# UNIVERSITY OF THE WITWATERSRAND
# JOHANNESBURG

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

## FACULTY OF HEALTH SCIENCES

*Trend of Pre-antiretroviral Therapy HIV-1 Drug Resistance in Kilombero and Ulanga Antiretroviral Cohort, South-Western Tanzania, for over 15 years (2005-2020).*

Alex NTAMATUNGIRO

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy

JANUARY 2024

## DECLARATION

I, **Alex Ntamatungiro** declare that this thesis is my original work and has not been submitted before for any degree or award at this or any other university. Where information has been derived from other sources, I confirm that this has been duly acknowledged. Parts of this work have been published as open access articles, I (and my co-authors) retained the copyright for that work.

Alex Ntamatungiro

17th January 2024

I dedicate this work to **my wife** Maria, and our **children** Ethan, and Alvin

## PUBLICATIONS ARISING FROM THIS RESEARCH PROJECT

1. **Ntamatungiro AJ**, Kagura J, Weisser M, Francis JM. Pre-treatment HIV-1 drug resistance in antiretroviral therapy-naive adults in Eastern Africa: a systematic review and meta-analysis. *J Antimicrobial Chemotherapy. 2022 Nov 28;77(12): 3231-3241.doi: 10.1093/jac/dkac338. PMID: 36225089.* https://pubmed.ncbi.nlm.nih.gov/36225089/

   **My contribution to the paper**
   Conceptualization of the study, generation of the hypothesis, conducting literature searches and articles reviews for inclusion eligibility, statistical analyses, synthesizing and presenting findings, writing all drafts of the paper, addressing co-authors' and peer reviewers' comments and checking proofs for the final accepted and published manuscript.

2. **Ntamatungiro AJ**, Anna Eichenberger, James Okuma, Fiona Vanobberghen, Robert Ndege, Namvua Kimera, Joel M. Francis, Juliana Kagura, Maja Weisser, on behalf of the KIULARCO Study Group: Transitioning to dolutegravir in a programmatic setting: Virological outcomes and associated factors in the Kilombero and Ulanga antiretroviral cohort in rural Tanzania: *Open Forum Infectious Diseases, Volume 10, Issue 7, July 2023, ofad321,https://doi.org/10.1093/ofid/ofad321. https://pubmed.ncbi.nlm.nih.gov/37520425*
   **My contribution to the paper**
   Conceptualization of the study, generation of the hypothesis, planning, and running all lab experiments, and statistical analyses, synthesizing and presenting findings, writing all drafts of the paper, addressing co-authors' and peer reviewers' comments and checking proofs for the final accepted and published manuscript.

3. **Ntamatungiro AJ**, Kagura J, Mnzava D, Ndege R, Okuma J, Vanobberghen F, Francis JM, Weisser M, on behalf of the KIULARCO Study Group: Recent HIV-1 infection and pre-treatment drug-resistance in recently infected, treatment-naïve persons in rural Tanzania: Submitted to the *Journal of Public Health* - http://jpubhealth.oupjournals.org (under review)**.**

   **My contribution to the paper**
   Conceptualization of the study, generation of the hypothesis, planning, and running all lab experiments, and statistical analyses, synthesizing and presenting findings, writing all drafts of the paper, and addressing co-authors' comments and checking proofs for the final submitted manuscript.

4. **Ntamatungiro AJ**, Francis Joel, Ndege Robert, Okuma James, Tschumi Nadine, Vanobberghen Fiona, Metzner Karin, Paris Daniel, Weisser Maja and Kagura Juliana on behalf of the KIULARCO Study Group: Trends in prevalence of the nucleoside/nucleotide reverse-transcriptase inhibitor (NRTI) associated mutations in newly diagnosed ART naive patients in the Kilombero and Ulanga Antiretroviral Cohort during the 15 years after ART rollout in Tanzania. To be submitted to the *Journal of AIDS.*

   **My contribution to the paper**

   Conceptualization of the study, generation of the hypothesis, planning, and running all lab experiments, and statistical analyses, synthesizing and presenting findings, writing all drafts of the paper, addressing co-authors' comments and preparing the final manuscript for submission.

## PRESENTATIONS ARISING FROM THIS RESEARCH PROJECT

1. **Ntamatungiro A**, Eichenberger A, Okuma J, Vanobberghen F, Ndege R, Kimera K, Francis J, Kagura J, Weisser. *Transitioning to dolutegravir: virological outcomes in an HIV cohort in rural Tanzania* (AS-EACS-20221-00296): <u>18th European AIDS Conference (EACS)</u> October 27-30, 2021, London, United Kingdom (ePoster presentation).

2. **Ntamatungiro A**, Eichenberger, Okuma J, Vanobberghen F, Mapesi H, Ndege R, Mnzava D, Kimera N, Weisser M. *Transitioning to Dolutegravir in a programmatic setting: Virological outcomes in the Kilombero and Ulanga Antiretroviral Cohort in rural Tanzania*: <u>9th Annual Joint Advanced Seminars Conference</u> 29th July 2022, Kampala, Uganda (Oral presentation).

3. **Ntamatungiro A**, Neumann k, METZNER K, PARIS D, and WEISSER M. *HIV-1 Drug Resistance Surveillance in Tanzania*: <u>Swiss TPH Virtual Symposium</u> 25th October 2022 (Oral presentation).

4. <u>**Ntamatungiro A**</u>, Francis JM, Mnzava D, Ndege R, Njau PS, Okuma J, Vanobberghen F, Kagura J, Weisser M, on behalf of the KIULARCO Study Group. *Recent HIV-1 Infection and Pre-Treatment HIV Drug-Resistance in Recently Infected Adults Initiating Antiretroviral Therapy in Rural Tanzania*: <u>Wits School of Public Health Research Day and CARTA Conference</u> 14-15 September 2023, Johannesburg, South Africa (Poster presentation).

## ABSTRACT

### Introduction

Pre-treatment HIV drug-resistance (PDR) may result in increased risk of virological failure and subsequently acquisition of new HIV drug resistant mutations. With recent increase in antiretroviral therapy (ART) coverage and periodic modifications of the guidelines for HIV treatment, monitoring changes in levels of PDR is critical, particularly in under-sampled areas, such as rural Tanzania. This PhD project aimed to determine the trend and patterns of PDR in the Kilombero and Ulanga antiretroviral cohort (KIULARCO), analyse the impact of recent HIV-1 infection, and dolutegravir rollout in rural Tanzania.

### Methods

The study comprised a systematic review and meta-analysis of primary studies about prevalence of PDR among ART-naive people living with HIV (PLHIV) (≥15 years old), published between 2017 and 2022. The data had to be in one or several of the countries of Eastern Africa, namely, Ethiopia, Kenya, Malawi, Rwanda, Mozambique, Tanzania, and Uganda. Thereafter, cross-sectional analyses of data on newly HIV-1-diagnosed ART-naïve adults (aged ≥ 15 years), enrolled in the on-going prospective clinic-based observational rural antiretroviral cohort-KIULARCO focusing on various aspects of PDR. Multivariate logistic regressions were used to determine the factors associated with recent HIV-1 infection, and viral suppression at 12-months in patients initiating dolutegravir-based ART in the KIULARCO.

### Results

Overall, the pooled prevalence estimate of any PDR was 10.0% (95% CI: 7.9%–12.0%, $I^2$ =88.9%) among 22 studies in the general adults' population, which was higher than the previously reported prevalence of 8.7% using data available until 2016 in the Eastern Africa region. PDR was mainly driven by non-nucleoside reverse transcriptase inhibitors (NNRTI); whereas the pooled prevalence of PDR to nucleoside reverse transcriptase inhibitors (NRTI) was 2.6% (95% CI: 1.8%–3.4%, $I^2$=69.2%). Remarkably, PDR to NRTIs in a sub-population of recently HIV-1 infected PLHIV in the KIULARCO was high at 12.5%. Also, there was a notable tendency to an increasing prevalence of PDR to NRTI, with the overall prevalence of 2.1% in the first five-year period (2005-2009) of the ART program in Tanzania, and 3.4 % in the most recent period (2019-2022). Moreover, there was no PDR to the dolutegravir co-administered NRTI in those with viremia ≥50 copies/mL, at one year, in patients initiating dolutegravir-based ART in the KIULARCO 2 years after dolutegravir roll. Notably, dolutegravir-based ART was associated with >2 times the odds of viral suppression compared to NNRTI-based ART with an adjusted odds ratio (aOR) of 2.10 (95% CI 1.12-3.94).

**Conclusions**

There is notable level of PDR to NRTI among general adults' population in Eastern Africa region, that was high among recently HIV-1 infected PLHIV in a representative rural Sub-Saharan Africa setting. Hence, routine surveillance of pre-existing resistance to the DTG co-administered NRTI remains particularly important, in resource-limited settings, to prevent risk of failure of newer antiretroviral agents such as dolutegravir, which would be detrimental to Tanzania and other low- and middle-income countries for the aim to "end AIDS by 2030". Our results underline the benefit of programmatic uptake of dolutegravir -based ART in low- and middle-income countries.

## ACKNOWLEDGEMENTS

First and foremost, I am extremely grateful to my supervisors, Dr. Juliana Kagura, Dr. Joel Francis, and Prof. Maja Weisser for their invaluable advice, continuous support, and patience during my PhD study. Their immense knowledge and plentiful experience have encouraged me in all the time of my academic research and daily life. I would also like to thank Dr. Fiona Vanobberghen, Dr. James Okuma, and Dr. Anna Eichenberger for their contributions to the KIULARCO platform and advice on my study. My gratitude extends to the Consortium for Advanced Research Training in Africa (CARTA) for the funding opportunity to undertake my studies at the the Faculty of Health Sciences, University of the Witwatersrand, I would like to thank all the members of the KIULARCO Study Group. Finally, I would like to express my sincere gratitude to my wife and my children. Without their tremendous understanding and encouragement in the past few years, it would be impossible for me to complete my study.

# TABLE OF CONTENTS

## LIST OF FIGURES



## LIST OF TABLES

*CHAPTER 3*



*CHAPTER 4*



*CHAPTER 5*



*CHAPTER 6*

## LIST OF ABBREVIATIONS

| | |
|---|---|
| ABC | Abacavir |
| ART | Antiretroviral Therapy |
| CDCI | Chronic Diseases Clinic |
| CTC | Care and Treatment Centre |
| d4T | Stavudine |
| ddI | didanosine |
| DTG | Dolutegravir |
| EFV | Efavirenz |
| FTC | Emtricitabine |
| HIV-1 | Human Immunodeficiency Virus type 1 |
| HIVdb | HIV Drug Resistance Database |
| HIVDR | HIV-1 Drug Resistance |
| HVL | HIV viral load |
| INSTI | Integrase Inhibitors |
| KIULARCO | Kilombero & Ulanga Antiretroviral Cohort |
| 3TC | Lamivudine |
| LMIC | Low- and Middle-Income Countries |
| NACP | National AIDS Control Care Programme |
| NGS | Next-Generation Sequencing |
| NNRTI | Non-Nucleoside Reverse Transcriptase Inhibitor |
| NRTI | Nucleoside Reverse Transcriptase Inhibitor |
| PCR | Polymerase Chain Reaction |
| PDR | Pre-treatment HIV-1 drug resistance |
| PI | Protease Inhibitors |
| PLHIV | People living with HIV. |
| PMTCT | Prevent mother-to-child transmission |
| PR | Protease |
| RHI | Recent HIV infection |
| RLS | Resources Limited Settings |
| RNA | Ribonucleic Acid |
| RT | Reverse transcriptase |
| RT-PCR | Reverse transcriptase polymerase chain reaction |
| SSA | Sub-Saharan Africa |

| | |
|---|---|
| TDF | Tenofovir Disoproxil Fumarate |
| TDR | Transmitted HIV-1 drug resistance |
| TLD | Tenofovir/Lamivudine/Dolutegravir |
| WHO | World Health Organization |

# PART I

# GENERAL INTRODUCTION

# CHAPTER 1

## 1.1. BACKGROUND OF STUDY

### 1.1.1 HIV burden and the evolution of antiretroviral therapy initiation guidelines

Currently about 38 million people are living with HIV globally and two thirds of these reside in Sub-Saharan Africa (SSA)(1). The HIV prevalence in Tanzania has stabilized at 4.7 % (about 1.7 million people living with HIV (PLHIV) in 2021) and new infections have been reduced to a still high number of 54 000 new HIV infections in 2021. The global epidemiology of HIV infection has changed markedly because of the rapid expanding access to antiretroviral therapy (ART). For example, by the end of 2021, 28.7 million people were accessing ART(2), up from 7.8 million [6.9–7.9 million] in 2010. Also, the ART coverage increased from 24% in 2010 to 75% in 2021(2). By the end of 2021 about 86% of PLHIV in Tanzania were receiving antiretroviral treatment(1).

There has been major improvement in antiretroviral medication with replacement of nucleoside analogues (NRTIs) with long-term toxicity like stavudine (d4T) and didanosine (ddI) to more favorable NRTIs like tenofovir disoproxil fumarate (TDF)(3). Until recently most low- and middle-income countries (LMIC) were using 2 NRTIs and 1 non-nucleoside reverse transcriptase inhibitor (NNRTI) as first line treatment recommended by World Health Organization (WHO)(4,5). The preferred regimen was tenofovir disoproxil fumarate (TDF) + lamivudine (3TC) or emtricitabine (FTC) + efavirenz (EFV) as a fixed dose combination.

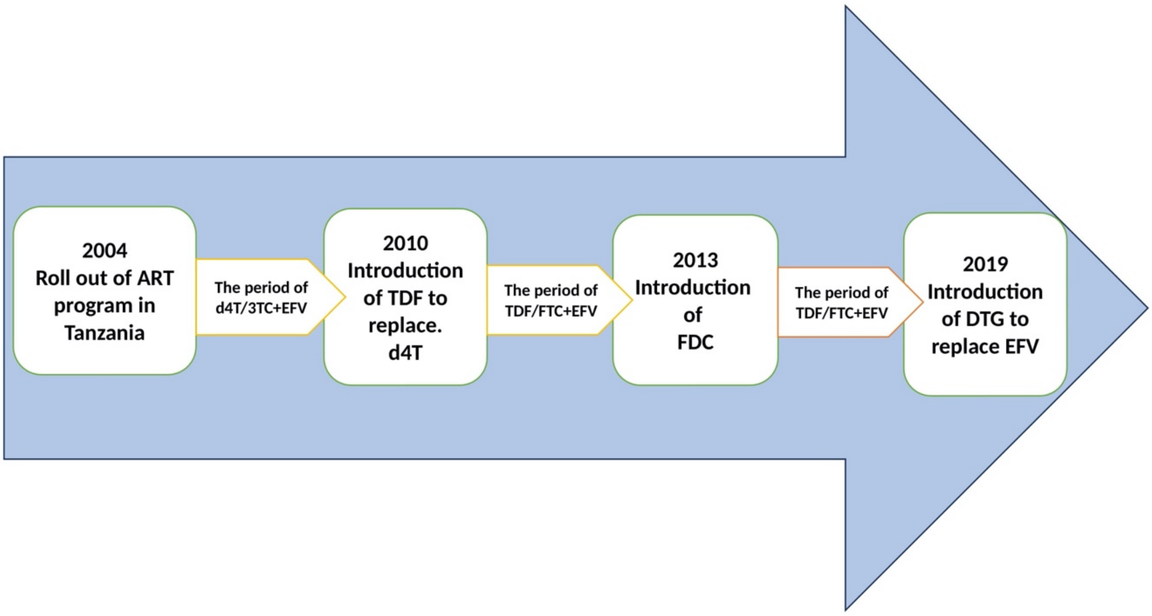



Figure 1.1 Evolution of first line ART in Tanzania

In many high-income countries, integrase-inhibitors as a new drug class have already replaced NNRTIs in first line treatment(6). Available since 2014, integrase inhibitors (INSTI) in several randomized clinical trials have shown faster viral suppression, higher genetic barrier to developing resistance and fewer side effects compared to NNRTIs in treatment-naïve and experienced patients(7,8). Dolutegravir (DTG) an integrase inhibitor for treatment of HIV infection became available to LMIC by the end of 2017, as the generic fixed dose combination tenofovir/lamivudine/dolutegravir (TLD), and since then has been rolled-out in many countries in SSA(9,10). Tanzania has been transitioning to dolutegravir-based first-line regimens since January 2019.

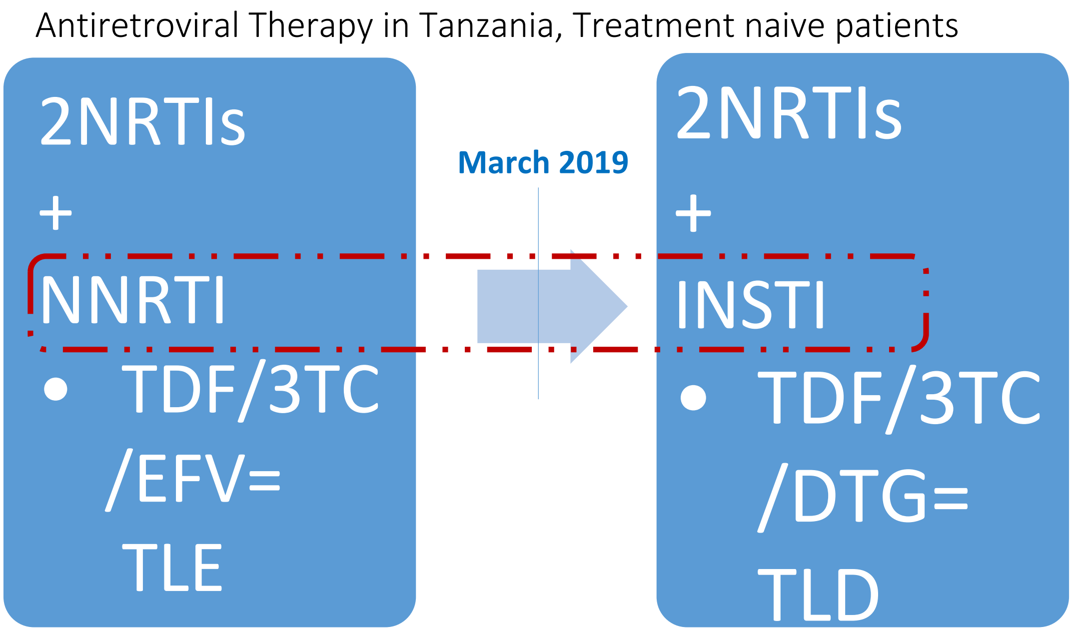



Figure 1.2 WHO recommended programmatic shift to integrase-based therapy.

### 1.1.2 HIV drug resistance

HIV drug resistance refers to the ability of viruses to continue multiplying even in presence of antiretroviral drugs that usually inhibits its replication. The phenomenon occurs mainly when the virus develops changes in its genetic information, referred to as “mutations”, which alter HIV proteins, that the virus uses to replicate, including the reverse transcriptase, protease, and integrase enzymes.

HIV drug resistance mutations may naturally emerge due to random errors occuring because of the high viral replication rates, or during episodes of incomplete viral suppression, occurring mainly due to suboptimal compliance and irregular drug taking behaviour, which allows the virus to replicate and develop new HIV drug resistant mutations over time. This is known as acquired drug resistance. In some cases, people acquire a strain of HIV that already has the mutations conferring resistance to antiretroviral (ARV), that is known as transmitted drug resistance (TDR). While TDR are resistances detected in patients with no prior history of HIV drug exposure, a synonym term "pre-treatment HIV drug resistance" (PDR) OR "pre-antiretroviral therapy" refers to resistance detected in ARV drug-naive people initiating ART or people with prior ARV drug exposure initiating or reinitiating first-line ART(11).

#### 1.1.2.1 Factors associated with development of HIV drug resistance

The main factor that can lead to uncontrolled viral replication, is poor adherence to ART(12). If ART are taken at sub-optimal level—and it is not diagnosed quickly, the virus can replicate and develop HIV drug resistant mutations. Other factors may include poor absorption: HIV drugs need to be taken consistently on schedule. Additionally, drugs must be absorbed properly into the bloodstream to an optimal drug level, to adequately inhibit the virus replication. Another factor is the pharmacokinetics profile of the antiretrovirals: which refers "to how the drugs are absorbed, distributed, broken down and eliminated from the body. Interactions between drugs can interfere with absorption. Some HIV medications require a booster to maintain high enough levels in the body (for example, protease inhibitors boosted with ritonavir)"(13,14).

#### 1.1.2.2 HIV drug resistance testing

HIV drug resistance can be tested by examining the virus genetic sequences to see whether it contains mutations on the drug targets regions of the viral genome, mainly in the reverse transcriptase, protease, and integrase enzymes. For example, the K65R mutation that reduces TDF and ABC susceptibility, is resulted from the lysine (K)-to-arginine (R) substitution at residue 65 (K65R) in human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT), results from a single nucleotide G-to-A transition (AAA to AGA)(15). Genotypic HIV drug resistance testing is achieved by using genetic analyzers equipment. The main sequencing methods are viral population-Sanger bases Sequencing (SBS), and high throughput deep sequencing method or Next Generations sequencing (NGS). Additionally, to archive genotypic resistance testing there need to be established laboratory protocols and bioinformatics tools for mutations annotations, such as the Stanford University's HIV Drug Resistance Database (HIVdb)(16,17). NGS compared to SBS allows for the detection of low-frequency mutation not identified by SBS. Also, NGS generates massive genomic data with large genome coverage compared to SBS, which allows details analysis of variants(18).

Phenotypic test is another method to test for the HIV drug resistance, that measures the behaviour of HIV in the presence of specific medications. A sample of HIV from a blood test is exposed to various concentrations of different drugs in a laboratory to see whether the virus can still replicate. Its ability to do so is compared against wild-type virus, which is known to be susceptible to all antiretrovirals(19).

### 1.1.3 The burden of pre-treatment HIV drug-resistance

Recent data from ART-naïve cohorts indicate varying trends in prevalence of PDR across regions globally. For instance, the analysis report of over 33,000 pooled sequences worldwide between 1996 and 2016, indicated an increasing trend of HIV drug resistance within non-B subtype virus populations(20). Importantly, evidence from European HIV-cohorts where HIV is predominantly subtype B, PDR reports indicate a stable at <7%, to a declining trend overtime(21–23). The declining prevalence of PDR in recent studies among western population, is likely to be explained by the routine virologic monitoring including HIV drug resistance testing, allowing early detection of HIV drug resistant mutations and switch to an active drug regimen, as well as an increasing usage of ART regimens with a higher genetic barrier to developing resistance, for example, the use of INSTI(24).

Conversely, available data from studies in SSA as in many other LMICs show a rise of PDR. A systematic review and meta-analysis involving ART-naïve adult populations in Eastern and Southern Africa reported a significantly increased PDR over time(11,25,26). However, the study declares several important limitations: For example, in Eastern Africa less than 30% of included studies come from rural settings, so the analysis reflects mostly the prevalence of PDR primarily in urban and peri-urban areas. Elsewhere, differences in HIV drug resistance burden between urban and rural settings have been documented(27,28). Also, the large amount of the included studies is not nationally representative, which makes extrapolation challenging. Similarly, sampling in the East African studies encompassed only studies published in 2005-2009, during which period ART represented in the current WHO guidelines, e.g., tenofovir-based ART were still relatively new in many LMICs.

In Tanzania like many other settings in SSA, data on PDR is scarce and geographically scattered(29–36). This situation may threaten the HIV control program, including the implementation of the test and treat policy, where newly diagnosed HIV patients are put on a lifelong ART. Elsewhere, evidence show that expansion of ART program appear to coincide with increase in PDR rates(37). Hence, a comprehensive analysis of the burden and trends of PDR for the period of ART rollout is of paramount importance.

As for the current ART regimen recommendation in most LMICs, there is high risk of some patients remaining on dolutegravir monotherapy. Despite of high genetic barrier to developing resistance of dolutegravir, resistance to dolutegravir monotherapy has been reported in some studies in western countries(38–40), where this drug has been in use for some-time. Similarly, it is essential to monitor the rate of emergence and spread of PDR to protease inhibitors (PI). This is because, under the current recommendation, HIV infected infants are started by default on PI based ART regimen. Thus, it's equally essential to monitor the trend of PI associated HIV drug resistant mutations, for effective implementation of the Prevention of Mother to Child Transmissions (PMTCT) programme program(41–43).

## 1.2 LITERATURE REVIEW

### 1.2.1 Introductory Information

The purpose of this literature review was to find out all published information related to the PDR and gaining insight on the rates of emergence and spread of HIV-1 drug resistant strains- among ART-naïve population, with the focus on SSA. The review was to establish the gap in knowledge on this topic, and assist focusing the research direction, to be able to generate useful scientific information that may substantially contribute to the existing body of knowledge on the topic among the scientific community. Furthermore, the literature review was aimed at familiarizing with various methodological approaches that has been employed to study the emergence of PDR among ART-naïve HIV infected populations, with the aim of selecting and employing the most appropriate method(s) for the current research study.

To archive the literature review, a systematic organized search notes was developed in a literature review matrix, as an invaluable writing resource for literature review chapter. The literature review matrix includes information about the author, year of publication, sampling year, country, features of study population, sample size, proportions (time-point prevalence) of HIV-1 drug resistance mutations by ART class and HIV-1 subtypes. Eligible studies had to report data about prevalence of pre-antiretroviral(s) therapy in adults (> 15years) with no prior history of ART exposure; quantitative studies involving genotyping of the reverse transcriptase with or without protease region of the pol gene and the intergrase gene.

Furthermore, in this chapter, the prevalence of PDR among newly diagnosed HIV patients in low- and middle-income countries (LMIC) is described, with a particular emphasis on Tanzania. We do so by describing the research gap, which is pertaining to the sparsity of PDR data in Tanzania as in other SSA. Such information is essential not only for any individual appropriate HIV management, but also for the national control program to be able to evaluate the control intervention(s), as HIV-1 drug-resistance may affect the control efforts, for both reducing HIV transmission, morbidity and mortality attributed to HIV(44,45)

The following section, include description of the research gaps, also the keys concepts and issues are explained, these includes TDR and PDR; Minority HIV-drug resistance mutations; Recent HIV infections with pre-ART drug resistant mutant virus, and dynamics of HIV-1 subtypes and circulating strains in Tanzania. The concept map is described in the next section, it depicts the conceptual relationship between different exposures and PDR status among HIV infected individuals. Thereafter, in the following sections, the various methods used to study the PDR are discussed. The overall focus of the study is on ART naïve PLHIV.

### 1.2.2 Research Gap

The ART programme in Tanzania has completed 17 years(35), a period in which the national guidelines for ART initiation have undergone periodic modifications based on the WHO recommendations. Likewise, there has been nearly 200 folds increase in the number of PLHIV on ART to date, from about 5000 PLHIV that were on ART at the beginning on the free ART program in 2005 in Tanzania(1). The rapid expansion of ART coverage may coincide with changes in the prevalence of HIV drug resistance among HIV infected ART-naïve population(37). Also, ART regimens may impact differently on the prevalence rates of HIV drug resistance among PLHIV(46). This is mainly because different ART regimens differ in their genetic barrier to developing resistance, pharmacokinetic-profile, and tolerability(47).

Even so, PDR data in Tanzania is scarce, mainly based in urban areas and come from studies with only small numbers of participants being evaluated in each study(29,30,34–36,48–52) Therefore, it is difficult to generalize the trends in prevalence of PDR in individual demographic and population groups. This situation could limit the effectiveness of HIV prevention approaches, such as those limiting onwards HIV transmission. This thesis aimed to improve availability of local evidence and support to adapt preventive measures and effective response to the threats of HIV drug resistance.

Additionally, as dolutegravir rollout continues in LMIC, information from this thesis contributes to evidence demonstrating the efficacy of dolutegravir alongside a compromised NRTI backbone under programmatic conditions in resources limited setting. Information generated in this study provides additional knowledge on the change in prevalence of PDR for over a full decade of ART rollout, in a representative African rural and semi urban setting, from the era of roll-out to the scaling up of ART in many places of Africa. Data generated are of great importance for the Tanzanian National AIDS Control Program, as well as for the region. The study contributes to the essential data needs for mitigating transmitted HIV-1 drug resistance in resources limited setting.

### 1.2.3 Key concepts

#### 1.2.3.1 Transmitted and pre-treatment HIV-1 drug resistance

HIV transmitted drug-resistance (TDR), or primary HIV drug-resistance, refers to drug resistance in HIV infected persons with no prior history of ART exposure, mainly acquired during initial infection with HIV drug-resistant variant(s), or "during subsequent re-infection with a resistant virus, referred to as superinfection"(11). Whereas pretreatment HIV drug-resistance (PDR) or pre-antiretroviral therapy is drug-resistance in HIV infected person prior ART initiation, which can either be a result of transmitted drug-resistant variant(s) or acquired during previous ART exposure, such as in women given ART to prevent mother-to-child transmission of HIV. PDR can arise either through transmission of drug-resistant HIV-1 variants from a person with acquired drug resistance, or by transmission of primary drug-resistant HIV from another ART naïve person(11).

PDR may compromise the effectiveness of the initial ART, as evidenced in previous systematic reviews, that the odds of virological failure is higher in patients with PDR mutant variants compared to their counterpart infected with wild-type virus only [45]. Likewise, maternal PDR may diminish efficacy of the regimen to prevent mother-to-child transmission (PMTCT) and future treatment options. Thus, information of the levels and trends of PDR is essential for monitoring of HIV control programs, such as PMCTC.

Pre-ART HIV drug-resistant variant(s) may revert to wild-type virus, or be overgrown by wild type variants, as such may exist at low levels(53–55). Hence PDR studies not considering patient populations with early HIV infection may underestimate the levels of mutations. Similarly, some HIV drug resistance associated mutations are known to confer viral fitness equally or more than the wild HIV variant(s). For example, the K103N mutations have been shown to persist for years even without drug pressure, which is not surprising that K103N is commonly detected in recently infected individuals(23). This may partly explain the majority of NNRTI transmitted mutations, observed among ART-naïve patients infected with HIV drug resistant variants.

Until recent, PDR was mainly driven by NNRTI associated HIV drug resistant mutations. In many setting in LMICs prevalence of PDR to NNRTIs exceeds 10%(56), which underscore the importance of WHO recommendation to replace NNRTI with a much more potent-the intergrase inhibitors, i.e., Dolutegravir-based ART regimen. Notably, NRTI associated PDR is reportedly rising, but in many setting to a level of about 5%. Similarly, PI associated PDR are reassuring low in LMICs. PI are characterized by relatively high genetic barrier to developing resistance, also until recent the usage of PI was relatively still very low, thus with recent increasing patients switched or started on PI-based regimen, there is need to systematically evaluate emergence and spread of mutations conferring resistance to PI.

1.2.3.2 HIV-1 drug-resistant minority variants

HIV-1 Minority variants are virus that exist in very low proportional within the viral population, that may go undetected when using standard viral population sequencing methods(57). For example, in a detection methods comparison study, HIV-1 drug-resistance prevalence rates of 8% and 31.3% were detected among recently infected HIV-1 patients using standard population-based Sanger sequencing and Next-Generation sequencing (NGS) respectively(18). Similarly, a pooled analysis of 10 studies involving ART-naïve patients with no detectable HIV-1 drug-resistance mutations using population based-Sanger Sequencing, showed that 14% harbored NRTI/NNRTI mutations by ultra-sensitive assay(18).

Clinical significance of minority HIV-1 variants is still an active area for investigation. However, some studies have shown that minority HIV drug resistant mutations prior to ART initiation are associated with more than two-folds increase in the risk of virologic failure [(55,58–62). For example, a retrospective samples analysis of the anonymized population in Uganda, reported 64% of failing patients without detectable HIV-1 drug resistance associated mutations as determined by population-based Sanger sequencing, harbored minority HIV drug resistant variants. Which may explain the continued virology failure observed in that population(63).

Likewise, Rupérez et al., while studying a population of pregnant women in Mozambique, observed that all emerged drug resistance associated mutations during pregnancy were minority mutants, underscoring the importance of minority HIV-1 variants(64). Nevertheless, such virologic/clinical effects of minority HIV-1 variants have not been apparent in some studies(65).

Minority HIV-1 drug resistant variants may go undetected with standard population-based genetic analysis methods. However, may emerge especially under drug pressure. Pimenta *et al*, while studying a population of HIV-1 infected ART-naive pregnant women in Sao Paulo, Brazil, did not find any HIV drug resistant mutations (66). A similar, recent study in Uganda did not detect HIV drug resistant mutations among ART-naive Ugandan women initiating Option B+ (67). The levels of pretreatment HIV drug resistant mutations in above studies, may have been underestimated. Firstly, the analytical technique employed detect the majority viral population, at about 20% of the virus population(18). Whereas HIV drug resistant mutations may exist at low levels, particularly in the absence of drug pressure. A similar study in Mozambique, found that 10% of pretreatment HIV drug resistant mutations among pregnant women using NGS, which is the genetic analysis method able to detects minority HIV drug resistant mutations to as lower threshold as 1% of the viral population. In this study, all the emerged HIV drug resistant mutations during pregnancy were minority variants, which underscore the importance of minority HIV drug resistant mutations (64).

#### 1.2.3.3 Recent HIV infections testing assays and their limitations.

Several assays and testing strategies based on HIV antigens, or HIV antibodies and HIV viral RNA have been developed over the years to distinguish recent from chronic HIV infections. The advantage of these assays is that they can be carried out retrospectively on stored blood samples from cross-sectional studies, and they are cheaper and simpler to perform than following cohort studies. However, there are several limitations of the assays that currently limit their widespread applicability, the most important of which relate to the misclassification of the tests- resulting in false recent HIV infection, estimation of the window period, which varies substantially by HIV subtype and host population and low reproducibility. To overcome these misclassification of tests, new HIV-1 recent infection testing algorithm for HIV-1 Limiting Antigen (LAg) Avidity test have included HIV viral load test, and antiretroviral test results(68).

#### 1.2.3.4 Recent HIV infection and drug resistance

Recent HIV infection (RHI) is considered the phase of ≤6 months after infection, during which anti-HIV antibodies become detectable(69). People with RHI may be more likely to transmit HIV to others due to relatively high HIV viral load (HVL) in blood and genital secretions, absence, or few HIV specific blocking antibodies, fewer viral quasispecies diversity and possibly high transmissible, and which may possibly contain HIV-1 drug resistant variants(70).

Data on PDR among recently HIV infected ART-naive patients from routine surveillance is crucial to inform interventions to control the HIV epidemic. However, prevalence estimates of HIV-1 drug resistance among recently HIV infected ART-naive patients in Tanzania has not yet been described. Previous studies show that HIV resistant variants are significantly higher in recently HIV infected population than in those with chronic HIV infections(70). For example, PDR prevalence of 20.9% vs 8.6 % was documented in recently HIV infected population and chronic HIV infection respectively(71). Also, a study in North-West India reported a moderate to high prevalence of PDR at 16% in patients with RHI, in setting where earlier studies have shown lower level of PDR prevalence(72). Likewise, a study in Rio de Janeiro, Brazil documented TDR prevalence rate of 16.3% among acutely/recently HIV-infected individuals, which was higher than those found in chronically HIV-infected individuals in Brazil(73).

Similarly, relatively higher prevalence of TDR was reported in a population-based household survey, that involve adult patients with RHI in 2012 in Kenya(74). Hypothetically, higher levels of PDR may be observed when targeting the recent HIV infected population, which is key consideration while estimating the burden of PDR.

#### 1.2.3.5 HIV-1 subtypes and circulating strains in Tanzania

The distribution and dynamics of HIV-1 viral subtype, matched to HIV drug resistant mutations or changing ART regimens over time has not been described in Tanzania, and in many other SSA countries. Previous studies conducted in Tanzania at the beginning of rollout of free ART in Tanzania have reported subtype A1 being the most prevalent, followed by subtype C(32,34,35) and D, CRF-CD and URF have been reported in the most recent studies(32). Nonetheless, these studies were only conducted in urban settings and had only small numbers of participants been evaluated. Therefore it is difficult to generalise the trends of HIV variants-linked to HIV drug resistance mutations or changing ART regimens over time, in individual demographic and population groups.

Likewise, previous studies have evidently demonstrated that certain HIV subtype are more prone to developing mutations. For instance, HIV subtype C is known to be more prone to develop K65R mutation, which confer intermediate to high level resistance to Tenofovir. Thus, it is essential to monitor the emergence and spread of the mutations such as K65R. Thus, molecular epidemiological studies of HIV subtypes linked to long-term clinical and epidemiological data from under-sampled areas such as rural Tanzania, are key component in assessing and addressing the threat of HIV drug resistance.

#### 1.2.3.6 Pre-treatment HIV drug resistance to the dolutegravir co-administered Nucleoside reverse transcriptase inhibitors

The current new first line treatment recommended by the WHO, the integrase inhibitor dolutegravir in a fixed dose combination with tenofovir/lamivudine (TLD). Dolutegravir is known for its high and rapid viral suppression and a high genetic barrier to developing resistance. As such, TLD is predicted to curb the number of new HIV infections and the spread of resistance – leading the way to control of the HIV pandemic.

Most evidence on efficacy of dolutergravir-based regimens comes from randomized controlled trials conducted in high-income countries, with a low background level of HIV-drug resistance. In resource-limited settings, with limited or absent viral load and HIV drug resistance testing, long delays in the management of uncontrolled viraemia, could facilitate emergence of resistance to the co-administered NRTIs and possibly dolutegravir.

Also, pre-existing HIV resistances to drugs used in combination might lead to dolutergravir functional monotherapy and potential for emergence of dolutergravir resistance(75). European cohorts have shown that, virological efficacy of dolutergravir monotherapy associates with virologic failures and risk of dolutegravir resistance(76). Analysis from the NADIA and DAWNING trial shows that no evident positive effect of inactive NRTI backbone used in combination with dolutegravir; Although observed emerging intermediate -or -high level dolutegravir resistance within the 48 months follow-up raises concern(77,78). Certainly, surveillance of pre-existing resistance to the co-administered NRTIs remains particularly important.

1.2.3.7 Trends of HIV-1 pretreatment HIV drug-resistant mutations

The wider coverage of ART may increase the rates of disorderly use of ART, resulting into increased rates of acquired HIV-DRM, which may in turn be transmitted to individuals who have never been exposed to ART(37). Thus, analysis of time-trend in PDR is essential to monitor the ART regimen and evaluate the effectiveness of the program.

Available data in SSA shows increasing trend of PDR among general adult population, which is mainly driven by NNRTI associated HIV drug resistant mutations (25,26,79). Also, there has been noticeable increase in NRTIs, particularly the Tenofovir associated HIV drug-resistance mutation(26). e.g., a study conducted in two clinics in Nairobi, Kenya in the year 2006 and 2014 respectively, reported a nearly tripled number of cases of K65R mutations(80). The reverse transcriptase (RT) mutation K65R results in a four-fold decrease in TDF susceptibility and is selected by TDF, didanosine (ddI), d4T and Abacavir (ABC)(81).

A pooled analysis of studies on PDR in South Africa shows a relatively stable prevalence of PDR at <5% until 2009, and afterward increased to 11.9% in 2015(26). Similarly, a study involving seven West African and 2 Asian countries, where the prevalence of pre-ART drug-resistance ranged from 9.6% -24.6%(82). On the contrary, data from European HIV cohorts shows a decline or rather stable prevalence of PDR, at <7% (21–23)Examples of such cohorts are United Kingdom (UK) national representative PDR database which show stable or declining prevalence rates of PDR(83,84). Similar trend has been documented by Sallam, et al while studying a population in Iceland between 1996-2012(22), and Machnowska et al, in German with a PDR prevalence declining between 1996-2017(85). The declining prevalence of PDR in recent studies among western population, may be explained by the routinely virologic monitoring, including HIV drug resistance testing and increasingly usage of ART regimen which have higher genetic barriers, e.g., intergrases. Most European HIV cohorts are characterized with increased genetic diversity of HIV variants, where HIV subtype other the traditionally mostly prevalent, subtype B are becoming increasingly(84,86).

### 1.2.4 Conceptual framework and concept maps

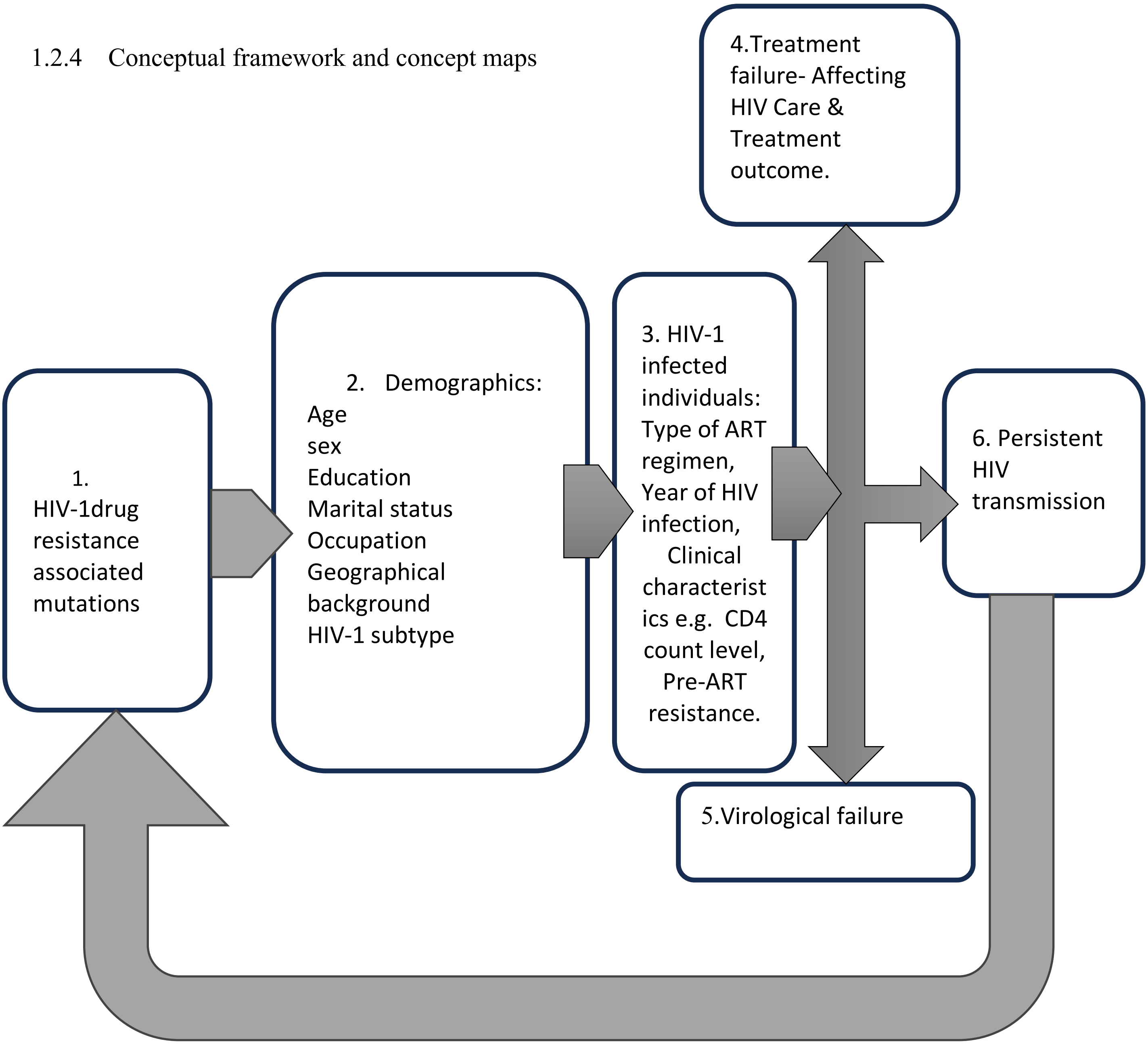



Figure 1.3 Concept map of pre-Antiretroviral Therapy HIV-1 drug resistance and associated factors.

The concept map depicts the conceptual relationship between different exposures and PDR status among HIV infected patients. The concept map show that different demographic factors may be associated with risk of being infected with a drug-resistance HIV virus, e.g. young age. Likewise, year of HIV infection may be associated with the coverage of ART, whereby increased ART access may increase use at uncontrolled level, thus increase circulation of resistant virus, which may infect an individual who have never been exposed to ART. The concepts map shows the risk of transmission of resistant virus.

### 1.2.5 Methods for studies of HIV drug resistance prevalence

Temporal analysis of HIV drug resistance associated mutation (HIV-DRM) among newly diagnosed HIV patients, enables the evaluation of HIV diversity overtime, including emergence of new HIV variants, e.g., the circulating recombinant forms (CRFs)(87), understanding the rate of change of burden of HIV drug resistant mutations and emergence of new HIV drug resistant mutations (88). Previous observational studies on molecular dynamics of pre-ART HIV-DRM have employed a variety of methods, including prospective observational methodological approach of newly diagnosed HIV infected patients. On the other hand, retrospective study methods, have been used to generate information by analyzing bio-banked specimen(89), and or analysis of data generated during routine treatment and care(90–92).

Methods for assessing prevalence and incidence of HIV-DRM are mainly quantitative. Quantitative prospective and retrospective methodological approaches have been used to determine the prevalence and incidence of HIV-DRM over-time. Machnowska et al, and Siemieniu et al, employed prospective and retrospective quantitative methods, respectively(23,93). In a prospective cohort study, Machnowska et al, studied the prevalence of transmitted HIV-DRM considering the changing landscape of HIV-1 subtypes in the German HIV-1 Seroconverter Study Cohort between 1996 and 2017, and assessed the proportion of study patients potentially at risk of ART-failure due to these transmitted HIV drug resistant mutations. Elsewhere, in a retrospective analysis Siemieniu et al., documented an increase in non-subtype B, with notable increase in subtype C and CRFS, in southern Alberta, Canada. However, the major limitation of such retrospective studies, is that there is frequently an absence of data on potential confounding factors as the data was recorded in the past. Similarly, it may be difficult to identify an appropriate exposed cohort and an appropriate comparison group.

Data from prospective studies can inform control programs on the rate of emergence of HIV-DRM in programmatic interventions, such as Option B+ which represent a challenge to PMTCT, and emergence of new circulating HIV strains. Prospective methodological approaches allow setting inclusion criteria and may enable to determine if HIV drug resistant mutations are lost during follow-up. Prospective longitudinal studies are expensive and may take long time to accomplish. Conversely, retrospective longitudinal analysis of bio-banked specimen or previously collected data, may provide invaluable information, within short period and at a low cost.

## 1.3 AIM OF THE PhD WORK

The overall aim of this thesis was to determine the trend and patterns of pre-Antiretroviral therapy HIV-1 drug resistance in Kilombero and Ulanga Antiretroviral Cohort South-western-Tanzania.

## 1.4 OUTLINES OF THIS PhD THESIS

As an introduction to the thesis, the first two chapters; Chapter 1 include a review of data on PDR, with focus on SSA, that were available before the start of the PhD research (before 2020); and Chapter 2 focus of the study methodology.

In chapter 3, A systematic review and meta-analysis was undertaken to assess the trends in PDR associated mutations in Eastern African countries, during a five-year period (2017-2022).

In chapter 4, A cross-sectional study was undertaken to determining the proportion of recent HIV-1 infection, its predictors, and PDR in recently HIV-1 infected persons in the Kilombero and Ulanga antiretroviral cohort, in rural Tanzania.

In chapter 5, A cross-sectional study was undertaken to measure the change in prevalence of PDR to NRTIs in the KIULARCO during the 15 years after ART rollout in Tanzania.

In chapter 6, A cross-sectional study was undertaken to assess the virological-outcomes at 12 months, and its predictors after initiating DTG-based ART, and prevalence of PDR among patients failing on DTG-based ART.

The final chapter 7, is a summary and general discussion of the main research findings of this thesis, followed by some concluding remarks.

**Appendices** are included after the references.

**Appendix 1** contains a copy of the ethical approval form for conducting my PhD study, **Appendix 2** contains the publication (paper I) in the *Journal of Antimicrobial Chemotherapy*, **Appendix 3** contains the publication (paper IV) in the Journal of *Open Forum Infectious Diseases.* **Appendix 4** contains the publication (paper II) under review in the *Journal of Public Health*.

# CHAPTER 2

## 2.0 Methodology

### 2.1 Research settings

The study consists of two parts;

Firstly, a systematic review and meta-analysis of primary studies reporting prevalence of pretreatment HIV-1 drug resistance (PDR) in antiretroviral therapy-naïve adults in seven Eastern Africa: Ethiopia, Kenya, Malawi, Rwanda, Mozambique, Tanzania, and Uganda (*paper I*).

Secondly, the studies in paper (II-IV) were embedded in the on-going prospective clinic-based observational Kilombero and Ulanga antiretrovirals cohort (KIULARCO) study of Peole living with HIV (PLHIV) in rural Tanzania. KIULARCO includes participants from two districts, namely, Kilombero and Ulanga in Morogoro region, South-Western Tanzania (Figure 4). The longitudinal follow-up of the cohort is conducted within the Tanzanian National AIDS Control Care Programme (NACP) framework. KIULARCO has been described in detail elsewhere(94–99). Briefly, this study was carried out within the Chronic Disease Clinic of Ifakara (CDCI), the HIV Care and Treatment Centre (CTC) of the St. Francis Referral Hospital in Ifakara, Southwestern Tanzania. CDCI is a joint project of the hospital, with the Ifakara Health Institute (IHI), the St. Francis Referral Hospital, the Swiss Tropical and Public Health Institute and the University Hospital Basel, Switzerland. CDCI was among the first rural CTCs in Tanzania started in 2005 to provide HIV care and free ART within the NACP framework. CDCI runs a patient cohort, the KIULARCO described above.

For more detailed information about patients, ethical permits, and statistical analysis, see respective papers.

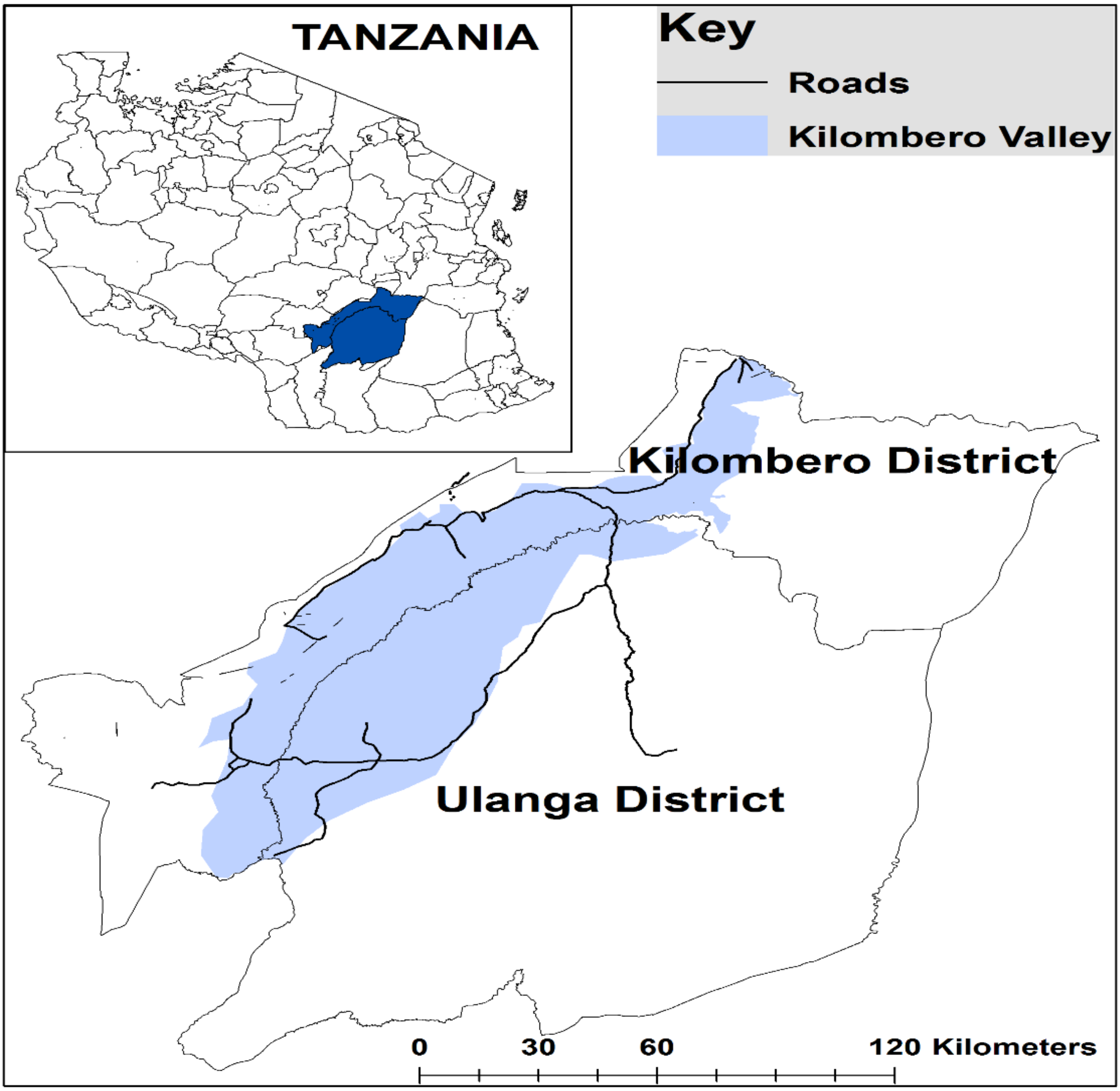



Figure 2.1. Study area for paper II-IV; Kilombero and Ulanga districts in Morogoro region, South-Western Tanzania.

## 2.2 Study population

**In paper I,** A Systematic Review and Meta-Analysis of eligible observational studies published between 1 January 2017 and 30 April 2022 about prevalence of PDR among ART-naive PLHIV (>15 years old)(56).

**In paper II- IV,** studies were be embedded in the KIULARCO of the IHI in Tanzania. KIULARCO is one of the largest long-term patients' cohorts in the East African region, started in 2005(96)(Figure 1). KIULARCO offers a clinical database and a biobank of yearly collected plasma samples collected during routine visits of patients in the past 20 years (have been stored in

-80°C freezers at the IHI laboratory in Ifakara, Tanzania), from more than 12,000 PLHIV within a population of about 600,00 inhabitants and an estimated 30,000 patients living with HIV/AIDS in the area (Figure 2). The longitudinal follow-up of the cohort is conducted within the Tanzanian National AIDS Control Care Programme (NACP) framework(96). Newly HIV-1-diagnosed adult individuals (aged ≥ 15 years) prior to ART initiation, who are enrolled in the KIULARCO were included. Study population detailed descriptions is provided in respective papers.

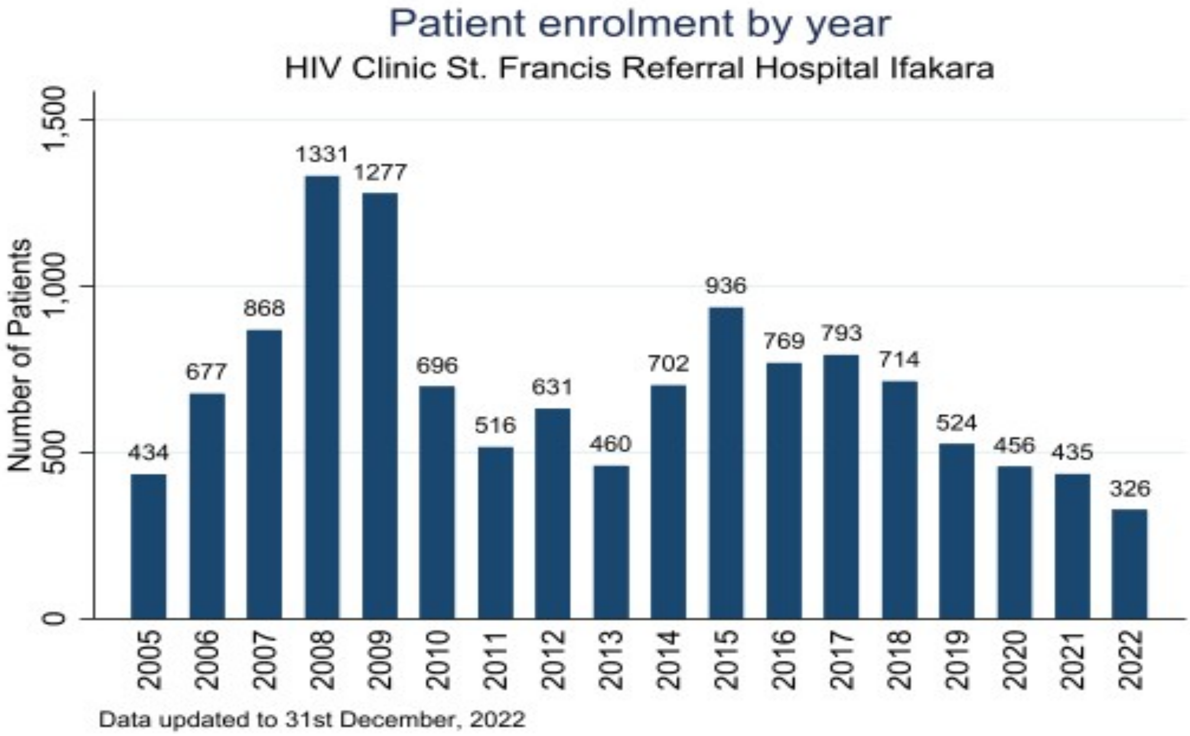



Figure 2.2. Patients' enrolment into the Kilombero and Ulanga Antiretroviral Cohort (KIULARCO) by year.

### 2.3 Sample size estimation

The estimated sample size (In paper III) was calculated based on the expected prevalence of pre-treatment HIV-1 drug resistance of 10%, with prevalence between 3.3% and 5% based on existing published data. Sampling period was, the first five-year period (2005-2009) of the ART program in Tanzania, in the most recent period (2019-2022), in each batch 130 randomly selected samples was included in the study. With this sample, the assumption was to be able to detect the prevalence ≥ 7 %, one sided 5% level of significance, with the power of 80% (Table 1). An addition of 20% for sequencing failure rate to ensure minimal sample size for power is met.

Table 1. Sample size calculations

| Established Prevalence | Expected Prevalence | Statistical Power | Calc. Sample Size* |
|---|---|---|---|
| 3.3%** | 7% | 80% | 310 |
| | | 90% | 389 |
| | 7.5% | 80% | 252 |
| | | 90% | 315 |
| | 10% | 80% | 122 |
| | | 90% | 149 |
| 5%*** | 7% | 80% | 1168 |
| | | 90% | 1519 |
| | 7.5% | 80% | 784 |
| | | 90% | 1013 |
| | 10% | 80% | 239 |
| | | 90% | 301 |

*the caculated sample sizes are calculated with a one-sided test.
**according to the last prevalence study in the KIULARCO cohort in 2009 [14]
***reported transmitted HIVDR prevalence across different sites in Africa [7]

In paper IV, sample size estimation was based on the assuming the rate of VF at 10%, a 95% confidence and 80% statistical power, sample size is 230 patients. Adding 20% sequencing failure rate minimal sample size is 276, rounded-up to 280 total sample size is required.

### 2.4 Study procedures

In paper I, a systematic review and meta-analysis of pre-treatment HIV-drug resistance (PDR) in antiretroviral therapy-naïve I reviewed all relevant articles, the supervisors independently reviewed 20% of randomly selected articles. Standardized data abstraction form was used to systematically collect data from scientific published articles. Core set of information retrieved from the articles, included bibliographic details, study design, inclusion and exclusion criteria, country and their main demographic, ART exposure history, the year(s) of sample collection, study type, study population, proportion of participants gender, and method for determining pre-treatment HIV-drug resistance (PDR), and reported prevalence of PDR. The systematic review protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) on 18/02/2022 (registration number CRD42022304207).

According to the KIULARCO protocol, patients who signed informed consent have blood samples drawn at routine clinic visits, prior to ART initiation and every 12 months thereafter. Plasma is cryopreserved at -80$^0$C for research purposes. In brief, data on demographics, clinical presentation, therapy outcome and adherence of ART and treatment outcome are being prospectively captured within an electronic database over the years. In paper II-IV, blood plasma samples of eligible patients were identified from the KIULARCO biobank, unfrozen and tested as detailed in respective papers.

## 2.5 Laboratory analysis

### 2.5.1 RNA Extraction

Briefly, RNA was extracted from 150 µl of plasma using the PureLink™ Viral RNA/DNA Mini Kit (Invitrogen™, Thermo Fisher Scientific, USA), according to the manufacturer's protocol(100).

### 2.5.2 Reverse Transcription and PCR

RNA extracts were retrotranscribed and amplified using the HIV-1 Genotyping Kit Amplification Module (Applied Biosystems™, Thermo Fisher Scientific, USA), according to the manufacturer's protocol(101). The PCR products were visualized on a gel visualization system (To confirm that the 2892 bp length of the pol gene) after being run in a 1% agarose gel at 130 V.

### 2.5.3 HIV Genotypic resistance testing

**In Paper II- IV,** Sequencing was conducted to determine the presence of HIV drug resistance-associated mutations in the *pol* gene.

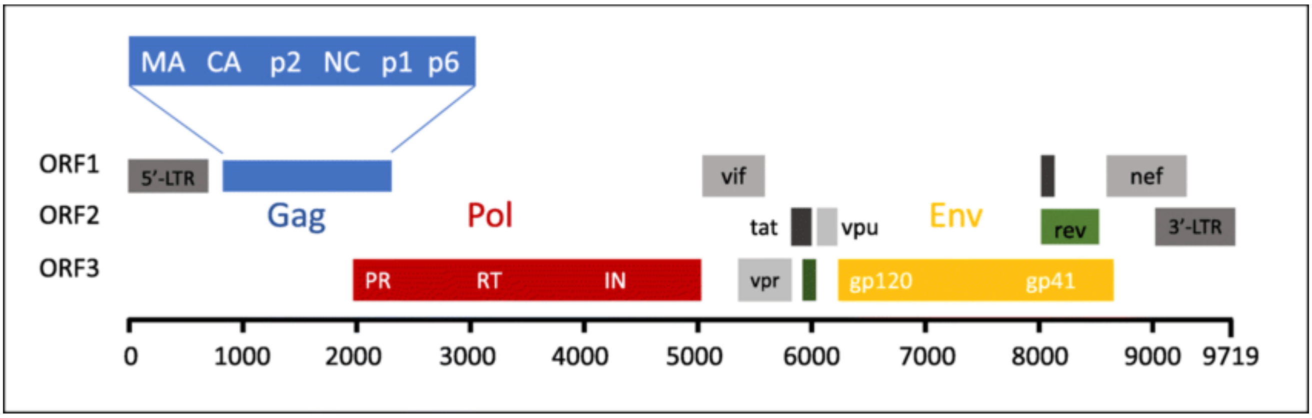



Figure 2.3 Schematic presentation of HIV-1 viral genome

Sequencing was performed using a validated in-house PCR protocol to determine the HIV-1 drug resistance-associated mutations for Reverse Transcriptase (RT), Protease (PR) and *Integrase* Inhibitor (INSTI) – mutations(42,95,99,102) A direct sequencing reaction was done using 6 overlapping primers (Table 1).

**Table 2. Sequencing primers information**

| # | Primer Name | Sequence |
|---|---|---|
| §1 | F-2150 | AGCCAACAGCCCCACCAG |
| 2 | F-2232 | GGAACTGTATCCTTTAGCTTCCC |
| 3 | F-2610 | GTTAAACAATGGCCATTGACAGAAGAAA |
| 4 | F-3007 | GGAAAGGATCACCAGCAATATTCCA |
| 5 | R-2605 | GGGCCATCCATTCCTGG |
| 6 | R-3010 | CCATCCCTGTGGAAGCACATTG |
| 7 | R-3541 | TTCTGTATTTCTGCTATTAAGTCTTTTGATGGGTCA |

## 2.6 Bioinformatics analysis

### 2.6.1 Sequence reads assembly, analysis, and annotation

**In Paper II-IV** sequence reads assembly and analysis was performed using BioEdit 7.2-program(103), each sequence was manually inspected to ensure sequence quality, and finally annotated according to the Stanford HIV Drug Resistance Database, current version (https://hivdb.stanford.edu/)(104)

### 2.6.2 Drug Resistance analysis

The assessment of HIV drug resistance associated mutations were interpreted according to the Stanford University's HIV Drug Resistance Database (HIVdb) Program version 9.2 (http://hivdb.stanford.edu). HIV drug resistant mutations conferring low, intermediate, or high-level resistance were considered. The reported protease and reverse transcriptase sequences are deposited in GenBank accession numbers are provided in respective papers.

## 2.7 Ethical approval

This study received ethics approval from the University of the Witwatersrand- Human Research (Medical) Ethics Committee. Ethical approval for the KIULARCO cohort has been obtained from

Ifakara Health Institute Review Board IHI/IRB/No: 16 – 2006 and the National Health Research Committee of the National Institute of Medical Research of Tanzania (NIMR/HQ/R.8c/Vol.I/378). Written informed consent for KIULARCO is sought from all participants ≥18years, and for children and adolescents aged <18 years from caregivers.

# PART 2

# EMPIRICAL CHAPTERS

# CHAPTER 3

**A systematic review and meta-analysis to assess the levels of pretreatment HIV-1 drug resistance in antiretroviral therapy- naïve in Eastern Africa (2017-2022).**

**Introduction**

This chapter describes the prevalence of pre-treatment HIV-1 drug resistance among ART-naïve adult general population in Eastern African countries, within a systematic review and meta-analysis, during a five-year period (2017-2022). Results are presented in research ***paper I***, entitled "Pre-Treatment HIV-1 Drug Resistance in Antiretroviral Therapy-Naïve Adults in Eastern Africa: A Systematic Review and Meta-Analysis", this has been published as an original article in the *Journal of Antimicrobial Chemotherapy.*

J Antimicrob Chemother
https://doi.org/10.1093/jac/dkac338

Journal of Antimicrobial Chemotherapy

# Pre-treatment HIV-1 drug resistance in antiretroviral therapy-naive adults in Eastern Africa: a systematic review and meta-analysis

**Alex J. Ntamatungiro [1,2]*, Juliana Kagura[2], Maja Weisser[1,3,4] and Joel M. Francis [5]**

*[1]Ifakara Health Institute, Ifakara, Tanzania; [2]Division of Epidemiology and Biostatistics, University of the Witwatersrand, Johannesburg, South Africa; [3]Division of Infectious Diseases and Hospital Epidemiology, University Hospital of Basel, Basel, Switzerland; [4]Swiss Tropical and Public Health Institute, Basel, Switzerland; [5]Department of Family Medicine and Primary Care, University of the Witwatersrand, Johannesburg, South Africa*

*Corresponding author. E-mail: antamatungiro@cartafrica.org

*Received 23 May 2022; accepted 12 September 2022*

**Background:** Pre-treatment HIV drug resistance (PDR) may result in increased risk of virological failure and acquisition of new resistance mutations. With recently increasing ART coverage and periodic modifications of the guidelines for HIV treatment, there is a need for an updated systematic review to assess the levels of the PDR among adults newly initiating ART in Eastern Africa.

**Methods:** We conducted a systematic search for studies published between 1 January 2017 and 30 April 2022 in the MEDLINE Complete and CINAHL Complete, searched simultaneously using EBSCOhost, and Web of Science. To determine the overall PDR prevalence estimates, we extracted data from eligible articles and analysed prevalence estimates using Stata 14.2.

**Results:** A total of 22 eligible observation studies were selected. The studies included a total of 5852 ART-naive people living with HIV. The overall pooled prevalence of PDR was 10.0% (95% CI: 7.9%–12.0%, $I^2=88.9\%$) and 9.4% (95% CI: 7.0%–11.9%, $I^2=90.4\%$) for NNRTIs, 2.6% (95% CI: 1.8%–3.4%, $I^2=69.2\%$) for NRTIs and 0.7% (95% CI: 0.3%–1.2%, $I^2=29.0\%$) for PIs. No major integrase strand transfer inhibitors (INSTI)-related mutations were identified.

**Conclusions:** We observed a moderate overall PDR prevalence among new ART initiators in this study. PDR to NNRTIs is more prevalent, underscoring the importance of the current WHO recommendation for replacement of NNRTIs by INSTIs. PDR to NRTIs was low but notable, which warrants continuous surveillance of pre-existing resistance to the dolutegravir co-administered NRTI in Eastern Africa.

**Background**

The Antiretroviral Therapy programme in countries in Eastern Africa: has been running for more than 15 years. Over these years, the guidelines for HIV care and treatment including ART initiation have undergone periodic modifications based on the WHO recommendations(105). Also, there has been remarkable increase in the number of people living with HIV (PLHIV) on ART to date. For example, globally the ART coverage increased from 24% in 2010 to 65% in 2018. Also 73% of PLHIV East and Southern Africa were on ART by the end of 2019(106). The scale-up of ART rollout may coincide with changes in the prevalence of pre-treatment HIV drug-resistance (PDR)(46). This is mainly due to the existing gaps in HIV preventions interventions, care, and *treatment* cascade, also potency of antiretroviral regimen and patient factors associated with emergence and spread of HIV drug-resistance mutations, such as inadequate adherence to ART(107,108)

Major improvement in antiretroviral medication in terms of safety and pill count have been achieved over the past 15 years of ART rollout worldwide and in low- and middle-income countries (LMIC) – e.g., replacement of NRTI with long-term toxicity like stavudine (d4T) and didanosine (ddI) to more favourable NRTIs like tenofovir disoproxil fumarate (TDF). Until recently, most LMIC were using 2 NRTI and 1 NNRTI as first line treatment according to the recommendations by the WHO. The preferred regimen was tenofovir disoproxil fumarate (TDF) + lamivudine (3TC) or emtricitabine (FTC) + efavirenz (EFV) as a fixed dose combination. Dolutegravir (DTG), one of the first developed integrase inhibitors for treatment of HIV infection became available to LMIC by the end of 2017, as the generic fixed dose combination tenofovir/lamivudine/dolutegravir (TLD). Since then, TLD has been rolled out in many countries in sub-Ssaharan Africa (SSA). It is worth noting that, ART regimens may impact differently on the prevalence rates of PDR. This is mainly because ART regimens differ in their genetic barrier to developing resistance, pharmacokinetic-profile, and tolerability

PDR may compromise ART efficacy resulting in increased risk of virological failure, acquisition of new HIV drug resistant mutations and need of ART switch(109,110). Previous review study using available data in eastern Africa, include studies published until 2016(11). However, this was a period during which ART was not widely available, also ART represented in the current WHO guidelines, such as Tenofovir-based ART was still relatively new in many LMIC. Thus, with increasing ART coverage and periodic modifications of the guidelines for HIV care and treatment including ART initiation, there is the need for an updated systematic review to assess the levels of the PDR associated mutations among adults newly initiating ART in eastern Africa countries from 2017 until 2022.

**Methods**

**Study design**

We conducted a systematic search for studies published during January 1,2017 to April 30, 2022, about prevalence of PDR among ART-naïve PLHIV (>15 years). The data had to be collected in one or several of the countries of eastern Africa (Ethiopia, Kenya, Malawi, Rwanda, Mozambique, Tanzania, and Uganda). The systematic review protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) on 18/02/2022 (registration number CRD42022304207).

**Search Strategy and Selection Criteria**

An electronic search was conducted from three databases: MEDLINE Complete and CINAHL Complete searched simultaneously using EBSCOhost and Web of Science. The search advanced search approach was carried out according to PRISMA guidelines (*Figure 1*)(111), using the following search terms: (HIV OR 'Human Immunodeficiency Viruses) AND ('Pre- Antiretroviral Therapy' OR 'Pre-Antiretroviral OR 'Pre-treatment' OR 'Pretreatment' OR antiretroviral OR ART OR drug OR antiviral OR ARV OR ART OR 'Nucleoside RT Inhibitor' OR NRTI OR NNRTI OR INSTI OR 'integrase inhibitors' OR 'protease inhibitors' OR 'non-nucleoside reverse transcriptase inhibitor' OR 'nucleoside reverse transcriptase inhibitor') AND ('HIV-1 Drug Resistance' OR 'transmitted HIV drug resistance' OR 'drug resistance' OR 'Resistance Mutations') AND (Burundi OR Ethiopia OR Kenya OR Malawi OR Rwanda OR Mozambique OR Tanzania OR Uganda OR eastern Africa). We screened studies for eligibility based on title and abstract. Eligible studies had to report original data about the proportion of PDR in a sample of PLHIV (>15 years), with no prior history of ART exposure. Also, studies had be published in English. Publications without primary data such as review articles and letters to editors were excluded. We included quantitative studies involving HIV-1 drug resistance mutations for Reverse Transcriptase (RT), Protease (PR) and, integrase strand transfer inhibitors *(INSTI)*- mutations.

**Study Selection process and Extraction**

Standardized data abstraction form was used to systematically collect data from journal articles. AJN and JMF independently screened articles for selection. This involved an assessment of articles based on titles and abstract. In the case of insufficient information in the title and abstract, the full text of the specific article was retrieved and assessed. JK was involved in case the two reviewers failed to reach a consensus to include the article. We recorded the source, year of sampling, country origin, gender proportion, study period, number of study participants, prevalence of PDR to NRTI, NNRTI, PI, INSTI and HIV subtypes.

## Data Analysis

The statistical analysis was done using STATA 14.2. We assessed heterogeneity using $I^2$ statistics, where $I^2 > 50\%$ heterogeneity was high. Studies quality assessment was done using JBI's critical appraisal tools. (https://jbi.global/critical-appraisal-tools). A funnel plot and Egger's test were applied to detect any publication bias. A *p*-value of <0.05 was considered statistically significant.

## Results

### Characteristics of Included studies

We identified 889 potential research articles from three databases according to our search strategy. A total of 22 observational studies were selected, representing a total of 5852 PLHIV with no prior history of ART exposure (Figure 1), with available resistance data derived from RT and/or PR and/or Integrase sequences in seven eastern African countries; Ethiopia, Kenya, Malawi, Rwanda, Mozambique, Tanzania, and Uganda (Table 1). All the research articles included in this study involved heterosexual patients' population, expect one research article that included men who have sex with men (MSM)(112).

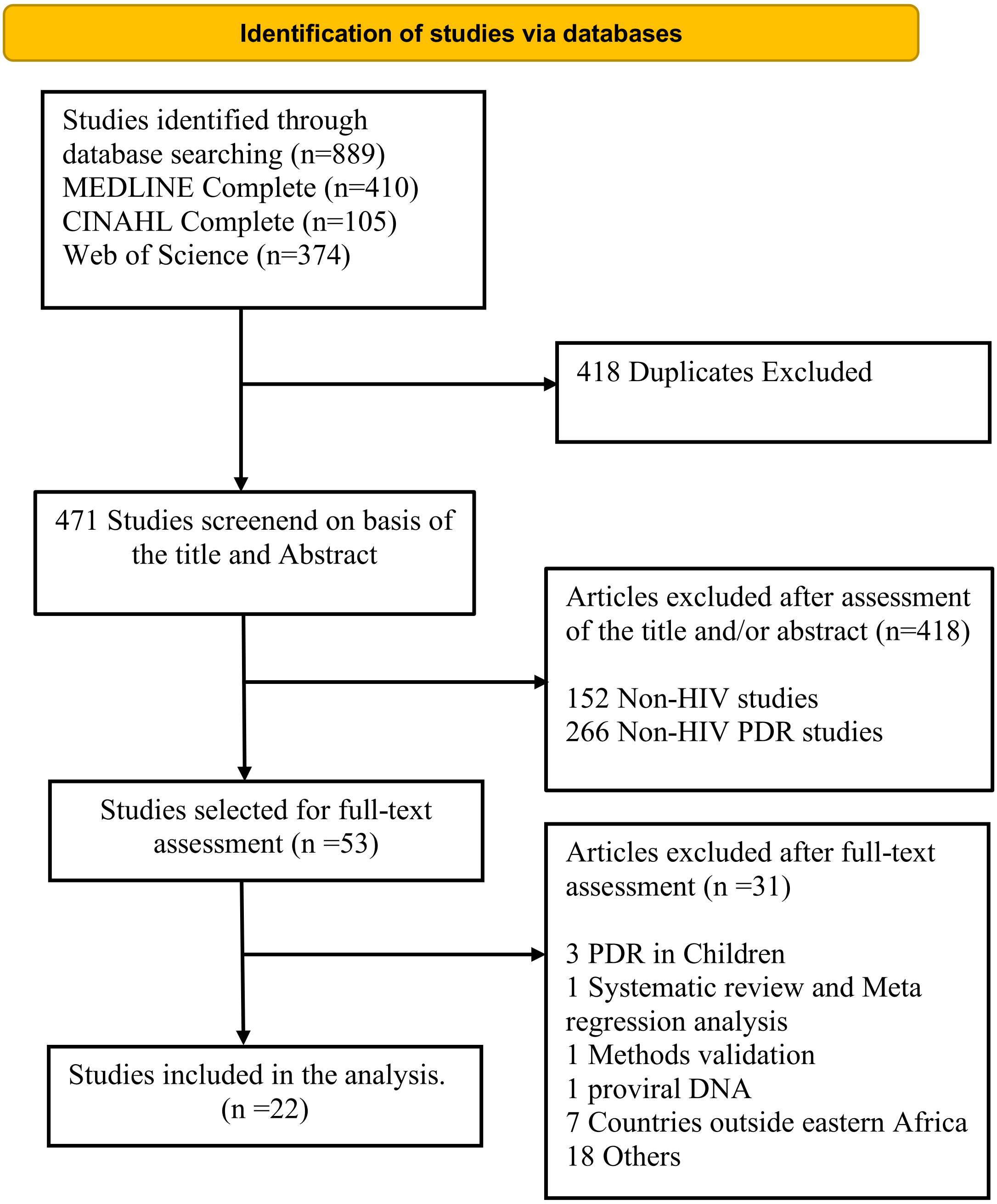



FIGURE 1 | Flow diagram of search results and screening

**Table 1:** **The characteristics of included datasets with reverse transcriptase and/or protease sequences from ART-naïve adults.**

| AUTHOR, PUBLICATION YEAR, REFERENCE | Year of Sampling | Country Origin | Study Population | Females (%) | Study Design | A Point Prevalence Estimate (%) | | | | GRT-Method | HIVDRM Identification-Method |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Any | NNRTI | NRTI | PI | | |
| **HASSAN *ET AL.*, (2019)(113)** | 2008 | Kenya | 50 | 72% | Retrospective hospital-based cohort study | 24 | 6.0 | 8.0 | 12 | NGS | *HIVdb* |
| **TELELE *ET AL.*, (2018)(114)** | 2009-2011 | Ethiopia | 461 | 58% | Prospective hospital-based cohort study | 3.9 | 1.5 | 2.0 | 0.4 | PBSS | HIVdb |
| **HASSAN *ET AL.*, (2018)(112)** | 2005-2017 | Kenya | 97 | 0 % | Prospective population-based cohort study | 8.2 | 7.2 | 2.1 | 0.0 | PBSS | HIVdb |
| **CROWELL *ET AL.*, (2020)(115)** | 2013- 2019 | Tanzania | 154 | 59.6% | Prospective hospital-based cohort study | 8.0 | 6 .0 | 3.0 | 1.0 | PBSS | HIVdb |
| | 2013-2019 | Kenya | 254 | 59.6% | Prospective hospital-based cohort study | 7.0 | 7.0 | 2.0 | 0.0 | | |
| | 2013-2019 | Uganda | 291 | 59.6% | Prospective hospital-based cohort study | 14 | 10.0 | 5.0 | 1.0 | | |
| **BARABONA *ET AL.*, (2019)(29)** | 2017 | Tanzania | 47 | 75.7% | Cross-sectional hospital-based study | 29.8 | 25.5 | 8.5 | 0.0 | PBSS | HIVdb |
| **RUDOVICK *ET AL.*, (2018)(30)** | 2013-2015 | Tanzania | 235 | 75.7% | Cross-sectional hospital- | 4.7 | 3.0 | 1.7 | 0.0 | PBSS | HIVdb |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | based study | | | | | | |
| **VON BRAUN *ET AL.*, (2018)(116)** | 2015 | Uganda | 152 | 62.8% | Cross-sectional hospital-based study | 5.9 | 5.3 | 3.3 | 0.0 | PBSS | HIVdb |
| **RUTSTEIN *ET AL.*, (2019)(117)** | 2012–2014 | Malawi | 45 | 39% | Prospective hospital-based cohort study | 24.4 | 20 | 6.7 | 0.0 | PBSS | HIVdb |
| **SILVERMAN *ET AL.*, (2017)(118)** | 2013–2014 | Kenya | 815 | 65.1% | Cross-sectional hospital-based study | 9.4 | 9 | 0.7 | 0.0 | PBSS | OLA |
| **MILNE *ET AL.*, (2020)(119)** | 2006-2010 | Kenya | 97 | 100% | Prospective hospital-based cohort study | 6.2 | . | . | . | PBSS | HIVdb |
| **ONYWERA *ET AL.*, (2017)(74)** | 2012 | Kenya | 87 | 72% | Cross-sectional population-based study | 9.2 | 6.9 | 4.6 | 1.2 | PBSS | HIVdb and WHO 2009 Mutation list |
| **CARNIMEO *ET AL.*, (2021)(120)** | 2017–2018 | Mozambique | 196 | 58.7% | Cross-sectional hospital-based survey | 16.8 | 14.8 | 4.6 | 0.5 | PBSS | HIVdb |
| | | | 258 | 66.7% | Cross-sectional hospital-based survey | 26.4 | 25.6 | 4.3 | 0.4 | PBSS | |
| **KIROS *ET AL.*, (2020)(121)** | 2018 | Ethiopia | 51 | 54.9% | Cross-sectional hospital-based study | 9.8 | 7.8 | 2.0 | 0.0 | PBSS | *HIVdb and IAS-USA 2019 Mutation list* |
| **WATERA *ET AL.*, (2021)(122)** | 2016 | Uganda | 335 | 61.5%* | Cross-sectional survey | 18.0 | 13.7 | 6.0 | 1.5 | PBSS | HIVbd |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **BECK *ET AL*., (2020)(123)** | 2006 | Kenya | 303 | 66.7% | Cross-sectional hospital-based cohort analysis | 2.6 | . | . | . | PBSS | OLA |
| | 2010 | Kenya | 97 | 100% | Cross-sectional hospital-based cohort analysis | 6.2 | . | . | . | | |
| | 2013–2014 | Kenya | 679 | 65.7% | Cross-sectional hospital-based cohort analysis | 4.4 | . | . | . | | |
| **MCCLUSKEY *ET AL*., (2019)(124)** | 2005-2013. | Uganda | 738 | 69% | Cross-sectional hospital-based cohort analysis | 3.5 | 2.8 | 1.0 | 0.0 | PBSS | HIVbd |
| **NEUHANN *ET AL*, (2020)(125)** | 2014-2015 | Malawi | 197 | 61.9% | Cross-sectional hospital-based cohort analysis | 14.2 | 13.7 | 0.5 | 0.0 | NGS | HIVdb |
| **ZHOU *ET AL*., (2018)(126)** | 2008 | Malawi | 20 | . | Cross-sectional hospital-based cohort analysis | . | 9 | 2 | 0 | NGS | . |
| **ARIMIDE *ET AL*., (2018)(127)** | 2011–2013 | Ethiopia | 67 | 87% | Cross-sectional hospital-based survey | 6.0 | 6.0 | 0.0 | 0.0 | PBSS | HIVdb |
| **NDASHIMYE *ET AL*., (2018)(128)** | 2007-2011 | Uganda | 87 | 100% | Retrospective hospital- | 8.7 | 8.7 | . | . | PBSS | HIVdb |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | based cohort study | | | | | | |
| **UMVILIGIHOZO *ET AL*., (2021)(129)** | 2005-2011 | Rwanda | 21 | . | Prospective hospital-based cohort study | 19 | 19 | . | . | PacBio RSII | HIVdb |
| **SANGEDA *ET AL*., (2019)(52)** | 2010 | Tanzania | 18 | . | Prospective hospital-based cohort study | 0.0 | 0.0 | 0.0 | 0.0 | PBSS | HIVdb |

**HIVdb**, HIV Drug Resistance Database; **IAS-USA**, International *AIDS Society-USA; NGS, Next Generation Sequencing; PBSS-Population based Sanger Sequencing*; data not analysed or not provided; **OLA**, Oligonucleotide Ligation Assay; **HIVDRM**, HIV drug resistance mutation; **GRT**, Genotypic Resistance Testing.

**The Overall Prevalence of Pretreatment Drug Resistance-Associated Mutations**

The overall prevalence of PDR was 10.0% (95% CI: 7.9%–12.0%, $I^2$ =88.9%) among 22 studies (*Figure 2*). Sub-analysis by country shows that the prevalence of PDR in Ethiopia 4.6% (95% CI: 2.4%–6.9%, $I^2$ =12.0%, in 3 studies), in Kenya 7.0% (95% CI: 4.6%–9.3%, $I^2$=79.6%, in 7 studies), in Malawi 17.6% (95% CI: 8.2%–27.1%, $I^2$ =54.9%, in two studies), in Mozambique 21.6% (95% CI: 12.2%–30.9%, $I^2$ =83.8%, in two studies), in Rwanda 19.0% (95% CI: 2.3%–35.8%, in one study), in Tanzania 10.8% (95% CI: 3.0%–18.6%, $I^2$ =86.0%, in three studies) and in Uganda 10.0% (95% CI: 4.1%–16.0%, $I^2$ =93.6%, in five studies). HIV-1 subtypes data is reported in 14(63.6%) of the selected studies; the presence of HIV-1 subtype in Ethiopia, Malawi and Mozambique are predominantly subtype C; in Kenya subtype A and D; in Tanzania subtype A, C and D, whereas in Uganda subtype A and D are prevailing.

Publication bias, as examined by the funnel plot and Egger's test, showed no publication bias in this analysis.

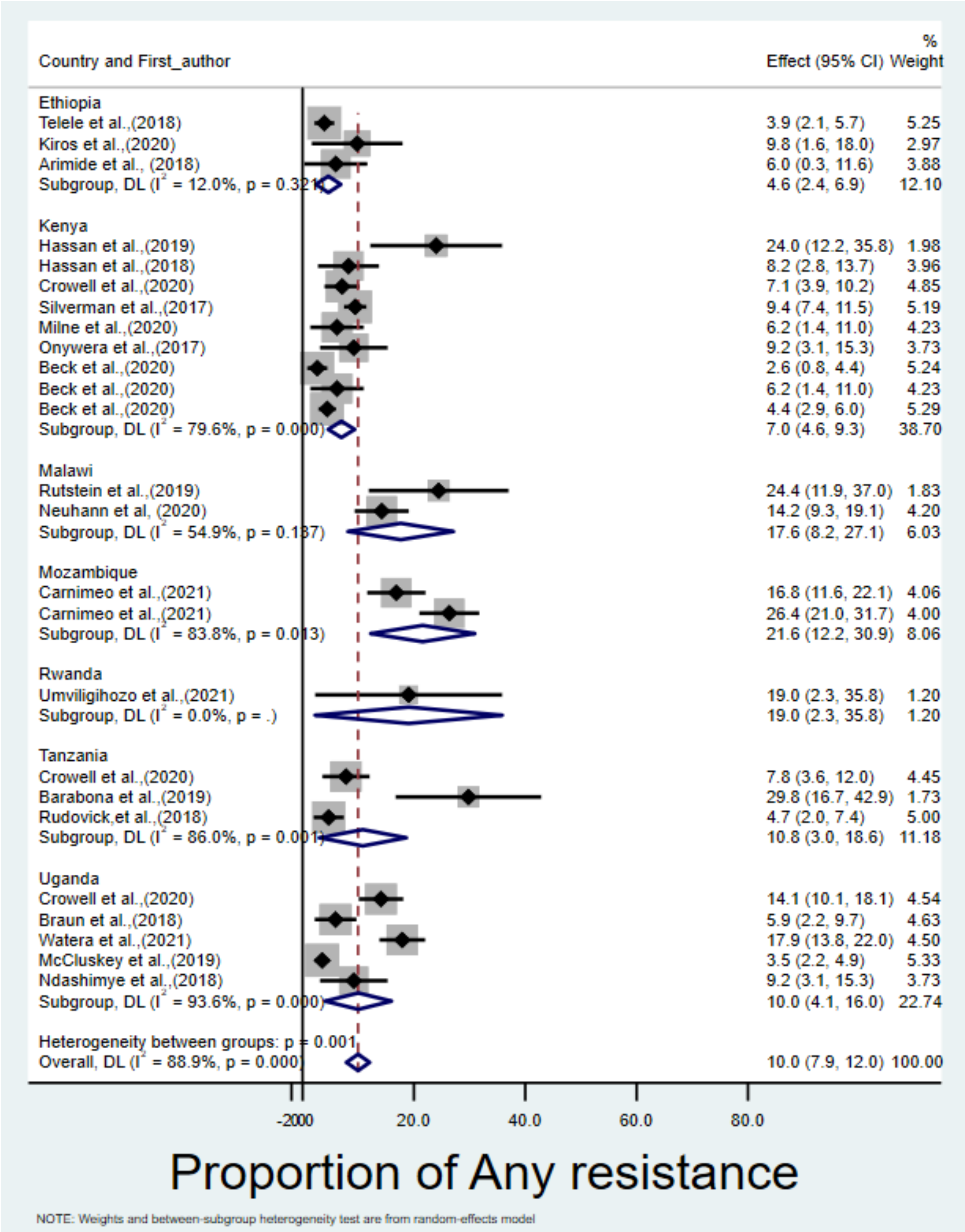



FIGURE 2 | Overall prevalence of PDR. Note: the red dotted line indicates the overall prevalence of PDR.

**PDR of RT inhibitors**

The prevalence of mutations conferring resistance to NNRTI was 9.4% (95% CI: 7.0%–11.9%, $I^2$=90.4%) in 21 of the included studies (*Figure 3*). NNRTI resistance reached highest level among different HIV-drugs categories. The prevalence of PDR to NRTI is 2.6% (95% CI: 1.8%–3.4%, $I^2$=69.2%, in 18 studies (*Figure 4*). Nonetheless, prevalence of PDR to NRTI above 5% have been reported in several countries, including 8.5% in Tanzania(29); 5.2%-6% in Uganda(122) and 6.7% in Malawi(117). Also, population with a high risk of PDR to NRTI are young adults (2.9% in a study from Uganda)(130), and people injecting drugs (15.5% in a study from Kenya)(43). *Supplementary Table S1*.

**PDR of PIs**

Overall, PI resistance was re-assuring low, 0.7% (95% CI: 0.3%–1.2%, $I^2$=29.0%) in 8 studies (*Figure 5*). High levels of PDR to PI were reported in studies detecting HIV drug resistant mutations using Next Generation Sequencing (NGS). For-example, PDR to PI of 12%and PDR to PI of 15% was documented by studies using NGS in in Kenya and Malawi respectively, whereas analysis of same samples in the later study by consensus sequencing no mutation associated with PI resistance was detected(113,126)

**PDR of INSTIs**

We identified four studies that examined HIV-1 INSTI associated resistance in patients prior to ART initiation, which found no participants had mutations associated with high levels of INSTI resistance.

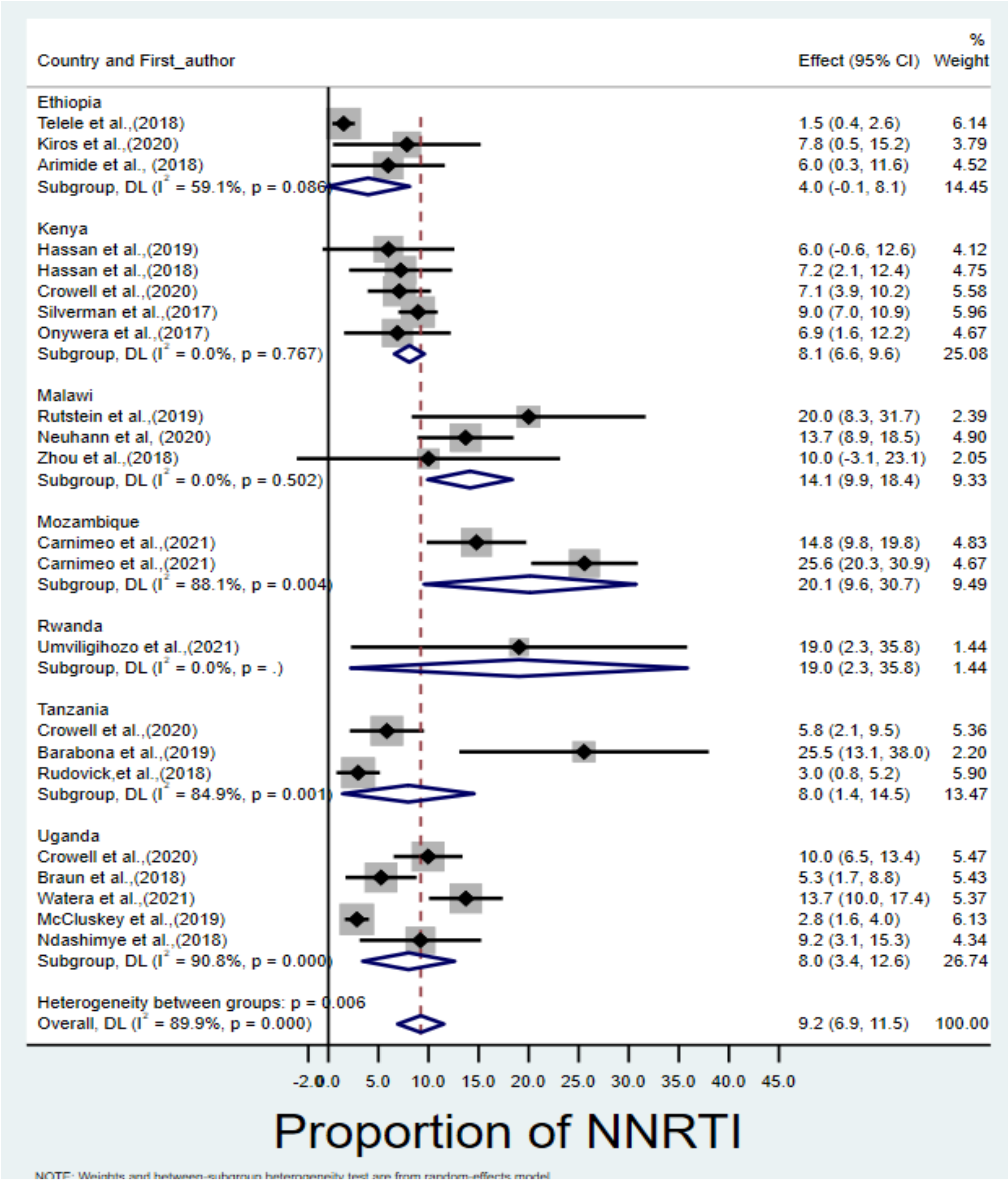



FIGURE 3 | Prevalence of PDR to Non-nucleoside reverse transcriptase inhibitor

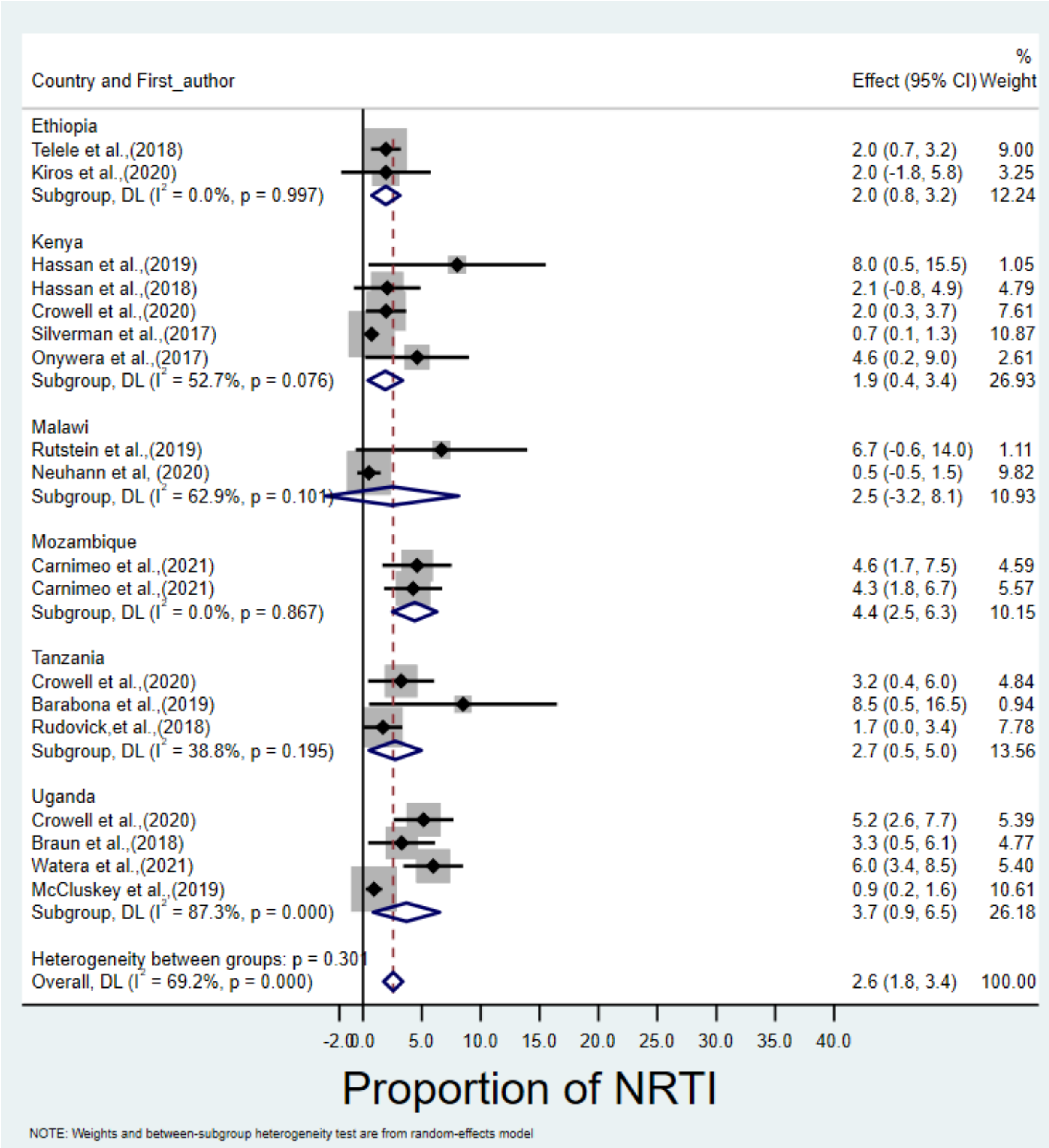



FIGURE 4 | Prevalence of PDR to nucleoside reverse transcriptase inhibitor

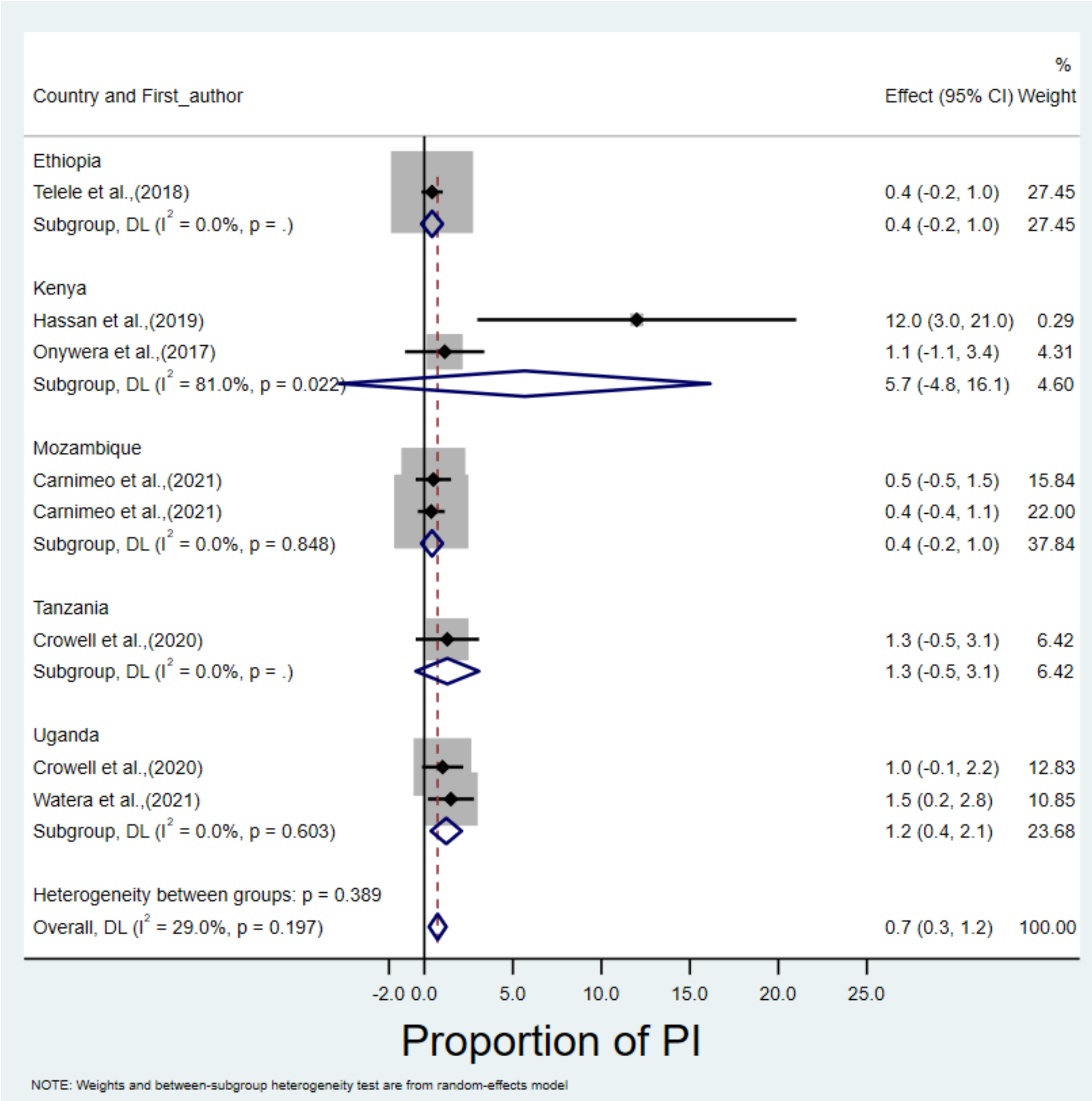



FIGURE 5 | Prevalence of PDR to protease inhibitor

**Discussion**

In this study, we investigated the overall prevalence of PDR in ART naïve adults living with HIV, in seven eastern African countries for over a period of five years. We observed an overall PDR prevalence of 10.0% (95% CI: 7.9%–12.0%); Whereas previous review study using available data in eastern Africa published until 2016, reported overall PDR prevalence estimates of 8.7%(11). Like our finding PDR was highest to the NNRTI drug class, particularly in more recent studies across the region, which underscores the importance of the current WHO recommendation, to replace NNRTI within the fixed dose combination by dolutegravir for HIV treatment, which has now been introduced in many SSA countries and other LMIC.

Good rates of virological suppression among patients with virological failure on NNRTI based first line ART- possibly as results of suboptimal adherence, have been observed following switching to dolutegravir- based ART(77). Conversely, analysis from the ADVANCE trial indicates that PDR to NNRTI is associated with lower viral suppression rates in patients initiating the dolutegravir based ART(131). Even though, the mechanism by which NNRTI-associated mutations affects dolutegravir-based therapy is still unclear, the widely spread of NNRTI-associated mutations among newly ART initiators in low- and middle-income countries raises concern over the long-term efficacy of dolutegravir-based therapies in resources limited settings, where access to viral load and HIV drug-resistance testing is still limited, and long delays in the management of uncontrolled viremia have been documented.

We observed low however notable prevalence of PDR to NRTI, which may pose risk for the currently preferred first-line ART regimen for HIV treatment in LMIC, which is the fixed dose combination of dolutegravir with tenofovir/lamivudine. HIV drug resistant mutations to NRTI may lead to de facto dolutegravir monotherapy with a risk of acquisition of dolutegravir resistance. Existing evidence, such as from the NADIA and DAWNING trial shows that no evident positive effect of inactive NRTI backbone used in combination with dolutegravir; Although observed emerging intermediate -or -high level dolutegravir resistance within the 48 months follow-up raises concern(77,78). Similarly, available data mainly from randomized clinical trials (RCT) have shown, that virologic efficacy of dolutegravir monotherapy has a sub-optimal potency, with virologic failures increasing significantly over time (37,38,127–129). Hence, there is need for continuous surveillance of pre-existing resistance to the co-administered NRTI. Of-note, prevalence of PDR to NRTI was slightly higher in studies reporting PDR in populations at high risk of HIV transmission, e.g., young-adults and people with intravenous drug use compared to the general adults' population(43,124,130). This observation warrants continuous monitoring of PDR to NRTI in populations at high risk of HIV transmission.

The overall PI resistance rate was re-assuring low, however notable PDR to PI were reported in studies detecting HIV drug resistant mutations using NGS(113,119), which underscores the importance of more sensitive technologies of PDR surveillance, as the minority variants are transmissible and associated with virologic failure(114) Surprisingly, the M46I/L mutation was detected in 3(7%) of the 45 f in Tanzania in 2005 (Supplementary *Table S1*), during the time ART, including PI was not widely available. The observed PI associated HIV drug resistant mutations are likely to be results of naturally occurring polymorphism, rather than transmitted resistance, though PDR or acquired resistance because of undisclosed pre-ART exposure cannot be ruled out completely(49). PDR prevalence data to HIV-1 INSTI in eastern Africa, as in other LMIC is rare(132).

In this study, Intergrase mutations were not found. However, as DTG is relatively new and is being widely rolled-out in eastern Africa, continuous surveillance studies are important to monitor emergence of resistance to DTG at the population level(124,132)

The current analysis extends the sampling period of currently available meta-analysis evidence to 2019(11). Nonetheless, our study has limitations. The variability of methods applied for PDR detection in different studies, may have resulted in underestimation of PDR prevalence rates. Many of the studies included used Sanger based sequencing (PBSS), which is limited to the presence of abundant HIV quasispecies of least 20%. However, HIV variants thresholds as low as 5% are common(114,126). Notably, in the absence of selective drug pressure, other mutations such M184V may quickly disappear from the dominant quasispecies(133). Thus, HIV variants existing at very low levels, may go undetected, especially in patients who have never been exposed to ART. Similarly, the use of different algorithms for HIV drug resistant mutations interpretation among selected studies e.g., HIV drug resistance based on the Stanford HIV drug resistance algorithm, and International Antiviral Society-(IAS)-USA mutations list, which might have resulted in the inclusion of mutation signatures that are excluded in the other algorithm(133). Even though we included studies that reported PDR in ART-naïve, we cannot completely rule out the undisclosed previous exposure to ART. PDR prevalence rates are generally higher in the population with prior exposure to ART as compared to those who have never been exposed to ART(11,120). A small proportion of included studies screened patients for recent HIV infection. Higher levels of PDR may be observed when targeting the acutely/recently HIV infected population, as documented in studies in Malawi and Rwanda(117,129). The possibility is that drug-resistant HIV may revert to wild-type or may be overgrown by the wild viral population(134).

**Conclusion**

We observed a moderate PDR prevalence among ART-naïve patients in this study. Resistance to NNRTI appears to be more prevalent compared to other ART classes, particularly in more recent studies across the region; Thus, underscoring the importance of the current WHO guideline recommending replacement of NNRTI by the integrase inhibitor dolutegravir, as a fixed combination with tenofovir disoproxil fumarate and lamivudine, as preferred first-line ART regimen. Similarly, the observed PDR to NRTI warrants continuous surveillance of pre-existing resistance to the backbone of the dolutegravir-based regimen in LMIC.

**Acknowledgements**

The authors are grateful to all published research that contributed to the data used in this Systematic Review and Meta-Analysis.

**Funding**

This research was supported by the Consortium for Advanced Research Training in Africa (CARTA). CARTA is jointly led by the African Population and Health Research Center and the University of the Witwatersrand and funded by the Carnegie Corporation of New York (Grant No—G-19-57145), Sida (Grant No:54100113), Uppsala Monitoring Centre and the DELTAS Africa Initiative (Grant No: 107768/Z/15/Z). The DELTAS Africa Initiative is an independent funding scheme of the African Academy of Sciences (AAS)'s Alliance for Accelerating Excellence in Science in Africa (AESA) and supported by the New Partnership for Africa's Development Planning and Coordinating Agency (NEPAD Agency) with funding from the Wellcome Trust (UK) and the UK government. The statements made and views expressed are solely the responsibility of the Fellow.

**Transparency declarations**

None to declare.

**Authors' contributions**

AJN conceptualized the study, performed the literature search, data extraction, data analysis, and wrote the first draft of the manuscript. JFM participated in the conceptualization of the study, aided in the literature search, data review and data analysis, MW and JK participated in the conceptualization of the study, interpretation of the findings and the writing of the final paper. All authors reviewed and approved the final version of the paper.

**Supplementary Data**

Table S1. Characteristics of datasets with Reverse Transcriptase (RT)/AND OR Protease (PR) sequences of ART-naïve adults in eight Eastern Africa Countries (2005-2022).

| *Author, publication year* | *Year of Sampling* | *Country origin* | *Study population* | *Females* | *A point prevalence estimate (%)* | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | **Any** | **NNRTI** | **NRTI** | **PI** |
| *Somi et al., (2008)* | 2005-2006 | Tanzania | 39 | 100% | 0.0 | 0.0 | 0.0 | 0.0 |
| *Nyombi et al., (2008)* | 2005 | Tanzania | 100 | 100% | 4.0 | 4.0 | 3.0 | 0.0 |
| *Kiwelu et al.,(2014)* | 2004-2007 | Tanzania | 45 | 100% | 7.0 | 4.0 | 4.0 | 7.0 |
| *Masimba et al.,(2013)* | 2005-2007 | Tanzania | 120 | 80% | 8.4 | 7.6 | 3.4 | 0.0 |
| | 2009 | Tanzania | 119 | 74% | 3.3 | 3.3 | 0.8 | 0.0 |
| *Hassan et al.,(2019)* | 2008 | Kenya | 50 | 72% | 24 | 6.0 | 8.0 | 12 |
| *Rusine et al.,(2012)* | 2007-2009 | Rwanda | 138 | 63% | 2.9 | 2.9 | 1.4 | 0.0 |
| *Rusine et al.,(2013)* | 2007-2010 | Rwanda | 109 | 52.3% | 3.6 | 3.6 | 1.8 | 0.0 |
| *Vairo, et al.,(2013)* | 2010 | Tanzania | 67 | 100% | 11.9 | 7.5 | 1.5 | 0.0 |
| *Telele et al.,(2018)* | 2009-2011 | Ethiopia | 461 | 58% | 3.9 | 1.5 | 2.0 | 0.4 |
| *Lee et al.,(2014)* | 2002-2004 | Uganda | 81 | 62% | 7.0 | 2.5 | 5.1 | 1.2 |
| | 2005-2010 | Uganda | 491 | 62% | 3.0 | 2.2 | 1.2 | 0.0 |
| *Hassan et al.,(2018)* | 2005-2017 | Kenya | 97 | 0 % | 8.2 | 7.2 | 2.1 | 0.0 |
| *Reynolds et al.,(2012)* | 2004–2009 | Uganda | 31 | 45% | 13 | 9.6 | 3.2 | 6.4 |
| *Onsongo et al.,(2016)* | . | Kenya | 63 | 62% | 0.0 | . | . | 0.0 |
| *Bartolo et al.,(2009)* | 2002-2004 | Mozambique | 104 | 43% | 5.9 | 1.5 | 6.0 | 0.0 |
| *Eshleman et al.,(2009)* | 1998-2003 | Uganda | 104 | 55.8% | 5.8 | 0.0 | 2.9 | 2.9 |
| *Bila et al.,(2013)* | 2007 | Mozambique | 47 | 100% | 6.3 | 0.0 | 4.3 | 2.1 |
| | 2009 | Mozambique | 82 | 100% | 8.2 | 8.2 | 0.0 | 0.0 |
| *Kamoto et al.,(2008)* | 2006 | Malawi | 34 | 100% | 0.0 | 0.0 | 0.0 | 0.0 |
| *Abegaz et al.,(2008)* | 2005 | Ethiopia | 39 | 100% | 0.0 | 0.0 | 0.0 | 0.0 |
| *Ndembi et al.,(2011)* | 2009-2010 | Uganda | 70 | 70.1% | 8.6 | 4.2 | 2.9 | 1.4 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Ndembi et al.,(2008)* | 2006- 2007 | Uganda | 37 | 100% | 0.0 | 0.0 | 0.0 | 0.0 |
| *Hamers et al., (2011)* | 2007-2009 | Uganda | 570 | 57% | 11.6 | . | . | . |
| | | Kenya | 404 | 57% | 4.7 | . | . | . |
| *Price et al., (2011)* | 2006-2009 | Uganda | 26 | 57% | 19 | . | . | . |
| | | Rwanda | 78 | 57% | 8 | . | . | . |
| | | Kenya | 49 | **15.6** | 4 | . | . | . |
| *Kantor et al.,(2014)* | 2006 | Kenya | 55 | 61% | 1.8 | 0.0 | 1.8 | 1.8 |
| *Kantor et al.,(2015)* | 2005–2007 | Malawi | 29 | 44% | 6.9 | 27 | 20. | 3.4 |
| *Chung et al.,(2016)* | 2006 | Kenya | 386 | 60% | 3.8 | 3.4 | 0.8 | 0.0 |
| | 2014 | Kenya | 567 | 66% | 10.9 | 10.2 | 3.2 | 0.0 |
| *Zeh et al., (2016)* | 2003-2005 | Kenya | 258 | 52% | 1.2 | 0.0 | 1.2 | 0.0 |
| *Mosha et al.,(2011)* | 2004-2005 | Tanzania | 44 | 77% | 9.0 | 9.0 | 7.0 | 0.0 |
| *Kassu et al., (2007)* | 2003 | Ethiopia | 92 | 60.9% | 3.2 | 2.2 | 1.1 | 0.0 |
| *Kasang et al.,(2011)* | . | Tanzania | 88 | 78% | 14.8 | 10.2 | 8.0 | 5.7 |
| *Nazziwa et al.,(2013)* | 2009-2011 | Uganda | 47 | 47% | 6.0 | 6.0 | 0.0 | 0.0 |
| *Hassan et al., (2013)* | 2008-2010 | Kenya | 182 | 75.8% | 1.1 | 0.0 | 0.5 | 0.5 |
| *Ssemwanga et al.,(2012)-(a)* | 2004-2010 | Uganda | 72 | . | 1.4 | 1.4 | 0.0 | 0.0 |
| *Ssemwanga et al.,(2012)-(b)* | 2008–2010 | Uganda | 40 | 100% | 2.6 | 2.5 | 0.0 | 0.0 |
| *Sigaloff et al., (2012)* | 2009-2010 | Kenya | 68 | . | 13 | 7.4 | 1.4 | 4.4 |
| *Kabondo et al.,(2012)* | 2009 | Malawi | 109 | 100% | 5.5 | 4.6 | 1.8 | 0.0 |
| *Reepalu et al., (2021)* | 2011-2015 | Ethiopia | 99 | 61% | 9.1 | 9.1 | 1.0 | 0.0 |
| *Mutagoma et al.,(2016)* | 2011 | Rwanda | 57 | 86% | 3.5 | 3.5 | 0.0 | 1.8 |
| *Crowell et al.,(2020)* | 2013- 2019 | Tanzania | 154 | 59.6% | 8.0 | 6 .0 | 3.0 | 1.0 |
| | 2013-2019 | Kenya | 254 | 59.6% | 7.0 | 7.0 | 2.0 | 0.0 |
| | 2013-2019 | Uganda | 291 | 59.6% | 14 | 10.0 | 5.0 | 1.0 |
| *Barabona et al.,(2019)* | 2017 | Tanzania | 47 | 75.7% | 29.8 | 25.5 | 8.5 | 0.0 |
| *Rudovick,et al.,(2018)* | 2013-2015 | Tanzania | 235 | 75.7% | 4.7 | 3% | 1.7 | 0.0 |
| *Braun et al.,(2018)* | 2015 | Uganda | 152 | 62.8% | 5.9 | 5.3 | 3.3 | 0.0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Rutstein et al.,(2019)* | 2012–2014. | Malawi | 45 | 39% | 24.4 | 20 | 6.7 | 0.0 |
| *Silverman et al.,(2017)* | 2013–2014 | Kenya | 815 | 65.1% | 9.4 | 9 | 0.7 | 0.0 |
| *Milne et al.,(2020)* | 2006-2010 | Kenya | 97 | 100% | 6.2 | . | . | . |
| *Reynolds et al.,(2016)* | 2012 –2013 | Uganda | 75 | 42% | 4 | 2.7 | 0.0 | 1.3 |
| *Abdissa et al., (2014)* | 2010–2012 | Ethiopia | 12* | . | 50 | 50 | 0.0 | 00 |
| *Onywera et al.,(2017)* | 2012 | Kenya | 87 | 72% | 9.2 | 6.9 | 4.6 | 1.2 |
| *Carnimeo et al.,(202)* | 2017–2018 | Mozambique | 196 | 58.7% | 16.8 | 14.8 | 4.6 | 0.5 |
| | | | 258 | 66.7% | 26.4 | 25.6 | 4.3 | 0.4 |
| *Kiros et al.,(2020)* | 2018 | Ethiopia | 51 | 54.9% | 9.8 | 7.8 | 2.0 | 0.0 |
| *Watera et al.,(2021)* | 2016 | Uganda | 335 | 61.5%* | 18.0 | 13.7 | 6.0 | 1.5 |
| *Kaleebu et al.,(2015)* | 2012-2013 | Uganda | 413 | 66.4% | 4.5 | 4.0 | 2.1 | 0.0 |
| *Beck et al., (2020)* | 2006 | Kenya | 303 | 66.7% | 2.6 | . | . | . |
| | 2010 | Kenya | 97 | 100% | 6.2 | . | . | . |
| | 2013–2014 | Kenya | 679 | 65.7% | 4.4 | . | . | . |
| *McCluskey et al.,(2019)* | 2005-2013 | Uganda | 738 | 69% | 3.5 | 2.8 | 1.0 | 0.0 |
| *Neuhann et al, (2020)* | 2014-2015 | Malawi | 197 | 61.9% | 14.2 | 13.7 | 0.5 | 0.0 |
| *Zhou et al.,(2018)* | 2008 | Malawi | 20 | . | . | 9 | 2 | 0 |
| *Petch LA et al., (2005)* | <2005 | Malawi | 21 | . | 0.0 | 0.0 | 0.0 | 0.0 |
| *Nyamache et al., (2011)* | 2005 | Kenya | 78 | 60.2% | 1.3 | 1.3 | 0.0 | 0.0 |
| *Boender et al.,2015* | 2007–2009 | Kenya | 424 | . | 3 | . | . | . |
| | | Uganda | 606 | . | 10 | . | . | . |
| *Kiptoo et al., (2013)* | 2005-2006 | Kenya | 188 | 100% | 3.2 | 1.6 | 1.1 | 0.5 |
| *Lihana et al.,(2009)* | 2005 | Kenya | 53 | 66% | 7.5 | 7.5 | 5.6 | 0.0 |
| *Cheriro et al., (2015)* | 2009 | Kenya | 41 | 68% | 7.3 | 1.0 | 2.0 | 1.0 |
| *Budambula et al.,(2015)* | . | Kenya | 32 | 56.3% | 3.1 | . | . | 3.1 |
| | | | 21 | 76.2 | 0.0 | . | . | 0.0 |
| *Arimide et al., (2018)* | 2011–2013 | Ethiopia | 67 | 87% | 6.0 | 6.0 | 0.0 | 0.0 |
| *Mulu et al., (2014)* | 2008-2009 | Ethiopia | 155 | 54.1% | 5.6 | 5.0 | 4.0 | 0.0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Mulu et al., (2015)* | 2008 | Ethiopia | 45 | 53 % | 0.0 | . | . | . |
| *Huruy et al., (2015)* | 2010 | Ethiopia | 83 | 38.6% | 7.2 | 3.6 | 1.2 | 2.4 |
| *Amogne et al.,(2016)* | 2008 | Ethiopia | 127 | 31% | 3.1 | 1.6 | 0.8 | 0.8 |
| *Vidal et al.,(2007)* | 2002 | Burundi | 119 | 59% | 0.8 | 0.8 | 0.0 | 0.0 |
| *Ndashimye et al (2018)* | 2007-2011 | Uganda | 87 | 100% | 8.7 | 8.7 | . | . |
| *Galluzzo et al., (2007)* | <2007 | Uganda | 97 | 100% | 7.2 | 3.1 | 4.1 | 0.0 |
| *Osman et al., (2013)* | 2010 | Kenya | 58 | . | 13.8 | 1.7 | 15.5 | 0.0 |
| *Parreira et al., (2006)* | 2003 | Mozambique | 131 | . | 12 | . | . | 0.8 |
| *Abreu et al., (2008)* | 2002 | Mozambique | 75 | 100% | 0.0 | 0.0 | 0.0 | 0.0 |
| *Gale et al., (2006)* | <2005 | Uganda | 187 | . | 0.0 | 0.0 | 0.0 | 0.0 |
| *Bila et al.,(2015)* | 209-2010 | Mozambique | 68 | 72% | . | 4.4 | 0.0 | 1.5 |
| *Vubil et al.,(2016)* | 2009-2010 | Mozambique | 95 | 0.0% | 5.3 | 4.2 | 1.1 | 0.0 |
| *Otecko et al.,(2016)* | 2007-2009 | Kenya | 100 | 73.7% | 0.05 | 0.03 | 0 | 0.03 |
| *Umviligihozo et al., (2021)* | 2005-2011 | Rwanda | 21 | . | 19 | 19 | . | . |
| *Sangeda et al.,(2019)* | 2010 | Tanzania | 18 | . | 0.0 | 0.0 | 0.0 | 0.0 |
| *Bellochi et al.,(2005)* | <2005 | Mozambique | 58 | . | . | 5.2 | 6.9 | 10.3 |

# CHAPTER 4

**Recent HIV-1 infection and pre-treatment drug-resistance in recently infected, treatment-naïve persons in rural Tanzania.**

## Introduction

This chapter describes the proportion of recent HIV-1 infection among newly diagnosed ART naïve people living with HIV and its predictors, and the prevalence of PDR in recently infected persons in rural Tanzania. This's a cross-sectional analysis for recent HIV Infection among newly diagnosed adults enrolled in the Kilombero and Ulanga Antiretroviral cohort between March 2019 and March 2022. Results are presented in research ***paper II***, entitled "Recent HIV-1 infection and pre-treatment HIV drug-resistance in recently infected adults initiating antiretroviral therapy in rural Tanzania.", this manuscript is under review at the *Journal of Public Health*.

Journal of Public Health

http://mc.manuscriptcentral.com/jph

# Recent HIV-1 infection and pre-treatment HIV drug-resistance in recently infected adults initiating antiretroviral therapy in rural Tanzania.

| | |
|---|---|
| Journal: | *Journal of Public Health* |
| Manuscript ID | Draft |
| Manuscript Type: | Original Article |
| Date Submitted by the Author: | n/a |
| Complete List of Authors: | Ntamatungiro, Alex; University of the Witwatersrand; Ifakara Health Institute 53, Department of Interventions and Clinical Trials<br>FRANCIS, Francis; University of the Witwatersrand, Department of Family Medicine and Primary Care<br>MNZAVA, Dorcas; Ifakara Health Institute 53, Department of Interventions and Clinical Trials; St Francis Referral Hospital<br>NDEGE, Robert; Ifakara Health Institute 53, Department of Interventions and Clinical Trials; St Francis Referral Hospital; University of Basel<br>NJAU, Prosper; National AIDS Control Programme (NACP)<br>OKUMA, James; Swiss Tropical and Public Health Institute; University of Basel<br>VANOBBERGHEN, Fiona; Swiss Tropical and Public Health Institute; University of Basel<br>KAGURA, Juliana; University of the Witwatersrand<br>WEISSER, Maja; Ifakara Health Institute 53, Department of Interventions and Clinical Trials; Swiss Tropical and Public Health Institute; University Hospital Basel4001 |
| Keywords: | Infectious disease, Public health, surveillance |
| | |

**Abstract**

**Background**: Recently HIV-1 infected patients remain an important threat to ending the HIV epidemic, contributing to high number of transmissions, and possibly spread of viral resistance. We determined the proportion of recent HIV-1 infection (RHI) among newly diagnosed people living with HIV (PLHIV). Additionally, we assessed the prevalence of pre-treatment HIV-1 drug resistance (PDR) in recently infected patients in rural Tanzania.

**Methods**: We conducted a cross-sectional analysis for RHI among newly diagnosed adults enrolled in the Kilombero and Ulanga Antiretroviral cohort between March 2019 and March 2022. RHI was tested using Asanté™ HIV-1 Rapid Recency® Assay, an immune-chromatographic, lateral-flow, rapid diagnostic test. Risk factors of RHI were determined using logistic regression. Genotypic resistance testing (GRT) was performed for RHI cases.

**Results**: Among 610 PLHIV, 24 (4%) had RHI. We found no factors associated with RHI. GRT was successful in 16/24 (67%) participants, and 5/16 (31%) had mutations associated with HIV-1 drug resistance: 4/16 (25%) for non-nucleoside reverse transcriptase inhibitors, 2/16 (13%) for nucleoside reverse transcriptase inhibitors and 1/16 (6%) for protease inhibitors.

**Conclusion**: The low observed prevalence of RHI in this referral hospital-based study, indicates the high number of late diagnoses. Since the observed proportion of recently HIV infected patients with PDR is based on a small sample size, we can’t really make any conclusion on this.

**Background**

Patients who are recently infected with HIV, defined as new infection within the last six months(69), continue to be an important threat to ending the AIDS epidemic, as early and undiagnosed HIV leads to the highest number of transmission and possibly spread of viral resistance(72). Previous studies estimate that 25-50% of new HIV transmissions are due to persons with recent HIV infections (RHI)(135). During the first six months of infection, the high uncontrolled viremia in blood and genital secretions, the absence of HIV-specific neutralizing antibodies and a small viral quasi-species diversity contribute to a high transmissibility of HIV(70). Thus, strategies for early identification, linkage into care and treatment of recently HIV infected patients will contribute to a major extent to the interruption of HIV transmission and hence reductions in new infections.

Integrase inhibitor (INSTI)-based first-line regimens, through their rapid viral suppression and high genetic barrier to developing resistance(1), have been predicted to curb the number of new HIV infections and the spread of resistance – leading the way to reach the UNAIDS target of reducing the number of adults acquiring HIV infection to fewer than 200,000 in 2030(136). Further, dolutegravir has fewer side effects compared to non-nucleoside reverse transcriptase inhibitors (NNRTI) in treatment-naïve and experienced patients(137). However, we do not yet know the potential impact of the massive rollout of dolutegravir in settings with a substantial prevalence of pre-treatment HIV-1 drug resistance (PDR) against nucleoside reverse transcriptase inhibitor (NRTI) backbone drugs that could lead to dolutegravir monotherapy increasing the risk of virological failure and acquisition of new HIV drug resistant mutations against INSTI(110).

Interestingly, comparative evidence shows that PDR is more prevalent in patients with RHI compared to those with long-term HIV infection(71–74,138). During the chronic phase of infection some mutant viral strains may be overgrown by the wild-type population, and thus exist at very low levels, remaining undetected, especially in patients who have never been exposed to antiretroviral therapy (ART)(139). In Tanzania, as in many sub-Saharan African (SSA) countries, these important data on PDR among the recent HIV infected populations are missing, as routine resistance testing at diagnosis of HIV infection is not implemented. We determined the proportion of RHI among newly diagnosed ART naïve PLHIV and its predictors, and the prevalence of PDR in recently infected persons in rural Tanzania.

## Methods

### Study design and population

This is a cross-sectional study of newly diagnosed PLHIV enrolled in the Kilombero and Ulanga Antiretroviral Cohort (KIULARCO) between March 2019 and March 2022.

### Study area

The study was carried out within the Chronic Disease Clinic of Ifakara (CDCI), the HIV Care and Treatment Centre (CTC) of the St. Francis Referral Hospital in Ifakara, Southwestern Tanzania. CDCI is a joint project of the hospital, the Ifakara Health Institute (IHI), the Swiss Tropical and Public Health Institute and the University Hospital Basel, Switzerland. CDCI was among the first rural CTCs in Tanzania started in 2005 to provide HIV care and free ART within the Tanzanian National AIDS Control Care Programme framework. CDCI runs a PLHIV cohort, the KIULARCO, which has been previously described in detail elsewhere(94,96).

### Inclusion criteria

For this study, we included newly HIV-1 diagnosed adult PLHIV (aged ≥ 15 years), who were enrolled in KIULARCO, have a signed informed consent, and had baseline plasma sample stored in a -80°C freezer.

### Study procedures

At enrolment into KIULARCO, participants receive a clinical baseline evaluation and blood samples are drawn before ART initiation. ART use at enrolment is assessed through a systematic questionnaire. No viral load is routinely done at enrolment. Six months after start of ART and 12 months thereafter, HIV viral load (HVL) is monitored according to Tanzania national guidelines. Besides routine analyses, plasma is cryopreserved at -80$^{0}$C for research purposes. From 934 eligible participants, 610 biobanked baseline samples were randomly selected due to budget constraints and available recent HIV assay kits. Demographic, clinical and laboratory data was extracted from the KIULARCO electronic database.

### Laboratory procedures

Plasma specimens were identified from the KIULARCO biobank, unfrozen and tested using Asanté™ HIV-1 Rapid Recency® Assay, an immune-chromatographic, lateral-flow, rapid diagnostic test(140), according to the manufacturer protocol (Sedia Biosciences, Portland, OR, USA). HVL was measured for all specimens with probable recent infection (tested positive using Asanté™ HIV-1 Rapid Recency® Assay) by quantifying HIV-1 RNA using the, Roche Cobas Ampliprep/Cobas TaqMan (Roche Diagnostics USA, Indianapolis, IN, USA).

HIV-1 genotypic resistance testing (GRT) was performed on specimens with RHI. GRT was performed using a validated in-house PCR protocol.[18–20] Briefly, RNA was extracted from 150 µl of plasma using the PureLink™ Viral RNA/DNA Mini Kit (Invitrogen™, Thermo Fisher Scientific, USA), according to the manufacturer's protocol(141). RNA extracts were retrotranscribed and amplified using the HIV-1 Genotyping Kit Amplification Module (Applied Biosystems™, Thermo Fisher Scientific, USA). A direct sequencing reaction was done using 6 overlapping primers, and assembly program (BioEdit 7.2) was used for sequence analysis. Mutations were interpreted according to the Stanford University's HIV Drug Resistance Database Program version 9.2 (http://hivdb.stanford.edu). HIV drug resistant mutations conferring low, intermediate, or high-level resistance were considered. The reported protease and reverse transcriptase sequences are available in GenBank accession OQ696322-OQ696337.

**Definitions and covariates**

Recent HIV infection is defined as new HIV infection within the last six months(69)

PLHIV that tested positive for recent HIV infection and had a HVL >1,000 copies/ml were classified as RHI while those with an HVL<1,000 copies/ml were classified as long-term infections (no RHI)(142,143).

Covariates measured were age, gender, marital status, disclosure status, highest education level, distance of residence from the clinic (estimated based on centre of ward of residence), tuberculosis status (positive if positive microscopy with acid-fast bacilli, positive XpertMTB/RIF® assay (Cepheid, Sunnyvale, CA, USA) in sputum or other extra-pulmonary sample, or chest radiograph suggestive of tuberculosis plus at least one symptom, physician diagnosis by ICD-10 code and prescription of anti-tuberculosis medication), body mass index (BMI), HIV WHO stage and CD4 cell count. The nearest recorded measurement of BMI, CD4 cell count, HIV WHO stage up to one month after enrolment was used.

**Statistical analysis**

Demographic characteristics were summarized using median and interquantile range (IQR) for continuous data, and frequencies and percentages for categorical data. The following variables were considered a priori as potentially associated with RHI: age, gender, marital status, disclosure status, highest education level, distance of residence to clinic, tuberculosis status, BMI, HIV WHO stage and CD4 cell count(144–146). Logistic regression models were used to assess the association between the above variables and RHI. Univariable and multivariable analysis with no model selection were performed. PDR analyses were descriptive. Analyses were performed using Stata version 15.0.

**Ethical Considerations**

Written informed consent of PLHIV willing to participate in KIULARCO are obtained at registration. This study received ethical approval from the University of the Witwatersrand-Human Research Ethics Committee (Medical) Clearance Certificate No.M210714. Yearly ethical approval for data and sample collection as well as analysis are sought from the Ifakara Health Institute institutional review board (IHI/IRB/No16-2006) and the Health Review Committee of the National Institute for Medical Research of Tanzania (NIMR/HQ/R.8c/Vol.I/378).

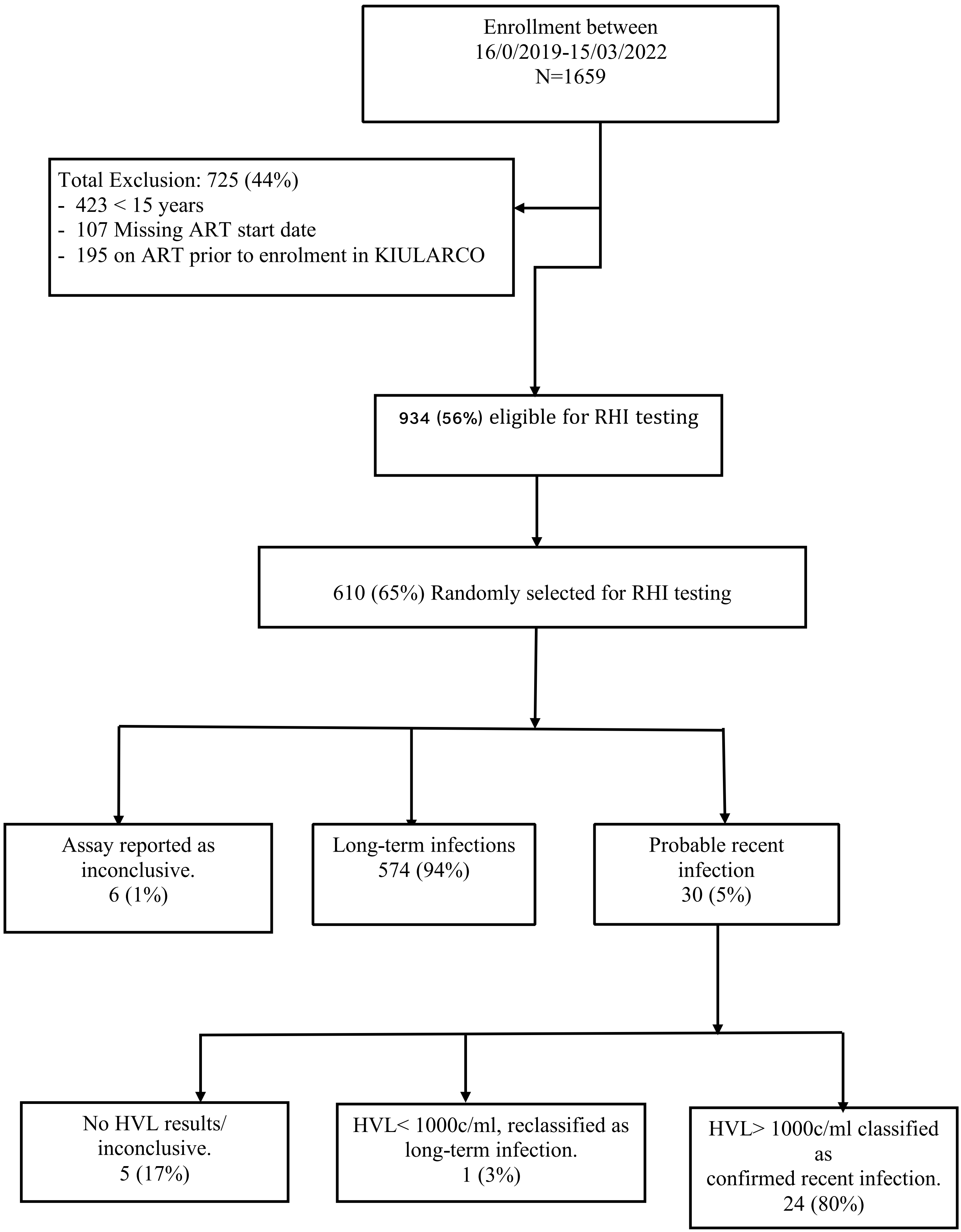
**Results**

**Baseline characteristics**

A total of 1659 PLHIV were enrolled into KIULARCO during the study period. We excluded 725 (44%) participants: 423 (58%) were <15 years old, 195 (27%) were previously on ART, and 107 (15%) had no ART start date, leaving 934 (56%) eligible PLHIV. We randomly selected 610 (65%) of the eligible PLHIV for this study.

Among the 610 PLHIV with a recent HIV infection test, 6 had an invalid result, bringing the total number of study participants to 604 (Figure 1). Of these, the median age was 38 years (IQR 30-47), 395 (65%) were female, 377 (62%) were married/cohabiting, 400 (66%) had disclosed their HIV status and 73 (12%) had no formal education. Clinically, 49 (8%) participants suffered from a tuberculosis co-infection, 70 (12%) were undernourished (BMI<18.5), 138 (23%) had a HIV WHO stage III/IV, and 226 (41%) had a CD4 cell count of <350 cells/µl (Table 1).

FIGURE 1: Study participants flowchart



**Recent HIV infection and associated factors**

Probable RHI was detected in 30 (5%) participants; Among those, 1(3%) participant had a HVL test result of <1000 copies/ml, thus reclassified as chronic infection, and 5(17%) participants had no HVL test result due to inadequate specimen, thus reclassified as inconclusive RHI test results; Whereas, 24 (80%) participants had HVL ≥1000 copies/ml, hence confirmed RHI, corresponding to an RHI prevalence of 4% (24/599). (Figure 1.).

Baseline characteristics among those with RHI were comparable to all study participants with exception of those with RHI having a lower HIV WHO stages I/II (4(17%) vs 138(23%)) and a higher CD4 cell count of ≥350 cells/µl (11(55%) vs 208 (38%)) (Table 1). We found no evidence of an association between risk factors and RHI (Table 2).

Table 1. Characteristics of study participants

| Socio-demographic | Recent HIV Infection (RHI) (N=24) | No RHI (N=575) | ALL (N=599) |
|---|---|---|---|
| **Age, years (median (IQR)** | 37 (30 – 47) | 38 (30 – 48) | 39 (30 – 47) |
| **Age, years, n (%)** | | | |
| **15 – 24** | 4 (17%) | 70 (12%) | 74 (12%) |
| **25 – 34** | 6 (25%) | 154 (27%) | 160 (27%) |
| **35 - 44** | 10 (42%) | 166 (29%) | 176 (29%) |
| **≥45** | 4 (17%) | 185 (32%) | 189 (32%) |
| **Gender, female, n (%)** | 16 (67%) | 375 (65%) | 391 (65%) |
| **Marital status, n (%)** | | | |
| **Married/cohabiting** | 15 (63%) | 359 (62%) | 374 (62%) |
| **Never married** | 2 (8%) | 38 (7%) | 40 (7%) |
| **Separated/divorced/widowed** | 7 (29%) | 178 (31%) | 185 (31%) |
| **Disclosed HIV status, n (%)** | | | |
| **No** | 8 (33%) | 186 (33%) | 194 (33%) |
| **Yes** | 16 (67%) | 379 (67%) | 395 (66%) |
| **Missing** | 0 (0%) | 10 (2%) | 10 (2%) |
| **Education, n (%)** | | | |
| **None** | 3 (13 %) | 69 (12 %) | 72 (12 %) |

| | | | |
|---|---|---|---|
| **Primary** | 19 (79%) | 442 (77%) | 461 (77%) |
| **Secondary and above** | 2 (8%) | 64 (11%) | 66 (11%) |
| **Distance of residence to clinic, n (%)** | | | |
| **<=1 km** | 17 (77%) | 328 (60%) | 345 (60%) |
| **2— <50 km** | 4 (18%) | 158 (29%) | 162 (28%) |
| **≥50 km** | 1 (5%) | 63 (12%) | 64 (11%) |
| **Missing** | 2 (8%) | 26 (5%) | 28 (5%) |
| | | Clinical | |
| **Tuberculosis status, n (%)** | | | |
| **Unlikely** | 23 (96%) | 514 (90%) | 537 (92%) |
| **Yes** | 1 (4%) | 48 (9%) | 49 (8%) |
| **Missing** | 0 (0%) | 13 (2%) | 13 (2%) |
| **Body Mass Index (BMI)[a], Kg/m$^2$, n (%)** | | | |
| **Underweight, <18.5** | 4 (18%) | 66 (12%) | 70 (12%) |
| **Normal, 18.5 - <25** | 10 (46%) | 302 (55%) | 312 (52%) |
| **Overweight, ≥25** | 8 (36%) | 178 (33%) | 186 (31%) |
| **Missing** | 2 (8%) | 29 (5%) | 31 (5%) |
| **HIV WHO stage, n (%)** | | | |
| **I/II** | 20 (83%) | 432 (83%) | 452 (77%) |
| **III/IV** | 4 (17%) | 134 (24%) | 138 (23%) |
| **Missing** | 0 (0%) | 9 (2%) | 9 (2%) |
| **CD4 count, cells/µl[a], n (%)** | | | |
| **<100** | 1 (5%) | 112 (22%) | 113 (21%) |
| **100 - 350** | 8 (40%) | 216 (41%) | 226 (41%) |
| **≥350** | 11 (55%) | 194 (37%) | 208 (38%) |
| **Missing** | 4 (17%) | 53 (9%) | 57 (10%) |

Results are numbers and column % of those with non-missing data; missing data rows are numbers and column %.
[a]BMI, HIV WHO stage and CD4 measurement closest to enrolment upto one month after.

Table 2. Risk factors associated with recent HIV infection

| Characteristics | Unadjusted OR (95% CI) [a] | Adjusted OR (95% CI) [ab] |
|---|---|---|
| **Age, years** | | |
| **15 – 24** | Reference | Reference |
| **25 – 34** | 0.68 (0.19 - 2.49) | 0.35 (0.05 - 2.37) |
| **35 - 44** | 1.05 (0.32 - 3.45) | 2.09 (0.41 – 10.6) |
| **45 +** | 0.37 (0.09 - 1.52) | 0.55 (0.08 – 3.77) |
| **Gender** | | |
| **Male** | Reference | Reference |
| **Female** | 1.06 (0.45 - 2.52) | 1.53 (0.38 – 6.20) |
| **Marital Status** | | |
| **Married/Cohabiting** | Reference | Reference |
| **Never Married** | 1.27 (0.28 - 5.77) | 2.57 (0.44 – 15.2) |
| **Separated/divorced/widowed** | 0.93 (0.38 - 2.34) | 1.46 (0.44 - 4.85) |
| **Disclosed HIV Status** | | |
| **No** | Reference | Reference |
| **Yes** | 0.97 (0.41 - 2.30) | 1.36 (0.41 – 4.56) |
| **Education** | | |
| **None** | Reference | Reference |
| **Primary** | 0.99 (0.29 - 3.45) | 0.50 (0.11 – 2.19) |
| **Secondary and above** | 0.73 (0.19 - 4.50) | 0.30 (0.02 – 3.67) |
| **Distance of residence to clinic** | | |
| **≤1km** | Reference | Reference |
| **2—<50km** | 0.48 (0.16 - 1.46) | 0.32 (0.08 - 1.32) |
| **≥50km** | 0.30 (0.04 - 2.31) | 0.37 (0.03 - 3.28) |
| **Tuberculosis status** | | |
| **Unlikely** | Reference | Reference |
| **Yes** | 0.47 (0.62 - 3.56) | 0.57 (0.05 – 7.30) |
| **Body Mass Index, Kg/m$^2$** | | |
| **Normal 18.5 - <25** | Reference | Reference |
| **Underweight, <18.5** | 1.85 (0.56 - 6.09) | 3.80 (0.81 – 18.0) |
| **Overweight, ≥25** | 1.37 (0.53 - 3.52) | 1.23 (0.35 – 4.31) |

| | | |
|---|---|---|
| **HIV WHO Stage** | | |
| **I/II** | Reference | Reference |
| **III/IV** | 0.65 (0.22 - 1.94) | 0.92 (0.17 – 4.95) |
| **CD4 count, cells/µl** | | |
| **<100** | Reference | Reference |
| **100 – 349** | 4.11 (0.51 - 33.3) | 4.26 (0.45 – 40.6) |
| **350+** | 6.25 (0.80 - 49.1) | 7.01 (0.71 – 69.1) |

[a]Odds Ratios (OR) and 95% confidence intervals (CI) obtained from logistic regression.

[b]Adjusted for baseline covariates shown, participants with missing data for any of the variables excluded, N=435.

Table 3. Distribution of Pretreatment Drug Resistance-Associated Mutations- and HIV-1 subtype(s) among recently HIV-1 infected people living with HIV.

| Sample ID | Sample date | Major NRTI HIV resistance mutations | Major NNRTI HIV resistance mutations | Major PI HIV resistance mutations | Sub-type |
|---|---|---|---|---|---|
| **101** | 05.07.2019 | None | K103N | None | C |
| **102** | 22.10.2021 | None | None | None | C |
| **103** | 11.06.2022 | K219Q | None | None | C+D |
| **104** | 05.07.2021 | None | K103N, K238T | None | A1 |
| **105** | 01.06.2020 | None | None | None | D |
| **106** | 04.06.2020 | None | None | None | C+D |
| **107** | 08.06.2020 | None | None | None | C |
| **108** | 30.07.2020 | M41L, K65R, M184V | K103N, Y181C | I54M, L90M | C |
| **109** | 08.10.2019 | None | None | None | C |
| **110** | 04.08.2020 | None | None | None | D |
| **111** | 06.07.2021 | None | None | None | C |
| **112** | 05.07.2021 | None | None | None | C |
| **113** | 27.11.2020 | None | None | None | D |
| **114** | 23.03.2021 | None | None | None | C |
| **115** | 15.9.2021 | None | E138K | None | A1 |
| **116** | 30.12.2021 | None | None | None | A1 |

**Pretreatment HIV-1 drug resistance mutations**

Among 24 PLHIV with RHI, genotypic results for PDR were available for 16 (67%) participants. Any PDR was observed in 5 (31%) participants. PDR for NNRTI, NRTI and PI were observed in 4 (25%), 2 (13%) and 1 (6%) participant, respectively (Table 3.). HIV-1 subtypes were A1 (n = 3 (19 %), C (n = 8 (50%), D (n = 3 (19%)) and CRF C+D (n = 2 (13%)).

## Discussion

### Main finding of this study

In this study on the prevalence of RHI among newly diagnosed PLHIV in a rural sub-Saharan setting, we observed a prevalence of 4.0%, which was lower compared to other studies in the region(145,147,148). This observation may imply that majority of PLHIV in this rural SSA setting present late for care and treatment, hence representing a bottleneck in curbing the number of new HIV infections. Additionally, we determined the prevalence of PDR among patients with RHI to be 31%. Though on a limited sample size, the observed high DR rates is concerning.

### What is already known on this topic?

In previous studies, female gender, young age, secondary and above education, and marital status have been reported as risk factors for RHI in SSA(145–150). However, in our analysis these factors were not found to be independently associated with RHI, likely due to small sample size of RHI cases. Elsewhere, factors such as commercial sex practice, contact with index case and illicit drug utilization have associated with RHI(147) However, these data are not systematically collected in the KIULARCO.

### What this study adds

Our results raise several important public health issues; firstly, the observed low prevalence of RHI, which may be this being a health facility-based study, in a referral hospital, where patients attend due to referral from lower-level health facilities, and or severe illness that may be due to advanced HIV disease. This suggests that the prevalence of RHI might be higher in the community and in patients attending lower-level health facilities. In a recent door-to-door testing among general population sample in rural Lesotho, a prevalence of 7% was documented(146) A comparable prevalence of 7.4% was observed in a study targeting fishing community along the shore of Lake Victoria in Tanzania(148).

Secondly, the observed high PDR rates among recently HIV infected PLHIV, suggest that targeting this population is a key consideration while estimating the burden of PDR(74). A comparable high PDR prevalence of 29.8% has been documented among self-reporting ART-naive urban population in Tanzania(29). Our study, however, characterized PDR among recently HIV infected PLHIV in a rural population. PDR prevalence was highest for NNRTI (25%); the NNRTI drug class is being replaced by the more potent integrase dolutegravir based ART in many countries in SSA. However, the widely spread PDR to NNRTI poses threat to the long-term efficacy of dolutegravir. Even though the mechanism is still unclear, PDR to NNRTI has previously been associated with subsequently long-term virologic failure on dolutergravir based ART(131). Prevalence of PDR to NRTI among recently HIV infected PLHIV was observed at 12.5%, which was higher than overall pooled estimate prevalence of PDR to NRTI of 2.5% that was documented in our recent systematic review on PDR prevalence studies among adults in Eastern African countries(56). High prevalence of PDR to NRTI, which is co-administered with dolutergravir in the current WHO recommended first-line ART, risks some patients remaining on functional monotherapy and potential for emergence of dolutergravir resistance(75). The observed high PDR rate to NRTI among recently HIV infected population poses a threat to public health.

**Limitations of this study**

To the best of our knowledge, this is the first study reporting PDR among recently HIV infected PLHIV in Tanzania. However, our study has several limitations, including the study being in a referral hospital may have underestimated prevalence of RHI. Secondly, a relatively small sample size of patients presenting with RHI and PDR, which limits further exploration of risk factors associated with PDR in the recent HIV infected population. Thirdly, we used the Asanté™ HIV-1 Rapid Recency® Assay, which – after initial documentation of a high sensitively and specificity(140)– showed in some studies limited accuracy(151). However, in this study we added viral load testing to reduce false-positive recent infections. Likewise visual scoring of faint bands may be subjective and lead to misdiagnosis of RHI. Another limitation is that PCR amplification and genotypic testing was only successful for 67% of patients with RHI, which might be due to poor storage quality. Finally, all genotypic data were obtained through standard Sanger sequencing; rates of HIV associated drug resistance mutations would possibly be higher if ultra-sensitive HIV-1 drug resistance testing by deep sequencing had been used.

## Conclusion

To conclude, we observed a prevalence of RHI of 4% in this hospital-based cross-sectional study in a rural setting, thus calling for strengthening strategies for early identification of HIV cases and linkage into care and treatment, to enable meeting of the UNAIDS' 95-95-95 goals. Since the observed proportion of recently HIV infected patients with PDR is based on a small sample size, we can't really make any conclusion on this.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Authors' contributions

AJN JMF, JK, MW conceptualized the study and designed the experiments. AJN, DM performed the experiments. AJN, JO, FV analysed the data AJN wrote the manuscript. JMF, DM, RN, PSN, JO, FV, JK, MW reviewed and approved the final manuscript.


## Acknowledgements

We are grateful to all patients and staff from the CDCI. We also would like to thank the funders of our clinic: the Ministry of Health of Tanzania, the Government of the Canton of Basel, the Swiss Tropical and Public Health Institute, and the Ifakara Health Institute. We are grateful to the National AIDS Control Programme for facilitating the donation of 600 Asanté™ HIV-1 Rapid Recency® tests. Our special thanks are extended to all members of the KIULARCO Study Group.

## Funding

This research was supported by the Consortium for Advanced Research Training in Africa (CARTA). CARTA is jointly led by the African Population and Health Research Center and the University of the Witwatersrand and funded by the Carnegie Corporation of New York (Grant No—G-19-57145), SIDA (Grant No:54100113), Uppsala Monitoring Centre and the DELTAS Africa Initiative (Grant No: 107768/Z/15/Z). The DELTAS Africa Initiative is an independent funding scheme of the African Academy of Sciences (AAS)'s Alliance for Accelerating Excellence in Science in Africa (AESA) and supported by the New Partnership for Africa's Development Planning and Coordinating Agency (NEPAD Agency) with funding from the

Wellcome Trust (UK) and the UK government. The funders had no rule in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

**Conflict of interest**

All authors report no potential conflicts.

# CHAPTER 5

**Trends in prevalence of the nucleoside reverse-transcriptase inhibitor associated mutations in newly diagnosed antiretroviral therapy-naive patients in the Kilombero and Ulanga Antiretroviral cohort in Tanzania.**

## Introduction

This chapter compares the levels of pre-treatment HIV-1 resistance to Nucleoside reverse transcriptase inhibitor (NRTI), among antiretroviral-naïve adults people living with HIV in in the Kilombero and Ulanga Antiretroviral cohort, in the first five-year period (2005-2009) of the ART program in Tanzania, and the most recent period (2019-2022). Results are presented in research ***paper III***, entitled "Trends in prevalence of the NRTI associated mutations in newly diagnosed antiretroviral therapy-naive patients in the Kilombero and Ulanga Antiretroviral Cohort in Tanzania.", this manuscript is to be submitted for publication in the journal AIDS.

# Trends in Prevalence of the Nucleoside/ Nucleotide Reverse-Transcriptase Inhibitor Associated Mutations in Newly Diagnosed ART-Naive Patients in the Kilombero and Ulanga Antiretroviral cohort, in Tanzania.

**NTAMATUNGIRO AJ**[1,2*], FRANCIS JM[5], NDEGE R[2,3], OKUMA J[4], VANOBBERGHEN F[4], PARIS D[4], WEISSER M[2,3,4,] and KAGURA J[1]

[1]Division of Epidemiology and Biostatistics; University of the Witwatersrand, 2050, Johannesburg, South Africa

[2]Department of Interventions and Clinical Trials, Ifakara Health Institute, 53, Ifakara, Tanzania

[3]University of Basel, 4001, Basel, Switzerland

[4]Swiss Tropical and Public Health Institute, 4123 Allschwil, Switzerland

[5]Department of Family Medicine and Primary Care; University of the Witwatersrand, 2050, Johannesburg, South Africa

[6]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel,4001, Basel, Switzerland

*Corresponding author.

E-mail: antmatungiro@ihi.or.tz

Submitted for publication *AIDS*

# AIDS

## Trends in Prevalence of the pre-treatment HIV-1 drug resistance to NRTI in the Kilombero and Ulanga Antiretroviral cohort, in Tanzania.

--Manuscript Draft--

| | |
|---|---|
| **Manuscript Number:** | |
| **Full Title:** | Trends in Prevalence of the pre-treatment HIV-1 drug resistance to NRTI in the Kilombero and Ulanga Antiretroviral cohort, in Tanzania. |
| **Article Type:** | Original paper (Basic Science) |
| **Keywords:** | Pre-treatment HIV-1 drug resistance, Nucleoside/ Nucleotide Reverse-Transcriptase Inhibitor, HIV-1, Antiretroviral Therapy, Tanzania. |
| **Corresponding Author:** | Alex John Ntamatungiro, Msc<br>Ifakara Health Institute<br>Dar es Salaam, TANZANIA, UNITED REPUBLIC OF |


| | |
|---|---|
| **Abstract:** | Objective:<br>To measure the change in prevalence of pre-treatment HIV-1 drug resistance (PDR) to NRTI in the Kilombero and Ulanga Antiretroviral cohort (KIULARCO), between the first five-year period (2005-2009) of the ART program in Tanzania, and in the most recent period (2019-2022).<br>Methods:<br>Genotypic resistance testing (GRT) was performed to establish PDR in HIV infected person (aged ³ 15 years old) prior ART initiation. Study period: 2005-2009 (the first five-year period of the ART program in Tanzania) and the most recent period (2019-2022). PLHIV were excluded if they were aged less than 15 years and had prior history of ART exposure. Statistical analyses were descriptively performed to compare the prevalence of PDR between the two study periods.<br>Results:<br>The study population comprised 357 ART-naïve PLHIV; 239 during “2005-2009” and 118 in “2019-2022” period, comprised of 66% and 63% female in “2005-2009” and “2019-2022, respectively. Mean age was 38 years in “2005-2009” and 41 years in “2019-2022. The prevalence of PDR to NRTI was 2.1 % “2005-2009” and 3.4% in “2019-2022” (P=0.063). Whereas the frequency of major NRTI associated mutations was 3.8% in “2009-2009” and 7.6% in “2019-2022” (p=0.063174).<br>Conclusions:<br>The prevalence of PDR to NRTI was higher in the most recent period (2019-2022) compared with the first five-year period (2005-2009) of the ART program in Tanzania. Certainly, there is need for continuous surveillance of PDR to NRTI remain important, to prevent risk of failure of newer antiretroviral agents such as dolutegravir, which would be detrimental to Tanzania and other low- and middle-income countries |

**Background**

The wider antiretroviral therapy (ART) coverage inevitably results in increasing rates of an ART use at uncontrolled levels, and risk of acquired HIV drug resistant mutations. This may in turn lead to an increased risk of transmitted drug resistance in individuals who have never been exposed to ART(152). In Sub Saharan Africa (SSA) available data shows increasing trend of pre-treatment HIV-1 drug resistance (PDR) among general adult population, which is mainly driven by non-nucleoside reverse transcriptase inhibitors (NNRTIs) HIV-1 drug resistance associated mutations(11,26,80). The new first-line treatment recommended by the WHO, the integrase inhibitor dolutegravir based ART which is being rolled-out in many countries in SSA, in Tanzania specifically since March 2019, is predicted to curb the number of new HIV-1 infections and the spread of resistance – owing to its high and rapid viral suppression and a high genetic barrier to developing resistance(9,47,75,153).

However, there has been notable increase in prevalence of PDR to Nucleoside reverse transcriptase inhibitor (NRTI), particularly in Eastern Africa(11,56), which may pose risk for the currently preferred first-line ART regimen for HIV treatment in Low- and middle-income countries, which is the fixed dose combination of dolutegravir with tenofovir/lamivudine. HIV drug resistant mutations to NRTI may lead to de facto dolutegravir monotherapy with a risk of acquisition of dolutegravir resistance(38,40,154). With this study, we therefore, measured the change in prevalence of PDR to NRTIs in the Kilombero and Ulanga Antiretroviral cohort (KIULARCO), during first five-year period (2005-2009) of the ART program in Tanzania, and in the most recent period (2019-2022).

**Methods**

**Study design**

We performed a cross-sectional analysis for PDR levels to NRTI among ART -naïve adults initiating ART in the KIULARCO, during the recent period (2019-2022), and compared to the previously published data from the same cohort, during the first five-year period (2005-2009) of the ART program in Tanzania(31).

**Setting and population**

We included newly HIV-1-diagnosed adult people living with HIV (PLHIV) (aged ≥ 15 years) prior to ART initiation, who are enrolled in the KIULARCO. For this study, we included 239 and

118 patients' plasma samples randomly selected for inclusion in "2005-2009" and in "2019-2022", respectively.

**Data collection**

Data for patient demographics, and on ART was extracted from OpenMRS, the KIULARCO electronic medical record system. Plasma specimens of eligible patients were retrieved from the KIULARCO biobank, unfrozen and tested.

**Laboratory analysis**

Genotypic HIV-1 resistance testing was performed using a validated in-house PCR protocol(42,95,99). Briefly, HIV-1 RNA was isolated using NucleoSpin RNA Virus, (MACHEREY-NAGEL, Germany), according to the manufacturer's protocol(100), from an equivalent of 150uL of pre-ART plasma samples enrolled in the KIULARCO. DNase-treated HIV-1 RNA was reverse-transcribed and amplified by two semi-nested PCRs. A direct sequencing reaction was done using 6 overlapping primers, and assembly program (BioEdit 7.2) was used for sequence analysis(103). Mutations were interpreted according to the Stanford University's HIV Drug Resistance Database Program version 9.2 (http://hivdb.stanford.edu)(104).

**Statistical analysis**

Data were analyzed statistically by STATA 15 using descriptive t-test to compare the prevalence of PDR between groups.

**Ethics approval**

Written informed consent of patients willing to participate in KIULARCO are obtained at registration. This study received ethical approval from the University of the Witwatersrand-Human Research Ethics Committee (Medical) Clearance Certificate No.M210714**.** Yearly ethical approval for data and sample collection as well as analysis are sought from the Ifakara Health Institute institutional review board (IHI/IRB/No16-2006) and the Health Review Committee of the National Institute for Medical Research of Tanzania (NIMR/HQ/R.8c/Vol.I/378).

**Results and Discussion**

Study-eligible participants initiating ART in the KIULARCO were tested for HIV-1 drug resistance to NRTI. A total of 357 patients' samples were analysed: 239 during the first five-year period (2005-2009) of the ART program in Tanzania, and 118 in the most recent period (2019-2022). The median age of those in "2005-2009" was slightly younger than in 2019-2022 (38 vs. 41 years). The proportion of women in "2005-2009" and in "2019-2022", was 66% and 63% respectively.

The overall prevalence of PDR to NRTI was 2.1 % and 3.4% in "2005-2009" and "2019-2022" ($p$=0.233), respectively. Whereas the overall frequency of major NRTI associated mutations was 3.8% in "2009-2009" and 7.6% in "2019-2022" ($p$=0.063174). The difference in observed prevalence of PDR to NRTI was not statistically significant, possibly due to small sample size. However, a rising trend of PDR to NRTI has previously been reported in the Eastern Africa regions, in a systematic review and meta-analysis using data available until 2016(11).

In "2005-2009", 9 mutations were detected in 5 participants at baseline (*Table 1*). The proportional of common discriminatory NRTI mutations were (M184V 4(1.7%), K65R 1(0.4%), L74LV 1(0.4%)); Whereas the proportional of the thymidine Analog Mutations, TAMs were (M41L 1(0.4%), K70R 1(0.4%), T215F 1 (0.4%)). In "2019-2022", 9 mutations to NRTI were detected in 4 participants at treatment initiation (Table 1) The proportional of common discriminatory NRTI mutations were (M184V 3 (2.0%), K65R 2(1.7%)); Whereas the proportional of the thymidine Analog Mutations, TAMs in 2005-2009 were (M41L 2(1.7%), K70R 1(0.9%), T215F 1 (0.9%) and (K219Q 1(0.9%)) (*Table 1*). Prevalence of K65R mutation associated with reduced susceptibility to tenofovir, was 0.4% in "2005-2009" and 1.7% in "2019-2022" ($p$=0.104). Elsewhere, a twice increased in prevalence of K65R mutation between to study period has been reported in across-sectional study neighboring country, Kenya(80).

In "2005-2009", subtype C was the most prevalent HIV-1 subtype with a frequency of 43.7%, followed by A1(29.4%) and D (16%). Likewise, in "2019-2022" subtype C was the most prevalent HIV-1 subtype with a frequency of (34.7%), followed by subtype D (28%)) and A1 (20.3%)) (Table 2.). The frequency of subtype D was significant high in "20019-2022" compared to "20019-2022" ($p$=0.004); Likewise, the recombinant form A1,01_AE ($p$=0.001). The significant of HIV-1 subtype on treatment outcome is largely still an area of active investigation. However, HIV-1 subtypes such as C is known to more prone to developing K65R mutations(81,155)

**Limitations**

The major limitation of our study is the small sample size due to time and budget constraints. Also, prior exposure to antiretrovirals was based on self-report and could be subject to misclassification. Further studies involving a higher number of participants and more sampling time points are needed to determine the trends of PDR, and identify real predictors associated with changes in prevalence of PDR to NRTI.

**Conclusion**

In summary, the findings show increasing tendency of PDR to NRTI during the study periods; This increased prevalence of PDR suggests that a considerable number of ART-naïve adults initiating dolutegravir based-ART in fixed dose combination of NRTI backbones, may be at risk of being on dolutegravir monotherapy and acquisition of dolutegravir resistance. These findings highlight a need for continuous surveillance of pre-existing resistance to the dolutegravir co-administered NRTI.

Table 1: Prevalence of pre-antiretroviral treatment HIV drug resistance mutations detected in antiretroviral-naïve adult in “2005-2009”(31) and “2019-2022”.

| Major NRTI Mutations | 2005-09 (N=239) n (%) | 20019-22 (N=118) n (%) | P-value (X; $H_0$: “20019-2022” ≥ “20019-2022”) |
|---|---|---|---|
| M184V | 4 (1.7) | 3(2.5) | 0.305 |
| T215F | 1 (0.4) | 1(0.9) | 0.278 |
| K65KR | 1 (0.4) | 2(1.7) | 0.104 |
| K70R | 1(0.4) | 1(0.9) | 0.278 |
| M41L | 1(0.4) | 2(1.7) | 0.104 |
| L74LV | 1(0.4) | - | - |
| K291Q | - | 1(0.9) | - |
| Total major NRTI | 9(3.8) | 9 (7.6) | 0.063 |
| No. of individuals with major NRTI mutations | 5 (2.1%) | 4 (3.4) | 0.233 |

Table 2: Prevalence of HIV-1 subtypes detected in antiretroviral-naïve adult in "2005-2009"(31) and "2019-2022".

| **HIV-1 subtypes** | **2005-09 (N=231) N (%)** | **20019-2022 (N=118) N (%)** | **P-value (X; $H_0$: "20019-2022" ≥ "20019-2022")** |
|---|---|---|---|
| A1 | 68 (29.4) | 24(20.3) | 0.034 |
| B | | 2 (1.7) | - |
| C | 101 (43.7) | 41(34.7) | 0.053 |
| D | 37 (16) | 33 (28.0) | 0.004 |
| A1,01_AE | 5 (2.2) | 11 (9.3) | 0.001 |
| CD | 11 (4.8) | 3(2.5) | 0.150 |
| A1D | 2 (0.9) | 3 (2.5) | 0.118 |
| BC | - | 1 (0.8) | - |
| A1, C | 5 (2.2) | - | - |
| A1, C, D | 1 (0.4) | - | - |
| A1,01_AE, C | 1 (0.4) | - | - |
| All recombinants | 25 (10.8) | 18 (15.3) | 0.113 |

**Notes**

**Acknowledgements**: We are thankful to all the participants of the KIULARCO cohort. We thank the staff of the Chronic Diseases Clinic of St Francis Referral Hospital in Ifakara for making this study possible.

**Sources of Funding**: TThis research was supported by the Consortium for Advanced Research Training in Africa (CARTA). CARTA is jointly led by the African Population and Health Research Center and the University of the Witwatersrand and funded by the Carnegie Corporation of New York (Grant No—G-19-57145), Sida (Grant No:54100113), Uppsala Monitoring Centre and the DELTAS Africa Initiative (Grant No: 107768/Z/15/Z). The DELTAS Africa Initiative is an independent funding scheme of the African Academy of Sciences (AAS)'s Alliance for Accelerating Excellence in Science in Africa (AESA) and supported by the New Partnership for Africa's Development Planning and Coordinating Agency (NEPAD Agency) with funding from the Wellcome Trust (UK) and the UK government. The statements made and views expressed are solely the responsibility of the Fellow.

**Authors' contributions:** AJN, JF, MW and JK conceptualized the study, designed the experiments, AJN, wrote the manuscript. AN, Performed the genotypic HIV-1 resistance testing. AJN analyzed the data and reviewed the manuscript. AN, JO, FV, DP, JF, MW and JK participated in the interpretation of the data, writing, reviewing, and approving the final manuscript. The corresponding author AJN is the guarantor of the paper.

**Conflicts of interest** There are no conflicts of interest.

# CHAPTER 6

**Virological-outcomes at one-year, and its predictors after initiating dolutegravir (DGT)-based antiretroviral therapy (ART), and prevalence of pretreatment HIV-1 drug resistance among patients failing on DTG-based ART in a rural cohort, in Tanzania.**

## Introduction

This chapter compares viral suppression in treatment-naïve patients at 12 months after initiating dolutegravir (DTG)-based versus non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART. Additionally, we assessed predictors associated with viral suppression and analysed pre-treatment HIV-1 drug resistance (PDR) and acquired resistance, side effects, and pregnancy outcomes in patients initiated on a DTG-based regimen. Results are presented in research ***paper IV***, entitled "Transitioning to dolutegravir in a programmatic setting: Virological outcomes and associated factors among treatment-naïve patients with HIV-1 in the Kilombero and Ulanga antiretroviral cohort in rural Tanzania.", this has been published as an original article in the *Journal of Open Forum Infectious Disease*

*Open Forum Infectious Diseases*
MAJOR ARTICLE

# Transitioning to Dolutegravir in a Programmatic Setting: Virological Outcomes and Associated Factors Among Treatment-Naive Patients With HIV-1 in the Kilombero and Ulanga Antiretroviral Cohort in Rural Tanzania

**Alex J. Ntamatungiro,[1,2,a] Anna Eichenberger,[3,a] James Okuma,[4] Fiona Vanobberghen,[4,5] Robert Ndege,[1,4,5] Namvua Kimera,[2] Joel M. Francis,[6] Juliana Kagura,[1,b] and Maja Weisser,[2,4,5,7,b] for the Kilombero and Ulanga Antiretroviral Cohort (KIULARCO) Study Group**

[1]Division of Epidemiology and Biostatistics, School of Public Health, University of the Witwatersrand, Johannesburg, South Africa, [2]Department of Interventions and Clinical Trials, Ifakara Health Institute, Ifakara, Tanzania, [3]Department of Infectious Diseases, Bern University Hospital, Bern, Switzerland, [4]Department of Medicine, Swiss Tropical and Public Health Institute, Basel, Switzerland, [5]University of Basel, Basel, Switzerland, [6]Department of Family Medicine and Primary Care, University of the Witwatersrand, Johannesburg, South Africa, and [7]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland

***Background.*** Virological outcome data after programmatic transition from non-nucleoside reverse transcriptase inhibitor (NNRTI)-based to dolutegravir (DTG)-based antiretroviral therapy (ART) regimens in sub-Saharan Africa (SSA) outside of clinical trials are scarce. We compared viral suppression and associated factors in treatment-naïve people living with HIV (PLHIV) starting DTG- based versus NNRTI-based ART.

***Methods.*** We compared virological suppression at 12 months, after treatment initiation in the two cohorts of participants aged ≥15 years, initiating DTG- and NNRTI-based ART. Drug resistance was assessed among participants with viremia ≥50 copies/mL on DTG.

***Results.*** Viral suppression was achieved for 165/195 (85%) and 154/211 (73%) participants in the DTG- and NNRTI- cohorts, respectively ($P = 0.003$). DTG-based ART was associated with >2 times the odds of viral suppression versus NNRTI-based ART (adjusted odds ratio, 2.10 [95% confidence interval {CI}, 1.12–3.94]; adjusted risk ratio, 1.11 [95% CI, 1.00–1.24]). HIV-1 genotypic resistance testing (GRT) before ART initiation was done in 14 of 30 viremic participants on DTG, among whom nucleoside reverse transcriptase inhibitor (NRTI), NNRTI, and protease inhibitors resistance was detected in 0 (0%), 2 (14%) and 1 (7%), respectively. No resistance was found in the 2 of 30 participants with available GRT at the time of viremia ≥50 copies/mL.

***Conclusions.*** Virological suppression at 1 year was higher in participants initiating DTG- versus NNRTI-based ART. In those with viremia ≥50 copies/mL on DTG-based ART, there was no pretreatment or acquired resistance to the DTG co-administered NRTIs, although the number of samples tested was small.

**Keywords.** HIV-1; ART-naive; dolutegravir; drug resistance; sub-Saharan Africa.

**Background**

Globally, almost 38 million people are living with HIV and two thirds of these reside in sub-Saharan Africa (SSA)(1). To end the HIV epidemic with the global 95-95-95 targets set by UNAIDS for 2025, one key element is access to antiretroviral therapy (ART) for all people with HIV (PWH)(156,157). Until recently, in most low- and middle-income countries (LMIC) first-line ART consisted of a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimen containing either nevirapine or efavirenz. However, in the last years a substantial increase of pretreatment drug resistance (PDR) for NNRTIs has been observed in SSA(109), in some countries exceeding the World Health Organization (WHO) recommended threshold of 10%(56), thus, compromising the ambitious target to end the HIV epidemic by 2030(158). PDR resulting in virologic failure (VF) might go undetected for long timespans in LMIC due to limited resources for viral load (VL) and resistance testing, leading to increased morbidity, mortality, and onward transmission of virus (159). Therefore, transition to integrase-inhibitors characterized by a faster viral suppression, a higher barrier to resistance and fewer side effects compared to NNRTIs within randomized controlled trials(160), has been advocated by many national HIV programs.

By the end of 2017, dolutegravir (DTG)-based therapy became available to LMIC as the generic fixed dose combination of tenofovir disoproxil fumarate, lamivudine and dolutegravir (TLD) at the favorable price of 75 USD per person per year(161). Since then, many LMIC have rolled out DTG-based ART. Initially, the WHO advised women of childbearing age to avoid DTG-based ART, when planning a pregnancy or not being on consistent contraception due to a signal for an increased risk of neural tube defects in the offspring of women on DTG-based ART at conception in the Tsepamo study in Botswana(162). Only after more data became available showing a considerably lowered risk of neural tube defects, the WHO recommended DTG-based ART for all adolescents and adults in July 2019.

So far, limited data outside of clinical trials is available on the clinical and virologic outcome after the transit from NNRTI-based to DTG-based regimens in SSA(12,137,153). Evaluation of the rollout of DTG-based ART under programmatic conditions in resource-limited regions is important given the challenges outlined above. With this study, we aimed to compare viral suppression in treatment-naïve patients at 12 months after initiating DGT-based versus NNRTI-based ART. Additionally, we assessed predictors associated with viral suppression and analysed

PDR mutations and acquired resistance, side effects, and pregnancy outcomes in patients initiated on a DTG-based regimen.

## Methods

### Study design and setting

This is a retrospective, observational study nested within the Kilombero and Ulanga antiretroviral cohort (KIULARCO), a prospective cohort of PWH. The cohort includes patients seen at the Chronic Diseases Clinic of Ifakara (CDCI), the care and treatment center for PWH of the Saint Francis Referral Hospital (SFRH), located in rural southeastern Tanzania. The cohort was established in 2005 and captures comprehensive demographic and clinical data, including ART use and monitoring, co-morbidities and treatment outcomes, with details described previously(94,96).

### Study population

Two groups of PWH aged ≥15 years attending KIULARCO were included in the analysis: a) treatment-naïve patients initiating NNRTI-based ART between 16$^{th}$ December 2016 and 15$^{th}$ December 2017 (referred to as the NNRTI cohort) and b) treatment-naïve patients initiating DTG-based ART between 16$^{th}$ March 2019 and 15$^{th}$ September 2020 (referred to as the DTG cohort). The separation of periods ensured that no patient in the NNRTI-cohort would have been switched to a DTG-based regimen by the time of outcome assessment, which could have had an impact on VL results.

We excluded patients initiating protease inhibitor (PI)-based therapy in both periods, patients starting NNRTI-based ART between 16$^{th}$ March 2019 and 15$^{th}$ September 2020, treatment-experienced patients (assessed in the baseline questionnaire by asking if they had ever received ART before), those not starting ART during the given time periods and those without written informed consent to KIULARCO (Figure 1). Reasons for excluding patients starting PI-based ART was that this is not the usual first-line therapy in Tanzania. Similarly, those initiating NNRTI-based ART during DTG roll-out were excluded as they were only few and would have only started on this therapy due to specific criteria.

### Data collection

Data for patient demographics, routine clinical information, and laboratory data as well as information on ART was extracted from OpenMRS, the KIULARCO electronic medical record system. As per routine care and according to the Tanzania National Guidelines for the Management of HIV and AIDS, patients receive a VL measurement at 6 and 12 months after treatment start, and once yearly thereafter for those with a VL <1000 copies/ml. At the same time point as VL measurements, blood samples are stored in a biobank at -80°C for research purposes.

### Laboratory measurements

VL measurement was done using the Abbott Real-time m2000 HIV-1 Assay (Abbott Laboratories, Chicago, Illinois), with a reportable range of 40-10,000,000 copies/mL for blood plasma. For this study, we performed HIV-1 genotypic resistance testing (GRT) from biobanked samples for those with an HIV VL ≥50 copies/mL. In addition, HIV-1 GRT was performed on samples available prior to treatment initiation for cases with a VL≥50 copies/mLat 12 months using cryopreserved samples, to determine PDR before ART initiation in the DTG cohort.

GRT was performed using a validated in-house PCR protocol to determine the HIV-1 drug resistance-associated mutations for Reverse Transcriptase (RT), Protease (PR) and Integrase Inhibitor (INSTI) – mutations(95,99). Briefly, RNA was be extracted from 150 µl of plasma using the PureLink™ Viral RNA/DNA Mini Kit (Invitrogen™, Thermo Fisher Scientific, USA), according to the manufacturer's protocol. RNA extracts were retrotranscribed and amplified using the HIV-1 Genotyping Kit Amplification Module (Applied Biosystems™, Thermo Fisher Scientific, USA)(101). A direct sequencing reaction was done using 6 overlapping primers, and assembly program (BioEdit 7.2) was used for sequence analysis. Mutations were interpreted according to the Stanford University's HIV Drug Resistance Database Program version 9.2 (http://hivdb.stanford.edu)(104). HIV drug resistant mutations conferring low, intermediate, or high-level resistance were considered. The reported protease and reverse transcriptase sequences are available in GenBank accession OQ627458-OQ627474.

### Definitions

Viral suppression was defined as <50 copies/ml and viremia as defined as ≥50 copies /ml, at one time-point measurement, and virological failure was defined as ≥1000 copies /ml after a minimum of 6 months on ART, based on two consecutive viral load measurements in 3 months (with adherence support following the first viral load test), in line with the most recent WHO guidelines(163). Loss to follow up (LTFU) was defined as being >60 days late for a scheduled visit.

Tuberculosis was recorded if within 3 months from enrolment, acid-fast bacilli, or a positive Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) from sputum or an extrapulmonary site were documented, or if anti-tuberculosis drugs with an ICD-10 code or clinical signs suggestive of tuberculosis were present. Unlikely tuberculosis was defined as no prescription of anti-tuberculosis drugs and no diagnosis of tuberculosis by ICD-10. For other cases, an indeterminate tuberculosis status was stated and treated as missing data.

**Study Outcomes**

The primary outcome was viral suppression at 12- months, allowing for a time window of 5-15 months with a preference for the measurement closest to 12 months after treatment start. The secondary outcomes of this study were prevalence of PDR and acquired resistance among patients presenting with viremia on DTG-based ART, defined as the presence of resistance associated mutations; plus, side effects and pregnancy outcomes in the DTG-cohort.

**Statistical methods**

Demographic characteristics were summarized using frequencies and percentages. We compared descriptively baseline characteristics between participants with and without a viral load result at 12 months. The proportion of participants with viral suppression in the DTG and NNRTI cohorts was compared using a chi square test. Factors associated with 12-month viral suppression were determined using logistic regression models, reporting odds ratios (OR) and 95% confidence intervals (CI). We also calculated adjusted risk ratios (aRR) after fitting the multivariable model with standard errors estimated using the delta method(164). The multivariable model incorporated the following covariates which were selected a priori: age, gender, marital status, disclosure of HIV status, education level, distance in kilometers of residence from the clinic, body mass index (BMI), HIV WHO stage, CD4 cell count, tuberculosis status and cohort (DTG-based or NNRTI-based ART). No model selection was done. We performed a sensitivity analysis restricting VL measurement to a time window of 9-15 months from treatment start. The analysis was repeated under the assumption that those LTFU were not virally suppressed, as such participants are likely to be off treatment and therefore not suppressed.

The prevalence of HIV-1 drug resistance, the proportion of patients who discontinued DTG-based ART and the proportion of women who conceived while on DTG-based ART and their pregnancy outcomes were described descriptively. All analyses were performed using Stata version 15 (StataCorp LP, College Station, TX, USA).

**Ethical considerations**

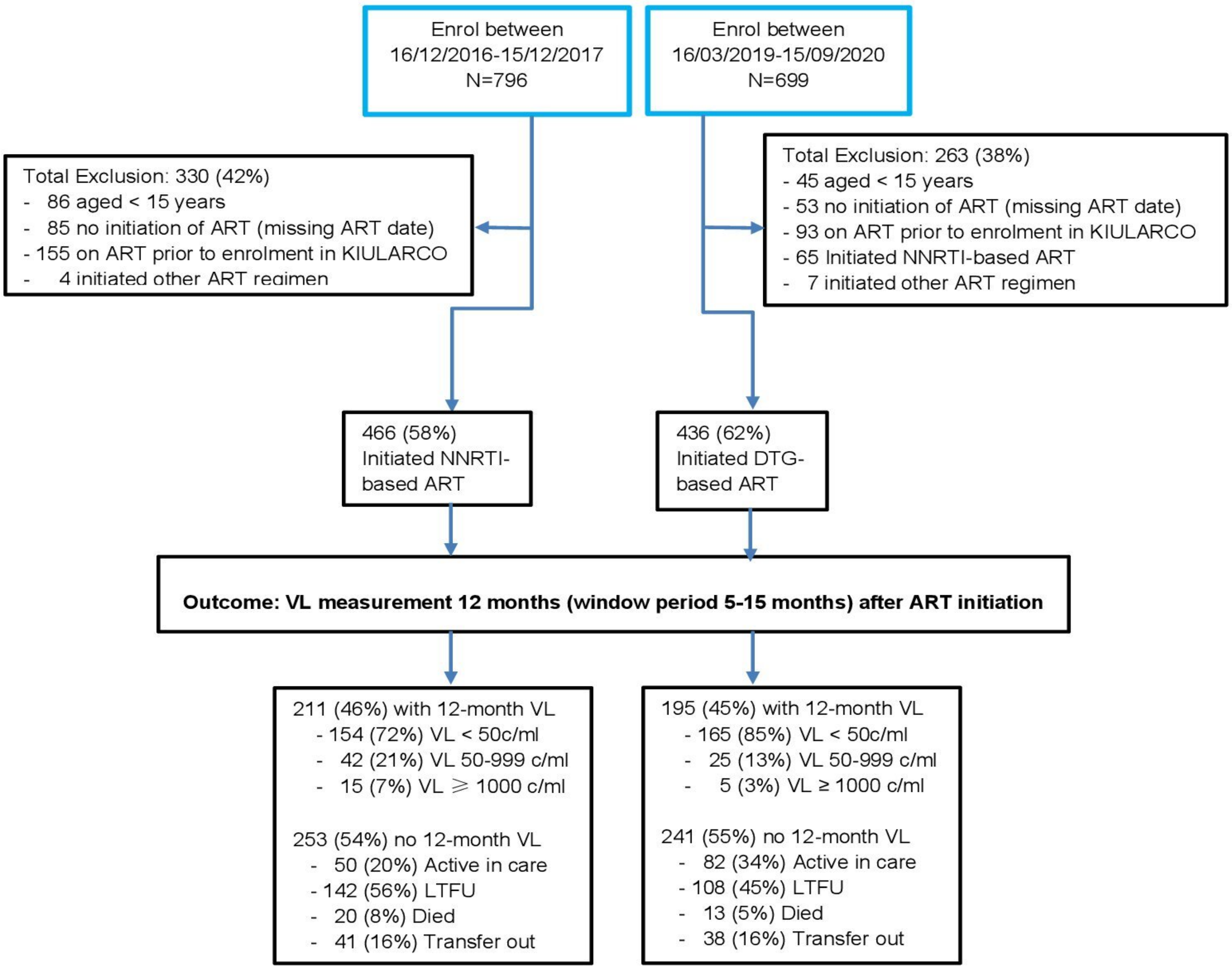
Written informed consent of patients willing to participate in KIULARCO are obtained at registration. This study received ethical approval from the University of the Witwatersrand-Human Research Ethics Committee (Medical) Clearance Certificate No.M210714**.** Yearly ethical approval for data and sample collection as well as analysis are sought from the Ifakara Health Institute institutional review board (IHI/IRB/No16-2006) and the Health Review Committee of the National Institute for Medical Research of Tanzania (NIMR/HQ/R.8c/Vol.I/378).

## Results

**Study Population and Baseline Characteristics**

There were 436 and 466 patients in the DTG- and NNRTI- cohorts, respectively (Figure 1.)
The combined median age in both cohorts was 38 years (IQR 30-45). In the DTG- and NNRTI- cohorts, 280 (64%) and 300 (65%) patients were female, 292 (67%) and 263 (57%) were married/cohabiting, and 290 (72%) and 318 (79%) had disclosed their HIV status, and 51 (12%) and 106 (25%) were resident ≥50 km from clinic, respectively. The clinical parameters were broadly comparable within the two groups, with a normal BMI (18.5-25 Kg/$m^2$) in 230 (57%) and 259 (62%), a WHO stage III/IV in 107 (25%) and 166 (39%), a CD4 cell count of ≥350 cells/ml in 142 (37%) and 134 (34%), and tuberculosis coinfection in 33 (8%) and 52 (11%) participants, respectively (Table 1).

FIGURE 1: Patient flowchart for those initiating DTG or NNRTI-based regimens




We compared patients with and those without VL tests at 12 months and found them to be broadly comparable with exception of those with 12 months VL more likely to have disclosed their HIV status (309 (81%) vs 301(70%), be resident <1 km from clinic (210 (54%) vs 213 (45%)) and less likely to be HIV WHO stage III/IV ((115 (29%) vs 159 (35%) and CD4 count ≥350 cells/µl ((118 (31%) vs 151 (38%). Those without 12-month VL were more likely to have missed baseline characteristics measurements compared to those with VL (Table S1).

**Table 1: Patients' characteristics at initiation of NNRTI- or DTG-based ART regimens**

| Patient characteristics | Initiated NNRTI-based ART 2016-2017 N=466 | Initiated DTG based ART 2019-2021 N=436 | *P-value* |
|---|---|---|---|
| **Socio-demographics** | | | |
| Age, years, n (%) | | | |
| 15 – 24 | 52 (11%) | 53 (12%) | 0.870 |
| 25 – 34 | 136 (29%) | 120 (28%) | 0.859 |
| 35 - 44 | 164 (35%) | 138 (32%) | 0.585 |
| ≥45 | 111 (24%) | 125 (29%) | 0.385 |
| Gender, Female, n (%) | 300 (65%) | 280 (64%) | 0.801 |
| Marital status, n (%) | | | |
| Married/Cohabiting | 263 (57%) | 292 (67%) | 0.015 |
| Never married | 63 (14%) | 28 (6%) | 0.270 |
| Separated/Divorced/Widowed | 137 (30%) | 116 (27%) | 0.598 |
| Disclosed HIV status, n (%) | | | |
| No | 85 (21%) | 113 (28%) | 0.260 |
| Yes | 318 (79%) | 290 (72%) | 0.044 |
| Missing | 60 (13%) | 33 (8%) | 0.464 |
| Education, n (%) | | | |
| None | 64 (14%) | 28 (6%) | 0.269 |
| Primary | 347 (75%) | 368 (84%) | 0.003 |
| Secondary and above | 52 (11%) | 40 (9%) | 0.753 |
| Distance of residence to clinic, n (%) | | | |
| <1 km | 182 (42%) | 240(56%) | 0.004 |
| 2— <50 km | 141 (33%) | 135 (32%) | 0.859 |
| ≥50 km | 106 (25%) | 51 (12%) | 0.062 |
| Missing | 34 (7%) | 10 (2%) | 0.554 |
| **Clinical** | | | |

| | | | |
|---|---|---|---|
| ART regimen, n (%) | | | |
| DTG-based | 0 (0%) | 436 (100%) | - |
| EFV-based | 456 (98%) | 0 (0%) | - |
| NVP-based | 9 (2%) | 0 (0%) | - |
| Other | 1 (0%) | 0 (0%) | - |
| Tuberculosis status, n (%) | | | |
| Unlikely | 405 (89%) | 395 (92%) | 0.148 |
| Yes | 52 (11%) | 33 (8%) | 0.650 |
| Missing | 6 (1%) | 8 (2%) | 0.881 |
| Body Mass Index (BMI)[b], Kg/$m^2$, n (%) | | | |
| Underweight (<18.5) | | | |
| Normal (18.5 - <25) | 66 (16%) | 52 (13%) | 0.647 |
| Overweight (≥25) | 259 (62%) | 230 (57%) | 0.260 |
| Missing | 63 (15%) | 123 (30%) | 0.025 |
| | 43 (9%) | 31 (7%) | 0.756 |
| HIV WHO stage[a], n (%) | | | |
| I/II | 260 (61%) | 316 (75%) | 0.001 |
| III/IV | 166 (39%) | 107 (25%) | 0.017 |
| Missing | 37 (8%) | 13 (3%) | 0.536 |
| CD4 count, cells/µl[a], n (%) | | | |
| <100 | 92 (24%) | 62 (16%) | 0.230 |
| 100 - 350 | 166 (42%) | 184 (47%) | 0.347 |
| ≥350 | 134 (34%) | 142 (37%) | 0.603 |
| Missing | 71 (15%) | 48 (11%) | 0.529 |

*Results are number and column % of those with non-missing data; missing data rows are number and column %.

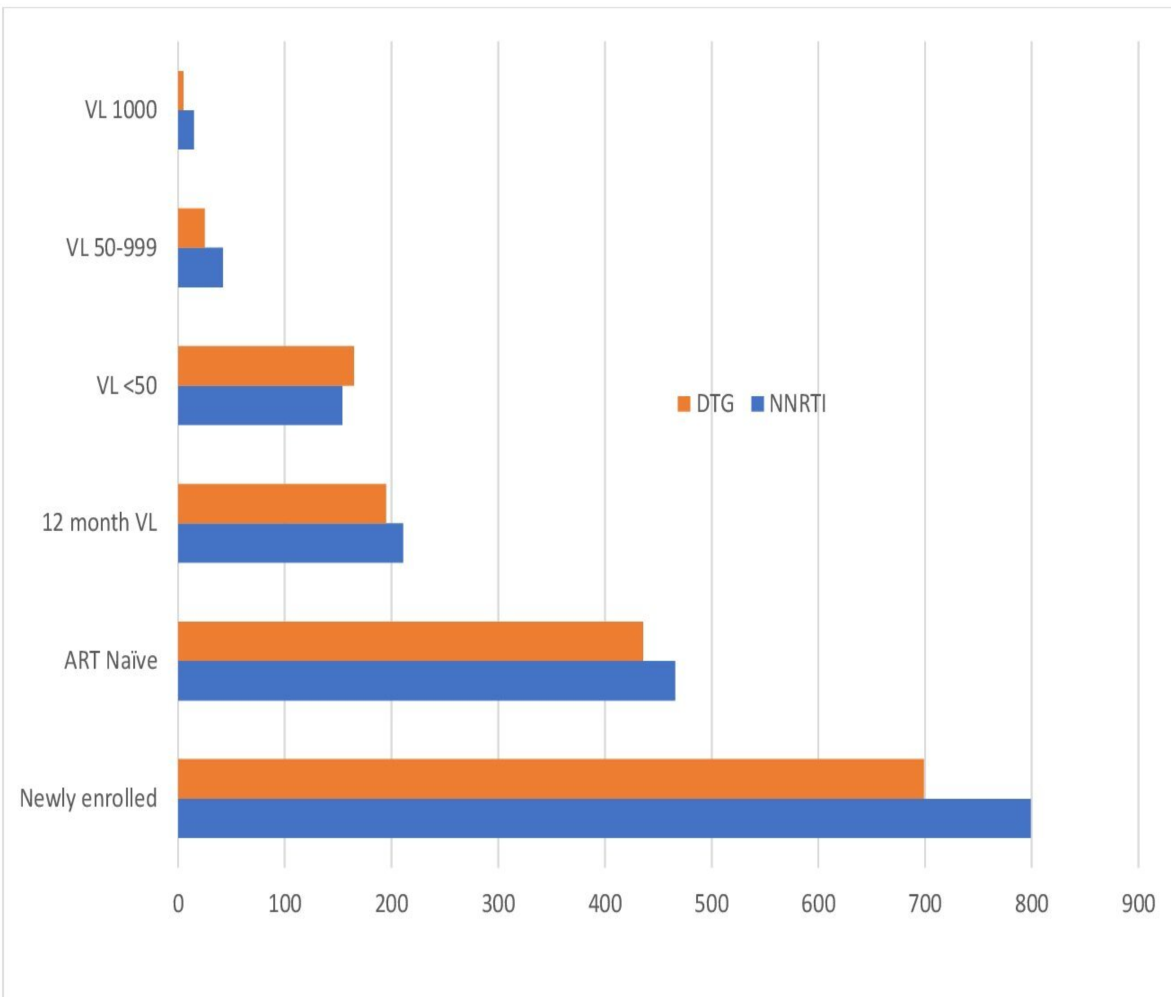
[a]Tuberculosis, BMI, HIV WHO stage and CD4 measurements closest to ART initiation within 6 months before and 3 months after.

**Virological Outcome**

VL data at 12 months after ART initiation were available for 195/436 (45%) and 211/466 (45%) participants in the DTG- and NNRTI-cohorts, respectively (Figure 2). Among those with VL results available, viral suppression was achieved for 165/195 (85%) and 154/211 (73%) participants in the DTG- and NNRTI- cohorts, respectively (p=0.003). For those with viremia in the DTG- and NNRTI-cohorts, 25/30 (83%) and 42/57 (74%) had a VL 50-999 copies/mL and

5/30 (17%) and 15/57 (26%) had VL ≥1000 copies/ml respectively. At one year following ART initiation, 108/436 (25%) patients in the DTG- cohort and 143/466 (31%) in the NNRTI-cohort were LTFU. (Figure 1). Assuming those LTFU were not virally suppressed, 165/303 (55%) and 154/353 (44%) achieved viral suppression in the DTG- and NNRTI-cohorts, respectively ($p<0.001$).

FIGURE 2: Virologic outcome at 12 months after initiation of reverse transcriptase inhibitor (NNRTI)- and dolutegravir (DTG)-based antiretroviral therapy (ART) in treatment naïve.



**Factors associated with virological suppression**

Results of the univariable and multivariable analyses are shown in Table 2. DTG-based versus NNRTI-based ART was independently associated with improved viral suppression (aOR 2.10, 95% CI 1.12-3.94 and aRR 1.11, 95% CI (1.00-1.24). In addition, the following factors were independently associated with viral suppression (Table 2): being separated/divorced/widowed (and to some extent married/cohabiting) versus never married, higher education level, and higher (worse) HIV WHO stage.

In sensitivity analysis restricting VL measurements to a time window of 9-15 months including 59/436 (14%) and 133/466 (29%) patients in the DTG- and NNRTI-cohorts, ART regimen is not associated with viral suppression, (aRR) 2.12 95% CI (0.82 – 5.49), compared to using 5-15 months VL windows above (Table S2).

**Table 2: Factors associated with viral suppression (HIV-1 RNA <50 copies/ml) at 12 months (5-15 month) among treatment-naïve patients initiating NNRT-based or DTG-based ART.**

| Characteristics | Unadjusted OR (95% CI) [a] | Adjusted OR (95% CI) [ab] | Adjusted RR (95% CI) [c] |
|---|---|---|---|
| **ART regimen** | | | |
| **NNRTI-based** | Reference | Reference | Reference |
| **DTG-based** | 2.16 (1.29 – 3.62) | 2.10 (1.12 – 3.94) | 1.11 (1.00 – 1.24) |
| **Age, years** | | | |
| **15 – 24** | Reference | Reference | Reference |
| **25 – 34** | 1.56 (0.62 – 3.89) | 1.81 (0.57 – 5.69) | 1.09 (0.87 – 1.37) |
| **35 - 44** | 1.05 (0.45 – 2.45) | 1.55 (0.51 – 4.73) | 1.03 (0.82 – 1.29) |
| **45 and above** | 1.23 (0.51 – 3.00) | 1.36 (0.43 – 4.31) | 0.99 (0.78 – 1.26) |
| **Gender** | | | |
| **Male** | Reference | Reference | Reference |
| **Female** | 0.65 (0.38 – 1.12) | 0.62 (0.32 – 1.21) | 0.96 (0.85 – 1.07) |

| | | | |
|---|---|---|---|
| **Marital Status** | | | |
| **Never** | Reference | Reference | Reference |
| **Married/Cohabiting** | 3.19 (1.51 – 6.74) | 2.03 (0.70 – 5.86) | 0.80 (0.58 – 1.10) |
| **Separated/divorced/widowed** | 2.94 (1.29– 6.71) | 3.32 (1.03– 10.7) | 1.04 (0.93 – 1.17) |
| **Disclosed HIV Status** | | | |
| **No** | Reference | Reference | Reference |
| **Yes** | 1.49 (0.81 – 2.71) | 1.58 (0.74 – 3.36) | 1.12 (0.95– 1.32) |
| **Education** | | | |
| **None** | Reference | Reference | Reference |
| **Primary** | 1.42 (0.65 – 3.09) | 1.79 (0.72 – 4.49) | 1.14 (0.90 – 1.45) |
| **Secondary and above** | 1.65 (0.57 – 4.79) | 4.72 (1.09– 20.4) | 1.31 (1.02– 1.70) |
| **Distance of residence to clinic** | | | |
| **≤1km** | Reference | Reference | Reference |
| **2—<50km** | 1.07 (0.60 – 1.89) | 1.27 (0.64 – 2.54) | 1.02 (0.91 – 1.15) |
| **≥50km** | 0.66 (0.33– 1.31) | 0.66 (0.30– 1.46) | 0.93 (0.78– 1.11) |
| **Tuberculosis status** | | | |
| **Unlikely** | Reference | Reference | Reference |
| **Yes** | 0.73 (0.32 – 1.64) | 1.21 (0.44 – 3.33) | 0.98 (0.81 – 1.19) |
| **Body Mass Index, Kg/m$^2$** | | | |
| **Underweight, <18.5** | 0.70 (0.35 – 1.41) | 0.86 (0.37 – 2.01) | 0.96 (0.81 – 1.15) |
| **Normal, 18.5 - <25** | Reference | Reference | Reference |
| **Overweight, ≥25** | 1.10 (0.62 – 1.95) | 1.46 (0.68 – 3.10) | 1.06 (0.93 – 1.20) |

| | | | |
|---|---|---|---|
| **HIV WHO Stage** | | | |
| **I/II** | Reference | Reference | Reference |
| **III/IV** | 2.06 (1.24 – 3.41) | 2.33 (1.17 – 4.65) | 1.14 (1.00 – 1.34) |
| **CD4 count, cells/µl** | | | |
| **<100** | Reference | Reference | Reference |
| **100 – 349** | 1.03 (0.54 – 1.96) | 0.70 (0.32 – 1.54) | 0.93 (0.81 – 1.06) |
| **350** | 2.13 (0.99 – 4.56) | 0.98 (0.39 – 2.45) | 1.00 (0.87 – 1.16) |

[a]Odds Ratios (OR) and 95% confidence intervals (CI) obtained from logistic regression.

[b]Adjusted for all variables shown in the table; patients with missing data excluded; N=330

[b]Adjusted risk ratios estimated from the multivariable logistic regression model with standard errors estimated using the delta method, adjusted for all variables shown in the table; patients with missing data excluded; N=330

**HIV-1 drug resistance associated mutations**

For patients with viremia at 12 months after starting DTG-based ART, blood plasma samples prior to treatment initiation were available for 25/30 (83%) patients. Of those, 14/25 (56%) samples were successfully PCR amplified and sequenced for PDR determination. In 2/14 (14%) samples we detected one mutation each, which were V108I and K103N mutations, known to be associated with NNRTI resistance. Additionally, 1/14 (7%) patient harbored the Q58E mutation, which is a PI-associated HIV drug resistant mutations. No mutations associated with NRTI were detected (Table 3).

Blood plasma samples at VL $\geq$ 50 copies/mL were available for 13/30 (43%) patients. PCR amplification and sequencing for determination of acquired and/or persisting HIV-1 drug resistance associated mutations was successful for only 2/13 (15%) patients. No mutations associated with HIV-1 drug resistance were detected (Table 3).

**<u>TABLE 3</u>: Patterns of mutations detected in the reverse transcriptase and protease region of the HIV-1 pol -sequences** in **participants on DTG -based ART with VL>=50 at 12 months.**

| Description | At baseline (*n* = 14)[a] | At 12 months (*n* = 2)[b] |
|---|---|---|
| Major NRTI HIV drug resistant mutations | 0 (0%) | 0 (0%) |
| Major NNRTI HIV drug resistant mutations | V108I 1 (7%)<br>K103N 1 (7%) | 0 (0%) |
| Major PI HIV drug resistant mutations | Q58E 1 (7%) | 0 (0%) |

[a] 14/25 (56%) samples were tested for pretreatment HIV-1 drug resistance associated mutations.

[b] Acquired HIV-1 drug resistance associated mutations was tested for only 2/13 (15%) patients at viremia VL ≥ 50 copies/mL

### Side effects and pregnancy outcomes

Seven (of 436, 2%) patients discontinued DTG-based due to side-effects. Of the women initiating DTG-based ART, thirty-three (12%) women had a documented pregnancy either at treatment initiation or during the follow-up period; 25 (76%) of those delivered a live born with no obvious birth defect, 3 (9%) had a reported abortion, 1 (3%) mother had a stillbirth, and 4 (12%) were LTFU before giving birth.

### Discussion

In this prospective real-world study from a rural setting in Eastern Africa, we compared the virological outcome of DTG- and NNRTI-based ART in treatment-naïve PWH. We found higher virological suppression rates on DTG-based ART compared to NNRTI-based ART at 12 months after initiation. The documented difference in viral suppression in our cohort was even higher than in the first trials evaluating DTG-containing first-line regimens in African settings(165,166). The cohorts are comparable as there were no major changes regarding the clinical practice guidelines during the timeframes, specifically ART initiation was recommended for all, regardless of CD4 count since October 2016 in Tanzania, and VL testing was recommended to monitor treatment success.

Furthermore, we assessed for the presence of PDR, among patients with viremia ≥50 copies/mL at months after ART initiation in the DTG-cohort. No PDR to NRTI was detected; PDR to NRTI and PIs was rare, albeit the subgroup for available sequencing data was small. Thus, the observed elevated VL might be due to delayed suppression after treatment start or poor adherence with treatment interruption(167). Analyses from randomized controlled trials (RCTs) and real-life data have shown good virological suppression of DTG-based ART among patients with PDR to NNRTI(77,78,168). In our study we did not systematically screen for PDR to DTG, as resistances to INSTI would not be expected during the beginning of DTG-based ART roll-out, as reported in recent studies in similar settings(128,132,169). Moreover, of 30 patients with detectable VL in the DTG-based cohort 25(83%) had low viremia (i.e., 50-999 copies/ mL), which may be a result of momentarily subtherapeutic drug levels due to suboptimal adherence(167).

The fact that we found no HIV-1 associated drug resistance mutations in PWH with a detectable VL on a DTG-based ART is reassuring. However, these results are mitigated by the fact that genotypic results were only available for a small subgroup of participants.

While the high proportion of patients with low viremia at 12 months in the DTG-based cohort might be due to early measurement with an expected further decrease, it remains worrisome, as most patients are expected to suppress viral load within 3 months and low viremia due to poor adherence may subsequently lead to VF and emergence of resistance(170). Even though resistance to DTG is still uncommon in this setting, evidence from other studies now shows that resistance to DTG may emerge over time, particularly following accumulation of resistance to the NRTIs back-bone(38–40,171). Hence vigilance and monitoring of low viremia among PWH on DTG-based therapy remain important.

More than half of the patients in both cohorts had no VL results in the given time span. One reason is the high rate of LTFU, which accounted for 56% and 44% of those without VL results in the NNRTI and the DTG-based cohorts, respectively. This rate is comparable to a previous report from 2005-2016 from the same clinic with LTFU rates of 41% in the first year after enrolment (97). Though some of these patients may have silently transferred and collected medication from another clinic, and therefore have a suppressed VL, others are likely to have suboptimal adherence, or stopped medication altogether. Importantly, viral suppression rate in the sensitivity analysis was still higher in the DTG-based cohort compared to the NNRTI-based cohort. Regardless, the high rate of LTFU in this rural setting in SSA remains a major concern of the treatment cascade and urgently needs a better understanding of patients' needs and adequate interventions(172).

Being on DTG-based ART was an important factor associated with improved viral suppression, which is in line with existing data from RCTs, that show excellent pharmacokinetic profile and tolerability of DTG, rapid viral suppression, and fewer side effects compared to NNRTIs in treatment-naïve and experienced patients(160). Similarly, in this study, we observed very few patients who discontinued DTG due to side effects. However more data on an extended time span is required and is the aim of future studies. Other factors associated with viral suppression were a higher education level and being separated/divorced/widowed. While other studies have indicated high odds of VF in clients who did not disclose their HIV status(173), we found no evidence of such an association. The association of virological suppression with advanced WHO clinical stages in our study might be due to patients feeling worse and therefore more likely to adhere to medication, also closer care and monitoring, and therefore better adherence(12). However, this outcome might also be affected by a reporting bias, as these patients are attending the clinic and are tested more frequently.

As in other settings, in the first year of DTG rollout in Tanzania, women of childbearing potential were given the choice to start either DTG-based or NNRTI-based ART, based on informed consent according to the WHO guidelines(174). Nevertheless, a number of women became pregnant while on DTG. Reassuringly, we did not document obvious birth defects in the 33 women with live births during the study period. One stillbirth and three abortions of unknown reasons were reported. While the numbers of this study are too low to draw firm conclusions, it is important to observe uptake among women and pregnancy outcomes as a large observational study from eleven LMICs has shown substantial disparities in the uptake of DTG affecting females of childbearing age (175). The benefit of rapid viral suppression in pregnant women is of uttermost importance to avoid viral transmission to their newborns.

To our knowledge, this is the first study addressing virological outcomes among treatment-naïve patients initiating DTG-based ART in Tanzania under programmatic conditions. Our study has several limitations. Most importantly, a large proportion of patients in both cohorts had no VL result in the given time span due to a high rate of LTFU. Another major limitation is unmeasured confounding as this is observational data. Further, many patients who were in active care also had no VL result. Most likely reasons were stockout of reagents or procedural challenges during VL testing implementation. In both timespans National guidelines recommended VL testing to monitor treatment success. The lack of available VL results might have led to overestimating the rates of viral suppression. Another limitation is that that many blood samples were not available for drug resistance testing. Furthermore, there was significant PCR amplification failure, especially in those with a low viral load, which might be due to poor samples storage quality. Finally, all genotypic data were obtained through standard Sanger sequencing; rates of HIV associated drug resistance mutations would possibly be higher if ultra-sensitive HIV-1 drug resistance testing by Next-Generation sequencing had been used.

**Conclusion**

Our results underline the benefit of programmatic uptake of DTG-based ART in LMICs. We did not find pretreatment resistance to the DTG co-administered NRTIs nor acquired resistance among viremic patients on DTG-based ART, although the number of samples tested was small. Continuous monitoring of pre-treatment and acquired resistance under programmatic condition during the roll out of DTG-based first-line is of utmost importance. LTFU remains high and needs further attention as it jeopardizes control of the HIV epidemic.

**Notes**

**Authors' contributions:** AJN, AE and MW conceptualized the study, designed the experiments, and wrote the manuscript. AN, NK performed the viral load and resistance testing. AN, AE, JO, FV analysed the data and reviewed the manuscript. AN, AE, JO, FV, RN, NK, JF, JK, MJ participated in the interpretation of the data, writing, reviewing, and approving the final manuscript. The corresponding author AN is the guarantor of the paper.

**Acknowledgements**: We are thankful to all the participants of the KIULARCO cohort. We thank the staff of the Chronic Diseases Clinic of St Francis Referral Hospital in Ifakara for making this study possible.

**Patient Consent Statement:** Written informed consent of patients willing to participate in KIULARCO are obtained at registration. This study received ethical approval from the University of the Witwatersrand-Human Research Ethics Committee (Medical) Clearance Certificate No.M210714**.** Yearly ethical approval for data and sample collection as well as analysis are sought from the Ifakara Health Institute institutional review board (IHI/IRB/No16-2006) and the Health Review Committee of the National Institute for Medical Research of Tanzania (NIMR/HQ/R.8c/Vol.I/378)

**Financial support.**: KIULARCO receives funds from the Ministry of Health and Social Welfare of Tanzania, the Government of the Canton of Basel, the Swiss Tropical and Public Health Institute, the Ifakara Health Institute, and the United States Agency for International Development (USAID) through the local implementer. AJN was supported by the Consortium for Advanced Research Training in Africa (CARTA). CARTA is jointly led by the African Population and Health Research Center and the University of the Witwatersrand and funded by the Carnegie Corporation of New York (Grant No—G-19-57145), Sida (Grant No:54100113), Uppsala Monitoring Centre and the DELTAS Africa Initiative (Grant No: 107768/Z/15/Z). The DELTAS Africa Initiative is an independent funding scheme of the African Academy of Sciences (AAS)'s Alliance for Accelerating Excellence in Science in Africa (AESA) and supported by the New Partnership for Africa's Development Planning and Coordinating Agency (NEPAD Agency) with funding from the Wellcome Trust (UK) and the UK government. The funders had no rule in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

**Potential Conflicts of Interest:** All authors report no potential conflicts.

**Data Availability Statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Table S1: Patients' characteristics at initiation for patients without and with 12-month viral measurement**

| Patient characteristics | Without 12-month VL N=496 | With 12-month VL N=406 |
|---|---|---|
| **Socio-demographics** | | |
| **Age, years, n (%)** | | |
| **15 – 24** | 67 (14%) | 39 (10%) |
| **25 – 34** | 150 (30%) | 106 (26%) |
| **35 - 44** | 155 (31%) | 147 (36%) |
| **≥45** | 125 (25%) | 113 (28%) |
| **Gender, Female, n (%)** | 311 (63%) | 271 (67%) |
| **Marital status, n (%)** | | |
| **Married/Cohabiting** | 301 (61%) | 256 (63%) |
| **Never married** | 56 (11%) | 36 (9%) |
| **Separated/Divorced/Widowed** | 140 (28%) | 113 (28%) |
| **Disclosed HIV status, n (%)** | | |
| **No** | 127 (30%) | 71 (19%) |
| **Yes** | 301 (70%) | 309 (81%) |
| **Missing** | 69 (14%) | 25 (6%) |
| **Education, n (%)** | | |
| **None** | 56 (11%) | 37 (9%) |
| **Primary** | 391 (79%) | 326 (81%) |
| **Secondary and above** | 50 (10%) | 42 (10%) |
| **Distance of residence to clinic, n (%)** | | |
| **<1 km** | 213 (45%) | 210 (54%) |
| **2— <50 km** | 150 (32%) | 126 (33%) |

| | | |
|---|---|---|
| **≥50 km** | 107 (23%) | 52 (13%) |
| **Missing** | 27 (5%) | 17 (4%) |
| **Clinical** | | |
| **Tuberculosis status, n (%)** | | |
| **Unlikely** | 436 (90%) | 369 (93%) |
| **Yes** | 51 (11%) | 30 (8%) |
| **Missing** | 10 (2%) | 6 (2%) |
| **Body Mass Index (BMI)[b], Kg/m$^2$, n (%)** | | |
| **Underweight (<18.5)** | 66 (15%) | 53 (13%) |
| **Normal (18.5 - <25)** | 254 (59%) | 236 (60%) |
| **Overweight (≥25)** | 112 (26%) | 106 (27%) |
| **Missing** | 65 (13%) | 10 (3%) |
| **HIV WHO stage[b], n (%)** | | |
| **I/II** | 290 (66%) | 286 (71%) |
| **III/IV** | 159 (35%) | 115 (29%) |
| **Missing** | 48 (10%) | 4 (1%) |
| **CD4 count, cells/µl[b], n (%)** | | |
| **<100** | 90 (22%) | 72 (19%) |
| **100 - 350** | 161 (40%) | 191 (50%) |
| **≥350** | 151 (38%) | 118 (31%) |
| **Missing** | 95 (19%) | 24 (6%) |

Results are number and column % of those with non-missing data; missing data rows are number and column %.

[b]Tuberculosis, BMI, HIV WHO stage and CD4 measurements closest to ART initiation within 6 months before and 3 months after.

**Table S2: Factors associated with viral suppression (HIV-1 RNA <50 copies/ml) at 12 months (+/- 3 months) among patients initiating NNRT-based or DTG-based ART.**

| Characteristics | Unadjusted OR (95% CI) [a] | Adjusted OR (95% CI) [ab] |
|---|---|---|
| **ART regimen** | | |
| **NNRTI-based** | Reference | Reference |
| **DTG-based** | 2.07 (0.93 – 4.65) | 2.12 (0.82 – 5.49) |
| **Age, years** | | |
| **15 – –4** | Reference | Reference |
| **25 – 34** | 1.11 (0.33 – 3.77) | 1.39 (0.32 – 5.93) |
| **35 - 44** | 0.75 (0.24 – 2.29) | 0.81 (0.20 – 3.28) |
| **45 and above** | 0.51 (0.16 – 1.62) | 0.33 (0.06 – 1.43) |
| **Gender** | | |
| **Male** | Reference | Reference |
| **Female** | 0.72 (0.34 – 1.52) | 0.36 (0.13 – 0.98) |
| **Marital Status** | | |
| **Never** | Reference | Reference |
| **Married/Cohabiting** | 3.04 (1.14 – 8.15) | 2.78 (0.71 – 10.9) |
| **Separated/divorced/widowed** | 2.05 (0.70– 6.00) | 2.68 (0.61– 11.7) |
| **Disclosed HIV Status** | | |
| **No** | Reference | Reference |
| **Yes** | 1.87 (0.81 – 4.32) | 1.90 (0.69 – 5.19) |
| **Education** | | |
| **None** | Reference | |
| **Primary** | 4.76 (1.58 – 14.30) | |
| **Secondary and above** | 8.25 (1.87 – 36.4) | |
| **Distance of residence to clinic** | | |
| **≤1km** | Reference | Reference |
| **2—<50km** | 1.08 (0.50 – 2.36) | 1.29 (0.52 – 3.21) |
| **≥50km** | 0.43 (0.67– 1.10) | 0.46 (0.16– 1.38) |
| **Tuberculosis status** | | |
| **Unlikely** | Reference | Reference |
| **Yes** | 0.87 (0.26 – 2.90) | 1.42 (0.32 – 6.41) |

| | | |
|---|---|---|
| **Body Mass Index, Kg/m$^2$** | | |
| **Underweight, <18.5** | 0.61 (0.22 – 1.68) | 0.74 (0.22 – 2.48) |
| **Normal, 18.5 - <25** | Reference | Reference |
| **Overweight, ≥25** | 1.01 (0.46 – 2.26) | 1.10 (0.40 – 3.04) |
| **HIV WHO Stage** | | |
| **I/II** | Reference | Reference |
| **III/IV** | 2.54 (1.29 – 5.01) | 2.19 (0.72 – 6.67) |
| **CD4 count, cells/µl** | | |
| **<100** | Reference | |
| **100 – 349** | 1.42 (0.59 – 3.41) | |
| **350** | 5.11 (1.66 – 15.7) | |

[a]Odds Ratios (OR) and 95% confidence intervals (CI) obtained from logistic regression.
[b]Adjusted for all variables shown in the table except education and CD4 count due to model instability; patients with missing data excluded; N=160

# PART 3

# GENERAL DISCUSSION

# CHAPTER 7

## Summary, Overall discussion, and Conclusions

Highlights of the key findings

1. The overall prevalence of pre-treatment HIV-1 drug resistance in Eastern Africa, using data available from 2017-2022, has surpassed 10%.
2. The prevalence of RHI in this referral hospital-based cross-sectional study in a rural setting is 4%
3. The proportional of pre-treatment HIV-1 drug resistance among RHI cases is alarmingly high.
4. There is increasing tendency of pre-treatment HIV-1 drug resistance to NRTI in the Kilombero and Ulanga antiretroviral cohort.
5. Virological suppression rate on DTG-based ART is high compared to NNRTI-based ART at 12 months after initiation.

This thesis is dedicated to epidemiological and public health studies related to pre-treatment HIV-1 drug resistance (PDR) in Eastern Africa, with the focus in under-sampled area of rural Tanzania. To measure the change in PDR as a key component in assessing and addressing the threat of HIV drug resistance in Tanzania as in other Sub-Saharan African (SSA) countries.
We assessed the levels of PDR among ART- naïve adults living with HIV in seven Eastern Africa countries (*In paper I*). The overall pooled prevalence estimate of any PDR was higher than previous reported in the region, that included PDR data available until 2016(11). The high PDR prevalence was mainly driven by non-nucleoside reverse transcriptase inhibitors (NNRTIs) resistance, which underlines the importance of the current WHO recommended programmatic shift to dolutegravir based- triple ART for HIV treatment initiation (176). Tanzania started the rollout of dolutegravir-based ART in March 2019, by switching treatment experienced patients and initiating newly HIV-1 diagnosed patients on dolutegravir based- triple ART(176).

Remarkably, though on a limited sample size, prevalence estimate of any PDR was as high as 31% in the sub-population with recent HIV-infection (RHI) (*In Paper II*). The observed high PDR prevalence in recent versus longer-standing infected individuals could possibly be explained by the fact, that a proportion of drug resistance associated mutations are overgrown by more replication-competent wild-type virus over time in chronically infected patients(177), especially in individuals who have never been exposed to ART, and thus not detected by standard genotypic viral resistance detection methods, e.g., population-based Sanger sequencing methods. This underlines the importance of intensifying strategies for PDR surveillance among recently HIV-infected patients in Tanzania.

We observed a low but notable prevalence of PDR to nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) below 5%, during a five-year period (2017-20220), in East-Africa (*In paper I*). However, prevalence of PDR to NRTIs > 5% was observed in a few individual studies, including those on PDR to NRTIs in HIV high-risk population groups, e.g., young adults and people with intravenous drug use(43,124,130). Notably, PDR to NRTI was up to 4 times more common among HIV recently infected sub-population (*In paper II*) than observed in the general population (*In paper I*)(56). Moreover, in the general ART-naïve adults' population in the Kilombero and Ulanga antiretroviral cohort (KIULARCO) (*In paper III*), there was a tendency increase in prevalence of PDR to NRTI over time; 2.1% in the first five-year period (2005-2009) of the ART program in Tanzania, 3.4% in the most recent period (2019-2022). Certainly, surveillance of PDR to NRTIs remains particularly important, as these backbone drugs remained the same throughout the change to dolutegravir first line treatment.

Of-note, no major PDR to dolutegravir (DTG)-associated mutation was observed during a five-year period (2017-2022) in Eastern Africa (*In paper I*). Similar observation has also been reported in West Africa(178,179),which further suggests effectiveness of dolutegravir-based regimens, and thus re-assuring for the ongoing DTG- based triple ART rollout in many Low- & middle-income countries(180). Nevertheless, accessory mutations associated with DTG resistance are common (128,180)(48), which - when accumulating with other mutations - may significantly reduce viral susceptibility to integrase inhibitors(180). Even though no major PDR to DTG was reported, DTG is relatively new in Eastern Africa, hence continuous surveillance is warranted.

In an observational study (*In paper IV*), virological suppression at 12 months was significantly higher in patients initiating DTG-based ART compared to NNRTI-based ART under a programmatic condition in a rural Tanzanian setting. DTG is known for its interesting pharmacokinetic profile, including rapid viremia suppression rates and high genetic barrier to developing resistance. However, existing weakness in the ART program functioning, coupled with patients' factors leading to inadequate adherence to ART, inevitably results in an increased risk of acquiring HIV-1 drug resistance mutations, which may in turn lead to an increased risk of PDR in individuals who have never been exposed to ART(152). Even though, we did not assess the emergence of resistance to DTG- in this study, DTG HIV resistance mutation have recently been reported among patients experiencing virologic failure in Tanzania (181), and neighbour country-

Malawi(168). Monitoring of emergence and spread of resistance to DTG remains important, particularly in resource-limited settings.

Moreover, PDR to NRTI have been previously reported as major risk factor for virological failure on DTG based ART, and subsequent emergence of DTG resistance, due to functional monotherapy in triple combination with inactive NRTIs(76,137,168,182). However, in this study (*In paper IV*), there were no HIV drug resistant mutations associated with PDR to DTG co-administered NRTI, among viraemic patients at one year on dolutegravir-based first-line ART. Though firm inference is difficult due to the small number of samples tested.

PDR to protease inhibitors (PI) was below 1% (*In paper I*). This is re-assuring as PI (mainly LPV/r plus a two-NRTI backbone) is still recommended as a preferred first-line for HIV treatment initiation in infants and children in Tanzania as in many other LMICs(176), and remains the second line treatment in adults upon treatment failure. Though on a small sample size, prevalence of PDR to PIs >5% was documented among ART-naïve recently HIV infected adult patients (*In paper II*), also among patients failing on DTG based triple ART with 3TC/TDF, at the time of ART initiation (*In paper IV*). Notably, prevalence of PDR to PIs >5% was reported in individual studies using high throughput deep genotypic resistance testing methods (*In paper I*)(113,119). These observations warrant continuous surveillance of PDR to PI, especially now that the costs for deep sequencing technologies are going down and accessibility is increasing in Tanzania.

The major conclusions from this PhD research.

1. There is a notable high prevalence of PDR in Eastern Africa, and tendency to increasing prevalence of PDR to NRTI particularly in the KIULARCO, as a representative rural SSA setting.
2. There is a high prevalence of PDR to NRTI among recently HIV-1 infected PLHIV, even though inference with small numbers is challenging.
3. High proportion of newly HIV-1 diagnosed PLHIV in this rural Tanzanian setting have chronic HIV-1 infection.
4. Evidence from individual studies in a systematic review shows high PDR to NRTI in populations at high risk of HIV transmission.
5. Finally, being on DTG-based ART was an important factor associated with improved viral suppression; No PDR to INSTI was found.

RECOMMEDANTION

1. Routine surveillance of pre-existing resistance to the DTG co-administered NRTI remains particularly important, in resource-limited settings, to prevent risk of failure of newer antiretroviral agents such as dolutegravir, which would be detrimental for Tanzania and other low- and middle-income countries for the aim to “end AIDS by 2030”.
2. Given that our data show low prevalence of recent HIV-1 infection among newly HIV-1 diagnosed PLHIV, implying that diagnosis of HIV is delayed in this rural Tanzanian setting. It’s important to strengthening strategies for early HIV cases identification and linkage into care and treatment.
3. Particular attention should be paid to people with recent HIV infection, given that our data show high prevalence of not only PDR to NNRTI but also NRTI- backbone mutations in this group. Also, PDR among recently HIV-1 infected population needs to be a consideration while estimating the burden of PDR in a population.
4. Strengthening surveillance of PDR in populations at high risk of HIV transmission (e.g., youth and young adults and people with intravenous drug use) is certainly important.
5. Since DTG is relatively new in Eastern Africa. Long-term continuous surveillance and monitoring of PDR to DTG is warranted.

**References:**


1. UNAIDS 2021 epidemiological estimates. Unaids. 2021.
2. Global HIV & AIDS statistics — Fact sheet | UNAIDS [Internet]. [cited 2022 Nov 24]. Available from: https://www.unaids.org/en/resources/fact-sheet
3. Mouton JP, Cohen K, Maartens G. Key toxicity issues with the WHO-recommended first-line antiretroviral therapy regimen. Expert Rev Clin Pharmacol [Internet]. 2016 Nov 1 [cited 2023 Apr 5];9(11):1493–503. Available from: https://pubmed.ncbi.nlm.nih.gov/27498720/
4. World Health Organisation. Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection. Geneva: World Health Organization [Internet]. 2013 [cited 2023 Apr 4];14(7):269. Available from: https://www.ncbi.nlm.nih.gov/books/NBK195400/
5. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. :269.
6. GUIDELINES. 2019 [cited 2023 Apr 4]; Available from: http://www.eacsociety.org
7. Stellbrink HJ, Reynes J, Lazzarin A, Voronin E, Pulido F, Felizarta F, et al. Dolutegravir in antiretroviral-naive adults with HIV-1: 96-week results from a randomized dose-ranging study. AIDS [Internet]. 2013 Jul 17 [cited 2021 Dec 27];27(11):1771–8. Available from: https://pubmed.ncbi.nlm.nih.gov/23807273/
8. Trottier B, Lake JE, Logue K, Brinson C, Santiago L, Brennan C, et al. Dolutegravir/abacavir/lamivudine versus current ART in virally suppressed patients (STRIIVING): a 48-week, randomized, non-inferiority, open-label, Phase IIIb study. Antivir Ther [Internet]. 2017 [cited 2021 Dec 27];22(4):295–305. Available from: https://pubmed.ncbi.nlm.nih.gov/28401876/
9. Wilson HI, Mapesi H. Rollout of dolutegravir-based antiretroviral therapy in sub-Saharan Africa and its public health implications. Pan Afr Med J [Internet]. 2020 Sep 1 [cited 2023 Apr 5]; 37:1–4. Available from: /pmc/articles/PMC7847221/
10. Dorward J, Lessells R, Drain PK, Naidoo K, de Oliveira T, Pillay Y, et al. Dolutegravir for first-line antiretroviral therapy in low-income and middle-income countries: uncertainties and opportunities for implementation and research. Lancet HIV [Internet]. 2018 Jul 1 [cited 2021 Dec 10];5(7): e400–4. Available from: https://pubmed.ncbi.nlm.nih.gov/29884404/
11. Gupta RK, Gregson J, Parkin N, Haile-Selassie H, Tanuri A, Andrade Forero L, et al. HIV-1 drug resistance before initiation or re-initiation of first-line antiretroviral therapy in low-income and middle-income countries: a systematic review and meta-regression analysis. Lancet Infect Dis [Internet]. 2018 Mar 1 [cited 2021 Dec 27];18(3):346–55. Available from: https://pubmed.ncbi.nlm.nih.gov/29198909/

12. Mehari EA, Muche EA, Gonete KA. Virological suppression and its associated factors of dolutegravir based regimen in a resource-limited setting: An observational retrospective study in Ethiopia. HIV/AIDS - Research and Palliative Care. 2021;13.
13. d'Vinci Interactive and POZ. HIV Drug Resistance Explained - POZ [Internet]. [cited 2023 Apr 5]. Available from: https://www.poz.com/basics/hiv-basics/hiv-drug-resistance
14. Bertagnolio S, De Luca A, Vitoria M, Essajee S, Penazzato M, Hong SY, et al. Determinants of HIV drug resistance and public health implications in low- and middle-income countries. Antivir Ther. 2012;17(6):941–53.
15. Parikh UM, Bacheler L, Koontz D, Mellors JW. The K65R Mutation in Human Immunodeficiency Virus Type 1 Reverse Transcriptase Exhibits Bidirectional Phenotypic Antagonism with Thymidine Analog Mutations. J Virol [Internet]. 2006 May 15 [cited 2023 Apr 5];80(10):4971. Available from: /pmc/articles/PMC1472090/
16. Zhang Y, Ma L. Application of high-throughput sequencing technology in HIV drug resistance detection. Biosaf Health. 2021 Oct 1;3(5):276–80.
17. Clutter DS, Jordan MR, Bertagnolio S, Shafer RW. HIV-1 drug resistance and resistance testing. Infection, Genetics and Evolution. 2016 Dec 1; 46:292–307.
18. Moscona R, Ram D, Wax M, Bucris E, Levy I, Mendelson E, et al. Comparison between next-generation and Sanger-based sequencing for the detection of transmitted drug-resistance mutations among recently infected HIV-1 patients in Israel, 2000-2014. J Int AIDS Soc [Internet]. 2017 [cited 2019 Jul 7];20(1):21846. Available from: http://doi.wiley.com/10.7448/IAS.20.1.21846
19. Resistance assays - Antiretroviral Resistance in Clinical Practice - NCBI Bookshelf [Internet]. [cited 2023 Apr 5]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK2252/
20. Zazzi M, Hu H, Prosperi M. The global burden of HIV-1 drug resistance in the past 20 years. PeerJ [Internet]. 2018 [cited 2023 Apr 5];2018(5). Available from: /pmc/articles/PMC5971836/
21. Tostevin A, White E, Dunn D, Croxford S, Delpech V, Williams I, et al. Recent trends and patterns in HIV-1 transmitted drug resistance in the United Kingdom. HIV Med [Internet]. 2017 Mar [cited 2019 Jun 2];18(3):204–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27476929
22. Sallam M, Şahin GÖ, Indriðason H, Esbjörnsson J, Löve A, Widell A, et al. Decreasing prevalence of transmitted drug resistance among ART-naive HIV-1-infected patients in Iceland, 1996-2012. Infect Ecol Epidemiol [Internet]. 2017 [cited 2019 Jun 2];7(1):1328964. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28649306

23. Machnowska P, Meixenberger K, Schmidt D, Jessen H, Hillenbrand H, Gunsenheimer-Bartmeyer B, et al. Prevalence and persistence of transmitted drug resistance mutations in the German HIV-1 Seroconverter Study Cohort. Mor O, editor. PLoS One [Internet]. 2019 Jan 16 [cited 2019 Aug 4];14(1): e0209605. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30650082

24. Bickel M, Hoffmann C, Wolf E, Baumgarten A, Wyen C, Spinner CD, et al. High effectiveness of recommended first-line antiretroviral therapies in Germany: a nationwide, prospective cohort study. Infection [Internet]. 2020 Jun 1 [cited 2023 Apr 5];48(3):453–61. Available from: https://link.springer.com/article/10.1007/s15010-020-01428-1

25. Gupta RK, Jordan MR, Sultan BJ, Hill A, Davis DHJ, Gregson J, et al. Global trends in antiretroviral resistance in treatment-naive individuals with HIV after rollout of antiretroviral treatment in resource-limited settings: a global collaborative study and meta-regression analysis. Lancet [Internet]. 2012 Oct 6 [cited 2019 Jun 2];380(9849):1250–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22828485

26. Chimukangara B, Lessells RJ, Rhee SY, Giandhari J, Kharsany ABM, Naidoo K, et al. Trends in Pretreatment HIV-1 Drug Resistance in Antiretroviral Therapy-naive Adults in South Africa, 2000–2016: A Pooled Sequence Analysis. EClinicalMedicine [Internet]. 2019 Mar 1 [cited 2019 Jul 7]; 9:26–34. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2589537019300422

27. Fokam J, Takou D, Teto G, Nforbih SE, Kome OP, Santoro MM, et al. Pre-treatment drug resistance and HIV-1 genetic diversity in the rural and urban settings of Northwest-Cameroon. PLoS One [Internet]. 2020 Jul 1 [cited 2023 Apr 5];15(7): e0235958. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0235958

28. Rossouw TM, Nieuwoudt M, Manasa J, Malherbe G, Lessells RJ, Pillay S, et al. HIV drug resistance levels in adults failing first-line antiretroviral therapy in an urban and a rural setting in South Africa. HIV Med [Internet]. 2017 Feb 1 [cited 2023 Apr 5];18(2):104–14. Available from: https://pubmed.ncbi.nlm.nih.gov/27353262/

29. Barabona G, Mahiti M, Masoud S, Mbelele P, Mgunya AS, Minja L, et al. Pre-treatment and acquired HIV drug resistance in Dar es Salaam, Tanzania in the era of tenofovir and routine viral load monitoring. J Antimicrob Chemother [Internet]. 2019 Oct 1 [cited 2021 Dec 24];74(10):3016–20. Available from: https://pubmed.ncbi.nlm.nih.gov/31273377/

30. Rudovick L, Brauner JM, Englert J, Seemann C, Plugaru K, Kidenya BR, et al. Prevalence of pretreatment HIV drug resistance in Mwanza, Tanzania. Journal of Antimicrobial Chemotherapy [Internet]. 2018 Dec 1 [cited 2021 Dec 24];73(12):3476–81. Available from: https://academic.oup.com/jac/article/73/12/3476/5084894

31. Masimba P, Kituma E, Klimkait T, Horvath E, Stoeckle M, Hatz C, et al. Prevalence of drug resistance mutations and HIV type 1 subtypes in an HIV type 1-infected cohort in Rural Tanzania. AIDS Res Hum Retroviruses. 2013 Sep 1;29(9):1229–36.
32. Vairo F, Nicastri E, Liuzzi G, Chaula Z, Nguhuni B, Bevilacqua N, et al. HIV-1 drug resistance in recently HIV-infected pregnant mother's naïve to antiretroviral therapy in Dodoma urban, Tanzania. BMC Infect Dis [Internet]. 2013 Dec 21 [cited 2019 Apr 15];13(1):439. Available from: https://bmcinfectdis.biomedcentral.com/articles/10.1186/1471-2334-13-439
33. Kasang C, Kalluvya S, Majinge C, Stich A, Bodem J, Kongola G, et al. HIV drug resistance (HIVDR) in antiretroviral therapy-naïve patients in Tanzania not eligible for WHO threshold HIVDR survey is dramatically high. PLoS One. 2011;6(8).
34. Nyombi BM, Holm-Hansen C, Kristiansen KI, Bjune G, Müller F. Prevalence of reverse transcriptase and protease mutations associated with antiretroviral drug resistance among drug-naïve HIV-1 infected pregnant women in Kagera and Kilimanjaro regions, Tanzania. AIDS Res Ther. 2008 Jun 21;5.
35. Somi GR, Kibuka T, Diallo K, Tuhuma T, Bennett DE, Yang C, et al. Surveillance of transmitted HIV drug resistance among women attending antenatal clinics in Dar es Salaam, Tanzania. Antivir Ther [Internet]. 2008 [cited 2019 Apr 15];13 Suppl 2:77–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18575194
36. Mosha F, Urassa W, Aboud S, Lyamuya E, Sandstrom E, Bredell H, et al. Prevalence of Genotypic Resistance to Antiretroviral Drugs in Treatment-Naive Youths Infected with Diverse HIV Type 1 Subtypes and Recombinant Forms in Dar es Salaam, Tanzania. AIDS Res Hum Retroviruses [Internet]. 2011 Apr 1 [cited 2019 Oct 15];27(4):377–82. Available from: http://www.liebertpub.com/doi/10.1089/aid.2010.0113
37. Karade S, Chaturbhuj DN, Sen S, Joshi RK, Kulkarni SS, Shankar S, et al. HIV drug resistance following a decade of the free antiretroviral therapy programme in India: A review. International Journal of Infectious Diseases [Internet]. 2018 Jan [cited 2019 Aug 4]; 66:33–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29128646
38. Blanco JL, Marcelin AG, Katlama C, Martinez E. Dolutegravir resistance mutations: lessons from monotherapy studies. Curr Opin Infect Dis [Internet]. 2018 Jun 1 [cited 2021 Dec 27];31(3):237–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29634660
39. Hocqueloux L, Raffi F, Prazuck T, Bernard L, Sunder S, Esnault JL, et al. Dolutegravir Monotherapy Versus Dolutegravir/Abacavir/Lamivudine for Virologically Suppressed People Living with Chronic Human Immunodeficiency Virus Infection: The Randomized Noninferiority MONotherapy of TiviCAY Trial. Clinical Infectious Diseases. 2019 Oct 15;69(9):1498–505.

40. Wijting IEA, Lungu C, Rijnders BJA, Van Der Ende ME, Pham HT, Mesplede T, et al. HIV-1 Resistance Dynamics in Patients with Virologic Failure to Dolutegravir Maintenance Monotherapy. J Infect Dis [Internet]. 2018 Jul 24 [cited 2021 Dec 27];218(5):688–97. Available from: https://pubmed.ncbi.nlm.nih.gov/29617822/
41. Obasa AE, Ambikan AT, Gupta S, Neogi U, Jacobs GB. Increased acquired protease inhibitor drug resistance mutations in minor HIV-1 quasispecies from infected patients suspected of failing on national second-line therapy in South Africa. BMC Infect Dis [Internet]. 2021 Dec 1 [cited 2023 Apr 5];21(1):1–8. Available from: https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-021-05905-2
42. Bircher RE, Ntamatungiro AJ, Glass TR, Mnzava D, Nyuri A, Mapesi H, et al. High failure rates of protease inhibitor-based antiretroviral treatment in rural Tanzania – A prospective cohort study. PLoS One. 2020 Jan 1;15(1).
43. Budambula V, Musumba FO, Webale MK, Kahiga TM, Ongecha-Owuor F, Kiarie JN, et al. HIV-1 protease inhibitor drug resistance in Kenyan antiretroviral treatment-naive and -experienced injection drug users and non-drug users. AIDS Res Ther [Internet]. 2015 Aug 15 [cited 2021 Dec 27];12(1):1–9. Available from: https://link.springer.com/articles/10.1186/s12981-015-0070-y
44. Hamers RL, Schuurman R, Sigaloff KCE, Wallis CL, Kityo C, Siwale M, et al. Effect of pretreatment HIV-1 drug resistance on immunological, virological, and drug-resistance outcomes of first-line antiretroviral treatment in sub-Saharan Africa: A multicentre cohort study. Lancet Infect Dis. 2012 Apr;12(4):307–17.
45. Phillips AN, Stover J, Cambiano V, Nakagawa F, Jordan MR, Pillay D, et al. Impact of HIV Drug Resistance on HIV/AIDS-Associated Mortality, New Infections, and Antiretroviral Therapy Program Costs in Sub–Saharan Africa. J Infect Dis [Internet]. 2017 May 1 [cited 2023 Apr 5];215(9):1362–5. Available from: https://academic.oup.com/jid/article/215/9/1362/3002848
46. Davy-Mendez T, Eron JJ, Brunet L, Zakharova O, Dennis AM, Napravnik S. New antiretroviral agent use affects prevalence of HIV drug resistance in clinical care populations. AIDS [Internet]. 2018 Nov 13 [cited 2021 Dec 27];32(17):2593. Available from: /pmc/articles/PMC6209541/
47. Inzaule SC, Hamers RL, Doherty M, Shafer RW, Bertagnolio S, Rinke de Wit TF. Curbing the rise of HIV drug resistance in low-income and middle-income countries: the role of dolutegravir-containing regimens. Lancet Infect Dis. 2019 Jul 1;19(7): e246–52.
48. Masoud S, Kamori D, Barabona G, Mahiti M, Sunguya B, Lyamuya E, et al. Circulating HIV-1 Integrase Genotypes in Tanzania: Implication on the Introduction of Integrase Inhibitors-Based Antiretroviral Therapy Regimen. AIDS Res Hum Retroviruses [Internet].

2020 Jun 1 [cited 2022 Oct 28];36(6):539–43. Available from: https://www.liebertpub.com/doi/10.1089/aid.2020.0021

49. Kiwelu IE, Novitsky V, Kituma E, Margolin L, Baca J, Manongi R, et al. HIV-1 pol Diversity among Female Bar and Hotel Workers in Northern Tanzania. PLoS One [Internet]. 2014 Jul 8 [cited 2021 Dec 24];9(7): e102258. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0102258

50. Masimba P, Kituma E, Klimkait T, Horvath E, Stoeckle M, Hatz C, et al. Prevalence of Drug Resistance Mutations and HIV Type 1 Subtypes in an HIV Type 1-Infected Cohort in Rural Tanzania. AIDS Res Hum Retroviruses [Internet]. 2013 Sep 19 [cited 2019 Oct 15];29(9):1229–36. Available from: http://www.liebertpub.com/doi/10.1089/aid.2011.0367

51. Kasang C, Kalluvya S, Majinge C, Stich A, Bodem J, Kongola G, et al. HIV Drug Resistance (HIVDR) in Antiretroviral Therapy-Naïve Patients in Tanzania Not Eligible for WHO Threshold HIVDR Survey Is Dramatically High. Kallas EG, editor. PLoS One [Internet]. 2011 Aug 19 [cited 2019 Oct 15];6(8): e23091. Available from: https://dx.plos.org/10.1371/journal.pone.0023091

52. Sangeda RZ, Gómes P, Rhee SY, Mosha F, Camacho RJ, Wijngaerden E Van, et al. Development of HIV Drug Resistance in a Cohort of Adults on First-Line Antiretroviral Therapy in Tanzania during the Stavudine Era. Microbiology Research 2021, Vol 12, Pages 847-861 [Internet]. 2021 Nov 12 [cited 2022 Mar 29];12(4):847–61. Available from: https://www.mdpi.com/2036-7481/12/4/62/htm

53. Geretti AM, Fox Z V., Booth CL, Smith CJ, Phillips AN, Johnson M, et al. Low-frequency K103N strengthens the impact of transmitted drug resistance on virologic responses to first-line efavirenz or nevirapine-based highly active antiretroviral therapy. J Acquir Immune Defic Syndr (1988). 2009 Dec;52(5):569–73.

54. Cong M er, Heneine W, García-Lerma JG. The Fitness Cost of Mutations Associated with Human Immunodeficiency Virus Type 1 Drug Resistance Is Modulated by Mutational Interactions. J Virol. 2007 Mar 15;81(6):3037–41.

55. Johnson JA, Geretti AM. Low-frequency HIV-1 drug resistance mutations can be clinically significant but must be interpreted with caution. Journal of Antimicrobial Chemotherapy [Internet]. 2010 Jul 1 [cited 2023 Apr 5];65(7):1322–6. Available from: https://academic.oup.com/jac/article/65/7/1322/789526

56. Ntamatungiro AJ, Kagura J, Weisser M, Francis JM. Pre-treatment HIV-1 drug resistance in antiretroviral therapy-naive adults in Eastern Africa: a systematic review and meta-analysis. Journal of Antimicrobial Chemotherapy [Internet]. 2022 Nov 28 [cited 2023 Apr

5];77(12):3231–41. Available from: https://academic.oup.com/jac/article/77/12/3231/6759804

57. Silver N, Paynter M, McAllister G, Atchley M, Sayir C, Short J, et al. Characterization of minority HIV-1 drug resistant variants in the United Kingdom following the verification of a deep sequencing-based HIV-1 genotyping and tropism assay. AIDS Res Ther [Internet]. 2018 Dec 8 [cited 2019 Aug 4];15(1):18. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30409215

58. Stella-Ascariz N, Arribas JR, Paredes R, Li JZ. The Role of HIV-1 Drug-Resistant Minority Variants in Treatment Failure. J Infect Dis [Internet]. 2017 Dec 1 [cited 2019 Jul 7];216(suppl_9): S847–50. Available from: https://academic.oup.com/jid/article/216/suppl_9/S847/4683196

59. Li JZ, Paredes R, Ribaudo HJ, Svarovskaia ES, Metzner KJ, Kozal MJ, et al. Low-frequency HIV-1 drug resistance mutations and risk of NNRTI-based antiretroviral treatment failure: a systematic review and pooled analysis. JAMA [Internet]. 2011 Apr 6 [cited 2019 Oct 15];305(13):1327–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21467286

60. Simen BB, Simons JF, Hullsiek KH, Novak RM, MacArthur RD, Baxter JD, et al. Low-Abundance Drug-Resistant Viral Variants in Chronically HIV-Infected, Antiretroviral Treatment–Naive Patients Significantly Impact Treatment Outcomes. Journal of Infectious Diseases [Internet]. 2009 Mar 1 [cited 2019 Oct 15];199(5):693–701. Available from: https://academic.oup.com/jid/article-lookup/doi/10.1086/596736

61. Paredes R, Lalama CM, Ribaudo HJ, Schackman BR, Shikuma C, Giguel F, et al. Pre-existing Minority Drug-Resistant HIV-1 Variants, Adherence, and Risk of Antiretroviral Treatment Failure. J Infect Dis [Internet]. 2010 Jan 26 [cited 2019 Oct 15];201(5):100126095936095–000. Available from: https://academic.oup.com/jid/article-lookup/doi/10.1086/650543

62. Mzingwane ML, Tiemessen CT, Richter KL, Mayaphi SH, Hunt G, Bowyer SM. Pre-treatment minority HIV-1 drug resistance mutations and long-term virological outcomes: is prediction possible? Virol J [Internet]. 2016 Dec 12 [cited 2019 Oct 15];13(1):170. Available from: http://virologyj.biomedcentral.com/articles/10.1186/s12985-016-0628-x

63. Kyeyune F, Gibson RM, Nanky I, Venner C, Metha S, Akao J, et al. Low-Frequency Drug Resistance in HIV-Infected Ugandans on Antiretroviral Treatment Is Associated with Regimen Failure. Antimicrob Agents Chemother. 2016.

64. Rupérez M, Noguera-Julian M, González R, Maculuve S, Bellido R, Vala A, et al. HIV drug resistance patterns in pregnant women using next generation sequence in Mozambique.

Ceccherini-Silberstein F, editor. PLoS One [Internet]. 2018 May 9 [cited 2019 Apr 15];13(5): e0196451. Available from: https://dx.plos.org/10.1371/journal.pone.0196451

65. Stekler JD, Ellis GM, Carlsson J, Eilers B, Holte S, Maenza J, et al. Prevalence and impact of minority variant drug resistance mutations in primary HIV-1 infection. PLoS One [Internet]. 2011 [cited 2019 Oct 15];6(12): e28952. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22194957

66. Pimenta ATM, Correa IA, Melli PPDS, Abduch R, Duarte G, Couto-Fernandez JC, et al. HIV-1 genetic diversity and resistance to antiretroviral drugs among pregnant women in Ribeirão Preto (SP), Brazil. Cross-sectional study. Sao Paulo Med J [Internet]. 2018 Mar [cited 2019 Apr 15];136(2):129–35. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-31802018000200129&lng=en&tlng=en

67. Machnowska P, Hauser A, Meixenberger K, Altmann B, Bannert N, Rempis E, et al. Decreased emergence of HIV-1 drug resistance mutations in a cohort of Ugandan women initiating option B+ for PMTCT. Paraskevis D, editor. PLoS One [Internet]. 2017 May 31 [cited 2019 Mar 11];12(5): e0178297. Available from: https://dx.plos.org/10.1371/journal.pone.0178297

68. Andrew C. Voetsch YTDPSSSSM et al.,. HIV-1 Recent Infection Testing Algorithm with Antiretroviral Drug Detection to Improve Accuracy of Incidence Estimates. J Acquir Immune Defic Syndr. 2921;87(1):73–80.

69. Buskin SE, Fida NG, Bennett AB, Golden MR, Stekler JD. Evaluating New Definitions of Acute and Early HIV Infection from HIV Surveillance Data. Open AIDS J [Internet]. 2014 Oct 2 [cited 2023 Jun 22];8(1):45. Available from: /pmc/articles/PMC4192836/

70. Metzner KJ, Scherrer AU, Preiswerk B, Joos B, Von Wyl V, Leemann C, et al. Origin of minority drug-resistant HIV-1 variants in primary HIV-1 infection. Journal of Infectious Diseases. 2013;208(7).

71. Yanik EL, Napravnik S, Hurt CB, Dennis A, Quinlivan EB, Sebastian J, et al. Prevalence of transmitted antiretroviral drug resistance differs between acutely and chronically HIV-infected patients. J Acquir Immune Defic Syndr (1988). 2012;61(2).

72. Chauhan CK, Lakshmi PVM, Sagar V, Sharma A, Arora SK, Kumar R. Primary HIV Drug Resistance among Recently Infected Cases of HIV in North-West India. AIDS Res Treat. 2019;2019.

73. Ferreira ACG, Coelho LE, Grinsztejn E, Jesus CS de, Guimarães ML, Veloso VG, et al. Transmitted drug resistance in patients with acute/recent HIV infection in Brazil. Brazilian Journal of Infectious Diseases. 2017;21(4).

74. Onywera H, Maman D, Inzaule S, Auma E, Were K, Fredrick H, et al. Surveillance of HIV-1 pol transmitted drug resistance in acutely and recently infected antiretroviral drug-naïve persons in rural western Kenya. PLoS One [Internet]. 2017 Feb 1 [cited 2021 Dec 27];12(2): e0171124. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0171124
75. Salou M, Butel C, Comlan AS, Konou AA, Tegueni K, Ehlan A, et al. Challenges of scale-up to dolutegravir-based regimens in sub-Saharan Africa. AIDS. 2020 Apr 1;34(5):783–7.
76. Wandeler G, Buzzi M, Anderegg N, Sculier D, Béguelin C, Egger M, et al. Virologic failure and HIV drug resistance on simplified, dolutegravir-based maintenance therapy: Systematic review and meta-analysis. F1000Res [Internet]. 2018 Apr 3 [cited 2021 Dec 10]; 7:1359. Available from: /pmc/articles/PMC6134332/
77. Aboud M, Kaplan R, Lombaard J, Zhang F, Hidalgo JA, Mamedova E, et al. Dolutegravir versus ritonavir-boosted lopinavir both with dual nucleoside reverse transcriptase inhibitor therapy in adults with HIV-1 infection in whom first-line therapy has failed (DAWNING): an open-label, non-inferiority, phase 3b trial. Lancet Infect Dis [Internet]. 2019 Mar 1 [cited 2022 Aug 5];19(3):253–64. Available from: http://www.thelancet.com/article/S1473309919300362/fulltext
78. Paton NI, Musaazi J, Kityo C, Walimbwa S, Hoppe A, Balyegisawa A, et al. Dolutegravir or Darunavir in Combination with Zidovudine or Tenofovir to Treat HIV. New England Journal of Medicine. 2021 Jul 22;385(4):330–41.
79. Gupta RK, Hill A, Sawyer AW, Cozzi-Lepri A, von Wyl V, Yerly S, et al. Virological monitoring and resistance to first-line highly active antiretroviral therapy in adults infected with HIV-1 treated under WHO guidelines: a systematic review and meta-analysis. Lancet Infect Dis [Internet]. 2009 Jul [cited 2019 Jun 16];9(7):409–17. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19555900
80. Chung MH, Silverman R, Beck IA, Yatich N, Dross S, McKernan-Mullin J, et al. Increasing HIV-1 Pre-Treatment Drug Resistance among Antiretroviral-Naïve Adults Initiating Treatment between 2006 and 2014 in Nairobi, Kenya. AIDS [Internet]. 2016 Jun 19 [cited 2021 Dec 24];30(10):1680. Available from: /pmc/articles/PMC4889515/
81. Xu HT, Martinez-Cajas JL, Ntemgwa ML, Coutsinos D, Frankel FA, Brenner BG, et al. Effects of the K65R and K65R/M184V reverse transcriptase mutations in subtype C HIV on enzyme function and drug resistance. Retrovirology [Internet]. 2009 Feb 11 [cited 2023 Apr 5];6(1):1–11. Available from: https://retrovirology.biomedcentral.com/articles/10.1186/1742-4690-6-14
82. Ngo-Giang-Huong N, Huynh THK, Dagnra AY, Toni T d'Aquin, Maiga AI, Kania D, et al. Prevalence of pretreatment HIV drug resistance in West African and Southeast Asian

countries. Journal of Antimicrobial Chemotherapy [Internet]. 2019 Feb 1 [cited 2019 Jul 7];74(2):462–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30418575

83. Olson A, Bannert N, Sönnerborg A, de Mendoza C, Price M, Zangerle R, et al. Temporal trends of transmitted HIV drug resistance in a multinational seroconversion cohort. AIDS [Internet]. 2017 Nov 14 [cited 2019 Jun 2];32(2):1. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29112061

84. Polly Clayden. Transmitted drug resistance in HIV positive pregnant women | HTB | HIV i-Base [Internet]. 2015 [cited 2019 Jun 16]. Available from: http://i-base.info/htb/28348

85. Machnowska P, Meixenberger K, Schmidt D, Jessen H, Hillenbrand H, Gunsenheimer-Bartmeyer B, et al. Prevalence and persistence of transmitted drug resistance mutations in the German HIV-1 Seroconverter Study Cohort. Mor O, editor. PLoS One [Internet]. 2019 Jan 16 [cited 2019 Jun 2];14(1):e0209605. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30650082

86. UK Collaborative Group on HIV Drug Resistance. The increasing genetic diversity of HIV-1 in the UK, 2002-2010. AIDS [Internet]. 2014 Mar 13 [cited 2019 Oct 15];28(5):773–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24257094

87. Hauser A, Hofmann A, Meixenberger K, Altmann B, Hanke K, Bremer V, et al. Increasing proportions of HIV-1 non-B subtypes and of NNRTI resistance between 2013 and 2016 in Germany: Results from the national molecular surveillance of new HIV-diagnoses. Zhang C, editor. PLoS One [Internet]. 2018 Nov 8 [cited 2019 Aug 4];13(11):e0206234. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30408827

88. Frentz D, Van de Vijver DA, Abecasis AB, Albert J, Hamouda O, Jørgensen LB, et al. Increase in transmitted resistance to non-nucleoside reverse transcriptase inhibitors among newly diagnosed HIV-1 infections in Europe. BMC Infect Dis [Internet]. 2014 Dec 21 [cited 2019 Aug 4];14(1):407. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25047543

89. Gibson KM, Steiner MC, Kassaye S, Maldarelli F, Grossman Z, Pérez-Losada M, et al. A 28-Year History of HIV-1 Drug Resistance and Transmission in Washington, DC. Front Microbiol [Internet]. 2019 Mar 8 [cited 2019 Oct 15]; 10:369. Available from: https://www.frontiersin.org/article/10.3389/fmicb.2019.00369/full

90. Fabeni L, Alteri C, Di Carlo D, Orchi N, Carioti L, Bertoli A, et al. Dynamics and phylogenetic relationships of HIV-1 transmitted drug resistance according to subtype in Italy over the years 2000–14. Journal of Antimicrobial Chemotherapy [Internet]. 2017 Oct 1 [cited 2019 Aug 4];72(10):2837–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29091206

91. Rutstein SE, Hosseinipour MC, Weinberger M, Wheeler SB, Biddle AK, Wallis CL, et al. Predicting resistance as indicator for need to switch from first-line antiretroviral therapy

among patients with elevated viral loads: development of a risk score algorithm. BMC Infect Dis [Internet]. 2016 Dec 13 [cited 2019 Aug 4];16(1):280. Available from: http://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-016-1611-2

92. Stirrup OT, Dunn DT, Tostevin A, Sabin CA, Pozniak A, Asboe D, et al. Risk factors and outcomes for the Q151M and T69 insertion HIV-1 resistance mutations in historic UK data. AIDS Res Ther [Internet]. 2018 Dec 16 [cited 2019 Aug 4];15(1):11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29661246

93. Siemieniuk RA, Beckthold B, Gill MJ. Increasing HIV subtype diversity and its clinical implications in a sentinel North American population. Can J Infect Dis Med Microbiol [Internet]. 2013 [cited 2019 Aug 4];24(2):69–73. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24421804

94. Vanobberghen F, Letang E, Gamell A, Mnzava DK, Faini D, Luwanda LB, et al. A decade of HIV care in rural Tanzania: Trends in clinical outcomes and impact of clinic optimisation in an open, prospective cohort. PLoS One [Internet]. 2017 Jul 1 [cited 2022 Oct 28];12(7). Available from: https://pubmed.ncbi.nlm.nih.gov/28719610/

95. Muri L, Gamell A, Ntamatungiro AJ, Glass TR, Luwanda LB, Battegay M, et al. Development of HIV drug resistance and therapeutic failure in children and adolescents in rural Tanzania: an emerging public health concern. AIDS [Internet]. 2017 [cited 2019 Oct 15];31(1):61–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27677163

96. Letang E, Kalinjuma AV, Glass TR, Gamell A, Mapesi H, Sikalengo GR, et al. Cohort profile: The Kilombero and Ulanga Antiretroviral Cohort (KIULARCO) - A prospective HIV cohort in rural Tanzania. Swiss Med Wkly [Internet]. 2017 Jul 4 [cited 2019 Oct 15];147(2728):w14485. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28695551

97. Kalinjuma A V., Glass TR, Weisser M, Myeya SJ, Kasuga B, Kisung'a Y, et al. Prospective assessment of loss to follow-up: incidence and associated factors in a cohort of HIV-positive adults in rural Tanzania. J Int AIDS Soc [Internet]. 2020 Mar 1 [cited 2022 Oct 28];23(3):e25460. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/jia2.25460

98. Gamell A, Luwanda LB, Kalinjuma AV, Samson L, Ntamatungiro AJ, Weisser M, et al. Prevention of mother-to-child transmission of HIV Option B+ cascade in rural Tanzania: The One Stop Clinic model. Charpentier C, editor. PLoS One [Internet]. 2017 Jul 12 [cited 2019 Apr 15];12(7):e0181096. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28704472

99. Ntamatungiro AJ, Muri L, Glass TR, Erb S, Battegay M, Furrer H, et al. Strengthening HIV therapy and care in rural Tanzania affects rates of viral suppression. Journal of

Antimicrobial Chemotherapy [Internet]. 2017 Jul 1 [cited 2019 Mar 7];72(7):2069–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28387865

100. Thermo Fisher Scientific Inc. PureLink™ Viral RNA/DNA Mini Kit (Invitrogen™, Thermo Fisher Scientific, USA), https://www.thermofisher.com/order/catalog/product/12280050 [Internet]. [cited 2024 Jan 12]. Available from: https://www.thermofisher.com/order/catalog/product/12280050

101. Thermo Fisher Scientific. HIV-1 Resistance Research - HIV-1 Genotyping Kit [Internet]. [cited 2024 Jan 12]. Available from: https://www.thermofisher.com/order/catalog/product/A32317

102. Gamell A, Muri L, … ANO forum, 2016 undefined. A case series of acquired drug resistance-associated mutations in human immunodeficiency virus-infected children: an emerging public health concern in rural Africa. academic.oup.com [Internet]. [cited 2021 Dec 8]; Available from: https://academic.oup.com/ofid/article-abstract/3/1/ofv199/2460550

103. Informer technologies. BioEdit [Internet]. [cited 2024 Jan 12]. Available from: https://bioedit.software.informer.com/

104. Stanford University. Stanford HIV Drug Resistance Database [Internet]. [cited 2024 Jan 12]. Available from: https://hivdb.stanford.edu/

105. Richardson ET, Grant PM, Zolopa AR. Evolution of HIV treatment guidelines in high- and low-income countries: Converging recommendations. Antiviral Res. 2014 Mar;103(1):88–93.

106. UNAIDS. UNAIDS DATA 2019. Available from: https://www.unaids.org/sites/default/files/media_asset/2019-UNAIDS-data_en.pdf

107. Vyankandondera J, Mitchell K, Asiimwe-Kateera B, Boer K, Mutwa P, Balinda JP, et al. Antiretroviral therapy drug adherence in Rwanda: Perspectives from patients and healthcare workers using a mixed-methods approach. AIDS Care - Psychological and Socio-Medical Aspects of AIDS/HIV. 2013 May 7;25(12):1504–12.

108. Heestermans T, Browne JL, Aitken SC, Vervoort SC, Klipstein-Grobusch K. Determinants of adherence to antiretroviral therapy among HIV-positive adults in sub-Saharan Africa: a systematic review. BMJ Glob Health [Internet]. 2016 [cited 2021 Dec 28];1(4). Available from: https://pubmed.ncbi.nlm.nih.gov/28588979/

109. Bertagnolio S, Hermans L, Jordan MR, Avila-Rios S, Iwuji C, Derache A, et al. Clinical Impact of Pretreatment Human Immunodeficiency Virus Drug Resistance in People Initiating Nonnucleoside Reverse Transcriptase Inhibitor-Containing Antiretroviral Therapy: A Systematic Review and Meta-analysis. J Infect Dis [Internet]. 2021 Aug 1 [cited 2021 Dec 24];224(3):377–88. Available from: https://pubmed.ncbi.nlm.nih.gov/33202025/

110. Boender TS, Hoenderboom BM, Sigaloff KCE, Hamers RL, Wellington M, Shamu T, et al. Pretreatment HIV Drug Resistance Increases Regimen Switches in Sub-Saharan Africa. Clinical Infectious Diseases [Internet]. 2015 Dec 1 [cited 2021 Dec 27];61(11):1749–58. Available from: https://academic.oup.com/cid/article/61/11/1749/333803

111. Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ [Internet]. 2015 Jan 2 [cited 2022 Mar 30];349. Available from: https://www.bmj.com/content/349/bmj.G7647

112. Hassan AS, Esbjörnsson J, Wahome E, Thiong'o A, Makau GN, Price MA, et al. HIV-1 subtype diversity, transmission networks and transmitted drug resistance amongst acute and early infected MSM populations from Coastal Kenya. PLoS One. 2018.

113. Hassan AS, Bibby DF, Mwaringa SM, Agutu CA, Ndirangu KK, Sanders EJ, et al. Presence, persistence and effects of pre-treatment HIV-1 drug resistance variants detected using next generation sequencing: A Retrospective longitudinal study from rural coastal Kenya. PLoS One [Internet]. 2019 Feb 1 [cited 2021 Dec 27];14(2): e0210559. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0210559

114. Telele NF, Kalu AW, Gebre-Selassie S, Fekade D, Abdurahman S, Marrone G, et al. Pretreatment drug resistance in a large countrywide Ethiopian HIV-1C cohort: a comparison of Sanger and high-throughput sequencing. Scientific Reports 2018 8:1 [Internet]. 2018 May 15 [cited 2021 Dec 7];8(1):1–10. Available from: https://www.nature.com/articles/s41598-018-25888-6

115. Crowell TA, Danboise B, Parikh A, Esber A, Dear N, Coakley P, et al. Pretreatment and Acquired Antiretroviral Drug Resistance Among Persons Living with HIV in Four African Countries. Clinical Infectious Diseases [Internet]. 2021 Oct 5 [cited 2021 Dec 27];73(7):e2311–22. Available from: https://academic.oup.com/cid/article/73/7/e2311/5891786

116. Von Braun A, Sekaggya-Wiltshire C, Bachmann N, Ssemwanga D, Scherrer AU, Nanyonjo M, et al. HIV-1 Drug resistance among Ugandan adults attending an urban out-patient clinic. J Acquir Immune Defic Syndr (1988) [Internet]. 2018 Aug 15 [cited 2021 Dec 24];78(5):566–73. Available from: https://journals.lww.com/jaids/Fulltext/2018/08150/HIV_1_Drug_Resistance_Among_Ugandan_Adults.12.aspx

117. Rutstein SE, Chen JS, Nelson JAE, Phiri S, Miller WC, Hosseinipour MC. High rates of transmitted NNRTI resistance among persons with acute HIV infection in Malawi: Implications for first-line dolutegravir scale-up. AIDS Res Ther [Internet]. 2019 Feb 22

[cited 2021 Dec 24];16(1):1–4. Available from: https://link.springer.com/articles/10.1186/s12981-019-0220-8

118. Silverman RA, Beck IA, Kiptinness C, Levine M, Milne R, McGrath CJ, et al. Prevalence of Pre-Antiretroviral-Treatment Drug Resistance by Gender, Age, and Other Factors in HIV-Infected Individuals Initiating Therapy in Kenya, 2013–2014. J Infect Dis [Internet]. 2017 Dec 19 [cited 2021 Dec 27];216(12):1569–78. Available from: https://academic.oup.com/jid/article/216/12/1569/4430536

119. Milne RS, Silverman RA, Beck IA, McKernan-Mullin J, Deng W, Sibley TR, et al. Minority and majority pre-treatment HIV-1 drug resistance associated with failure of 1st-line NNRTI ART in Kenyan women. AIDS [Internet]. 2019 May 1 [cited 2021 Dec 24];33(6):941–51. Available from: /pmc/articles/PMC6635101/

120. Carnimeo V, Pulido Tarquino IA, Fuentes S, Vaz D, Molfino L, Tamayo Antabak N, et al. High level of HIV drug resistance informs dolutegravir roll-out and optimized NRTI backbone strategy in Mozambique. JAC Antimicrob Resist [Internet]. 2021 Apr 8 [cited 2021 Dec 27];3(2). Available from: https://academic.oup.com/jacamr/article/3/2/dlab050/6274495

121. Kiros M, Alemayehu DH, Geberekidan E, Mihret A, Maier M, Abegaz WE, et al. Increased HIV-1 pretreatment drug resistance with consistent clade homogeneity among ART-naive HIV-1 infected individuals in Ethiopia. Retrovirology [Internet]. 2020 Sep 29 [cited 2021 Dec 27];17(1):1–10. Available from: https://retrovirology.biomedcentral.com/articles/10.1186/s12977-020-00542-0

122. Watera C, Ssemwanga D, Namayanja G, Asio J, Lutalo T, Namale A, et al. HIV drug resistance among adults initiating antiretroviral therapy in Uganda. Journal of Antimicrobial Chemotherapy. 2021;

123. Beck IA, Levine M, McGrath CJ, Bii S, Milne RS, Kingoo JM, et al. Pre-treatment HIV-drug resistance associated with virologic outcome of first-line NNRTI-antiretroviral therapy: A cohort study in Kenya. EClinicalMedicine. 2020 Jan 1; 18:100239.

124. McCluskey SM, Lee GQ, Kamelian K, Kembabazi A, Musinguzi N, Bwana MB, et al. Increasing Prevalence of HIV Pretreatment Drug Resistance in Women But Not Men in Rural Uganda During 2005–2013. AIDS Patient Care STDS [Internet]. 2018 Jul 1 [cited 2021 Dec 27];32(7):257. Available from: /pmc/articles/PMC6034395/

125. Neuhann F, De Forest A, Heger E, Nhlema A, Scheller C, Kaiser R, et al. Pretreatment resistance mutations and treatment outcomes in adults living with HIV-1: A cohort study in urban Malawi. AIDS Res Ther [Internet]. 2020 May 20 [cited 2021 Dec 7];17(1):1–7. Available from: https://aidsrestherapy.biomedcentral.com/articles/10.1186/s12981-020-00282-3

126. Zhou Z, Tang K, Zhang G, Wadonda-Kabondo N, Moyo K, Rowe LA, et al. Detection of minority drug resistant mutations in Malawian HIV-1 subtype C-positive patients initiating and on first-line antiretroviral therapy. Afr J Lab Med [Internet]. 2018 [cited 2021 Dec 27];7(1):2225–2002. Available from: /pmc/articles/PMC6018132/
127. Arimide DA, Abebe A, Kebede Y, Adugna F, Tilahun T, Kassa D, et al. HIV-genetic diversity and drug resistance transmission clusters in Gondar, Northern Ethiopia, 2003-2013. PLoS One [Internet]. 2018 Oct 1 [cited 2021 Dec 27];13(10):e0205446. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0205446
128. Ndashimye E, Avino M, Kyeyune F, Nankya I, Gibson RM, Nabulime E, et al. Absence of HIV-1 Drug Resistance Mutations Supports the Use of Dolutegravir in Uganda. AIDS Res Hum Retroviruses [Internet]. 2018 May 1 [cited 2022 Mar 29];34(5):404–14. Available from: https://pubmed.ncbi.nlm.nih.gov/29353487/
129. Umviligihozo G, Muok E, Nyirimihigo Gisa E, Xu R, Dilernia D, Herard K, et al. Increased Frequency of Inter-Subtype HIV-1 Recombinants Identified by Near Full-Length Virus Sequencing in Rwandan Acute Transmission Cohorts. Front Microbiol [Internet]. 2021 Oct 7 [cited 2022 Mar 29];12:734929. Available from: /pmc/articles/PMC8529237/
130. Ndembi N, Hamers RL, Sigaloff KCE, Lyagoba F, Magambo B, Nanteza B, et al. Transmitted antiretroviral drug resistance among newly HIV-1 diagnosed young individuals in Kampala. AIDS. 2011;
131. Siedner MJ, Moorhouse MA, Simmons B, de Oliveira T, Lessells R, Giandhari J, et al. Reduced efficacy of HIV-1 integrase inhibitors in patients with drug resistance mutations in reverse transcriptase. Nat Commun. 2020 Dec 1;11(1).
132. McCluskey SM, Kamelian K, Musinguzi N, Kigozi S, Boum Y, Bwana MB, et al. Pre-treatment integrase inhibitor resistance is uncommon in antiretroviral therapy-naive individuals with HIV-1 subtype A1 and D infections in Uganda. AIDS [Internet]. 2021 Jun 1 [cited 2021 Dec 27];35(7):1083–9. Available from: https://journals.lww.com/aidsonline/Fulltext/2021/06010/Pre_treatment_integrase_inhibitor_resistance_is.9.aspx
133. Mulu A, Lange T, Liebert UG, Maier M. Clade homogeneity and Pol gene polymorphisms in chronically HIV-1 infected antiretroviral treatment naive patients after the roll out of ART in Ethiopia. BMC Infect Dis [Internet]. 2014 Mar 22 [cited 2021 Dec 27];14(1):1–9. Available from: https://bmcinfectdis.biomedcentral.com/articles/10.1186/1471-2334-14-158
134. Onsongo S, Abidi SH, Khamadi S, Shah R, Kageha S, Ojwang P, et al. Prevalence of Transmitted Drug Resistance Mutations in HIV-1-Infected Drug-Naive Patients from Urban and Suburban Regions of Kenya. https://home.liebertpub.com/aid [Internet]. 2016 Feb 26

[cited 2021 Dec 24];32(3):220–5. Available from: https://www.liebertpub.com/doi/abs/10.1089/aid.2015.0026

135. Marzel A, Shilaih M, Yang WL, Böni J, Yerly S, Klimkait T, et al. HIV-1 Transmission During Recent Infection and During Treatment Interruptions as Major Drivers of New Infections in the Swiss HIV Cohort Study. Clinical Infectious Diseases [Internet]. 2016 Jan 1 [cited 2022 Jun 1];62(1):115–22. Available from: https://academic.oup.com/cid/article/62/1/115/2462685

136. Cahn P, Madero JS, Arribas JR, Antinori A, Ortiz R, Clarke AE, et al. Dolutegravir plus lamivudine versus dolutegravir plus tenofovir disoproxil fumarate and emtricitabine in antiretroviral-naive adults with HIV-1 infection (GEMINI-1 and GEMINI-2): week 48 results from two multicentre, double-blind, randomised, non-inferiority, phase 3 trials. Lancet [Internet]. 2019 Jan 12 [cited 2021 Dec 10];393(10167):143–55. Available from: https://pubmed.ncbi.nlm.nih.gov/30420123/

137. McCluskey SM, Pepperrell T, Hill A, Venter WDF, Gupta RK, Siedner MJ. Adherence, resistance, and viral suppression on dolutegravir in sub-Saharan Africa: Implications for the TLD era. Vol. 35, AIDS. 2021.

138. Ferreira ACG, Coelho LE, Grinsztejn E, Jesus CS de, Guimarães ML, Veloso VG, et al. Prevalence of primary resistance among acutely/recently HIV infected patients in Rio de Janeiro, Brazil. The Brazilian Journal of Infectious Diseases. 2017;(x x).

139. Barbour JD, Hecht FM, Wrin T, Liegler TJ, Ramstead CA, Busch MP, et al. Persistence of primary drug resistance among recently HIV-1 infected adults. AIDS. 2004;18(12).

140. Yufenyuy EL, Detorio M, Dobbs Id T, Patel Id HK, Id KJ, Vedapuri S, et al. Performance evaluation of the Asante Rapid Recency Assay for verification of HIV diagnosis and detection of recent HIV-1 infections: Implications for epidemic control. PLOS Global Public Health [Internet]. 2022 May 3 [cited 2022 Nov 23];2(5):e0000316. Available from: https://journals.plos.org/globalpublichealth/article?id=10.1371/journal.pgph.0000316

141. PureLink™ Viral RNA/DNA Mini Kit [Internet]. [cited 2023 Jul 7]. Available from: https://www.thermofisher.com/order/catalog/product/12280050

142. Reshma Kassanjee CDPMPBGMSNFSMKEMKMMAPJNMSJLFMHEGK and AW. Viral Load Criteria and Threshold Optimization to Improve HIV Incidence Assay Characteristics - A CEPHIA Analysis. AIDS. 2016;30(15):2361–71.

143. Parekh B, Detorio M, Shanmugam V, Yufenyuy E, Dobbs T, Kim A, et al. Performance Evaluation of Asante TM Rapid Recency Assay for HIV Diagnosis and Detection of Recent Infection: Potential for Surveillance and Prevention. [cited 2023 Jun 1]; Available from: www.cdc.gov

144. Nazziwa J. Transmission clusters and evidence of HIV-1 transmitted drug resistance among recently infected ART-naive individuals from Ugandan fishing communities of Lake Victoria. Tropical Medicine and International Health. 2012;

145. Parmley LE, Harris TG, Hakim AJ, Musuka G, Chingombe I, Mugurungi O, et al. Recent HIV Infection Among Men Who Have Sex with Men, Transgender Women, and Genderqueer Individuals with Newly Diagnosed HIV Infection in Zimbabwe: Results from a Respondent-Driven Sampling Survey. AIDS Res Hum Retroviruses. 2022 Nov 1;38(11):834–9.

146. Mohloanyane T, Olivier D, Labhardt ND, Amstutz A. Recent HIV infections among newly diagnosed individuals living with HIV in rural Lesotho: Secondary data from the VIBRA cluster-randomized trial. PLoS One. 2022 Nov 1;17(11 November).

147. Alemu T, Ayalew M, Haile M, Amsalu A, Ayal A, Wale F, et al. Recent HIV infection among newly diagnosed cases and associated factors in the Amhara regional state, Northern Ethiopia: HIV case surveillance data analysis (2019-2021). Front Public Health. 2022 Nov 15;10.

148. Panga OD, Joachim A, Samizi FG, Gitige CG, Moremi N, Simeo J, et al. Prevalence, recent infection and predictors of HIV infection in fishing community along the shore of Lake Victoria in Tanzania. J Public Health (Bangkok) [Internet]. 2022 Dec 1 [cited 2023 May 22];44(4):881–90. Available from: https://academic.oup.com/jpubhealth/article/44/4/881/6297159

149. Bernasconi D, Tavoschi L, Regine V, Raimondo M, Gama D, Sulgencio L, et al. Identification of recent HIV infections and of factors associated with virus acquisition among pregnant women in 2004 and 2006 in Swaziland. Journal of Clinical Virology. 2010 Jul;48(3):180–3.

150. Mermin J, Musinguzi J, Opio A, Kirungi W, Ekwaru JP, Hladik W, et al. Risk factors for recent HIV Infection in Uganda. JAMA. 2008 Aug 6;300(5):540–9.

151. Galiwango RM, Ssuuna C, Kaleebu P, Kigozi G, Kagaayi J, Nakigozi G, et al. Short Communication: Validation of the Asante HIV-1 Rapid Recency Assay for Detection of Recent HIV-1 Infections in Uganda. AIDS Res Hum Retroviruses. 2021 Dec 1;37(12):893–6.

152. Drescher SM, Von Wyl V, Yang WL, Böni J, Yerly S, Shah C, et al. Treatment-Naive Individuals Are the Major Source of Transmitted HIV-1 Drug Resistance in Men Who Have Sex with Men in the Swiss HIV Cohort Study. Clinical Infectious Diseases [Internet]. 2014 Jan 15 [cited 2023 Apr 12];58(2):285–94. Available from: https://academic.oup.com/cid/article/58/2/285/335263

153. Phillips AN, Venter F, Havlir D, Pozniak A, Kuritzkes D, Wensing A, et al. Risks and benefits of dolutegravir-based antiretroviral drug regimens in sub-Saharan Africa: a modelling study. Lancet HIV [Internet]. 2019 Feb 1 [cited 2022 Oct 27];6(2): e116–27. Available from: http://www.thelancet.com/article/S2352301818303175/fulltext

154. Trevillyan JM, Hoy JF. Dolutegravir monotherapy as maintenance ART bites the dust. Lancet HIV. 2017 Dec 1;4(12): e531–2.

155. Smit E, White E, Clark D, Churchill D, Zhang H, Collins S, et al. An association between K65R and HIV-1 subtype C viruses in patients treated with multiple NRTIs. Journal of Antimicrobial Chemotherapy [Internet]. 2017 Jul 1 [cited 2019 Jun 16];72(7):2075–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28379449

156. 2025 AIDS TARGETS - UNAIDS [Internet]. [cited 2022 Oct 27]. Available from: https://aidstargets2025.unaids.org/

157. Political Declaration on HIV and AIDS: On the Fast Track to Accelerating the Fight against HIV and to Ending the AIDS Epidemic by 2030 | UNAIDS [Internet]. [cited 2022 Oct 27]. Available from: https://www.unaids.org/en/resources/documents/2016/2016-political-declaration-HIV-AIDS

158. Goal 3 | Department of Economic and Social Affairs [Internet]. [cited 2022 Oct 27]. Available from: https://sdgs.un.org/goals/goal3

159. Mesic A, Spina A, Mar HT, Thit P, Decroo T, Lenglet A, et al. Predictors of virological failure among people living with HIV receiving first line antiretroviral treatment in Myanmar: retrospective cohort analysis. AIDS Res Ther [Internet]. 2021 Dec 1 [cited 2022 Oct 27];18(1):1–12. Available from: https://aidsrestherapy.biomedcentral.com/articles/10.1186/s12981-021-00336-0

160. Mondi A, Cozzi-Lepri A, Tavelli A, Rusconi S, Vichi F, Ceccherini-Silberstein F, et al. Effectiveness of Dolutegravir-Based Regimens as Either First Line or Switch Antiretroviral Therapy: Data from the ICONA Cohort. SSRN Electronic Journal. 2020.

161. Barton-Knott S. New high-quality antiretroviral therapy to be launched in South Africa, Kenya and over 90 low-and middle-income countries at reduced price Negotiated pricing agreements have lowered costs and will improve access to quality treatment for people living with HIV.

162. Zash R, Holmes L, Diseko M, Jacobson DL, Brummel S, Mayondi G, et al. Neural-Tube Defects and Antiretroviral Treatment Regimens in Botswana. New England Journal of Medicine [Internet]. 2019 Aug 29 [cited 2022 Oct 27];381(9):827–40. Available from: https://www.nejm.org/doi/full/10.1056/nejmoa1905230

163. WHO. WHO. 2021. Consolidated guidelines on HIV prevention, testing, treatment, service delivery and monitoring: recommendations for a public health approach.

164. Edward C. Norton Morgen M. Miller Lawrence C. Kleinman. Computing Adjusted Risk Ratios and Risk Differences in Stata. The Stata Journal: Promoting communications on statistics and Stata. 2013;13(3).

165. Zash R, Jacobson DL, Diseko M, Mayondi G, Mmalane M, Essex M, et al. Comparative safety of dolutegravir-based or efavirenz-based antiretroviral treatment started during pregnancy in Botswana: an observational study. Lancet Glob Health [Internet]. 2018 Jul 1 [cited 2022 Oct 28];6(7): e804–10. Available from: http://www.thelancet.com/article/S2214109X18302183/fulltext

166. Venter WDF, Moorhouse M, Sokhela S, Fairlie L, Mashabane N, Masenya M, et al. Dolutegravir plus Two Different Prodrugs of Tenofovir to Treat HIV. undefined. 2019 Aug 29;381(9):803–15.

167. Castillo-Mancilla JR, Morrow M, Coyle RP, Coleman SS, Zheng JH, Ellison L, et al. Low-Level Viremia Is Associated with Cumulative Adherence to Antiretroviral Therapy in Persons With HIV. Open Forum Infect Dis [Internet]. 2021 Sep 1 [cited 2022 Oct 28];8(9). Available from: /pmc/articles/PMC8465325/

168. Schramm B, Temfack E, Descamps D, Nicholas S, Peytavin G, Bitilinyu-Bangoh JE, et al. Viral suppression and HIV-1 drug resistance 1 year after pragmatic transitioning to dolutegravir first-line therapy in Malawi: a prospective cohort study. Lancet HIV [Internet]. 2022 Aug 1 [cited 2022 Oct 28];9(8):e544–53. Available from: https://pubmed.ncbi.nlm.nih.gov/35905753/

169. Henerico S, Lyimo E, Makubi AN, Magesa D, Desderius B, Mueller A, et al. Primary resistance against integrase strand transfer inhibitors in integrase strand transfer inhibitor-naive patients failing first- and second-line ART in Tanzania. Journal of Antimicrobial Chemotherapy. 2022 Sep 14;

170. An J, Lao Y, Tang S, Lou J, Li T, Dong X. The Impact of Low-Level Viraemia on Virological Failure—Results from a Multicenter HIV Antiretroviral Therapy Cohort Study in Yunnan, China. Front Med (Lausanne) [Internet]. 2022 Jul 4 [cited 2022 Oct 28];9. Available from: https://www.readcube.com/articles/10.3389%2Ffmed.2022.939261

171. Braun DL, Scheier T, Ledermann U, Flepp M, Metzner KJ, Böni J, et al. Emergence of resistance to integrase strand transfer inhibitors during dolutegravir containing triple-therapy in a treatment-experienced patient with pre-existing m184v/i mutation. Viruses. 2020 Nov 1;12(11).

172. Ehrenkranz P, Rosen S, Boulle A, Eaton JW, Ford N, Fox MP, et al. The revolving door of HIV care: Revising the service delivery cascade to achieve the UNAIDS 95-95-95 goals. PLoS Med [Internet]. 2021 May 1 [cited 2022 Dec 12];18(5):e1003651. Available from: https://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.1003651

173. Meshesha HM, Nigussie ZM, Asrat A, Mulatu K. Determinants of virological failure among adults on first-line highly active antiretroviral therapy at public health facilities in Kombolcha town, Northeast, Ethiopia: a case–control study. BMJ Open [Internet]. 2020 Jul 1 [cited 2022 Oct 28];10(7):e036223. Available from: https://bmjopen.bmj.com/content/10/7/e036223

174. NATIONAL AIDS CONTROL PROGRAMME. THE UNITED REPUBLIC OF TANZANIA. https://differentiatedservicedelivery.org/Portals/0/adam/Content/NqQGryocrU2RTj58iR37uA/File/NATIONAL_GUIDELINES_FOR_THE_MANAGEMENT_OF_HIV_AND_AIDS_2019.pdf [Internet]. 2019 [cited 2022 Nov 7]; Available from: https://differentiatedservicedelivery.org/Portals/0/adam/Content/NqQGryocrU2RTj58iR37uA/File/NATIONAL_GUIDELINES_FOR_THE_MANAGEMENT_OF_HIV_AND_AIDS_2019.pdf

175. Romo ML, Patel RC, Edwards JK, Humphrey JM, Musick BS, Bernard C, et al. Disparities in Dolutegravir Uptake Affecting Females of Reproductive Age with HIV in Low- and Middle-Income Countries After Initial Concerns About Teratogenicity: An Observational Study. Ann Intern Med [Internet]. 2022 Jan 1 [cited 2022 Nov 6];175(1):84. Available from: /pmc/articles/PMC8808594/

176. MINISTRY OF HEALTH CD, CHILDREN- NATIONAL AIDS CONTROL PROGRAMME. 7 th Edition APRIL 2019. [cited 2021 Dec 10]. NATIONAL GUIDELINES FOR THE MANAGEMENT OF HIV AND AIDS. Available from: https://www.differentiatedservicedelivery.org/Portals/0/adam/Content/NqQGryocrU2RTj58iR37uA/File/NATIONAL_GUIDELINES_FOR_THE_MANAGEMENT_OF_HIV_AND_AIDS_2019.pdf

177. S G Deeks TWTLRHMHJDBNSHCJPJMMMKHRMG. Virologic and immunologic consequences of discontinuing combination antiretroviral-drug therapy in HIV-infected patients with detectable viremia. N Engl J Med . 2001 Feb;15;(344(7):):472-80.

178. Djeneba B Fofana HDIGMKS et al. Prevalence of HIV-1 Natural Polymorphisms and Integrase-Resistance-Associated Mutations in African Children. Viruses. 2023 Feb;16(15(2)):546.

179. Ezechiel Ngoufack Jagni Semengue DASI et al. Baseline integrase drug resistance mutations and conserved regions across HIV-1 clades in Cameroon: implications for transition to dolutegravir in resource-limited settings. Journal of Antimicrobial Chemotherapy. 2021 May;76(5):1277–85.

180. Dawit Assefa Arimide ZISKZAGFASSPBPM. Pre-Treatment Integrase Inhibitor Resistance and Natural Polymorphisms among HIV-1 Subtype C Infected Patients in Ethiopia. Viruses. 2022 Mar;30(14(4)):729.
181. Doreen Kamori GBJRWM et al. Emerging integrase strand transfer inhibitor drug resistance mutations among children and adults on ART in Tanzania: findings from a national representative HIV drug resistance survey. Journal of Antimicrobial Chemotherapy. 2023 Mar;78(3):779–87.
182. Kantor R, Team for the ACTG (ACTG) AS, Smeaton L, Team for the ACTG (ACTG) AS, Vardhanabhuti S, Team for the ACTG (ACTG) AS, et al. Pretreatment HIV Drug Resistance and HIV-1 Subtype C Are Independently Associated with Virologic Failure: Results From the Multinational PEARLS (ACTG A5175) Clinical Trial. Clinical Infectious Diseases [Internet]. 2015 May 15 [cited 2021 Dec 24];60(10):1541–9. Available from: https://academic.oup.com/cid/article/60/10/1541/338500

**Appendix**

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R49 Mr A Ntamatungiro

## HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
## CLEARANCE CERTIFICATE NO. M210714

| | |
|---|---|
| **NAME:** (Principal Investigator) | Mr A Ntamatungiro |
| **DEPARTMENT:** | School of Public Health<br>Medical School<br>University |
| **PROJECT TITLE:** | *Trend of pre-antiretroviral therapy HIV-1 drug resistance in Kilombero and Ulanga antiretroviral cohort, South-Western Tanzania, for over 15 years (2005-2020)* |
| **DATE CONSIDERED:** | 2021/07/30 |
| **DECISION:** | Approved unconditionally |
| **CONDITIONS:** | Prior condition satisfied 2021/09/22 |
| **NOTE:** | If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za> |
| **SUPERVISOR:** | Drs J Kagura, J Francis and M Weisser |
| **APPROVED BY:** | Dr CB Penny, Chairperson, HREC (Medical) |
| **DATE OF APPROVAL:** | 2021/08/26 |

**This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.**

**DECLARATION OF INVESTIGATORS**

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **July** and therefore reports and re-certification will be due in the month of **July** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator

2021/09/22
Date

**EACS, London, October 27-30, 2021**

# Transitioning to Dolutegravir: Virological outcomes in an HIV cohort in rural Tanzania

**Alex Ntamatungiro[2], Anna Eichenberger[1,2,4,5], James Okuma[4,5], Fiona Vanobberghen[4,5], Herry Mapesi[2,3,4], Fabian Franzeck[3,5], R. Ndege[2], D. Mnzava[2], N Kimera[2], T Glass[4,5], T Klimkait[6], DH Paris[4,5], M Battegay[3,5], Maja Weisser[2,3,4,5], on behalf of the KIULARCO Study Group**

1 Department of Infectious Diseases, Bern University Hospital, Bern, Switzerland, 2 Ifakara Health Institute, Ifakara, Tanzania, 3 Department of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland, 4 Swiss Tropical and Public Health Institute, Basel, Basel, Switzerland, 5 University of Basel, Basel, Switzerland, 6 Molecular Virology, Department of Biomedicine, University of Basel, Basel, Switzerland

## Background
Dolutegravir-based antiretroviral therapy (ART) is being rolled out in many lower- and middle-income countries (LMICs) due to its better tolerability, efficacy and higher resistance barrier than non-nucleoside reserve transcriptase inhibitors (NNRTI). Real-life data are crucial to monitor the success and risks of transitioning to new regimens. We assessed virological outcomes after dolutegravir rollout in rural Tanzania.

## Methods
The Kilombero and Ulanga Antiretroviral Cohort (KIULARCO) enrols consenting patients attending the Chronic Diseases Clinic of Ifakara, a rural care and treatment centre for HIV-infected people in south-eastern Tanzania. We compared viral suppression (HIV-1 RNA <50 copies/mL) 12 months after treatment start (window 5-15 months) between adults (≥15 years) initiating dolutegravir-based ART in March 2019 - March 2020 (dolutegravir cohort) versus those initiating NNRTI-based ART in December 2016 - December 2017 (NNRTI cohort). Patients >60 days late for a scheduled visit were defined as loss to follow-up (LTFU). We assessed factors associated with 12-months viral suppression using logistic regression models.

## Results
Viral load (VL) measurements were available for 170/297 (57%) participants in the dolutegravir cohort and 210/463 (45%) in the NNRTI cohort, with patients' characteristics shown in table 1. Viral suppression was achieved for 144 (85%) and 151 (72%) in the dolutegravir and NNRTI cohorts, respectively (chi2 p=0.003). 73/297 (25%) in the dolutegravir and 142/463 (31%) in the NNRTI cohorts were LTFU. Assuming those LTFU were not virally suppressed, 144/243 (59%) and 151/352 (43%) achieved viral suppression in the dolutegravir and NNRTI cohorts, respectively (chi2 p<0.001). Adjusted for confounders, DTG-based ART was associated with >2 times the odds of viral suppression versus NNRTI-based ART (table 2).

## Conclusion
Virological suppression at one year after treatment start was significantly higher in patients initiating DTG-based ART compared to NNRTI-based regimens. This underlines the importance of programmatic uptake of DTG-based ART in LMICs. LTFU remains high and needs further attention as it potentially jeopardizes control of the HIV epidemic.

**9th Annual Joint Advanced Seminars Conference 29th July 2022, Kampala, Uganda (Oral presentation).**

## Transitioning to Dolutegravir in a programmatic setting: Virological outcomes in the Kilombero and Ulanga Antiretroviral Cohort in rural Tanzania

**AUTHORS:** NTAMATUNGIRO A[1,8*], EICHENBERGER A[1,2,3,4], OKUMA J[3,4], VANOBBERGHEN F[3,4], MAPESI H[2,3,4], NDEGE R[2,6], MNZAVA D[2], KIMERA N[2], WEISSER M[2,3,4,5], on behalf of the KIULARCO Study Group

*[1]Ifakara Health Institute, Ifakara, Tanzania, [2]Department of Infectious Diseases, Bern University Hospital, Bern, Switzerland [3]Swiss Tropical and Public Health Institute, Basel, Switzerland [4]University of Basel, Basel, Switzerland [5]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland6. [6]St. Francis Referral Hospital, Ifakara, Tanzania, [7]Molecular Virology, Department of Biomedicine, University of Basel, Basel, Switzerland. [8]Division of Epidemiology and Biostatistics; University of the Witwatersrand, Johannesburg, SA*

*Corresponding Author: Email: antamatungiro@cartafrica.org Tel No.: +255 785 421 404*

**Background**: Clinical and virologic outcome data after programmatic transition from non-nucleoside reverse transcriptase inhibitor (NNRTI)-based to dolutegravir (DTG)-based antiretroviral treatment (ART) regimens in sub-Saharan Africa outside of clinical trials is scarce. Here we evaluate virological outcome of DTG-based ART after the first year of rollout in Ifakara, rural Tanzania.

**Method:** Within the prospective Kilombero and Ulanga Antiretroviral Cohort (KIULARCO), we analyzed in a nested study virological suppression, defined as HIV-1 RNA <50 copies/mL at 5-15 months after treatment initiation in treatment-naive people living with HIV aged ≥ 15 years, initiating NNRTI-based ART from 16th December 2016 to 15th December 2017 and DTG-based ART from 16th March 2019 to 15th September 2020. Factors associated with viral suppression were assessed using logistic regression.

**Results:** Viral suppression was achieved for 165/195 (85%) and 154/211 (73%) in the DTG-based and NNRTI-based cohorts, respectively (p=0.003). 108/436 (25%) in the DTG-based ART and 143/466 (31%) in the NNRTI-based cohorts were LTFU within this first year. Assuming those LTFU were not virally suppressed, 144/243 (59%) and 108/241 (44%) achieved viral suppression in the DTG-based ART and NNRTI-based cohorts, respectively (p<0.001). DTG-based ART was associated with >2 times the odds of viral suppression versus NNRTI-based ART.

**Conclusion:** Virological suppression at one year after treatment start was significantly higher in patients initiating DTG-based ART compared to NNRTI-based regimens. This underlines the benefit of programmatic uptake of DTG-based ART in rural sub-Saharan Africa.

**<u>Wits School of Public Health Research Day and CARTA Conference</u> 14-15 September 2023, Johannesburg, South Africa (Poster presentation).**

**Recent HIV-1 infection and pre-treatment HIV drug-resistance in recently infected adults initiating antiretroviral therapy in rural Tanzania.**

NTAMATUNGIRO AJ[1,2*], FRANCIS JM[3], MNZAVA D[2,4], NDEGE R[2,4,5,6], NJAU PS[7], OKUMA J[5,6], VANOBBERGHEN F[5,6], KAGURA J[2], WEISSER M[2,5,6,8] on behalf of the KIULARCO Study Group

[1]Department of Interventions and Clinical Trials, Ifakara Health Institute, 53, Ifakara, Tanzania
[2]Division of Epidemiology and Biostatistics; University of the Witwatersrand, 2050, Johannesburg, South Africa
[3]Department of Family Medicine and Primary Care; University of the Witwatersrand, 2050, Johannesburg, South Africa
[4] St. Francis Referral Hospital, 73, Ifakara, Tanzania
[5]Swiss Tropical and Public Health Institute, 4123, Allschwil, Switzerland
[6]University of Basel, 4001, Basel, Switzerland
[7] National AIDS Control Programme, 784, Dodoma, Tanzania
[8]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel,4001, Basel, Switzerland

[*] Corresponding Author:
Email: antamatungiro@ihi.or.tz

**Background**: Recently HIV-1 infected patients remain an important threat to ending the HIV epidemic, as early and undiagnosed HIV leads to the highest number of transmissions and possibly spread of viral resistance. We determined the proportion of recent HIV-1 infection (RHI) among newly diagnosed people living with HIV (PLHIV). Additionally, we assessed the prevalence of pre-treatment HIV-1 drug resistance (PDR) in recently infected PLHIV in rural Tanzania.

**Methods**: We conducted a cross-sectional analysis for RHI among newly diagnosed adults enrolled in the Kilombero and Ulanga Antiretroviral Cohort between March 2019 and March 2022. Risk factors of RHI were determined using logistic regression. Genotypic resistance testing (GRT) was performed for RHI cases.

**Results**: Among 604 PLHIV, 24 (4%) had RHI. We found no factors associated with RHI. GRT was successful in 16/24 (67%) participants, and 5/16 (31%) had mutations associated with HIV-1 drug resistance: 4/16 (25%) for non-nucleoside reverse transcriptase inhibitors, 2/16 (13%) for nucleoside reverse transcriptase inhibitors and 1/16 (6%) for protease inhibitors.

**Conclusion**: The low observed prevalence of RHI in this referral hospital-based study, indicates the high number of late diagnoses., and highlights the need to further strengthen strategies for early HIV diagnosis and linkage to care. Although on a limited sample-size, the observed high proportion of recently HIV infected patients with PDR is a serious public health concern.