

Conservation %

Consensus sequence FLWDMGYDLHLSTYLE.LHPDKWTVQPIKLPEKESWTVNDIQKLVCQKYTEISRYV.RNGKTKLGKLNWASQEKFIY.PGIKVKQLCKLLRGAKA.TPQGSQSLTDVV.LTEE

| 2860 | 2870 | 2880 | 2890 | 2900 | 2910 | 2920 | 2930 | 2940 | 2950 | 2960 | 2970 |

A464 ---
A454 ---
A429 R.-L..-------.-....K..-----------------------V--------.....---..-P....-L....I....---...V.T..E
A247 ..-.......-....K....V...E-----------------------V--------.....---..-P....K....---...V.P..E
A235 ---.S-------Q-QFVER.-----------RRK-----------------EK--FQKL.L.IHTILQC.L-R.K--------I----A.DG
A375 ---
A434 ..-.....-....E...E....-----------------------V--------.....---..-P.V.K....---.....T..E
A515 PLD-.L-------T.S---------MDSTAYSAARKRQ.DCQ-HTEVS------GKIEL..SDLPRD------SKAIM-TP..NQS------TNRSNTANR
A141 D.-LYF-------K-FPN-SC-DCASKIKARNG.PKG-TMAIDRRKDKSINRYL-RNGKGGKNLK...-K---SI----QHSNI.YKEKRQY------M------EE
A408 ..-.....-....V...E....-----------------------V--------..-Q....K....---.....P..E
A354 ..-.....-....E...EH....-----------------------V--------..-P....IK....---...V.P..E
A427 H.-L..-......H.I.D....-----------------------V--------..-P....K....I....---...V.T..E
A395 ..-.......V..D....-----------------------V--------..-P....K....---...V.T..E
A423 H.-L..-....E..D.E....-----------------------V--------..-....K....K....---...V.T..E
A226 ---
A327 RLSTL.-------Q-N-SQE.MA.GLNNGH-QKKK-KH-..F-------V--------Q.WKRKEK---LQELDL.IHTILQY.Q-R.K--------IA----.NG
A259 ..-.....-....K...E....-----------------------V--------..-....K....R.I....---.....P..K
A392 ..-.....-....M....-----------------------V--------..-....K....C...T....---...EV.P...
A368 ..-.....-....V..D.E....-----------------------V--------..-....K....I..T....---...EV.P..K
A401 ..-.....-....K...E....-----------------------V--------..-S....K....---.....T..E
A437 ..-.....-....N..H....-----------------------V--------..-....K....---...V.T..E
A472 ..-.......N....-----------------------V--------..-....K....---...V.T..E
A252 ..-.....-....M..D.E....-----------------------V--------.N-IGQVK---FM-Q.S.-SSCVNS.GEP.H------QI-YH-LR
A381 ..-.......E...E....-----------------------V--------..-....K....---.....P..E
A477 ..-.......VK....-----------------------V--------..-P.Q.K....T....---.....T..K
A365 ..-.......E....-----------------------V--------..-P....K....---.....EV.T..E
A119 ---
A342 ..-.......V..D....-----------------------V--------..-S....K....---.....IP..E
A274 ---
A263 ---
A421 ---
A271

	2970	2980	2990	3000	3010	3020	3030	3040	3050	3060	3070	3080

Conservation %

Consensus sequence: LTEEARELEL..AENREILKDP.PPVHGVYYDPK....P..VGRGRRGSYLRSPNKSRVLRGGSRGAASGV.SPSG..VV.A......S.......WRRKEK.Q.LALKIH.

Ref_HIV1_RT_KT758282.1	LTAEA-ELEL--AENREILKEP---
A277	....-....--E.....INT.--
A255	--
A460	ALT.EAN-NWQKK.Q-DLVSN--
A348	..E..-....--.....VF--
A315	..E..-....--.....D.---VHGVYYQI--
A386	..D..-....--.....D.---VHGVYYDP--
A136	--
A231	..E..-....--.....VT---SRWSV--
A530	--
A306	..E..-....--...X-.---XAGVYYDL--
A383	ID-GSRIR-I--GRKQRDS.R.---VHGVYYDP-------------------------------------
A312	ID-GS-RIRI--GR------.Q---GDSKSPGNDQ-----------------------------------
A490	..E..-....--.....F-.NQ---YMSV-S--QK------------------------------------
A240	--
A336	--
A234	..E..-....--.....D.---VHGVYLTQNL--------------------------------------
A282	--
A299	--
A168	--
A308	..E..-....--.....SQ---YMVYYP-K--
A469	..E..-....--.....S---VHGYIQTK---
A482	..E..-....--.....D.---VHGVY-KI--
A379	TD-GSRK-NW--QKTGK...-.---VVRILRS--------------------------------------
A416	ID-GSRIRIG--R.QGDFKRPC---AWSILS-I-------------------------------------
A447	IN-GSRIRVG--RKQGNSKRPC---AWGIL-PL-------------------------------------
A505	--
A305	....-....--.....NNL---CMGIYDPSKD--------------------------------------
A372	TD-RSRN-NW--QKTGK..I-S---QSGVVDER-S-----------------------------------
A507	..E..-....--......---VHGVYYTQDL--
A519	..E..-....--.....TR---------Y---
A464	

Conservation %

Consensus sequence LTEEARELEL..AENREILKDP.PPVHGVYYDPK....P..VGRGRRGSYLRSPNKSRVLRGGSRGAASGV.SPSG..VV.A......S.......WRRKEK.Q.LALKIH.

A464	-------
A454	-------
A429	..E..-...,--.......D.---VHGVY-D-
A247	..E..-...,--.......T.---VHGYIIQ-RQ
A235	.DG.N---I.-ENLTK.L---R---TSGKFN-EYR
A375	-------
A434	..E..-........R,.---VHGVYYPSKD
A515	ANR------RSR.GTGRK-GDS---KRTSTWSVLS
A141	-EESS-RFQRAQ-K.SRL.GSS---TRNTASSGFR
A408	..E..-...,--.......D.---VHGVYYDP-D
A354	..E..-...,--.......D.---VHGVYFQN
A427	..E..-.I..--.......D.---VHGVYYHQKT
A395	..E..-...,--.......D.---VHGVYYDP
A423	..E..-...,--.......D.---VHGVYY-P
A226	-------
A327	.NG-G-N--I--S-ESS.KEHK---TFGKFN-EYR
A259	..K..-...,--.......N.---VHGVYY---X
A392	.........,--.......---VHGVYY---T
A368	..K..-...,--.......D.---VHGVYY---K
A401	..E..-...,--.......D.---VHGVYY---P
A437	..E..-...,--.......D.---VHGVYY---D
A472	..E..-...,--.......D.---VHGVYY---T
A252	-LRKQ-N-NW--QRTGRF-.TL---CMGY-
A381	..E..-...,--.......N.---VHGVYY---P
A477	..K..-...,--.......D.---VHGVYY---T
A365	..E..-...,--.......D.---VHGVYY---D
A119	-------
A342	..E..-...,--..........---VHGVYY---T
A274	-------
A263	-------
A421	-------

Appendix XIII: Research papers: Kabogo *et al.*, 2017 and Kabogo *et al.*, 2018.