HIV-1 viral load outcomes and the evolution of drug-resistance in low-income settings without virological monitoring

David Ian Dolling

Submitted to University College London

for the degree of Doctor of Philosophy

Institute of Clinical Trials and Methodology

2017

Declaration

I, David Ian Dolling, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

WHO guidelines recommend viral load monitoring for all HIV-1 positive patients on antiretroviral therapy (ART). However, few low-income countries have virological monitoring widely available, and patients may remain on virologically failing regimens. This could compromise future ART through the accumulation of drug resistance mutations and result in worse long-term clinical outcomes.

The DART trial was conducted in Uganda and Zimbabwe and compared clinically driven monitoring with or without routine CD4 measurement in ART-naïve adult patients. Annual plasma viral load was retrospectively measured for 1,762 patients. This thesis investigates how no laboratory monitoring impacts virological failure and the development of drug resistance.

Time to persistent virological failure was analysed, and analytical weights were calculated to correct for non-random sampling. The long-term durability of first-line ART was remarkable; 21% of patients on an NRTI-NNRTI regimen and 40% on a triple-NRTI regimen experienced persistent virological failure by 240 weeks. Routine CD4 monitoring did not reduce virological failure.

Deaths after 48 weeks of ART are widely assumed to be due to virological failure or non-adherence. Analyses revealed that a surprisingly high number of these deaths (40%) occurred without virological criteria for treatment switch being met. Routine CD4 monitoring reduced the rate of death with virological failure but did not impact deaths with virological suppression.

Cross-sectional analyses quantified HIV-1 drug resistance at the end of first-line ART. On NRTI-NNRTI regimens, 88% had NRTI resistance, and 66% had NNRTI resistance. Routine CD4 monitoring did not reduce the prevalence or extent of drug resistance. The order and rate of HIV-1 drug resistance mutations were explored using repeated genotypes within patients. On NRTI-NNRTI regimens, NRTI and NNRTI mutations developed at a rate of 0.96 and 0.21 per year respectively. Mutagenic tree models demonstrated that ART regimen influenced the order and rate in which mutations occurred.

Acknowledgements

I am extremely grateful to my supervisors, David Dunn and Ruth Goodall, who offered me guidance and support throughout my PhD. Thanks for your thoughtful insights. Whenever we had our PhD meetings, I always left feeling energised and motivated.

I would like to thank the DART trial team, the DART Virology group and all the DART patients for allowing me to use their data. Thanks to Moira Spyer who coordinated the virology and resistance testing. I am grateful to the Medical Research Council for the studentship which supported me during this PhD.

A special thanks to my parents for encouraging me to follow my dreams. I am indebted to my partner Tat, for her love, support, understanding and proof-reading.

Table of Contents

Acronyms

ABC	Abacavir
aCSHR	Adjusted Cause-Specific Hazard Ratio
AE	Adverse Event
ALT	Alanine aminotransferase test
aSHR	Adjusted Subdistribution Hazard Ratio
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
BMI	Body Mass Index
CDM	Clinical Disease Monitoring only
CI	Confidence Interval
CRF	Circulating Recombinant Form
CROI	Conference on Retroviruses and Opportunistic Infections
CSH	Cause-Specific Hazard
CT	Continuous Treatment
DALY	Disability-adjusted Life Years
DART	Development of Antiretroviral Therapy in Africa
DNA	Deoxyribonucleic acid
FBC	Full Blood Count
HAART	Highly Active Antiretroviral Therapy
HBV/HCV	Hepatitis B/C Virus
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
IDI	Infectious Diseases Institute
IQR	Interquartile Range
IRR	Incidence Rate Ratio
JCRC	Joint Clinical Research Centre
LCM	Laboratory and Clinical Monitoring
MRC	Medical Research Council
NA	Not Applicable
NNRTI	Non-nucleoside Reverse Transcriptase Inhibitor
NORA	Nevirapine or Abacavir trial
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NVP	Nevirapine
OHFS	Optimal HAART Feasibility Study
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PEP	Post Exposure Prophylaxis
PI	Protease Inhibitor
PrEP	Pre-exposure Prophylaxis
REGA	Not an acronym
RNA	Ribonucleic Acid
RR	Rate Ratio

SAE	Serious Adverse Event
SARA	The Boosted Protease Inhibitor Monotherapy as Maintenance Second-line Antiretroviral therapy in Africa
SD	Standard Deviation
SH	Subdistribution Hazard
STI	Structured Treatment Interruption
TAM	Thymidine Analogue Mutation
TB	Tuberculosis
TDF	Tenofovir Disoproxil Fumarate
UK	United Kingdom
VF	Viral Failure
VL	Viral Load
WHO	The World Health Organisation

List of Tables

List of Figures

1 Introduction

1.1 HIV infection

1.1.1 HIV life cycle

Human immunodeficiency virus (HIV) is a retrovirus, specifically a lentivirus, which replicates by infecting human immune system cells. The main targets for infection are CD4+T lymphocytes, otherwise known as CD4 cells. The CD4 cells send signals identifying cells to be destroyed by CD8 killer cells. HIV replicates by initially binding to the CD4 receptor and then fusing with the cell, releasing HIV Ribonucleic Acid (RNA) and various enzymes into the cell (steps 1 and 2 of Figure 1). An enzyme called *reverse transcriptase* changes RNA to DNA (step 4) and another enzyme, called *integrase*, integrates this into the DNA within the nucleus of the CD4 cell (step 6). Infected cells may lie dormant for several years before activating. Once active, the virus uses the CD4 cell to create more of its genetic material. The *protease enzyme* then assembles these long strands into a new virus.

During the process of HIV replication there is a gradual decline of CD4 cells through:

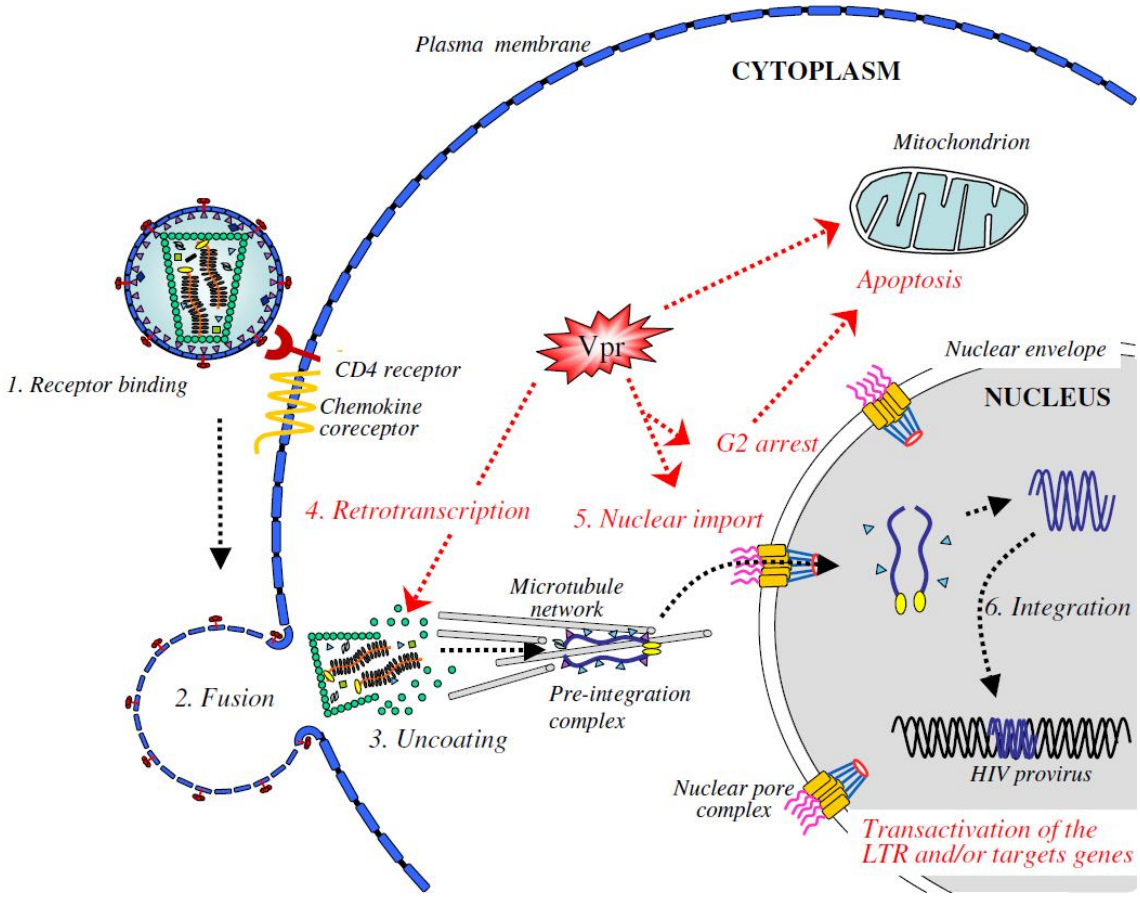
(i) The direct killing of cells as part of the HIV replication cycle.

(ii) Increased "programmed death" of cells infected with HIV, known as apoptosis.

(iii) Pyroptosis, a highly inflammatory form of programmed cell death, triggered by cells abortively infected with HIV. Pyroptosis attracts more CD4 cells to the area and is thought to account for 95% of CD4 cell deaths [1].

(iv) CD8 cells killing infected cells.

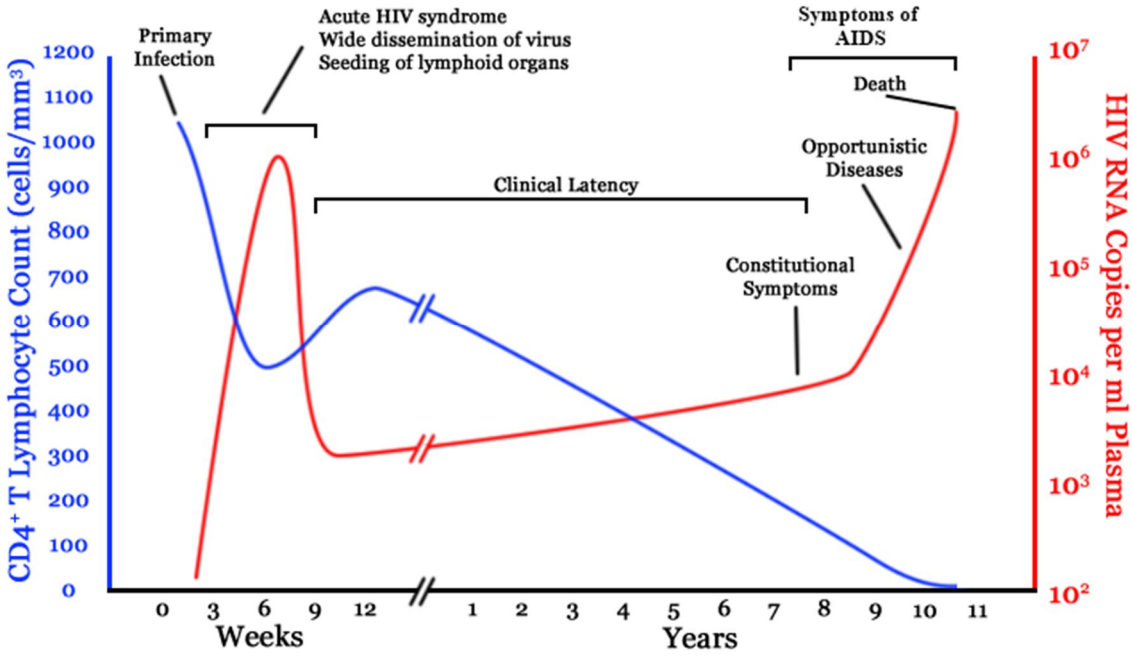
Figure 1: HIV-1 life cycle [2]



1.1.2 The course of HIV infection

As a person's CD4 cells are lost they become increasingly susceptible to opportunistic infections and other diseases; without antiretroviral therapy (ART) this will eventually lead to death. In HIV-positive people, this is known as Acquired Immune Deficiency Syndrome (AIDS). Figure 2 displays the course of a typical HIV infection without treatment. Patients move through several stages of HIV infection, classified by the World Health Organisation (WHO). Starting with stage I (where a patient is asymptomatic with a CD4 cell count greater than 500 copies/mL) to stage IV or AIDS (where there are CD4 cell count less than 200 copies/mL or severe symptoms, which can include rare cancers such as Kaposi's sarcoma and diseases like candidiasis of the lungs). The introduction of HIV treatment has transformed HIV from a lethal disease to a chronic one, and life expectancy is approaching that of non-infected individuals [3].

Figure 2: Natural history of HIV during untreated infection [4]



1.1.3 Transmission of HIV

HIV spreads through the transfer of bodily fluids such as blood, semen, vaginal fluid and breast milk. Unprotected sexual intercourse and direct blood contacts are the primary means of HIV-1 infection. HIV prevention programmes encourage the use of condoms, more frequent HIV testing and needle exchange programmes. Antiretroviral drugs have been shown to reduce the risk of HIV infection after a possible exposure (post-exposure prophylaxis; PEP) and also as pre-exposure prophylaxis (PrEP) [5]. Currently, antiretroviral therapy is also thought to be the best control of the transmission of HIV. The HPTN 052 study [6] demonstrates that antiretroviral therapy limits the transmission of HIV in serodiscordant couples if the HIV-positive patient has a suppressed viral load.

1.1.4 Antiretroviral Therapy

Treatment for HIV infection currently consists of highly active antiretroviral therapy (HAART), a combination of three or more drugs from at least two different classes of antiretroviral agents. Antiretroviral classes each affect a different part of the HIV-1 replication cycle. Nucleoside reverse transcriptase inhibitors (NRTIs) interfere with the

retrotranscription process by being taken up instead of the natural nucleotide, thereby stopping the viral DNA chain from continuing. Popular drugs in this class include lamivudine (3TC), abacavir (ABC), zidovudine (ZDV or AZT) and emtricitabine (FTC). Nucleotide reverse transcriptase inhibitors (NtRTIs) act similarly and are considered part of the same drug class; tenofovir (TDF) is the main drug of this type. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) disrupt the same part of the HIV life cycle but work by binding directly to the reverse transcriptase enzyme and are considered a different drug class. Commonly used NNRTIs include nevirapine (NVP), efavirenz (EFV) and etravirine (ETR). Protease inhibitors (PIs) block the protease enzyme from breaking up proteins. HIV still replicates, but the resulting virions are unable to mature so cannot infect new cells. PIs such as lopinavir (LPV) and atazanavir (ATV) are often used with a small dose of an additional PI, ritonavir (RTV), to "boost" the levels of the main PI in the blood and extend dosing intervals. Table 1 displays the most common antiretroviral drugs and abbreviations. In high-income countries, additional drug classes are also available, such as integrase inhibitors and fusion inhibitors. Currently, these are not recommended in low-income settings because they are more expensive and difficult to store compared to other drug classes.

1.1.5 HIV-1 drug resistance

HIV replicates rapidly, approximately 10^{10} new virions per day in an untreated individual [7]. The replication process of HIV is error prone compared to DNA as there is no "proofreading," a result of being constructed from RNA. This results in approximately one error per 10,000 to 30,000 nucleotide incorporations [8] and subsequently a wide variety of natural mutations develops. While many mutations will lead to virions unable to properly replicate or function, some will have mutations which confer drug resistance. These mutations have changed the structure of enzymes, such as reverse transcriptase and protease so that an

antiretroviral compound will not bind to the enzyme as effectively. While HIV can rapidly develop mutations to reduce susceptibility to a single drug, it is extremely unlikely to simultaneously develop mutations which reduce susceptibility to three or more drugs. This is particularly the case if these drugs are from several classes and this forms the basis for HAART.

Table 1: Antiretroviral drugs and their abbreviations

Class	Generic drug name	Abbreviation
NRTI	Abacavir	ABC
NRTI	Emtricitabine	FTC
NRTI	Didanosine	DDI
NRTI	Lamivudine	3TC
NRTI	Stavudine	D4T
NRTI	Tenofovir	TDF
NRTI	Zidovudine	ZDV or AZT
NNRTI	Efavirenz	EFV
NNRTI	Etravirine	ETR
NNRTI	Nevirapine	NVP
NNRTI	Rilpivirine	RPV
PI	Atazanavir	ATV
PI	Darunavir	DRV
PI	Fosamprenavir	FPV
PI	Indinavir	IDV
PI	Lopinavir	LPV
PI	Nelfinavir	NFV
PI	Ritonavir	RTV
PI	Saquinavir	SQV
PI	Tipranavir	TPV

HIV with drug resistant mutations can replicate more effectively in the presence of antiretroviral drugs. If a person stops taking a drug or changes antiretroviral regimen, then wild-type virus (HIV without drug resistance mutations) may replicate more efficiently and outgrow drug-resistant virus. Drug-resistant viruses are often undetectable in plasma when outgrown by wild-type virus. However, they are not lost completely and are archived in cells within the body [9]. Archived viruses can rapidly become the majority virus if antiretroviral therapy affected by these mutations is resumed. Treatment guidelines in high-income countries [10, 11] recommend performing resistance testing while a patient remains on their

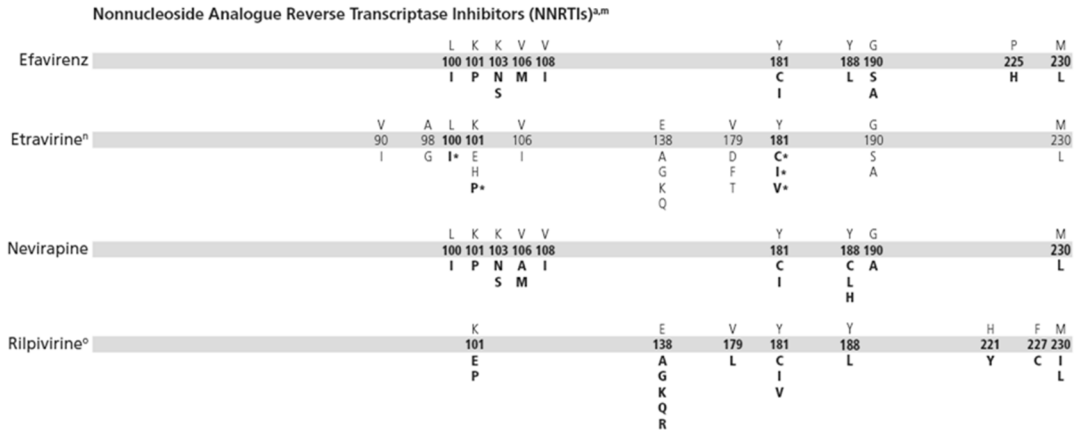
failing antiretroviral regimens. Not all mutations directly reduce susceptibility to treatment. Compensatory mutations may develop, which improve the replication of the resistant virus, while not directly influencing the interaction between the structure of the enzyme and antiretroviral drugs [12].

Most mutations involve the substitution of one amino acid for another. They are named using abbreviations for the amino acid changes that occur and the location of the amino acid codon. For instance, the K65R mutation identifies that the wild-type amino acid lysine (K) is substituted with arginine (R) at the codon numbered 65. Some mutations involve the insertion or deletion of an amino acid at a particular location; these are denoted using nomenclatures such as K69ins and D67del respectively.

HIV-1 drug resistance testing uses polymerase chain reaction (PCR) to produce large quantities of HIV-1 gene from plasma samples. The relevant HIV genes are sequenced and create an amino-acid sequence of the *pol* gene. This process produces a consensus sequence and differs from single-genome sequencing where a single molecule is amplified. Consensus or bulk sequencing report a mixture of bases at positions and can detect minority variants where they comprise more than 20% of the viral population. This consensus sequence can then be compared to lists of previously identified drug resistance mutations to determine whether a virus is susceptible to a particular drug.

There are several drug resistance interpretation algorithms available, including the IAS-USA lists [13] and the Stanford University HIVdb algorithm [14]. The IAS-USA lists are known as single mutation tables (Figure 3), where just one major mutation for an antiretroviral drug can reduce virological response.

Figure 3: Example of IAS-USA mutation list for NNRTIs



An alternative approach is the Stanford University HIVdb algorithm; this scores each mutation per antiretroviral drug according to the extent to which it reduces susceptibility. A higher score indicates a greater loss in susceptibility. Scores may be negative for some mutations, showing the increased susceptibility of HIV with this mutation to an antiretroviral agent. Also, certain combinations of mutations have associated scores. For each antiretroviral drug scores are cumulated, and a virus is classified as being either susceptible, potentially low-level resistant, low-level resistant, intermediate resistant or high-level resistant. Figure 4 displays examples of these scores.

Figure 4: Example of Stanford HIVdb scoring method

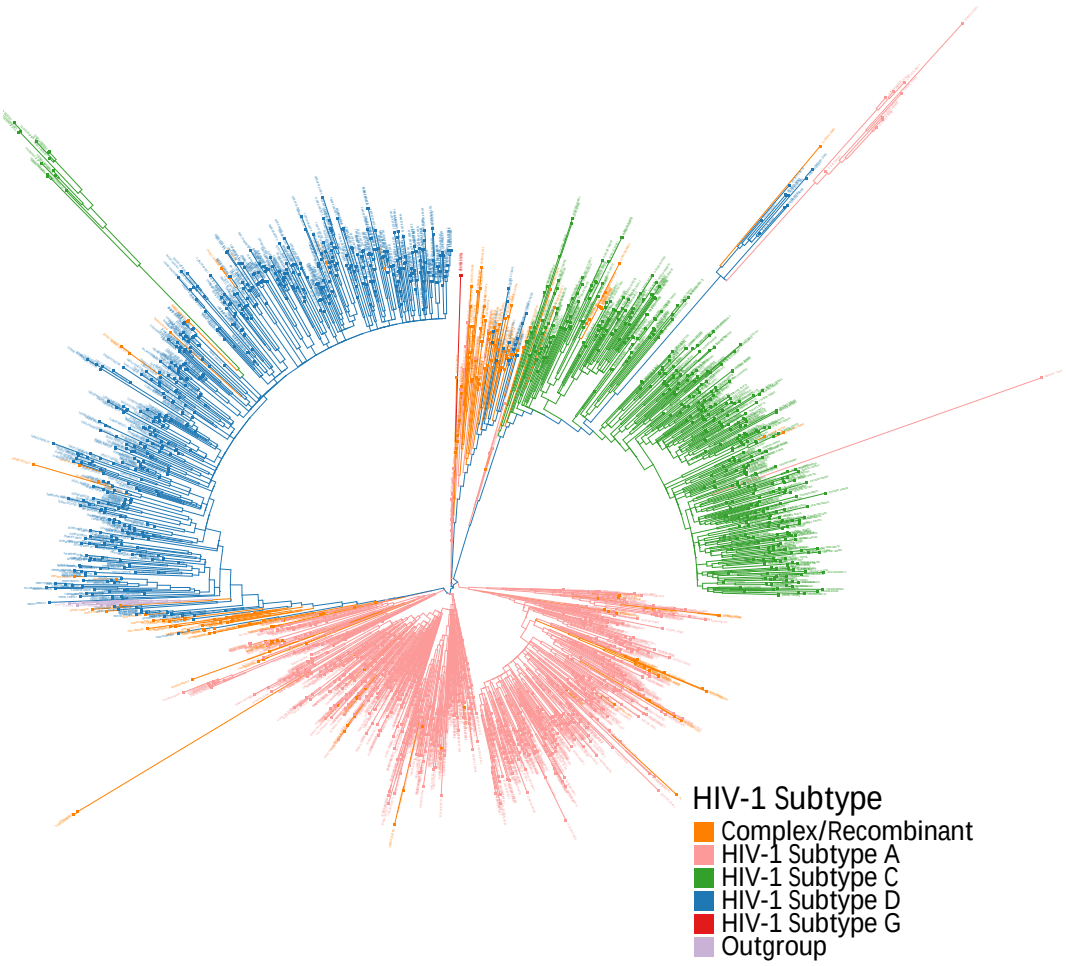
Position	Cons	AA	EFV	NVP	ETR	RPV
90	V	I	0	0	0	0
98	A	G	10	30	10	15
100	L	I	45	45	30	60
100	L	V	30	30	10	15
101	K	E	15	30	15	30
101	K	P	60	60	45	60
101	K	Q	0	0	0	0
101	K	H	15	15	10	10
101	K	N	0	0	0	0
103	K	N	60	60	0	0
103	K	R	0	0	0	0

1.1.6 HIV-1 subtype

Genetically distinct viral strains of HIV-1 are classified into nine distinct viral subtypes and many hybrid viruses, formed when two viruses of

different subtypes mix after meeting in a cell. These hybrid viruses currently include 79 circulating recombinant forms, many unique recombinant forms and other less well characterised complex structures [15]. Genetically divergent strains have biological differences which may impact the development of drug-resistance [16], susceptibility to antiretroviral therapy [17] and the rate of disease progression [18, 19]. The gold standard for classifying HIV-1 subtypes is a phylogenetic analysis of the full-length genome. In a clinical setting, a phylogenetic analysis of the *pol* region can be used to classify HIV-1 subtype by using software such as REGA [20]. Figure 5 depicts a phylogenetic tree using DART data featuring HIV-1 subtype and is discussed in Chapter 4.

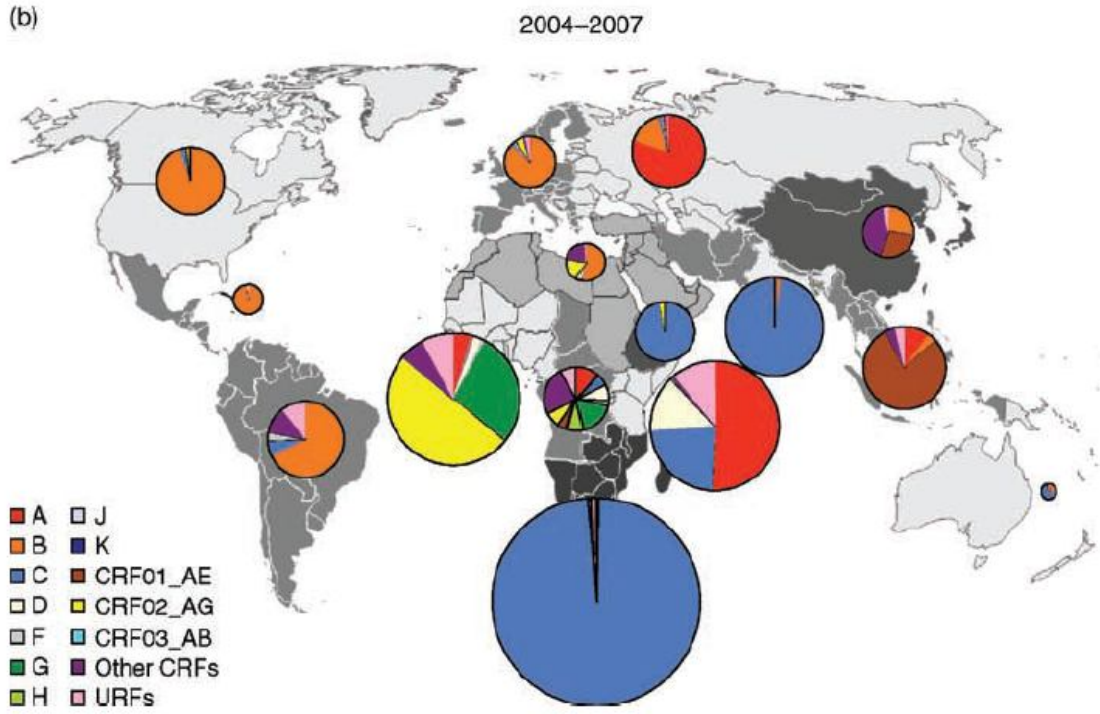
Figure 5: Phylogenetic tree of HIV-1 pol gene



Subtype B virus is the dominant form in regions such as Europe and the Americas and has consequently been the most extensively investigated.

Globally, Subtype C is the most prevalent, accounting for nearly half of all HIV infections [21]. Figure 6 presents the global distribution of HIV subtypes in 2004 to 2007 (taken from Hemelaar et al. [21]). Each pie chart size corresponds to the relative number of people living with HIV in each region. Subtypes A and D in Uganda and subtype C in Zimbabwe [22] are the most prevalent HIV subtypes in the countries where the DART trial was conducted.

Figure 6: Global distribution of HIV subtypes



1.2 HIV treatment in low-income settings

Globally, there are 35.3 (95% CI: 32.2-38.8) million people estimated to be living with HIV in 2012 [23]. Approximately 25.0 (23.5-26.6) million HIV-1 positive people live in sub-Saharan Africa with a prevalence of 4.7% (4.4-5.0%), and 3.9 (2.9-5.2) million people in South and Southeast Asia with a prevalence of 0.3% (0.2-0.4%). In sub-Saharan Africa, 10.3 (9.9-10.9) million people are likely to need antiretroviral therapy based on WHO 2010 guidelines [24], and 6.9 million are reported to be on antiretroviral therapy, giving a coverage of 68% (65-72%). Treatment availability has

expanded rapidly in Sub-Saharan Africa; seven times as many people were receiving antiretroviral therapy in 2012 compared to 2005.

The World Health Organisation (WHO) has released a series of guidelines designed for the scale-up of HIV in low-income settings with a public health approach [24-27]. The WHO's adult guidelines make recommendations concerning when antiretroviral therapy should be started, which antiretrovirals should be used, the frequency of clinical and laboratory monitoring of patients on antiretroviral therapy, and the surveillance of HIV-1 drug resistance mutations.

Additionally, guidelines define the criteria for the clinical stages of HIV disease. WHO stage 3 events include conditions such as severe unexplained weight loss, pulmonary tuberculosis, explained persistent diarrhoea, persistent unexplained fever, persistent oral candidiasis and severe bacterial infections. WHO stage 4 events include HIV wasting syndrome, Kaposi's sarcoma, Pneumocystis pneumonia, chronic herpes simplex infection, extrapulmonary tuberculosis and HIV encephalopathy.

Table 2 summarises the changes in recommendations over time. More recent recommendations have expanded the eligibility criteria for starting antiretroviral therapy, with a gradual increase in the CD4 cell count at which antiretroviral therapy should be initiated. The recommended first-line regimens have altered, with stavudine no longer included and tenofovir now being favoured. Guidelines have consistently advised that second-line regimens should use an NRTI plus a boosted PI regimen, but recent research has clarified individual PIs. Triple-NRTI regimens were initially considered a viable alternative in many situations where NNRTIs were not possible; they were also considered advantageous by conserving NNRTI and PI classes for second-line regimens. However, the increase in the number of available antiretroviral drugs means that patients requiring tuberculosis (TB) or hepatitis treatment can now start antiretroviral

therapy without using contraindicated medication. Before 2010, the guidelines emphasised that laboratory monitoring was not a requirement for starting antiretroviral therapy. More recent guidelines have noted that laboratory monitoring plays a key role, even in low-income settings. Monitoring CD4 cell counts every six months and viral load every twelve months while on antiretroviral therapy is now recommended. Drug resistance tests at treatment failure for individual patient management are not recommended. Instead, the WHO supports monitoring of drug resistance through surveillance programmes in low-income settings.

WHO guidelines recommend that patients receive regular viral load testing to diagnose and confirm treatment failure [27]. Despite this, the real life situation in low-income settings remains that viral load measurements are typically not performed in real time. A Médecins Sans Frontières survey [28] found that while 39 of the 52 low and middle-income countries surveyed had guidelines recommending viral load testing on antiretroviral therapy, only a minority (8 of 52) of countries had testing widely available.

The UNAIDS 2020 [29] aim, known as "90-90-90", is for 90% of HIV-positive patients to be diagnosed, 90% of these to be on treatment and for 90% to be virally suppressed. A variety of strategies are required to eradicate HIV, but UNAIDS note that this will be impossible without HIV treatment for all.

Table 2: Summary of changes in WHO recommendations for the treatment of adults with HIV infection

	2003 WHO Guidelines [25]	2006 WHO Guidelines [26]	2010 WHO Guidelines [24]	2013 WHO Guidelines [27]
When to start	Adults and adolescents should start ART when: • WHO stage 4 disease: irrespective of CD4 • WHO stage 3, with consideration of CD4 cell count<350 cells/mm^3 • WHO stage 1 or 2 with CD4≤200 cells/mm^3	Adults and adolescents should start ART when: • WHO stage 4 disease: irrespective of CD4 • WHO stage 3, if CD4 is available consider if CD4 cell count<350 cells/mm^3 and before CD4 cell count<200 cells/mm^3 • WHO stage 1 or 2: if CD4<200 cells/mm^3	Adults and adolescents should start ART when: • WHO stage 3 or 4 disease: irrespective of CD4 • WHO stage 1 or 2: if CD4<350 cells/mm^3 • Active TB/HBV infection: irrespective of CD4	Adults and adolescents should start ART when: • CD4 cell count≤500 cells/mm^3 • Active TB/HBV/pregnant/ serodiscordant relationship: irrespective of CD4
Recommended first-line regimens	• (D4T or ZDV)+3TC+(NVP or EFV) • ZDV+3TC+NVP should be used with lab monitoring	Primarily recommends: • (ZDV or TDF)+(3TC or FTC)+(EFV or NVP) If pregnant: • ZDV+3TC+NVP TB co-infection: • (ZDV or TDF)+(3TC or FTC)+EFV HBV co-infection: • TDF+(3TC or FTC)+EFV	Primarily recommends: • (ZDV or TDF)+(3TC or FTC)+(EFV or NVP) If pregnant: • ZDV+3TC+(EFV or NVP) TB co-infection: • (ZDV or TDF)+(3TC or FTC)+EFV HBV co-infection: • TDF+(3TC or FTC)+(EFV or NVP)	Adults (including pregnant women, TB/HBV coinfection): • TDF+(3TC or FTC)+EFV Alternatively: • ZDV+(3TC or FTC)+(EFV or NVP) • TDF+(3TC or FTC)+NVP In special circumstances regimens containing ABC, d4T and boosted PIs may be used
Recommended second-line regimens	• (TDF or ABC)+DDI+ (LPV/r or SQV/r)	• DDI or TDF+(ABC or 3TC) (±ZDV)+PI/r	A boosted PI plus two NRTIs backbone should be used ATV/r and LPV/r are recommended as boosted PIs If d4T or ZDV have been used in the first-line, switch to TDF+(3TC or FTC) backbone If TDF has been used in first-line switch to ZDV+3TC	Preferred regimen: • ZDV+3TC+(LPV/r or ATV/r) Alternative regimen: • TDF+(3TC or FTC)+(LPV/r or ATV/r)

	2003 WHO Guidelines [25]	2006 WHO Guidelines [26]	2010 WHO Guidelines [24]	2013 WHO Guidelines [27]
Triple-NRTI first-line regimens	A lower tier recommendation than regimens containing NNRTIs but should still be considered, particularly when NNRTIs cannot be used because of intolerance/drug resistance and PIs are not available or when co-administered with anti-TB therapy.	A triple-NRTI regimen should be considered as an alternative first-line ART regimen in situations where NNRTI options provide additional complications and to preserve the PI class for second-line treatment (e.g. in women with CD4 counts of 250–350 cells/mm^3; coinfection with viral hepatitis or tuberculosis; severe adverse reactions to NVP or EFV, infection with HIV-2). Recommended triple-NRTI combinations are ZDV+3TC+ABC and ZDV+3TC+TDF	The triple nucleoside regimens ZDV+3TC+ABC or ZDV+3TC+TDF should be used for individuals who are unable to tolerate or have contraindications to NNRTI-based regimens, particularly in the following situations: • HIV/TB coinfection • pregnant women • chronic viral hepatitis B • HIV-2 infection	No longer specifically mentioned in a separate section, discussed as an option for HIV-2 infection but a regimen containing a boosted PI such as LPV is preferred.
Clinical and laboratory monitoring	*Primary health care centre* • Rapid HIVab • Haemoglobin if ZDV is considered • Pregnancy test • TB sputum smear • District hospital, above plus: • FBC and differential • CD4+cell count • ALT *Regional referral centres* • All of above • Full serum chemistries • Viral load testing	Recommends clinical assessment as primary tool for monitoring At diagnosis: • Clinical assessment of HIV stage, weight, concomitant medical conditions and medicines. • CD4 where possible • Pregnancy test • Haemoglobin if ZDV is considered • HIV confirmation Before ART: • Clinical monitoring • CD4 count every six months	Laboratory monitoring is not a prerequisite for initiating ART. At diagnosis: • HBsAg is desirable • CD4 is recommended Before ART: • CD4 is recommended Patients on ART: • Haemoglobin if ZDV • Creatinine clearance if TDF • ALT for NVP • CD4 is recommended At clinical failure: • Viral load is desirable • CD4 is recommended	Clinical assessment and laboratory tests play a key role before ART is initiated and then monitoring their treatment response. At diagnosis, recommended: • HIV serology, CD4 cell count, TB screening At diagnosis, desirable: • HBsAg, HCV serology, STI screening Before ART, recommended: • CD4 cell count every 6 – 12 months Initiating ART, desirable: • Haemoglobin if ZDV, Pregnancy test, Blood pressure, Urine dipstick, eGFR and serum creatinine if TDF, ALT for NVP Initiating ART, recommended: • CD4 cell count

	2003 WHO Guidelines [25]	2006 WHO Guidelines [26]	2010 WHO Guidelines [24]	2013 WHO Guidelines [27]
Clinical and laboratory monitoring		Patients on ART: • Monitor clinical progression at 2,4, 8, 12 and 24 weeks then every 6 months • Routine monitoring of CD4 every 6 months, if available • Viral load measurement not recommended in low-income settings		Patients on ART, desirable: • Urine dipstick and serum creatinine if TDF Patients on ART, recommended: • CD4 cell counts every 6 months, viral load, at 6 months and then every 12 months At treatment failure, desirable: • HBV serology • At treatment failure, recommended: • CD4 cell count, HIV viral load
Surveillance of HIV-1 drug resistance	Drug resistance genotyping is not on the near-term or mid-term horizon for individual patient management in low-income settings, but country programmes are encouraged to develop or participate in drug resistance surveillance and monitoring programmes to assist with planning at the population level.	Recommends the continued use of surveillance programmes to assess transmitted drug resistance and resistance at treatment failure. Notes that the use of standard highly active ART regimens should help in choosing second-line regimens.	Monitoring surveys to assess the emergence of HIV drug resistance and associated factors in patients treated for at least 12 months Surveillance for transmitted drug resistance among newly infected individuals	Recommends the use of early warning indicators to help identify deficits in programme performance that favour the emergence of HIV drug resistance. Also, recommends that countries undertake surveillance of HIV drug resistance and provides specific guidance on how to do the surveys required.

1.3 The DART Trial

1.3.1 Trial design

The Development of Antiretroviral Therapy in Africa (DART) trial was a randomised non-inferiority trial [30]. It principally investigated whether routine laboratory monitoring was necessary in HIV-1 positive patients who were starting antiretroviral therapy for the first time. The trial was open-label and conducted in three centres in Uganda and one centre in Zimbabwe. In all analyses, the Joint Clinical Research Centre, Kampala is combined with a satellite centre, the Infectious Disease Institute, Mulago. Research ethics committees in Uganda, Zimbabwe and the UK approved the DART trial.

The DART trial randomly allocated 3,321 adults to receive either laboratory and clinical monitoring (LCM), or clinically driven monitoring (CDM) only. Randomisation was stratified by centre, pre-therapy CD4 (0-99, 100-199 cells/mm^3) and initial antiretroviral therapy regimen received. All patients had a full blood count (haemoglobin, white cells, and platelets), lymphocyte subset count (CD4 and CD8), and liver and renal tests (urea, bilirubin, creatinine and aspartate aminotransferase/alanine amino-transferase) conducted at screening, weeks four, twelve and then every twelve weeks. However, these results were only returned to patients in the CDM arm if requested for clinical reasons (after review and authorisation by each centre's project leader). A plasma sample was stored at each of these visits. Patients could switch treatment to second-line antiretroviral therapy in both groups if a new or recurrent WHO stage 4 event occurred or at the clinician's discretion if a WHO stage 3 event occurred. In the LCM arm, patients could switch treatment if there was a confirmed CD4 cell count less than 100 cells/mm^3 (less than 50 cells/mm^3 before July 2006). Switching treatment before patients had received antiretroviral therapy for 48 weeks was strongly discouraged.

Co-primary endpoints were a diagnosis of a new WHO stage 4 event or death. An independent endpoint review committee, blinded to randomised group and CD4 cell count, judged WHO stage 4 events and the cause of death against pre-specified criteria. The committee accepted 780 of the 992 (79%) reported WHO stage 4 events.

Patients in DART received a first-line regimen of co-formulated zidovudine-lamivudine (ZDV-3TC) plus either tenofovir disoproxil fumarate (TDF), abacavir (ABC) or nevirapine (NVP).

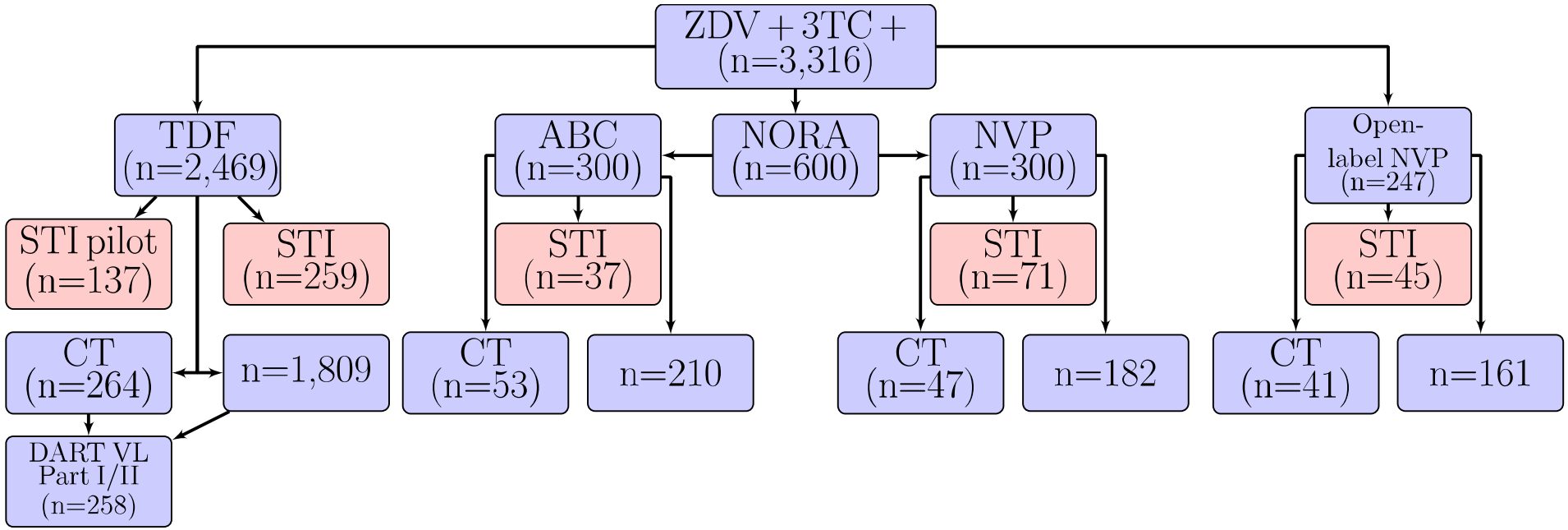
1.3.2 The main trial results

The main trial results [30] demonstrated that overall survival after five years was 88%. There was 87% (95% CI: 85-88%) survival in the CDM arm and 90% (95% CI: 88-91%) survival in the LCM arm. The trial found that 1,346 (81%) patients on CDM, versus 1,295 (78%) on LCM, were still on first-line antiretroviral therapy at their last clinic visit. A new WHO stage 4 event or death occurred in 459 (28%) CDM patients compared to 356 (21%) LCM patients. This corresponds to a relative hazard ratio (HR) of 1.31 (95% CI: 1.14-1.51). The pre-defined non-inferiority margin was 1.18 so the upper but not lower 95% confidence interval crossed this threshold. The trial team concluded that the high survival rate demonstrated that antiretroviral therapy could be delivered safely in low-income settings without routine laboratory monitoring. Clinically driven monitoring was not shown to be non-inferior, although it was also not formally shown to be inferior. Using routine CD4 cell count monitoring led to a small but significant benefit in disease progression and mortality.

1.3.3 DART substudies

A flow chart for the DART trial substudies is shown in Figure 7 (next page).

Figure 7: Flow chart of DART trial and substudy design



CT=Continuous Treatment

STI=Structured Treatment Interruption

1.3.3.1 NORA

The Nevirapine or Abacavir (NORA) substudy was conducted in two clinics in Uganda and was a randomised, double-blinded, phase II toxicity trial [31]. Six hundred participants were randomised between co-formulated zidovudine-lamivudine and either abacavir with nevirapine placebo or abacavir placebo with nevirapine, for 24 weeks. Participants continued on their randomised arm after 24 weeks but received open-label drug. Randomisation was stratified by centre, pre-ART CD4 count and monitoring randomisation. The primary endpoint was any serious adverse event (SAE) considered to be related to nevirapine or abacavir after 24 weeks of treatment. In the main NORA results, 20 events were deemed to be related to drug, 6 (2.0%) abacavir and 14 (4.7%) nevirapine, giving a hazard ratio of 0.42 (95% CI: 0.16-1.09, p-value=0.06). This substudy concluded that abacavir could be used more widely in low-income settings without major safety concerns.

1.3.3.2 The structured treatment interruption substudy

The structured treatment interruption (STI) substudy [32] evaluated whether intermittent antiretroviral therapy could be used to reduce treatment costs and long-term toxicity. Treatment interruptions can be guided by either CD4 count or of a fixed length. This substudy compared fixed length STIs and continuous treatment (CT) using a nested randomisation within the main DART trial. A pilot study informed the design of the main STI substudy. Participants who had CD4 cell counts greater than or equal to 250 cells/mm^3 at 28 weeks received one or two twelve week treatment interruptions. The pilot study recommended raising the CD4 threshold for inclusion and increasing the length of continuous treatment before randomisation for the substudy.

Participants in either arm of the main trial who had CD4 cell counts greater than or equal to 300 cells/mm^3 at 48 or 72 weeks were eligible for the main STI substudy. Participants were randomised to continuous

treatment or repeated twelve-week cycles on and off therapy, initiating these at weeks 52 and 76. Randomisation was stratified by centre, weeks since starting antiretroviral therapy, and randomised monitoring strategy. However, randomisation was not stratified by the antiretroviral regimen patients received. This led to a chance imbalance for patients in the NORA substudy which complicates interpretation beyond the main substudy results. A greater proportion of participants on a nevirapine-containing regimen were randomised to STI (n=71; 24%) than continuous treatment (n=47; 16%) compared to those on an abacavir-containing regimen [STI (n=37; 12%) and CT (n=53; 18%)]. The primary outcome of the substudy was progression to a new WHO stage 4 event, death, or a serious adverse event not solely related to HIV.

The STI randomisation was stopped after the second meeting of the Data Safety and Monitoring Committee on the 15th March 2006. Rates of death were similar in the two groups, but the incidence of first new WHO stage 4 event or death was higher in patients receiving a STI (n=24; 6.4 per 100 person-years) compared to CT (n=9; 2.4 per 100 person-years). This gave a HR of 2.73 (95% CI: 1.27-5.88; p-value=0.007). There was a sharp decrease in CD4 cell counts during a STI and an overall net decrease in patients who underwent a STI, even after restarting antiretroviral therapy. Eight weeks after restarting antiretroviral therapy, CD4 cell counts were still lower than before the interruption and each successive STI led to worse CD4 recover. The substudy concluded that patients should remain on continuous treatment due to the greater rates of disease progression.

1.3.3.3 Second-line substudies

DART's other substudies, SARA and OHFS, examined second-line treatment options and are described by Gilks et al. [33] and Ivan et al. [34]. Virological response and HIV-1 drug resistance on second-line treatment are not investigated in this thesis, so these results are not summarised.

1.3.4 DART Virology substudy

1.3.4.1 History

The main DART trial did not initially have the resources to retrospectively test the thousands of stored samples to determine viral load or to perform genotyping. Limited pharmaceutical funding generated viral load results, described below, for a small number of patients on tenofovir (known as part I/II) and NORA patients during the first 48 weeks of follow-up.

In April 2007, a UK grant was awarded for a proposal with the objective of developing an evidence-based approach to public health in low-income settings. Using a "walk backwards" ("walkback") testing algorithm it estimated that approximately 4,500 viral load samples (including approximately 600 baseline samples) would be necessary. Genotypic resistance testing would be performed on approximately 1,800 samples with viral load (VL) greater than 1,000 copies/mL. The grant was extended from June 2011 to August 2015 after several delays were experienced related to the complexity of the undertaking. Identifying and retrieving aliquots from the repository of over 60,000 stored samples was challenging after more than a decade since the trial's conclusion. This was often due to incomplete and sub-optimal record systems for these samples, frequently heavily reliant on individual staff member's expertise.

There were three main grant objectives. First, to determine the evolution of HIV-1 drug resistance in the absence of virological monitoring. The identification of the cross-resistance mutations reducing susceptibility to NRTIs at virological failure could then inform which NRTIs should subsequently be used in second-line antiretroviral therapy regimens. Second, to evaluate simple markers of resistance during first-line antiretroviral therapy, these could guide treatment switch in low-income settings. Third, to study virological determinants of disease progression during first-line therapy in the absence of virological monitoring. Laboratory work was conducted in Uganda and Zimbabwe to support

capacity building. Sequencing was not possible in Zimbabwe, so samples had to be retrieved and sent to sites in Uganda.

I have contributed to the testing process by identifying potential replacement viral load samples within the database, compiling these into lists for testing or shipment at individual sites, monitoring the completeness of testing and data entry. After viral load testing was complete and the data analysed, the time points of first virological failure and the last time point on first-line antiretroviral therapy were identified for genotypic testing. Frequently, plasma had either degraded, was low volume or viral load level was too low to amplify. In these cases successfully genotyping required attempts to amplify multiple plasma samples. I recorded which samples had definitively failed and generated genotype replacement lists for centres. I am extremely grateful to all the staff who conducted the viral load testing and HIV-1 genotyping, acknowledged in Appendix A.

1.3.4.2 Previous DART virological results

Prior to the additional viral load testing performed for the DART virology substudy; several analyses had been conducted retrospectively examining stored plasma samples. These analyses have consisted of cross-sectional investigations of virological response and patterns of HIV-1 drug resistance.

1.3.4.3 Part I/II

Three hundred patients on tenofovir were included in an earlier substudy (Part I/II) which examined longitudinal changes in viral load from initiating antiretroviral therapy to week 48 [35]. Patients in Part I/II were initially sampled after the first 100 patients had been enrolled in the study. This was based on the next 50 consecutively enrolled patients with a CD4 cell count of either 0 to 99 cells/mm^3 or 100 to 199 cells/mm^3. 300 patients were selected, equally divided between centres. Stored plasma samples from pre-ART, weeks 4, 12, 24, 36 and 48 were assayed for HIV RNA and week 24 samples were genotyped if HIV viral load was greater than 1,000

copies/mL. In total, 1,689 out of 1,800 (94%) samples were available for analysis. The analysis approach was cross-sectional and found that 79% and 72% had a viral load less than 400 copies/mL at weeks 24 and 48 respectively. In total, 36 (12%) patients achieved viral load less than 400 copies/mL, then had a confirmed rebound. Higher pre-ART CD4 cell count, female gender and being older were all found to be predictive of HIV viral load less than 50 copies/mL at week 48. There was no evidence that pre-ART HIV viral load was predictive. In total, 20 out of 53 (38%) patients with viral load greater than 1,000 copies/mL at week 24 were genotyped. Of these, 18 of 20 (90%) had major NRTI resistance mutations and the two patients without NRTI resistance had both interrupted antiretroviral therapy. The investigators concluded that a triple-NRTI regimen containing tenofovir demonstrated good antiviral efficacy and that this could be a useful first-line regimen in low-income settings.

1.3.4.4 NORA

Ndembi et al. [36] investigated stored plasma samples pre-ART and at weeks 4, 12, 24 and 48 for the 600 patients in the NORA substudy. Patients with viral load greater than 1,000 copies at week 48 had their week 48 and pre-ART samples genotyped for HIV-1 drug resistance mutations. A multivariate logistic regression model was used to identify pre-therapy factors associated with viral load less than 50 copies/mL at week 48. In total, 2,815 out of 3,000 (94%) possible samples were analysed. There was no evidence of a difference in the reduction of HIV viral load levels between abacavir and nevirapine prior to week 24. However, at weeks 24 and 48 a higher proportion of patients on nevirapine had viral loads less than 50 copies/mL (77% versus 62%; $p<0.001$). Furthermore, fewer patients on nevirapine had HIV viral load greater than 1,000 copies/mL at week 48. Pre-therapy CD4, RNA and initial antiretroviral therapy were strongly predictive while age, gender and WHO stage were not predictive of viral suppression. The mean residual activity of nevirapine-containing

regimens was lower than abacavir regimens, despite thymidine analogue mutations (TAMs) typically being more common in the abacavir group.

Munderi et al. [37] examined data from the NORA substudy for differences in immunology, virology, and clinical events during the first 48 weeks of the study. Alongside the previously reported differences in virology, the study showed that after 48 weeks of treatment the mean CD4 cell count increase was 147 cells/mm^3 for patients on abacavir compared to 173 cells/mm^3 for patients on nevirapine (p=0.006). However, 20 patients on abacavir developed new or recurrent WHO 4 events or died compared to 32 patients receiving nevirapine (HR=0.60; 95% CI: 0.34-1.05; p=0.07). Including WHO stage 3 events, 48 patients on abacavir and 68 on nevirapine had an event (HR=0.67; 95% CI 0.46-0.96; p=0.03). Munderi et al. [37] concluded that, despite the clear virological and immunological benefit of nevirapine over abacavir, this was not reflected in clinical outcomes at week 48 and required further investigation.

Other studies examining patients from the NORA substudy have used a longitudinal approach and extended the retrospective testing of plasma samples to week 96. Gupta et al. [38] investigated changes in viral load between weeks 48 and 96 and performed resistance testing on week 96 samples with a viral load greater than 1,000 copies/mL. In total, 107 patients who were randomised to an STI were excluded from analysis. Analyses were weighted to account for this bias. At week 96, there remained a difference in viral load between the randomised treatments; on abacavir 160 out of 226 (71%) had a viral load less than 1,000 copies/mL versus 156 out of 180 (87%) on nevirapine. However, this study also observed that patients suppressed at week 48 were more likely to remain so if they received nevirapine compared to abacavir (149 out of 156; 96% compared to 148 out of 180; 82%; p=0.003). The authors determined that 19 out of 70 (27%) patients, with viral load greater than 1,000 copies/mL at week 48, re-suppressed by week 96 and that this did not differ by

monitoring randomisation. The drug resistance analyses showed that patients with virological failure in the nevirapine arm typically had NNRTI resistance at week 96 (95%) but that the proportion with M184V (90% abacavir, 89% nevirapine) and three or more TAMs (49% abacavir, 42% nevirapine) did not differ by treatment.

1.3.4.5 TREAT

Ugandan patients in DART were eligible for a viral load test shortly after trial closure as part of a national programme. Patients enrolled in either OHFS or SARA were excluded since the viral load was already being measured retrospectively for these studies. Kityo et al. [39] examined this cross-sectional Ugandan data for the proportion of patients who switched to second-line antiretroviral therapy and the proportion with viral suppression (viral load less than 400 copies/mL) at trial closure on first and second-line regimens. Among 1,207 first-line patients, 80% were suppressed at trial closure and within the 242 second-line patients, 90% were suppressed at trial closure. There was evidence for a small difference between the main study monitoring strategies; 76% were suppressed in CDM versus 83% in LCM (Difference=7.1% ;95% CI: 2.5-11.5%). Multivariate analyses demonstrated the superior virological outcome in first-line patients on nevirapine (91% suppressed) compared to tenofovir (78% suppressed) and abacavir (79% suppressed).

1.3.4.6 STI substudy

McCormick et al. [40] examined 18 patients who underwent four full twelve week STI cycles. They found that mean HIV RNA off treatment ranged from 4.5 to 4.7 $\log_{10}$ copies/mL and that once treatment was resumed this declined to 2.3 to 2.6 $\log_{10}$ copies/mL. No K65R mutations were detected, and only one patient was found to have the M184V mutation. The authors concluded that there was a low risk that treatment interruptions would lead to HIV-1 drug resistant mutations developing.

1.3.4.7 Summary

To date, analyses of data from DART have typically been cross-sectional and have excluded patients who have switched treatment, died or been lost to follow-up before this time. There has only been a limited comparison of changes in viral load over time between antiretroviral regimens and monitoring strategies. Furthermore, the majority of the longitudinal analyses have focused on the randomised NORA patients and have not been able to make direct comparisons to patients on triple-NRTI regimens containing tenofovir. Finally, a longitudinal analysis of data in DART between weeks 96 and the end of the trial has not been performed.

1.4 Thesis objective

This thesis uses retrospectively collected virological and genotypic data from the DART trial to research how the absence of virological monitoring impacts virological failure and the development of HIV-1 drug resistance.

Chapter 2 investigates the durability of virological suppression after 48 weeks of antiretroviral therapy. Deaths that occur on first-line antiretroviral therapy have their virological status determined, and predictors explored in Chapter 3. In Chapter 4, HIV-1 drug resistance at the last time point on first-line antiretroviral therapy is evaluated. This data are assessed to investigate potential second-line regimens which retain the most susceptibility. Chapter 5 explores the development of HIV-1 drug resistance mutations from the time of first virological failure, found in Chapter 2, to the last time point. In Chapter 6, a summary of findings, limitations and potential additional research is presented.

2 The durability and predictors of virological suppression

2.1 Introduction

The cost and logistics of monitoring HIV viral load have led to limited routine use within clinics in low-income settings. Clinical and immunological criteria are often used as an alternative to determine when treatment should be switched; however, these criteria frequently lead to both unnecessary treatment switches and patients remaining on a regimen with virological failure [41]. This could compromise a patient's immune response and may potentially limit the efficacy of subsequent regimens if HIV-1 drug resistance develops.

Many experts have questioned whether it is ethical to provide antiretroviral therapy without laboratory monitoring. Alongside these concerns the competing priorities for limited resources needs to be considered. Enhanced laboratory monitoring could be at the expense of maximising the number of patients receiving antiretroviral therapy. Furthermore, enhanced laboratory monitoring leads to an increase in the use of expensive second-line regimens, yet potentially does not return the greatest public health benefit.

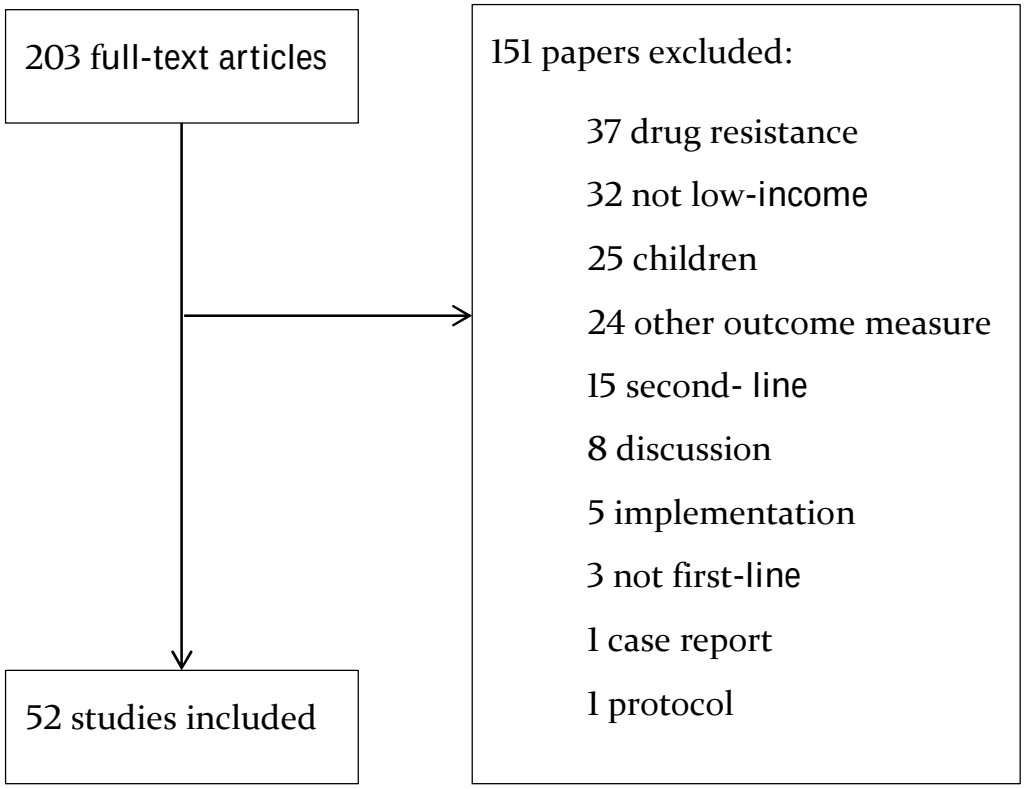
A major parameter when considering the value of laboratory monitoring is the rate of virological failure. Virological failure is a proxy for HIV disease progression and is frequently used to evaluate the relative performance of new antiretroviral drugs. It is a vital factor in cost-effectiveness models which evaluate the optimal monitoring strategy in low-income settings by comparing clinical, CD4 cell count, and viral load monitoring individually and as combinations. Cost-effectiveness models also evaluate a variety of monitoring testing frequencies and a range of cut-offs for determining the value at which patients should switch to a second-line regimen [42].

2.1.1 Virological suppression in low-income settings

A literature review was conducted by searching PubMed and updated on August 5th, 2016 for English-language publications investigating virological failure on first-line antiretroviral therapy in low-income countries. The search terms were "HIV" AND a term for virological failure ("virological failure" OR "viral failure" OR "virological suppression" OR "viral suppression") AND terms for low-income countries ("resource-limited" OR "resource-low" OR "resource-constrained" OR "sub-Saharan" OR "low-income" OR "low-" OR "LMIC" OR Uganda OR Zimbabwe OR "South Africa") AND ("first-line" OR "started ART").

This search (Figure 8) identified a total of 203 publications (listed in Appendix B and selectively summarised in this section), of which 52 studies were conducted in untreated adults (more than 15 years old) where a reported outcome was the incidence or prevalence of virological failure after 48 weeks of continuous first-line antiretroviral therapy.

Figure 8: Search strategy



Systematic reviews have examined the durability of virological suppression in low-income settings. Barth et al. [43] used 89 journal articles and conference abstracts, including 63,684 first-line patients from eighteen countries, to describe virological suppression in Sub-Saharan Africa. The proportion with virological suppression after six months of antiretroviral therapy was 78% (10,351 out of 13,288 patients). Suppression was defined by a range of viral load values (forty-seven reports used less than 400 copies/mL, six used less than 500 copies/mL and six used either less than 200 or less than 300 copies/mL). Virological suppression after one year was equivalent to six months (7,413 out of 9,794; 76%) and declined after two years (3,840 out of 5,690; 67%). Data after three years of antiretroviral therapy was scarce (available in only five papers), and virological suppression remained at 67% (837 out of 1,332).

Boender et al. [44] conducted an updated systematic review and meta-analysis by including papers up to the 14th May 2013. They examined 2,391 research papers and conference abstracts to find research examining virological outcomes on first-line antiretroviral therapy in low and middle-income countries. In total, 163 studies were used to examine virological suppression at up to 60 months of antiretroviral therapy. At least 90% of participants in these studies were on a dual-class regimen containing an NNRTI. Analyses were either on-treatment (OT) or intention-to-treat (ITT) where all participants who were lost to follow-up, died or stopped antiretroviral therapy were assumed to have experienced virological failure. Patients who switched to second-line were not classified as virological failures in either analysis. In the on-treatment analysis, 13 cohorts had data available at 48 months of antiretroviral therapy. The random-effects meta-analysis estimated that 88.6% (95% CI: 84.2-93.0%) were virologically suppressed. This dropped to 85.5% (95% CI: 77.5-93.5%) at 60 months based on data from six cohorts. In the intention-to-treatment analysis, four cohorts had data at 48 months, and 61.8% (95% CI: 44.0-79.7%) were

virologically suppressed. There was no intention-to-treat data available after 60 months of antiretroviral therapy.

The cohorts included in Boender et al. [44] with on-treatment analyses conducted after 60 months of antiretroviral therapy or at 48 months in intention-to-treat analyses are summarised in Table 3. All cohorts had regular viral load and CD4 cell count monitoring, typically every six months. In total, the intention-to-treat analyses had 504 patients available for analysis at 48 months. There was substantial uncertainty in the proportion of patients virologically suppressed at this time point.

Several important papers in this chapter's systematic review were not included in either of the prior systematic reviews. Mermin et al. [45] compared viral load monitoring (with CD4 and clinical monitoring), CD4 monitoring (with clinical monitoring) and clinical monitoring only in 1,094 Ugandan patients starting antiretroviral therapy for the first time with over three years of follow-up. During the study period, 61 out of 1,094 (6%) patients experienced virological failure (two consecutive values greater than 500 copies/mL). This study also demonstrated that patients may switch treatment without virological failure when treatment switch is determined by clinical criteria alone; two of the seventeen (12%) switches in this arm occurred without failure compared to zero of the eleven (0%) in the other two arms.

Haas et al. [46] used data from 297,825 patients in 16 countries in east, south and west Africa from the international epidemiological database to evaluate AIDS (IeDEA) to determine the cumulative probability of treatment switch by monitoring strategy. They report that with routine viral load monitoring the rate of confirmed virological failure (two consecutive viral loads greater than 1,000 copies/mL) was 4.23 per 100 person-years and that the cumulative probability of confirmed virological failure at five years was 15%.

Table 3: Summary of previous analyses of virological suppression in low-income settings at 48 or 60 months

Authors	Number of patients	Treatment	Monitoring	Analysis time point	Analysis performed	Result
Boulle et al. [47]	12,587 (44 at 48 months)	• 2 NRTI+NNRTI (100%)	Six-monthly CD4 counts and viral load testing	48 months	OT and ITT	91% OT and 63% in ITT were virologically suppressed after 48 months
Boulle et al. [48]	7,323 (148 at 60 months)	• D4T+3TC+EFV (44%) • D4T+3TC+NVP (39%) • ZDV+3TC+EFV (9%) • ZDV+3TC+NVP (8%)	Six-monthly viral load and CD4 cell count testing	60 months	OT	84% were virologically suppressed at 60 months.
Bussmann et al. [49]	633 (60 at 60 months)	• ZDV+3TC+EFV (51%) • ZDV+3TC+NVP (44%)	CD4+ cell counts and plasma viral load levels were initially obtained three-monthly and after November 2006, six-monthly	60 months	OT	After 60 months 98% remained virologically suppressed
Duong et al. [50]	1,572 (505 at 60 months)	•NRTIs+EFV (37%) •NRTIs+NVP (13%) •NRTIs+PIs (47%) •Triple-class regimen (3%)	CD4+ cell count and plasma viral load were conducted at baseline, 3 months and then six-monthly.	60 months	OT	Approximately 94% at 60 months of antiretroviral therapy

Fatti et al. [51]	66,953 (983 at 60 months)	• D4T+3TC+EFV (65%) • D4T+3TC+NVP (23%) • TDF+3TC+EFV (7%)	CD4 cell count and plasma viral load were measured six-monthly.,	60 months	OT	79% virologically suppressed after 60 months
Hamers et al. [52]	3,291 (172 at 48 months, 19 at 60 months)	•NNRTI-based (90%) •PI-based (10%)	All TASER sites have viral load testing. 8/13 PASER-M sites have plasma viral load testing. Testing frequency unclear.	48 and 60 months	ITT and OT	88% suppressed at 60 months in OT. 48% suppressed after 48 months in ITT.
He et al. [53]	437 (85 at 60 months)	•2 NRTI+NNRTI (100%)	Measurements taken but frequency unclear	60 months	OT	69% virologically suppressed at 60 months.
Manosuthi et al. [54]	140 (87 at 48 months)	• D4T+3TC+NVP	Plasma viral load and CD4+ counts measured every 24 weeks	48 months	ITT	By 48 months, 54% were virologically suppressed.
Soe et al. [55]	244 (201 at 48 months)	•NVP based (95%) •EFV based (5%) •Predominantly D4T+3TC (96.6%)	CD4+ counts measured twice per year and plasma viral load levels at least once a year	48 months	ITT	At 48 months, 81% were virologically suppressed

Castelnuovo et al. [56] collected ten-year outcome data from a cohort of 559 patients at the Infectious Disease Institute, Uganda. CD4 cell count and viral load measurements were made every six months. Patients initiated antiretroviral therapy with a dual-class regimen containing either stavudine, lamivudine and nevirapine (74%) or zidovudine, lamivudine and efavirenz (26%). Of these patients, 439 (79%) achieved viral suppression and the cumulative ten-year probability of treatment failure was 0.38 (95% CI: 0.34-0.43).

2.1.2 Choice of NRTI drugs

The ACTG 5202 study [57] was a four-arm randomised trial which compared blinded abacavir + lamivudine to tenofovir + emtricitabine in a dual-class regimen with a third drug of either ritonavir-boosted atazanavir or efavirenz. The study was stratified at randomisation by a screening HIV viral load of less than or greater than 100,000 copies/mL. During the first efficacy review, the independent data monitoring committee recommended stopping comparison in the high HIV viral load stratum because there was a shorter time to virological failure (viral load greater than 1,000 copies/mL) for patients in the abacavir + lamivudine arms. In the lower viral load screening stratum, the study did not find a difference in time to virological failure between atazanavir and efavirenz regimens (atazanavir HR: 1.25; 95% CI: 0.76-2.05, efavirenz HR: 1.23; 95% CI: 0.77-1.96). However, there was earlier regimen modification and safety events in the abacavir + lamivudine arm. This study was conducted with real-time monitoring of CD4, viral load and other safety markers and this may have contributed to the earlier regimen modification in the abacavir arm.

Other studies have also observed no difference in efficacy between tenofovir and abacavir. The BICOMBO study [58] found no difference in efficacy between tenofovir and abacavir. Furthermore, the HEAT study [59] compared abacavir + lamivudine + ritonavir-boosted lopinavir to tenofovir + emtricitabine + ritonavir-boosted lopinavir in 688 antiretroviral therapy-

naïve patients in a double-blinded placebo-controlled randomised study and showed no difference between abacavir and tenofovir. At week 48, 68% on abacavir + lamivudine and 67% on tenofovir + emtricitabine had viral load less than 50 copies/mL. Similarly, at week 96, the regimens remained non-inferior (60% compared to 58% respectively; p=0.60). There was no difference reported in efficacy by pre-treatment viral load.

Other studies have demonstrated that the combination of tenofovir + emtricitabine is superior to abacavir + lamivudine. The ASSERT study [60] recruited 385 patients and both arms used efavirenz as the third drug. The study found that 59% of patients on abacavir + lamivudine + efavirenz had viral load less than 50 copies/mL at week 48 compared to 71% in the tenofovir + emtricitabine + efavirenz arm (difference 95% CI: 2-21%). With a virological suppression definition of less than 400 copies/mL, 67% on abacavir + lamivudine + efavirenz versus 77% on tenofovir + emtricitabine + efavirenz (95% CI for difference: 1-18%) were suppressed at week 48. Treatment response was reduced in patients with pre-treatment viral load greater than 100,000 copies/mL but did not differ by regimen.

A meta-analysis of trials [61] investigated this difference between tenofovir + emtricitabine and abacavir + lamivudine where a ritonavir-boosted PI was used. This meta-analysis included three trials with a head-to-head comparison and nine trials with one of the regimens. The primary endpoint was virological suppression (less than 50 copies/mL) at week 48 and patients on abacavir were found to have a lower HIV response rate (69% suppressed) compared to tenofovir (76% suppressed; p=0.0015). Pre-treatment viral load was predictive of viral suppression, and there was evidence for a difference between abacavir and tenofovir with a viral load less than (p=0.02) and greater than 100,000 copies/mL (p=0.05).

2.1.3 Response to triple-NRTI regimens

Significant differences in the virological response of patients on different triple-NRTI regimens were observed in the double-blinded placebo controlled ACTG 5095 trial [62], 1,147 antiretroviral therapy-naïve patients were randomised to receive either: zidovudine + lamivudine + abacavir, zidovudine + lamivudine + efavirenz, or zidovudine + lamivudine + abacavir + efavirenz. The data and safety monitoring board discontinued the triple-NRTI arm early, after deciding it was unlikely that it could be demonstrated to be non-inferior to regimens containing efavirenz with an NRTI backbone. Virological failure occurred in 21% of the triple-NRTI regimen compared to 11% in the pooled efavirenz-containing regimens after a median of 32 weeks. Furthermore, virological failure occurred earlier in patients on the triple-NRTI regimen. No significant differences in CD4 response were found between the two types of regimen.

In contrast, the ACTION study [63] investigated a triple-NRTI regimen and recruited 279 participants with viral loads between 5,000 and 200,000 copies/mL and CD4 cell count greater than 100 cells/mm^3. Participants were randomised in an open-label setting to receive either zidovudine + lamivudine + abacavir or zidovudine + lamivudine + atazanavir. The triple-NRTI regimen was found to be non-inferior at 48 weeks with 62% versus 59% achieving viral suppression less than 50 copies/mL. The results were similar in the 230 patients with pre-ART viral load less than 100,000 copies/mL, but the triple-NRTI regimen was not found to be non-inferior in participants with pre-ART viral load greater than 100,000 copies/mL (39% versus 60% suppressed).

Gallant et al. [64] compared tenofovir and efavirenz with an NRTI backbone of abacavir + lamivudine; they recruited 340 antiretroviral therapy-naïve patients and found an unacceptably high rate of non-response in the triple-NRTI regimen. Non-response was defined as (i) a less than two $\log_{10}$ decrease from baseline by week eight, (ii) HIV viral load

rebound greater than or equal to one log_{10} copies/mL above the nadir, (iii) for subjects with two consecutive viral loads less than 50 copies/mL, a confirmed rebound of greater than 400 copies/mL. Non-response occurred in 50 of the 102 (49%) patients in the tenofovir arm compared to 5 of the 92 (5%) in the efavirenz arm ($p<0.001$).

Mugavero et al. [65] used the ART-CC cohort collaboration to compare data from cohorts to the results from the ACTG 5095 trial. The primary outcome measure was viral load greater than 200 copies/mL at 24 weeks. Analyses were adjusted for the year antiretroviral therapy was initiated, age, gender, prior AIDS diagnosis, CD4 count and HIV viral load at the start of antiretroviral therapy. There were 4,610 patients in the cohort collaboration, 1,694 on abacavir and 2,916 on efavirenz. Adjusted estimates found that patients on efavirenz had an odds ratio of 0.46 (95% CI: 0.37-0.57) compared to patients receiving abacavir. This was similar to the results found in the ACTG 5095 study itself, where there was an odds ratio of 0.53 (95% CI: 0.36-0.79). This supports the conclusions of the ACTG 5095 trial that efavirenz-containing regimen are superior to a triple-NRTI regimen containing abacavir.

Trial and cohort data advise that early (the first 48 weeks of antiretroviral therapy) virological response is inferior in triple-NRTI regimens compared to NRTI-NNRTI regimens. The long-term virological response has not been investigated, nor has HIV-1 drug resistance in patients who remain on triple-NRTI regimens for sustained periods despite virological failure.

2.1.4 Objective

The main aim of this chapter is to describe the virological durability of first-line antiretroviral therapy among DART trial participants. Virological data over more than three years of follow-up in low-income settings are scarce and are usually in settings where viral load monitoring is routinely available. The data from DART provide valuable insight on the efficacy of first-line antiretroviral treatment in the absence of virological monitoring.

The selection of patients for the DART virology substudy is described in Section 2.2.1. Section 2.2.3 describes the statistical methods employed to account for potential selection bias. Section 2.3.3 summarises HIV-1 viral load around the time of death and provides the motivation to investigate this further in Chapter 3. Section 2.3.4 describes virological status at the time of treatment switch. The distribution of time to virological failure and the predictors of failure are examined in Sections 2.3.6 and 2.3.7. The findings from this chapter are summarised and compared to other studies in Section 2.4.

2.2 Methods

2.2.1 Virology Substudy

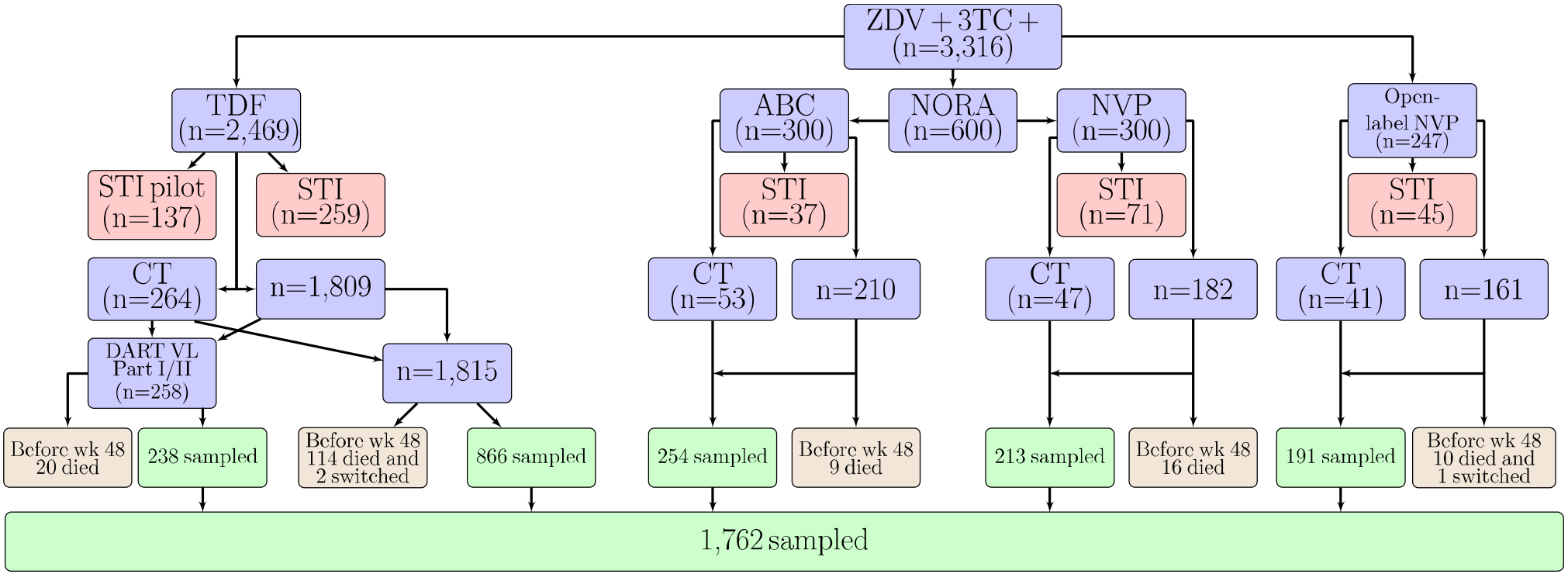
Only a small proportion of the approximately 60,000 plasma samples collected from 3,316 DART patients could be tested due to limited funds. The sampling scheme described in this section is illustrated in Figure 9 (below). Patients were selectively sampled in favour of patients whose virological response was likely to be of the most current interest. Analytical techniques were used in analyses to account for potential selection bias (Section 2.2.3).

Firstly, patients randomised to receive a structured treatment interruption were excluded as these are not recommended in treatment guidelines and are known to result in spikes of viremia during off-cycles (Section 1.3.3). All patients who died or switched treatment prior to 48 weeks of antiretroviral therapy were also excluded from sampling. This was based on the rationale that most early deaths (many occurred in the first twelve weeks) were due to advanced immunodeficiency rather than treatment failure. Deaths in DART during the first year are described by Walker et al. [66].

Apart from these exclusions, all patients who received either nevirapine or abacavir were selected for analysis. This maximised the power to compare different drug regimens. In total, 254 patients randomised to abacavir, 213

patients randomised to nevirapine and 191 patients who received open-label nevirapine were selected. A proportion of patients who received tenofovir were sampled. Firstly, all patients on tenofovir who died after 48 weeks were sampled. Secondly, a random sample of 90 patients who switched treatment during the study (30 from each centre) was chosen. Thirdly, all patients from the earlier virology substudy (part I/II) were sampled because these patients had existing viral loads and genotyping already conducted for pre-ART and week 48 [35]. Finally, all Ugandan patients with a first-line viral load results available from the TREAT study [39] were sampled. This group consisted of 442 patients with a viral load less than 200 copies/mL at the end of the trial and 140 with a detectable viral load. An additional random sample of 70 Zimbabwean patients, who were on first-line treatment at the end of trial follow-up and who had been recruited before 1st January 2004, was also chosen to achieve approximately 70 patients with a detectable viral load from each treatment centre.

Figure 9: Sampling flow chart for the DART virology substudy



Plasma samples for viral load testing were selected using a walkback procedure; beginning with plasma from the date of either treatment switch to second-line antiretroviral therapy, death, or the last time point in the trial. Patients with a suppressed viral load less than 200 copies/mL at this time point did not have further retrospective annual viral load testing conducted. If the viral load was greater than or equal to 200 copies/mL, then a sample collected 48 weeks prior to this was also tested. This process was repeated until either a sample where viral load less than 200 copies/mL was found or until plasma from week 48 was tested. If a sample could not be located, or an assay failure occurred, the closest available alternative sample (usually within twelve weeks) was used as an alternative. For some designated samples a viral load result was already available from NORA or part I/II substudies; in this case, the original value was used rather than repeating the test. Additional viral load results available through other substudies, yet not designated for testing by this process, were not utilised in analyses in order to avoid potential bias. Pre-ART viral loads were conducted for all patients, apart from Ugandan patients selected using TREAT study data with a suppressed viral load.

Through this selection process, 1,762 patients were analysed. Of these patients, 1,320 had additional retrospective testing conducted (662 patients on tenofovir, 404 on nevirapine and 254 on abacavir).

2.2.2 Viral load testing

Viral load assays were performed at several centres: Joint Clinical Research Centre (JCRC), Kampala; Uganda Virus Research Institute, Entebbe; the Infectious Diseases Institute (IDI), Mulago; The University of Zimbabwe-University of California San Francisco Collaborative Research Program (UZ-UCSF) laboratory, Harare; and the University of Zimbabwe, Harare. Four different types of assay were used; (i) The Roche Cobas Amplicor HIV-1 Monitor Version 1.5 with standard procedure (dynamic range of 400-750,000 copies/mL), (ii) The Roche Cobas Amplicor HIV-1 Monitor

Version 1.5 with ultrasensitive procedure (50-100,000 copies/mL), (iii) Roche Taqman v1.0 (40-10,000,000 copies/mL) and (iv) Roche Taqman v2.0 (20-10,000,000 copies/mL).

In total, 3,373 viral load tests were conducted; 576 (17%) were derived by the Roche Amplicor (standard), 969 (29%) by the Roche Amplicor (ultrasensitive) assay, 814 (24%) by the Roche TaqMan v1.0 assay, and 1,014 (30%) by the Roche TaqMan v2.0 assay. The Roche Taqman v1.0 and v2.0 assays were exclusively performed after 2011 at Entebbe (n=317 and 515), IDI, Kampala (n=28) and JCRC, Kampala (n=469 and 499), none were conducted in Harare. Roche Amplicor v1.5 and v1.5 ultrasensitive assays were performed at all testing sites: Entebbe (n=109 and n=4 respectively), JCRC, Kampala (n=24 and n=503), Harare (n=90 and n=418), IDI, Kampala (n=106) and Zvitambo (n=247 and n=44).

Do et al. [67] have conducted research comparing these different viral load assays. They find that using a cut-off for virological suppression of 50 copies/mL leads to significant discordance between the Roche Amplicor standard assay, the Taqman v1.0 (92% agreement; $p<0.01$) and the Taqman v2.0 (92% agreement; $p=0.02$). However, these differences are attenuated when a cut-off of 200 copies/mL is used (Taqman v1.0 agreement=96%, $p=0.05$; Taqman v2.0 agreement=95%, $p=0.16$). Therefore, the 130 viral load tests performed using the Roche Amplicor standard assay found to be at the lower limit of quantification (e.g. less than 400 copies/mL) were treated in analyses as also being at less than 200 copies/mL to avoid discrepancy between assays.

2.2.3 Weighting

Analytical weights were calculated for each patient, based on the inverse probability that a patient was sampled for analysis [68], to correct for selection bias. This created a pseudo-population of all patients alive and on continuous first-line antiretroviral therapy at week 48. This pseudo-population contains 3,007 patients and excludes the 169 patients who died,

3 patients who switched treatment and 137 patients from the STI pilot study who interrupted treatment prior to week 48.

Weights were generated to adjust for several sampling processes:

(i) Exclusion of patients randomised to receive a STI.

(ii) Complete sampling of all deaths.

(iii) Complete sampling of patients from the part I/II substudy.

(iv) Incomplete and unequal sampling of patients on tenofovir.

It was important to include all sampling factors within these models to ensure results are unbiased. For example, if switching antiretroviral therapy treatment regimen was ignored then weights generated for patients on tenofovir might lead to a pseudo-population being less likely to switch than the real DART population.

To adjust for process (i) for patients on abacavir or nevirapine, a logistic regression model was fitted within each antiretroviral therapy regimen with sampling as the outcome variable and using the following covariates: treatment centre, randomisation date (treated as continuous), trial monitoring strategy (LCM or CDM) and CD4 cell count at week 48. Weights were calculated for each subject as the inverse of their fitted value from this model, essentially the inverse probability of each patient being sampled. Patients on nevirapine or abacavir who died were excluded from the logistic regression analysis and were given a weight of one to account for process (ii).

Similarly, because patients on tenofovir who either died (process ii) or were in the part I/II substudy (process iii) were guaranteed to be included by the sampling method these patients were excluded from logistic regression models and given a weight of one. To adjust for process (i) patients on tenofovir in the STI substudy had weights calculated using a logistic regression model with identical covariates to that used to adjust for process (i) for patients on abacavir or nevirapine. Through this method, weights

were only generated for patients randomised to continuous treatment. These patients were considered to be the closest possible match to patients who received a STI. To adjust for process (iv), patients on tenofovir who were not in the STI substudy had a logistic regression model fitted with the previously described covariates and an additional term of treatment switch, with an interaction effect with treatment centre. With this approach, each patient is included in only one model.

As an example, the fitted model for the probability of being selected through process (iv) is displayed in Table 4. Patients who switched treatment were approximately equally likely to be selected for sampling at each treatment centre. Patients who remained on first-line antiretroviral therapy were more likely to be chosen for the virology substudy if they were from Uganda. This is a result of patients in the TREAT study being selected. The difference between Entebbe and Kampala is a further effect of this study, which preferentially conducted viral load testing on patients at Kampala compared to Entebbe.

Table 4: Model for patients alive on TDF, not in the STI substudy (n=753)

Factor	Odds Ratio	95% CI	p-value
Week 48 CD4 (100 cells/mm^3)	0.93	0.77-1.13	0.466
Monitoring randomisation			
LCM	1.00	-	-
CDM	1.10	0.86-1.41	0.446
Randomisation date	1.01	1.00-1.02	0.027
Interaction between centre and treatment switch			
Entebbe # No	1.00	-	-
Entebbe # Yes	0.27	0.16-0.44	<0.001
Kampala # No	3.23	2.22-4.68	<0.001
Kampala # Yes	0.19	0.12-0.30	<0.001
Harare # No	0.17	0.11-0.25	<0.001
Harare # Yes	0.27	0.17-0.41	<0.001

A summary of the actual number of virology substudy patients in each group and the sum of the total weights assigned to these patients (excluding deaths, switches and treatment interruptions before 48 weeks)

are displayed in Table 5. Summary statistics for the weights are presented in Table 6. Weights for patients on tenofovir were the most variable, a result of the incomplete sampling of these patients. Patients who received tenofovir in the structured treatment interruption substudy had the largest weights assigned due to this group being the most sparsely sampled.

Table 5: Summary of weights assigned

Initial ART	STI randomisation	ART Switch	# of patients analysed	Sum of weight assigned
TDF	In STI study	NA	155	522.1
TDF	Not randomised	No	753	1134.7
TDF	Not randomised	Yes	196	538.9
NVP	In STI study	NA	88	203.6
NVP	Not randomised	NA	316	316.0
ABC	In STI study	NA	53	89.9
ABC	Not randomised	NA	201	201.0
Total			1,741	3,006.1

Table 6: Weight summary statistics

Initial ART	STI randomisation	ART Switch	Mean	SD	Min	Max
TDF	In STI study	NA	3.37	1.94	1.00	11.32
TDF	Not randomised	No	1.51	1.00	1.00	5.46
TDF	Not randomised	Yes	2.75	1.33	1.00	4.99
NVP	In STI study	NA	2.31	0.33	1.61	3.02
NVP	Not randomised	NA	1.00	0.00	1.00	1.00
ABC	In STI study	NA	1.70	0.18	1.42	2.12
ABC	Not randomised	NA	1.00	0.00	1.00	1.00

2.2.4 Assumptions of viral load testing and analyses

The following analyses assume that if a patient is virologically suppressed, then they are suppressed throughout the trial up to this time point. At the last time point, 1,134 of the 1,762 (64%) patients had a viral load less than 200 copies/mL. For a further 178 patients, the walkback process was stopped before the week 48 time point was reached, this suppressed viral load was in the 48 week period prior to the last time point for 66 patients. This could indicate that the virological failure analysed was a "blip" and not indicative of persistent virological failure.

The assumption of continued virological suppression was checked by analysing viral loads generated through other DART substudies. In these samples, 115 patients had 207 detectable viral loads where a later measurement on the same regimen was less than 200 copies/mL. Many of these specimens (107/207; 52%) were from week 48, indicating patients who were slow to achieve initial virological suppression. The remaining 100 viral loads had a mean (SD) of 58,374 (198,342) copies/mL, implying intermittent viremia.

2.2.5 Statistical Methods

Time to virological failure, defined as the first viral load measurement greater than or equal to 200 copies/mL after 48 weeks of antiretroviral therapy, was analysed using adjusted Kaplan-Meier estimators [69, 70] and Cox regression models incorporating the analytical weights [71]. In these analyses, patients were considered to be at risk of virological failure after 36 weeks since randomisation. This is the earliest time point at which virological failure could be detected with the walkback viral load testing method (a 12 week sampling window was allowed and week 48 was the earliest time point targeted). Patients were censored at treatment switch or death if they had not virologically failed by this time (assumption probed in Section 2.3.5). Cox regression models included the following baseline covariates from the time antiretroviral therapy was initiated: monitoring

randomisation, gender, age, pre-ART CD4, pre-ART viral load, tuberculosis in the 12 months prior to enrollment, adherence in the previous 48 weeks and initial antiretroviral therapy received. Patients who received nevirapine in the open-label study in Zimbabwe and the NORA substudy were combined in analyses. Adherence was included as a time-dependent covariate summarising the estimated adherence in each 48 week period, as measured by the proportion of visits where pill counts indicated greater than 95% drug possession ratio. Drug possession ratio was defined as the days' supply of drugs delivered minus the days' supply of drugs returned divided by the number of days between clinic visits [72]. Adherence could be considered a potential mediator of other covariates (for instance gender and age) so the inclusion was tested in sensitivity analyses. Cox models were stratified by trial centre, thus allowing a separate baseline hazard for each centre. Due to the strong correlation between initial antiretroviral therapy regimen and the date of randomisation, the latter could not be included in the models. Excluding the date of randomisation may lead to some confounding. Non-proportionality was investigated using Schoenfeld residuals.

Patients from the TREAT substudy with suppressed viral load at the end of the study (452/1,762; 26%) were missing pre-ART viral load. Missing viral loads on the $\log_{10}$ scale were multiply imputed 30 times using a linear regression model which included all potential prognostic factors (terms of Cox model) and outcome variables (Nelson-Aalen estimator for time to virological failure, censoring indicator) [73] to avoid a loss in efficiency. This assumes that missing values do not depend on unobserved variables conditional on the observed data, such as the outcome variable. Analyses were performed on each imputed dataset, and the imputation-specific coefficients were combined using Rubin's rules [74]. Pre-ART CD4 cell count, age and adherence were not categorised to avoid a loss in power and

were included in multiple imputation analyses as fractional polynomials [75]. All analyses were conducted using Stata 14.1 [76].

2.2.6 Sensitivity Analyses

Sensitivity analyses were conducted with virological failure definitions of greater than or equal to 1,000 copies/mL or 10,000 copies/mL. An additional sensitivity analysis assumed that censored patients experienced virological failure to determine the impact of censoring on the analysis.

2.3 Results

2.3.1 Baseline Characteristics

Baseline characteristics of the 1,762 DART virology substudy patients were compared to all 3,316 patients in DART (Table 7). The substudy sample was not designed to be completely representative of the overall trial but achieved similar characteristics for the monitoring randomisation, gender and pre-ART CD4. The complete sampling of patients on nevirapine and abacavir ensured these patients were proportionally over-represented compared to patients who received tenofovir. There were comparatively fewer patients from Harare due to the use of TREAT viral loads.

Table 7: Comparison of baseline characteristics

Factor	DART Trial	VL substudy
Monitoring randomisation		
LCM	1,656 (50%)	882 (50%)
CDM	1,660 (50%)	880 (50%)
Gender		
Male	1,160 (35%)	587 (33%)
Female	2,156 (65%)	1,175 (67%)
Pre-ART CD4 (Cells/mm^3)		
0-50	1,109 (33%)	585 (33%)
50-100	785 (24%)	440 (25%)
100-150	759 (23%)	400 (23%)
150-200	663 (20%)	337 (19%)
Pre-ART viral load (Copies/mL)		
Missing	-	452 (26%)
<30,000	-	132 (10%)
30,000 - 100,000	-	181 (10%)
100,000 - 300,000	-	373 (21%)

300,000 – 700,000	-	340 (19%)
>700,000	-	284 (16%)
Initial ART		
TDF	2,469 (74%)	1,104 (63%)
NVP	547 (16%)	404 (23%)
ABC	300 (9%)	254 (14%)
TB in previous 12 months	831 (25%)	421 (24%)
Centre		
Entebbe, Uganda	1,020 (31%)	543 (31%)
Kampala, Uganda	1,297 (39%)	809 (46%)
Harare, Zimbabwe	999 (30%)	410 (23%)

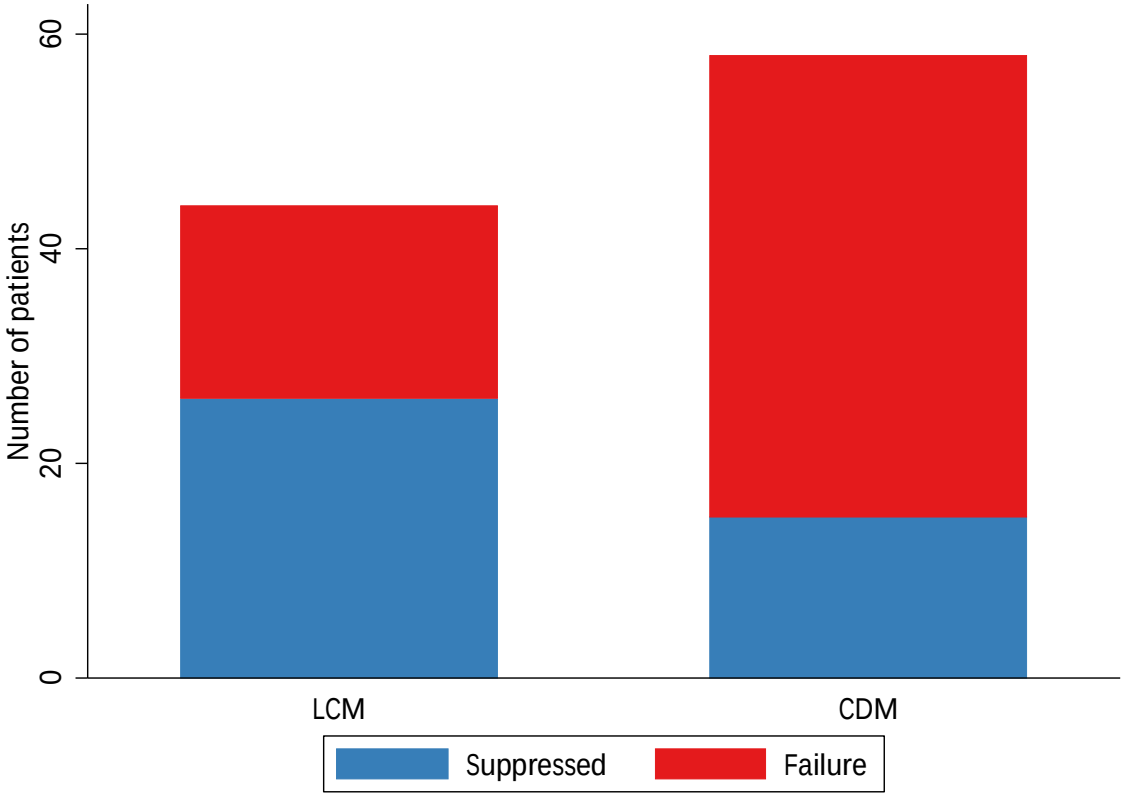
2.3.2 Missing viral loads

A total of 3,555 plasma samples were designated for retrospective viral load testing. A result was obtained for 3,373 (95%) samples, including replacements. Of the 1,762 patients in the virology substudy, 1,619 (92%) had a complete set of viral load data, as defined by the testing schedule and 1,741 (99%) had one or more viral loads available for analysis.

2.3.3 Virological status at the time of death

On continuous first-line antiretroviral therapy, 112 deaths occurred after 48 weeks (63 in the CDM arm and 49 in the LCM arm). These deaths occurred a median (IQR) of 116 (76-190) weeks after randomisation. Viral load, measured within 182 days of death, was available for 102 of the 112 patients (91%). In total, 61 of these 102 (60%) patients who died on first-line antiretroviral therapy had viral load greater than 200 copies/mL at death. These comprised of 18 of the 44 (41%) patients in the LCM arm and 43 of the 58 (74%) in the CDM arm ($p<0.001$) (Figure 10). Virological status at death is investigated further in Chapter 3.

Figure 10: Virological status at death by monitoring randomisation



2.3.4 Virological status at the time of treatment switch

During DART, there were 672 treatment switches to second-line antiretroviral therapy. In the main analysis [30], switches are shown to be more common in the LCM arm than the CDM arm (HR=1.19; 95% CI: 1.02-1.39; p=0.03), particularly during the second and third years after starting antiretroviral therapy.

In the virology substudy, 291 patients who switched treatment were available for analysis. There were 153 (53%) treatment switches in the LCM arm and 138 (47%) in the CDM arm. Treatment switch occurred a median (IQR) of 160 (114-200) weeks after randomisation. Viral loads, measured in the 182 days prior to treatment switch, were available for 267 of the 291 (92%) patients and 192 of the 291 (66%) had a viral load from the same day. Overall, 128 of the 145 (88%) switches in the LCM arm were with virological failure and 94 of the 122 (77%) in the CDM arm (p=0.02). For those who switched treatment without virological failure, viral load was typically less than 50 copies/mL (40 of the 45; 89%). Table 8 gives the

reported reasons for treatment switch. Only five treatment switches without virological failure were due to a CD4 criterion, and these all occurred in the LCM arm per trial protocol. Many treatment switches (44%) were due to new or recurrent WHO stage 4 events, where switching was recommended irrespective of CD4 count [27]. Switching due to a new or recurrent WHO 4 event was more common in the CDM arm than the LCM arm. A complete description of CD4 cell count in DART at the time of treatment switch is reported by Gilks et al. [77].

Table 8: Reasons for treatment switch among patients who switched to second-line ART without virological failure

Reason for switch	LCM	CDM	Total
New WHO 4 event	4 (24%)	16 (57%)	20 (44%)
Recurrent WHO 4 event	3 (18%)	1 (4%)	4 (9%)
2 × CD4≤50(100)	5 (29%)	0 (0%)	5 (11%)
Multiple WHO 3	0 (0%)	3 (11%)	3 (7%)
Single WHO 3	2 (12%)	8 (29%)	10 (22%)
Other CD4	3 (18%)	0 (0%)	3 (7%)
Total	17	28	45

2.3.5 Censoring at death or switch without virological failure

In total, 41 deaths and 45 treatment switches occurred without virological failure. In the following analysis of time to virological failure, patients are censored at their last virological measurement. While such an analysis could be biased, the extent of this is limited by the relatively small number of patients (86/1,741; 5%). This potential bias is quantified using sensitivity analyses in Section 2.3.8.

2.3.6 Durability of virological suppression

Overall, 609 of the 1,741 (35%) patients experienced virological failure (viral load greater than or equal to 200 copies/mL at 48 weeks or later). The time to virological failure by monitoring randomisation and initial antiretroviral therapy received are displayed in Figure 11 and Figure 12 respectively. According to the Kaplan-Meier survival function, 37% of patients were estimated to have virological failure by week 240 (Table 9). Findings at week 288 are limited to patients who received tenofovir.

Figure 11: Weighted Kaplan-Meier curves by monitoring randomisation

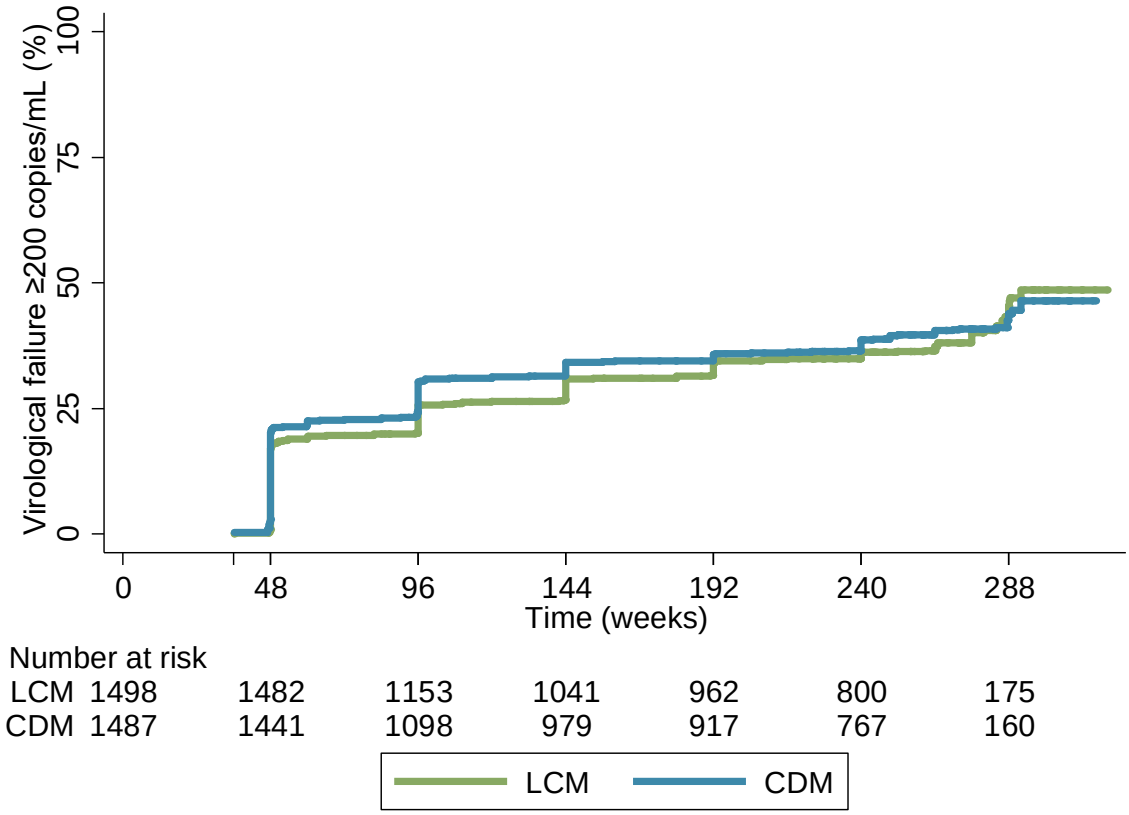


Figure 12: Weighted Kaplan-Meier curves by initial ART

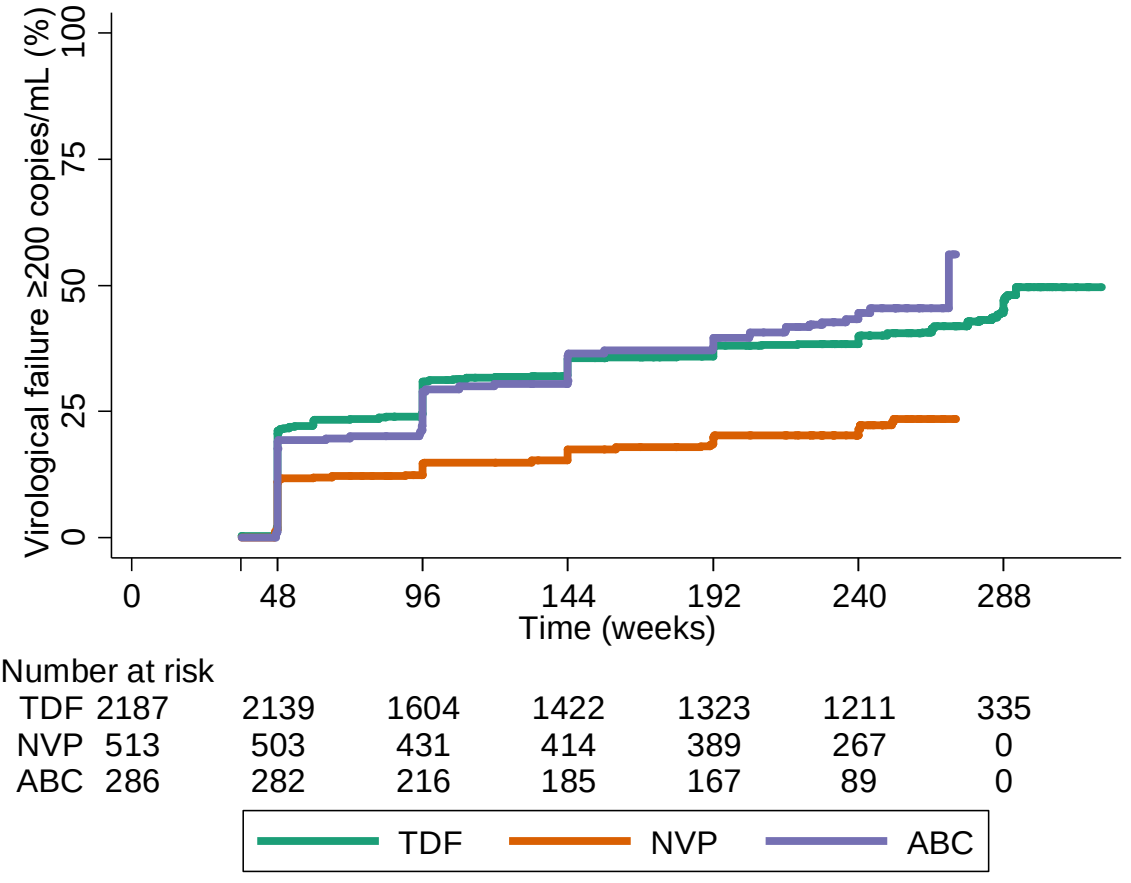


Table 9: Kaplan-Meier failure estimates by week and ART

Week	TDF	NVP	ABC	Overall
48	21%	11%	18%	19%
96	31%	15%	29%	28%
144	35%	18%	36%	32%
192	38%	20%	40%	35%
240	40%	21%	45%	37%
288	47%	-	-	45%

Virological failure rates over time, unadjusted for differences in baseline covariates, for the different initial antiretroviral therapy regimens are presented in Table 10.

Table 10: Virological failure rate (100 person-years) (95% CI)

Time (t, weeks)	TDF	NVP	ABC
0<t≤48	22.3 (19.3-25.9)	11.9 (9.1-15.8)	19.1 (14.7-25.3)
48<t≤96	14.7 (11.8-18.6)	4.5 (2.8-7.8)	15.0 (10.2-22.9)
96<t≤144	7.2 (5.0-10.9)	3.6 (2.2-6.5)	10.4 (6.3-18.2)
144<t≤192	3.9 (2.1-8.1)	3.0 (1.6-6.2)	6.1 (3.1-14.1)
192<t≤240	3.7 (2.1-7.0)	1.9 (0.9-4.9)	8.5 (4.6-17.3)
240<t≤288	9.2 (5.8-15.4)	8.5 (2.4-48.4)	10.1 (2.2-95.5)

2.3.7 Predictors of virological failure

Predictors of time to virological failure were analysed using Cox regression analyses (Table 11). There was no evidence that monitoring strategy influenced the time to virological failure (p=0.25). However, both gender and age were strong predictors of virological failure. Female patients had a 21% lower incidence of virological failure (p=0.01) and each additional 10-year increase in a patient's age reduced incidence of virological failure by 27% (p<0.001). Each additional 100 cell increase in patient's pre-antiretroviral therapy CD4 cell count reduced incidence of virological failure by 36% (p<0.001). Pre-antiretroviral therapy viral load did not affect the incidence of virological failure (p=0.89). There was no evidence of non-linearity in the multivariable fractional polynomial model, and there was no indication of non-proportionality for either monitoring strategy (p=0.83) or initial antiretroviral therapy (p=0.25). Compared with the tenofovir reference group, patients who received a nevirapine-containing

regimen had a 51% (p<0.001) lower incidence of virological failure and patients prescribed abacavir have a 28% (p=0.03) higher incidence of virological failure. Adherence was associated with virological failure, with 11% lower incidence of virological failure for every 10% increase in the proportion of visits with drug possession ratio greater than 95% (p<0.001). It should be noted that adherence could act as a mediation factor for other variables included in the model, such as initial ART, and the inclusion of this variable was investigated in sensitivity analyses in Section 2.2.6.

Table 11: Cox model of time to virological failure

Factor	Uni HR	95% CI	p-value	Multi HR	95% CI	p-value
Monitoring randomisation						
LCM	1.00	-	-	1.00	-	-
CDM	1.07	0.89-1.29	0.45	1.11	0.93-1.34	0.25
Gender						
Male	1.00	-	-	1.00	-	-
Female	0.80	0.66-0.96	0.02	0.79	0.65-0.95	0.01
Initial ART						
TDF	1.00	-	<0.001	1.00	-	<0.001
NVP	0.49	0.38-0.62	-	0.49	0.38-0.62	-
ABC	1.18	0.95-1.46	-	1.28	1.02-1.59	-
TB in 12 months prior to enrolment	1.13	0.91-1.40	0.26	1.07	0.87-1.33	0.52
Age (per 10 years older)	0.73	0.64-0.83	<0.001	0.73	0.64-0.84	<0.001
Pre-ART CD4 (per 100 cells/mm^3 higher)	0.60	0.50-0.71	<0.001	0.64	0.54-0.75	<0.001
Pre-ART viral load (log_{10} copies/mL)	1.02	0.86-1.20	0.81	1.02	0.85-1.21	0.89
Adherence in previous 48 weeks* (per 10% higher)	0.89	0.84-0.93	<0.001	0.89	0.84-0.94	<0.001

This Cox model was extended to explore the incidence of virological failure over time in 48 week periods (Table 12). Based on these results, follow-up time was divided into 0 to 96 weeks and 96 to 288 weeks to allow for easier interpretation (Table 13). Up to week 96, patients on nevirapine had a significantly lower incidence of virological failure than patients receiving tenofovir (HR=0.44). After week 96, patients on nevirapine continued to have a lower incidence of virological failure (HR=0.61), although the

difference was not as large. In contrast, patients receiving abacavir had a similar incidence of virological failure to those on tenofovir up to week 96 (HR=1.02). After week 96, patients on abacavir had an approximately 2.5-fold higher incidence of virological failure.

Table 12: Cox regression analysis investigating initial ART over time

Time (t, weeks)	TDF	NVP	ABC
36<t≤48	1.00	0.54 (0.39-0.74)	1.03 (0.76-1.41)
48<t≤96	1.00	0.27 (0.15-0.46)	1.10 (0.68-1.78)
96<t≤144	1.00	0.46 (0.24-0.90)	2.02 (1.06-3.85)
144<t≤192	1.00	0.68 (0.26-1.77)	2.19 (0.87-5.55)
192<t≤240	1.00	0.59 (0.21-1.62)	3.38 (1.41-8.08)
240<t≤288	1.00	3.48 (0.59-20.46)	9.82 (1.43-67.36)

Table 13: Cox regression analysis with collapsed time periods

Time (t, weeks)	TDF	NVP	ABC
36<t≤96	1.00	0.44 (0.33-0.59)	1.02 (0.78-1.33)
96<t≤288	1.00	0.61 (0.38-0.98)	2.47 (1.58-3.87)

Previous research suggests that abacavir-based regimens may be more prone to virological failure for pre-ART viral loads greater than or equal to 100,000 copies/mL. In a test for interaction there was no evidence of an association between initial antiretroviral therapy and a pre-ART viral load greater than or equal to 100,000 copies/mL in this data (p=0.23; Table 14). Patients on abacavir-based regimens had a higher incidence of virological failure with a pre-ART viral load≥100,000 copies/mL (HR=1.55; 95% CI: 0.89-2.68) but neither this nor the main effect of pre-ART viral load≥100,000 copies/mL were statistically significant.

Table 14: Cox model of time to virological failure with baseline viral load and initial ART interaction

Factor	HR	95% CI	p-value
Monitoring randomisation			
LCM	1.00	-	-
CDM	1.13	0.94-1.36	0.18
Gender			
Male	1.00	-	-
Female	0.79	0.65-0.95	0.01
Age (Decades)	0.73	0.64-0.83	<0.001
Pre-ART CD4 (per 100 cells/mm^3)	0.64	0.54-0.76	<0.001
Adherence in previous 48 weeks (per 10%)	0.89	0.85-0.94	<0.001
Initial ART			
TDF	1.00	-	0.04
NVP	0.53	0.32-0.86	-
ABC	0.91	0.55-1.48	-
Pre-ART VL≥100,000 copies/mL	1.25	0.93-1.70	0.14
Initial ART#Pre-ART VL≥100,000 copies/mL			
TDF	1.00	-	0.23
NVP	0.92	0.52-1.61	-
ABC	1.55	0.89-2.68	-
TB in previous 12 months	1.06	0.86-1.32	0.58

The relationship between CD4 cell count and virological failure over the time on antiretroviral therapy was investigated (Table 15). The results showed that evidence for an effect of pre-ART CD4 cell count on the incidence of virological failure was limited to the first 96 weeks of treatment. During the first 48 weeks of antiretroviral therapy, each additional 100 cell/mm^3 increase in pre-antiretroviral therapy CD4 cell count led to a 41% decrease in the incidence of virological failure. After 96 weeks, there was no evidence of an effect of pre-ART CD4 cell count on the incidence of virological failure (p=0.88). More than 85% of patients who started a regimen containing nevirapine with a baseline CD4 count greater than 100 cells/mm^3 remained virologically suppressed at 240 weeks.

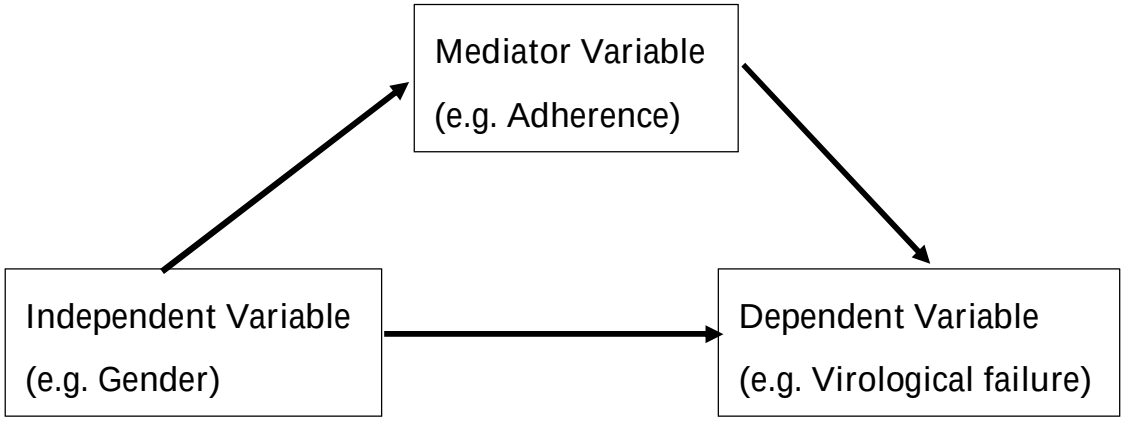
Table 15: Cox model investigating pre-ART CD4 cell count over time

Time (Weeks)	Pre-ART CD4 (per 100 cells/mm^3)	p-value
36<t≤48	0.59 (0.44-0.78)	<0.001
48<t≤96	0.48 (0.31-0.74)	0.001
96<t≤288	0.97 (0.67-1.41)	0.88

2.3.8 Sensitivity Analyses

Firstly, sensitivity analyses were used to explore the potential mediator effect of adherence. A mediator variable, displayed in Figure 13, is influenced by the independent variable which in turn influences the dependent variable. There may remain a residual direct causal relationship between the independent and dependent variables. Including adherence in the model could either weaken or strengthen the effects of other variables. This was explored in Table 16; the exclusion of adherence had no material effect on any of the other regression coefficients.

Figure 13: Example of a mediation relationship



Sensitivity analyses were also conducted to determine the effect of alternative virological failure thresholds (Table 17). Increasing the threshold of virological failure, resulted in a small increase in the difference between patients who received clinically driven monitoring only versus patients with laboratory and clinical monitoring. Nonetheless, the difference remained non-significant (p=0.11) for the highest VL threshold, confirming the conclusion of no evidence of an effect of monitoring randomisation. The comparison of tenofovir and nevirapine as initial

antiretroviral therapy was unaffected by altering the virological failure threshold. However, patients who received abacavir were found to have a similar incidence of virological failure to patients who received tenofovir when a threshold for virological failure of 10,000 copies/mL was used (HR=1.00). This implies that a disproportionate number of patients on abacavir fail with a viral load between 200 to 10,000 copies/mL compared to other antiretroviral regimens.

The impact of censoring was investigated by assuming that the 86 patients who died or switched treatment without virological failure would have virologically failed at this time point (Table 18). This assumption led to a higher incidence of virological failure in patients with clinical disease monitoring only compared to laboratory and clinical monitoring (HR=1.16; 95% CI: 0.98-1.37), reflecting the greater proportion of patients with laboratory and clinical monitoring who died or switched treatment with virological suppression. This sensitivity analysis reduced the impact of gender; a result of a greater proportion of female patients dying or switching treatment with virological suppression. The findings for initial antiretroviral therapy remained similar to Section 2.3.7.

Table 16: Cox model without adherence in previous 48 weeks

Factor	With adherence Adjusted HR	95% CI	p-value	Without adherence Adjusted HR	95% CI	p-value
Monitoring Randomisation						
LCM	1.00	-	-	1.00	-	-
CDM	1.11	0.93-1.34	0.25	1.12	0.93-1.34	0.23
Gender						
Male	1.00	-	-	1.00	-	-
Female	0.79	0.65-0.95	0.01	0.77	0.63-0.93	<0.001
Initial ART						
TDF	1.00	-	<0.001	1.00	-	<0.001
NVP	0.49	0.38-0.62	-	0.49	0.38-0.63	-
ABC	1.28	1.02-1.59	-	1.28	1.02-1.61	-
TB in 12 months prior to enrolment	1.07	0.87-1.33	0.52	1.07	0.86-1.34	0.52
Age (per 10 years older)	0.73	0.64-0.84	<0.001	0.72	0.63-0.83	<0.001
Pre-ART CD4 (per 100 cells/mm^3 higher)	0.64	0.54-0.75	<0.001	0.64	0.54-0.76	<0.001
Pre-ART viral load (log_{10} copies/mL)	1.01	0.85-1.21	0.89	1.02	0.85-1.22	0.83
Adherence in previous 48 weeks* (per 10% higher)	0.89	0.84-0.94	<0.001	-	-	-

Table 17: Cox model results for alternative virological failure definitions

	VL≥200 cps/mL			VL≥1,000 cps/mL			VL≥10,000 cps/mL		
Factor	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
Monitoring randomisation									
LCM	1.00	-	-	1.00	-	-	1.00	-	-
CDM	1.11	0.93-1.34	0.25	1.14	0.94-1.37	0.19	1.18	0.96-1.45	0.11
Gender									
Male	1.00	-	-	1.00	-	-	1.00	-	-
Female	0.79	0.65-0.95	0.01	0.78	0.64-0.95	0.01	0.77	0.62-0.95	0.01
Initial ART									
TDF	1.00	-	<0.001	1.00	-	<0.001	1.00	-	<0.001
NVP	0.49	0.38-0.62	-	0.49	0.38-0.63	-	0.50	0.38-0.65	-
ABC	1.28	1.02-1.59	-	1.23	0.97-1.55	-	1.00	0.76-1.31	-
TB in previous 12 months	1.07	0.87-1.33	0.52	1.08	0.86-0.94	0.49	1.15	0.91-1.46	0.24
Age (per 10 years older)	0.73	0.64-0.84	<0.001	0.70	0.62-0.80	<0.001	0.69	0.60-0.80	<0.001
Pre-ART CD4 (per 100 cells/mm^3)	0.64	0.54-0.75	<0.001	0.60	0.50-0.72	<0.001	0.52	0.43-0.64	<0.001
Pre-ART viral load (log_{10} copies/mL)	1.01	0.85-1.21	0.89	1.02	0.85-1.23	0.80	1.02	0.83-1.25	0.87
Adherence in previous 48 weeks (per 10%)	0.89	0.84-0.94	<0.001	0.89	0.84-0.94	<0.001	0.88	0.82-0.94	<0.001

Table 18: Cox model assuming all deaths and switches occurred with virological failure

	Deaths/switch no VF			Deaths/switch also VF		
Factor	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
Monitoring randomisation						
LCM	1.00	-	-	1.00	-	-
CDM	1.11	0.93-1.34	0.25	1.16	0.98-1.37	0.08
Gender						
Male	1.00	-	-	1.00	-	-
Female	0.79	0.65-0.95	0.01	0.86	0.73-1.03	0.10
Initial ART						
TDF	1.00	-	<0.001	1.00	-	<0.001
NVP	0.49	0.38-0.62	-	0.49	0.39-0.61	-
ABC	1.28	1.02-1.59	-	1.20	0.98-1.46	-
TB in previous 12 months	1.07	0.87-1.33	0.52	1.08	0.89-1.31	0.45
Age (per 10 years older)	0.73	0.64-0.84	<0.001	0.79	0.70-0.89	<0.001
Pre-ART CD4 (per 100 cells/mm^3)	0.64	0.54-0.75	<0.001	0.69	0.59-0.80	<0.001
Pre-ART viral load ($\log_{10}$ copies/mL)	1.01	0.85-1.21	0.89	0.98	0.84-1.14	0.78
Adherence in previous 48 weeks (per 10%)	0.89	0.84-0.94	<0.001	0.89	0.85-0.93	<0.001

2.4 Discussion

2.4.1 Durability of virological suppression

Overall, this analysis found that 63% of patients starting antiretroviral therapy for the first time in a low-income setting remained virologically suppressed at 240 weeks. For patients who remained alive on a first-line NRTI-NNRTI regimen, 79% were virologically suppressed at 240 weeks.

These findings extend a previous cross-sectional analysis of the DART trial investigating virological suppression at trial closure [39] by accounting for loss to follow-up, treatment switches and deaths and by describing the time of virological failure. In the part I/II substudy, 28% of patients had viral load greater than 400 copies/mL at week 48 [35]. An earlier comparison of abacavir and nevirapine [37] found that 25% of patients receiving abacavir and 23% of patients receiving nevirapine had a viral load greater than 400 copies/mL at week 48. The durability of virological suppression after 240 weeks of antiretroviral therapy was higher than expected based on these earlier DART analyses. This is a result of the lower rate of virological failure after the first 48 weeks of antiretroviral therapy.

The analysis in this chapter excluded deaths, treatment switches and treatment interruptions prior to week 48, so the findings show more favourable rates of virological suppression than if the overall DART population had been studied. However, the population examined is the most relevant to the current HIV-1 positive population initiating antiretroviral therapy for the first time in low-income settings. For example, an analysis including patients with treatment interruption would be irrelevant now that planned treatment interruptions are widely avoided. Furthermore, the majority of the excluded early deaths in DART occurred in patients initiating HAART with a CD4 cell count less than 50 cells/mm^3 [66]. This situation is now less common in low-income settings, where the median CD4 cell count at HAART initiation has increased from 80 to 145 cells/mm^3 between 2002 and 2009 [78].

The walkback approach will lead to an underestimation of patients who have experienced virological failure of any duration. Specifically, a patient with virologically failure at week 48 but who re-suppresses from week 96 onwards is treated as being continually virologically suppressed. Research by Gupta et al. [38] in the NORA substudy found that 27% of patients with viral loads greater than 1,000 copies/mL at week 48 re-suppressed by week 96. This analysis focuses on persistent virological failure, where the clinical consequences are clear, rather than intermittent virological failure, where interpretation is more complex. The proportion of patients with virological suppression found in this chapter will appear superior to other studies which have true time to event data, where patients are censored at the time virological failure is first detected.

A similar overall proportion of patients in this analysis remained virologically suppressed after three and four years of antiretroviral therapy compared to Barth et al. [43] and Boender et al. [44] where 67% and 62% remained virologically suppressed respectively. However, a greater proportion of patients on NRTI-NNRTI regimens in this analysis (79%) were suppressed at these time points compared to these studies. Furthermore, 85% of patients with a pre-ART CD4 cell count between 100 to 200 cells/mm^3 were suppressed on NRTI-NNRTI regimens.

The level of virological response in DART is remarkable compared to these meta-analyses where cohorts included at least real-time CD4 cell count monitoring, and sometimes real-time viral load monitoring. In the DART trial, virological response was observed over a longer follow-up time in patients starting therapy with lower pre-antiretroviral therapy CD4 cell counts and with 84% of patients initiating a triple-NRTI regimen. Furthermore, this analysis included virological failure in patients who subsequently switched to second-line regimens. One possible explanation for the extent of virological suppression is that the level of care received in DART, a randomised trial, might be superior to that found in routine

clinical settings. It could be argued that this might make these findings less generalizable to low-income settings; however, this also demonstrates that high levels of suppression are possible without laboratory monitoring, providing that drugs are readily available, without stock-outs.

Cost-effectiveness studies evaluating alternative monitoring strategies require assumptions about the long-term effectiveness of antiretroviral therapy treatment in low-income settings. Keebler et al. [42] use three independent models [79-81] to evaluate a range of monitoring strategies to inform the WHO 2013 guidelines for low-income settings. The first model by Phillips et al. [79] uses an estimate for the risk of virological failure (greater than 500 copies/mL) after five years of 28%. Virological failure after 240 weeks for patients in DART on an NRTI-NNRTI regimen in DART was 21%, so the assumed rate of virological failure rate used in the Phillips et al. synthesis model could be reduced. The second model by Estill et al. [81] assumes that failure (greater than 500 copies/mL) after one year would be approximately 10% without virological monitoring, in this chapter the rate in patients on a NRTI-NNRTI regimen containing nevirapine was 11%. Braithwaite et al. [80] model the trajectory of CD4 and viral load based on the current effectiveness of the antiretroviral therapy regimen. However, they do not explicitly describe the expected rate of virological failure after five years, so a comparison cannot be made.

2.4.2 Predictors of virological failure

There was no evidence that monitoring randomisation influenced the rate of virological failure. While CD4 monitoring could result in immunologically failing patients switching treatment earlier, immunological failure is usually found to be a consequence of virological failure. Since the majority of treatment switches occurred with virological failure, the lack of an effect of trial monitoring strategy on the rate of virological failure was expected.

There was a higher incidence of virological failure in patients receiving abacavir compared to tenofovir. When analysed for an interaction with pre-ART viral load there was higher incidence with pre-ART viral load greater than 100,000 copies/mL but this did not reach statistical significance. There was a lower incidence of virological failure for patients receiving a NRTI-NNRTI regimen compared to either triple-NRTI regimen during the first 96 weeks of the trial. However, after 96 weeks there was no evidence of a difference between triple-NRTI regimen and NRTI-NNRTI regimens. Comparisons by initial antiretroviral therapy regimen are not protected by randomisation, raising the possibility of bias, although analyses controlled for known potential confounders. While these findings are not as rigorous as those from a randomised comparison, they support the randomised trial results discussed in Sections 2.1.2 and 2.1.3. The ACTG 5095 trial demonstrated that NRTI-NNRTI regimens (zidovudine + lamivudine + efavirenz) were associated with a lower likelihood (OR=0.46) of viral load greater than 200 copies/mL at week 24 compared to a triple-NRTI regimen (zidovudine + lamivudine + abacavir). This chapter establishes that achieving and maintaining virological suppression on triple-NRTI regimens is possible after five years, but NRTI-NNRTI regimens are superior and should be recommended for first-line antiretroviral therapy.

Abacavir was found to have a higher incidence of virological failure than tenofovir, particularly after 96 weeks of antiretroviral therapy. However, there was no evidence of an interaction with pre-ART viral load greater than 100,000 copies/mL. Previous studies [57, 60, 61] comparing abacavir to tenofovir in NRTI-NNRTI regimens demonstrate a difference during the first 96 weeks of antiretroviral therapy. Sax et al. [57] find a shorter time to virological failure for patients on abacavir compared to tenofovir in the high pre-ART viral load stratum of more than 100,000 copies/mL (HR=2.46, 95% CI=1.20-5.05 for abacavir- + lamivudine + efavirenz versus

tenofovir + emtricitabine + efavirenz). They found no evidence for a difference in the failure rate in the low pre-ART viral load group (HR=1.23; 95% CI=0.77-1.96). A systematic review and meta-analysis [82] of six trials finds no evidence for a difference between abacavir and tenofovir at week 48 in terms of virological suppression in either high (Rate Ratio (RR)=0.96; 95% CI=0.90-1.03) or low pre-ART viral load strata (RR=1.01; 95% CI=0.99-1.03). Overall, the DART trial was not able to demonstrate a statistically significant interaction between abacavir and pre-ART viral load. Nonetheless, treatment guidelines [83] which recommend that abacavir is only an acceptable NRTI backbone in patients who start antiretroviral therapy with a pre-ART viral load less than 100,000 copies/mL should still be followed.

In the DART trial, older patients were less likely to experience virological failure (HR for failure=0.73 per decade). Other research has shown that older patients are more likely to achieve a virological response to HAART [84, 85], although often with a poorer immunological response. Paredes et al. [86] demonstrated that older patients were at a decreased hazard for virological failure (Relative hazard=0.86 per year older). In contrast, other studies [87, 88] have found no effect of age on virological failure. It is often speculated that older patients have better adherence to HAART than younger patients [89]; however, reported adherence in DART during the first year was not found to differ by age or gender [72]. Furthermore, the multivariate analyses which included a measurement for adherence still found evidence for an effect of age. Other studies have also demonstrated an effect of older age on lower viral load after controlling for adherence [90]. Adherence is often difficult to reliably measure, with no “gold standard” [91], so some residual confounding may account for the influence of age with younger patients possibly over reporting adherence. Alternatively, a decreased metabolism with age could contribute to these

findings. From this data, it is recommended that younger patients require more careful monitoring on HAART.

Female patients were shown to have significantly lower incidence of virological failure than male patients (HR=0.79 per decade). In a recent systematic review of gender differences in HIV outcomes, Castilho et al. [92] reported a decreased risk of death in female patients (RR=0.72; 95% CI: 0.69-0.75) and a decreased risk of immunologic failure for female patients compared to male patients (RR=0.83; 95% CI: 0.70-0.96). They did not find sufficient evidence for a decreased risk of virological failure in female patients (RR=0.93; 95% CI: 0.85-1.01). Castelnuovo et al. [56] found no evidence of a difference in the ten-year probability of treatment failure by gender. Male patients had a probability of 0.37 (95% CI: 0.32-0.43) compared to 0.45 (95% CI: 0.36-0.53; p=0.08). Like age, the influence of gender remained after accounting for differences in adherence during the previous 48 weeks. This could be a result of inaccurate adherence measurements, although some pharmacological effects could account for this difference such as female patients having a decreased metabolism.

In this analysis, pre-antiretroviral therapy CD4 was predictive of virological failure during the first 96 weeks of antiretroviral therapy but pre-ART viral load was not. This is supported by a systematic review conducted by Skowron et al. [93] which show that across 30 studies of NRTI+NNRTI regimens there was evidence of a correlation (p=0.01) between pre-ART CD4 cell count and virological suppression (<200-500 copies/mL) after twelve months of antiretroviral therapy. However, they found no evidence that pre-ART viral load was correlated (p=0.22). Patients in DART entered the trial with a pre-ART CD4 cell count less than 200 cells/mm^3, so this trial provides no evidence for patients initiating antiretroviral therapy at CD4 cell counts above 200 cells/mm^3. Nevertheless, our findings support guidelines recommending initiating HAART at higher CD4 cell counts.

The time of virological failure was interval censored but treated in analyses as the time where failure occurred. Analysing interval censored data in this way may underestimate the incidence of virological failure. A variety of statistical methods are available which handle interval-censored data [94] but currently none of the available statistical software implementations can work with data that requires both analytical weights and multiple imputation. A less sophisticated alternative approach could have analysed the midpoint of the censoring interval [95], but this was not applied in this dataset because the fixed sampling time points would have simply shifted the analysis forward by 24 weeks.

2.4.3 Treatment switch

The majority of treatment switches occurred with virological failure. After excluding switches made due to a new or recurrent WHO stage 4 event there was no difference between the main trial monitoring strategies and the proportion of patients switching while virologically suppressed.

Previous research [96] in the DART trial has found that WHO stage 4 events are more common in the CDM arm than the LCM arm at high CD4 counts. The authors speculate that this may be due to a CD4-dependent reporting bias where patients with similar clinical events but a high CD4 cell count are more likely to have this clinical event determined to be a WHO stage 4 event in the CDM arm where CD4 cell count is unknown. This explanation may also explain the imbalance by monitoring strategy for WHO stage 4 events at treatment switch without virological failure.

The findings on virological failure at treatment switch adds to the research of Gilks et al. [77], where retrospective viral loads were only available for 55% of the DART participants who switched treatment. The findings in this chapter support the conclusion that viral load "tie-breaker" tests would be of limited value to confirm clinical or immunological failure. In the majority of cases where treatment switch has been indicated, virological failure has occurred. Patients with poor immunological response but

suppressed viral load (less than 400 copies) can still benefit by switching to a second-line regimen despite virological suppression [77], so the results of a "tie-breaker" test in either direction may not change clinical decision making.

2.4.4 Conclusions

Recent WHO guidelines [27] recommend routine viral load monitoring is performed after six months of antiretroviral therapy and then every twelve months where possible. This chapter has shown that many patients on a NRTI-NNRTI regimen can have durable virological suppression after five years without virological monitoring. This suggests that a consistent drug supply enabling high levels of adherence is more crucial for success on therapy than upgrading clinical and laboratory infrastructure. Focus in low-income settings should remain on expanding access to antiretroviral therapy to patients with higher pre-ART CD4 cell counts. Nonetheless, laboratory monitoring may be required to reach the UNAID's "90-90-90" target for virological suppression in 90% of patients on ART.

Our analysis adds to the existing research by being the first to feature an intention to treat analysis of virological failure in low-income settings beyond 48 months on antiretroviral therapy and includes patients without laboratory monitoring. The results for NRTI-NNRTI regimens can inform cost-effectiveness models of laboratory monitoring with additional long-term data. A substantial proportion of patients who died after 48 weeks on first-line antiretroviral therapy did so while virologically suppressed (45%). This is surprising because deaths after 48 weeks of antiretroviral therapy have typically been thought to be a result of virological failure, so this is investigated further in Chapter 3.

3 Predictors of death with and without virological failure after one year of first-line ART

3.1 Introduction

HAART is estimated to have averted 7.6 million deaths between 1995 and 2013, including 4.8 million in sub-Saharan Africa [97]. However, there remained 1.5 million AIDS-related deaths in 2013 [97]. Granich et al. [98] calculated that 90% of global HIV mortality occurred in sub-Saharan Africa, Asia and the Pacific. In high-income settings, patients who start HAART with a high CD4 cell count are expected to have near normal life expectancy [99, 100]. However, life-expectancy may remain lower in low-income settings due to higher background mortality, lower CD4 cell count at treatment initiation and an increased risk of infectious diseases. There is approximately a threefold higher mortality during the first year of HAART in sub-Saharan Africa compared to high-income countries [101].

3.1.1 Predictors of late mortality in low-income settings

Predictors of mortality in resource-rich [102-106] and low-income settings [107-114] have been extensively studied. These studies [103, 104, 107, 112, 113] have typically concluded that current (time-dependent) CD4 cell counts are the strongest predictors of late mortality, although the estimated effect size varies between studies. Typically, a gradient of effect is observed; Lawn et al. [113] found that patients with a current CD4 cell count of less than 50 cells/mm^3 had an Incidence Rate Ratio (IRR) of 11.63 when compared to the reference group of greater than 500 cells/mm^3, whereas patients with 50-99 cells/mm^3 had an IRR of 4.93.

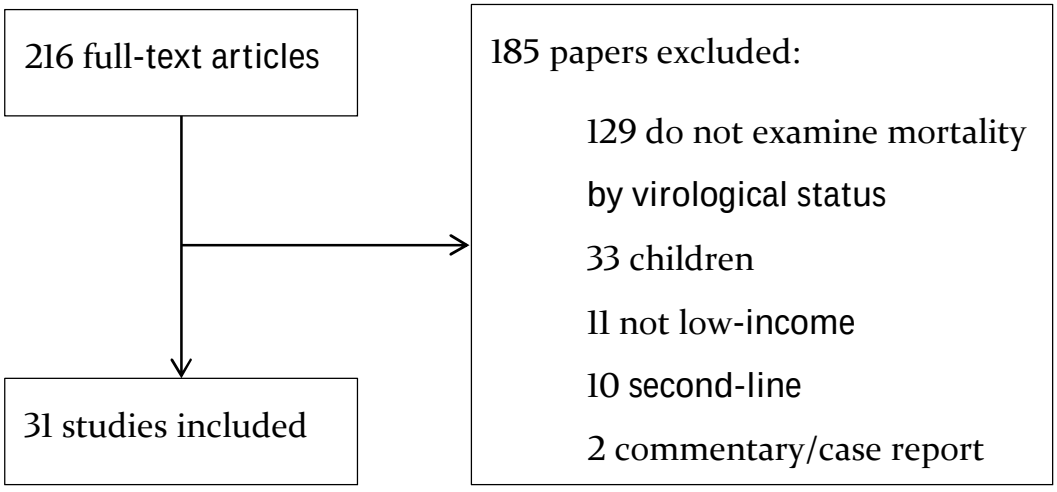
Other factors shown to be predictive of late mortality include older age [107-114], male gender [107-109, 111, 112, 114], higher current (time-dependent) viral load [107-111, 113, 114], higher baseline viral load [112],

baseline WHO stage 3 or 4 events [107-110, 112, 113], anaemia [111, 114] and lower body mass index [107, 110-112].

3.1.2 Virological status at late mortality

A literature review was conducted using PubMed, on the 19th April 2016, to find English language publications investigating the risk of death by virological status in low-income settings. Search terms for HIV, low-income settings (resource* or "sub-Saharan" or "low-income" or Uganda or Zimbabwe or "South Africa"), death (death or mortality) and virological response ("virological failure" or "virological suppression" or "virological response" or "viral suppression" or "viral failure" or "viral response" or "viral load suppression" or "viral load failure" or "viral load response" or "virologic suppression" or "virologic failure" or "virologic response" or "HIV RNA suppression" or "HIV RNA failure" or "HIV RNA response") were used. The search identified 216 publications and 31 were found to be relevant (Figure 14). Notable results are discussed in this section; the full list is available in Appendix C.

Figure 14: Search strategy



Mermin et al. [45] randomised 1,094 participants in rural Uganda starting a first-line NRTI-NNRTI regimen to either a viral load monitoring arm (clinical monitoring, quarterly CD4 counts and viral load measurements), CD4 arm (clinical monitoring and CD4 counts) or clinical arm (clinical monitoring alone). In total, 126 participants died, and 60 deaths (48%)

occurred during the first three months. Mortality was higher in the clinical arm than either the viral load arm (4.9 vs 3.7 per 100 person-years; aHR: 1.57; 95% CI: 1.00-2.46) or the CD4 arm (4.9 vs 4.0 per 100 person-years; aHR: 1.43; 95% CI: 0.92-2.21). In the clinical arm, two of the seventeen (12%) participants who experienced virological failure (greater than 500 copies/mL) died; the other 47 deaths in clinical arm occurred without virological failure being detected.

Keiser et al. [115] compared patients from the IeDEA Collaboration in South Africa, who received routine viral load monitoring to patients in Malawi and Zambia, who received CD4 cell count monitoring. Mortality was lower in South Africa (HR=0.58; 95% CI: 0.50-0.66; p<0.001) after three years (4.3%; 95% CI: 3.9-4.8) than Malawi and Zambia (6.3%; 95% CI: 6.0-6.5), despite a lower median CD4 cell count at the time of ART initiation (93 compared to 132 cells/mm^3; p<0.001). Analyses suggested that differences in background mortality accounted for approximately 20% of the observed difference and the rest was the result of earlier switches to second-line ART in patients with routine viral load monitoring.

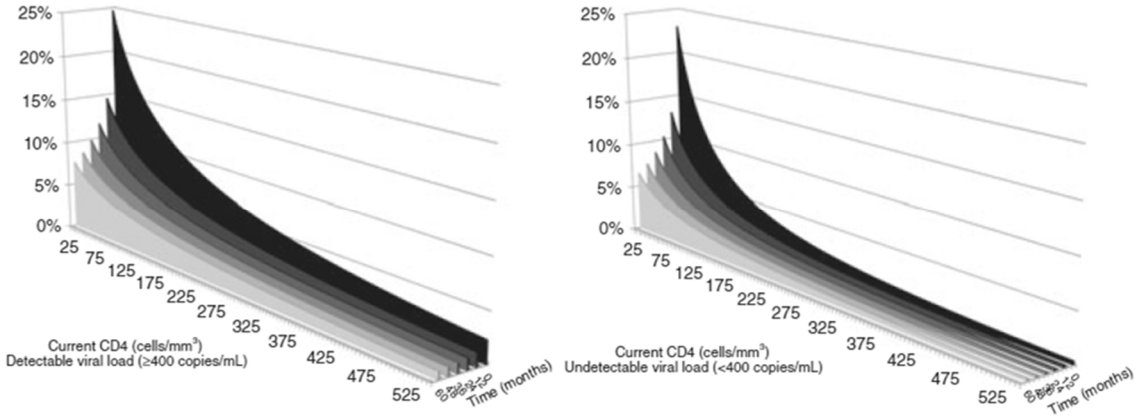
Petersen et al. [109] examined the risk of mortality among Ugandan and South African patients with virological failure (greater than or equal to 400 copies/mL). The relative odds of death were significantly lower for patients with a higher CD4 cell count at the most recent measurement (0.53; 95% CI: 0.37-0.76 per 100 cells/mm^3) and at CD4 nadir (0.56; 95% CI: 0.36-0.88 per 100 cells/mm^3). Additionally, there was higher mortality for patients with a greater CD4 decline since virological failure (1.17; 95% CI: 1.09-1.27 per 10% decline).

Hoffmann et al. [112] analysed 15,060 patients from South Africa who started HAART. CD4 count and viral load were monitored every six months, and 2,658 patients died during follow-up. Low CD4 count and a lack of virological suppression were associated with mortality after more than twelve months on HAART. Patients with time-dependent CD4 cell

count less than or equal to 50, 51-100, 101-200 and 201-350 cells/mm^3 had an aHR of 6.7, 4.5, 2.5 and 1.6 for mortality respectively ($p<0.01$) compared to patients with greater than 350 cells/mm^3. Patients with a time-dependent viral load greater than or equal to 400 copies/mL had an aHR of 5.6 (4.4-7.0) for mortality. In total, 100 of the 543 (18%) deaths after one year of HAART had a suppressed viral load at their last measurement (recorded in the six months prior to death).

Brennan et al. [110] used data from a prospective South African cohort of 14,932 antiretroviral therapy-naïve patients to investigate predictors of 1,985 mortalities. They also examined the interaction between CD4 cell count and viral loads, which were monitored annually. Brennan et al. divided follow-up into twelve month periods, with one paired measurement per patient in each period. A Poisson model was used to determine predictors of mortality, with an interaction between CD4, viral load and the time on antiretroviral therapy. Among patients who died, the viral load at the start of the twelve-month period was less than 400 copies/mL for 1,195 (60%) patients. Figure 15 displays the predicted mortality by this three-way interaction and demonstrates that the effect of CD4 count was strongest during the first-year and decreased over time. At lower CD4 cell counts and in patients who had an unsuppressed viral load there was increased mortality. The authors concluded that time-dependent CD4 cell count was the main predictor of late mortality, but that there was a strong interaction between viral suppression and time on treatment.

Figure 15: Predicted mortality by the current CD4 cell count (x-axis) and time on ART (z-axis) for patients with a suppressed viral load



3.1.3 Cause of death

The cause of death among patients on HAART has been extensively examined in high-income settings [116], but data are more limited for sub-Saharan Africa. Within Senegal, Etard et al. [117] investigated the cause of death in 93 patients who died on first-line antiretroviral therapy. The leading cause of death was mycobacterial infections (n=17), neurotropic infections (n=17) and septicaemia (n=17). However, a distinction was not made between deaths which occurred during the first year (n=47; 51%) and later. Castelnuovo et al. [118] determined the cause of death in 99 patients who died during the first 36 months of treatment in Kampala, Uganda. The majority (n=80) of deaths occurred during the first year and in total 76 deaths were thought to be HIV-related. The leading causes of HIV-related deaths were central nervous system infections (18; 25%), active tuberculosis (13; 16%), Kaposi sarcoma (7; 10%) and *Pneumocystis jiroveci* pneumonia (5; 7%). Further studies in Uganda by Moore et al. [119] investigated 112 deaths on antiretroviral therapy from a cohort of 1,132 patients in Tororo. The majority of deaths occurred during the first year of therapy (n=79; 71%). The overall leading cause of death was tuberculosis (21% of deaths), followed by oral or oesophagal candidiasis (15%), cryptococcal disease (12%), *Pneumocystis jiroveci* pneumonia (8%) and Kaposi sarcoma (6%).

The DART trial has previously reported on the 179 deaths (5.4%) which occurred during the first year of antiretroviral therapy [66]. The most common causes of early death were septicaemia or meningitis (36; 20%), extrapulmonary cryptococcus (18%), non-WHO stage 4 brain disease (16; 9%) and tuberculosis (14; 8%). Higher mortality during the first year was strongly associated with lower baseline CD4 cell count ($p<0.01$). Viral load testing on stored samples had not yet been conducted at the time this paper was published.

In summary, there are limited data on the cause of death, particularly beyond the first year on treatment. As access to HAART improves in low-income settings, reducing long-term mortality is of increasing importance.

3.1.4 Objective

It is widely assumed that deaths in low-income settings among HIV-infected individuals who are receiving antiretroviral therapy (apart from early deaths) are mainly due to virological failure or non-adherence. However, data from low-income settings without routine viral load monitoring are limited and a patient's virological status at death is likely to differ in these settings. Studies comparing laboratory monitoring strategies can use retrospective virological data to determine the virological status at death among patients who received no laboratory monitoring. This is likely to be informative for low-income countries determining the value of increasing the availability of laboratory monitoring. The relationship between mortality, time-dependent CD4 and viral load is complex and has not been studied in detail.

In Section 2.3.3, 40% of patients who died on first-line antiretroviral therapy after 48 weeks of antiretroviral therapy were found to be virologically suppressed at a measurement close to death. This was investigated further and work in progress was presented at the 2016 Conference on Retroviruses and Opportunistic Infections (CROI) (Appendix D) [120]. However, analyses of the predictors of death with

virological suppression were performed conditional on the occurrence of death after 48 weeks of ART. Ignoring other competing events, such as treatment switch after 48 weeks of ART, could give misleading results because patients who switch treatment are not able to die on first-line ART. The aim of this chapter is to determine predictors of deaths with and without virological failure after more than one year of first-line antiretroviral therapy in the DART trial population.

The statistical methods required to account for censoring due to treatment switch and to include time-dependent CD4 cell count in analyses are described in Section 3.2. Section 3.3.1 summarises the virological and immunological status of late deaths on first-line antiretroviral therapy and examines patient's reported cause of death. Section 3.3.2 investigates predictors of death by virological status. The implications of this research are summarised in Section 3.4.

3.2 Methods

3.2.1 Viral load testing

Viral load testing was conducted on all patients who died on continuous first-line antiretroviral therapy. For this analysis, viral load samples within 26 weeks of the time of death were used. If a sample was unavailable, but a viral load sample after 48 weeks of antiretroviral therapy indicated virological failure (viral load greater than or equal to 200 copies/mL), then virological failure was assumed. This approach was based on the rationale that patients with virological failure after 48 weeks of antiretroviral therapy were likely to remain viraemic. This assumption was required for only ten (10%) patients using viral load measurements from a median (Q10-Q90) of 40 (26-119) weeks prior to death. Three patients with virological suppression, recorded prior to week 26, did not have this observation carried forward. Among these patients, virological failure could reasonably still occur, so they were treated as lost to follow-up at their time of death.

3.2.2 Statistical Methods

3.2.2.1 Competing risks models

Competing events are outcomes (such as different causes of death) where the occurrence of one will prevent another event from occurring. All patients in the DART trial were included in analyses to determine predictors of death with virological suppression (<200 copies/mL) on first-line antiretroviral therapy. Treatment switch or interruptions were considered to act as competing events. Similarly, deaths with virological failure were also considered as competing events because patients cannot then go on to die with virological suppression. Methods to analyse competing events data include cause-specific hazard and subdistribution hazard models (also known as cumulative incidence models) [121].

Cause-specific hazards (CSH) describe "the instantaneous rate of occurrence of a given event among patients still event free at time, t" [122]. This can be calculated by a naïve analysis where observations with events other than the cause of interest are censored, and a standard Cox proportional hazard model is fitted. Covariate effects in cause-specific hazard models describe the association of a covariate with an event where competing events only contribute by removing individuals from the risk-set. Therefore, covariate effects in cause-specific hazard models report an increase or decrease of instantaneous hazard rate conditional on individuals having not had the event of interest by time t [123].

Subdistribution hazard (SH) models describe the "probability of occurrence of a given event, by time t" [122], in the presence of all other events. The risk-set for the cumulative incidence of a given event at time t includes both patients who have not had the event of interest yet and patients who have failed from other events. In the DART trial, it may seem counterintuitive to keep patients in the analysis dataset who switch to second-line antiretroviral therapy in the risk-set, since they cannot possibly die on first-line antiretroviral therapy. However, doing so has been shown

to be a mathematically valid construction of the relationship between a covariate and the cumulative incidence [121]. Covariate effects in a subdistribution hazard model can be considered as an effect on the cumulative incidence function that reflects the direct association of a covariate with an event and the contribution of other events removing patients from the risk-set.

Under the assumption of administrative censoring only, the subdistribution hazard can be modelled with a Cox proportional hazards model. In this model patients censored due to other events have their failure time replaced with their administrative censoring time. In the DART trial, only 234 (7%) participants were lost to follow-up before December 31st, 2008, and completeness of nurse and doctor visits were high (more than 97%). Therefore, simulation studies indicate that this assumption should have a small effect on parameter estimates and confidence interval coverage [124].

Latouche et al. [122] as well as Bakoyannis and Touloumi [124] argue that to understand data with competing events, both types of model need to be considered simultaneously. Differences between these models have been investigated in simulation studies [125], and covariate effects may differ substantially between models if dependent competing events exist. In particular, if a cause-specific model demonstrates that male gender has no impact on the cause-specific hazard of death, but a large effect on the cause-specific hazard of treatment switch, then there could still be an effect of gender on the cumulative incidence of death. This is a result of the number of male patients at risk of death being reduced by those switching treatment. In extreme cases, a higher cause-specific hazard of an event can be reversed and lead to a lower subdistribution hazard if the cause-specific hazard of a competing event is sufficiently strong [125]. Finding consistent and significant effects for both cause-specific hazard and subdistribution hazards provides stronger evidence of a causal association than finding an

effect for one measure only. Latouche et al. [122] recommend that results from both models should be reported with clear distinction and terminology and this proposal is followed for the results presented later.

3.2.2.2 Joint models

Previous studies have clearly established that time-dependent CD4 cell count is a strong predictor of late mortality. However, standard time to event models, such as the Cox model, can give misleading results if the time-dependent covariates are not considered to be "external" to the model. External covariates are those where "the value of the covariate at time point t is not affected by the occurrence of an event at time point u, with t>u" [126]. Examples include the time-dependent age of a patient, which is not related to whether a patient switches treatment. In contrast, patients who experience a decline in immunological function are more likely to die or switch treatment. As a consequence, there are likely to be fewer CD4 measurements and a sharper rate of decline. To correctly include longitudinal data, a more sophisticated approach such as a joint model of longitudinal and survival data is necessary [127].

Joint models simultaneously use a mixed effect model for the longitudinal process and a time to event model, such as the Cox model, to handle informative censoring. Both models have shared parameters which are used to account for the associations between outcomes [128]. Joint model methodology is described in detail by Rizopoulos [129].

Briefly, a standard mixed effects model is fitted for the longitudinal outcome, $y_i(t)$ such that:

$$y_i(t) = m_i(t) + \varepsilon_i(t)$$

Where $m_i(t) = x_i^T(t)\beta + z_i^T(t)b_i$ and $\varepsilon_i(t) \sim N(0, \delta^2)$. β terms are the unknown fixed effect parameters and b_i are the vector of random effects which are normally distributed with mean zero and covariance matrix D. x_i and z_i are the design matrices for the fixed and random effects respectively.

Then for the event process:

$$h_i(t|M_i(t), w_i) = h_0(t)\exp[\gamma^T w_i + \alpha m_i(t)]$$

Where $h_0(t)$ is the baseline risk function, w_i is a vector of the baseline covariates, γ the corresponding vector of regression coefficients and α the parameter for the association with the longitudinal outcome.

When the focus of a joint model is the interpretation of the event model, care should be taken to produce a good estimate of the longitudinal process. CD4 trajectories are a highly non-linear process [130], and Rizopoulos [127] recommends that high-order polynomials or splines are used in these cases for functions of time. Joint models have previously been used alongside competing risk analyses with cause-specific hazard [131, 132] and subdistribution hazard models [133].

3.2.2.3 Analysis

An overall death rate on first-line antiretroviral therapy excluding the initial 48 weeks of antiretroviral therapy was calculated using person-years spent on first-line antiretroviral therapy and by individually weighting patients using the analytical weights presented in Chapter 2. Person-years were further divided into time spent with suppressed and non-suppressed viral load. Person-years with suppressed viral load were cumulated from week 48 up until the first time point of death, treatment switch, loss to follow-up, virological failure or the end of the trial. Person-years with detectable viraemia were counted from the time virological failure was first detected after week 48 to the first of death, treatment switch, loss to follow-up or the end of the trial. This allowed the estimation of a mortality rate per 1,000 person-years with viraemia.

For patients who died, a mixed effect generalised linear model with a log link was fitted for CD4 cell count data measured during the 48 weeks prior to death. Time was treated as the number of weeks prior to death, leading to what Kurland et al. [134] describe as a terminal decline model which

appropriately handles drop-out due to death. Patients were treated as levels within the model with random intercepts and linear trajectories. Fixed effects included monitoring randomisation, gender, initial antiretroviral regimen, centre and baseline body mass index (BMI) and each was tested for an interaction with the linear term of time prior to death. Fixed effects with a p-value>0.2 were removed from the model in a backwards stepwise approach with the objective of achieving a parsimonious model, motivated by the limited number of deaths available for analysis.

Separate competing risk models were fitted to examine deaths with virological failure and deaths with virological suppression on continuous first-line antiretroviral therapy after 48 weeks. Competing risks included death before week 48, initiating a structured treatment interruption and switching treatment to second-line antiretroviral therapy at any time. These analyses were extended using a joint model to determine the influence of time-dependent CD4 cell count.

Covariates considered in univariate analyses included monitoring randomisation, gender, initial antiretroviral therapy regimen, tuberculosis at enrolment, centre, age at baseline, baseline BMI, baseline WHO stage and baseline CD4 cell count where baseline is the closest value prior to the time of antiretroviral therapy initiation. Continuous variables were included in the model as fractional polynomials [135] to avoid the biologically implausible and statistically inefficient use of dichotomisation [136]. The proportionality of hazards were investigated using Schoenfeld residuals in cause-specific hazard analyses and the log-minus-log of the subdistribution hazard. If non-proportionality was observed, the baseline hazard was stratified by these covariates. The cumulative incidence function was calculated after fitting each model.

The JM package [127] in R [137] was used to fit joint models. In the joint model, the square root of CD4 cell count was modelled as a function of

time since randomisation using a natural cubic spline. Monitoring randomisation, gender, age, centre and baseline CD4 cell count were included in the model, each with an interaction with trial time. CD4 cell count was censored at the first event to occur; death, treatment interruption or switching to second-line antiretroviral therapy. The association between the longitudinal model of CD4 and the survival model was parameterised as the true underlying value of the CD4 cell count, estimated from the longitudinal model. The decision to switch treatment, one of the competing risks in this analysis, is strongly influenced by time-dependent CD4 cell count in the LCM arm; therefore, we excluded monitoring randomisation from the joint model and included it only as an interaction with time-dependent CD4 cell count. The baseline risk function was estimated using a spline function. The JM package obtains the maximum likelihood estimate by maximising the log-likelihood function where the integrals are approximated using the adaptive Gauss-Hermite rule. Cumulative predicted incidences for different patients, conditional on their longitudinal CD4 measurements, were computed using a Monte Carlo estimate from the fitted joint models.

3.3 Results

3.3.1 Virological status at death

There were a total of 382 deaths during DART, of which 172 occurred within 48 weeks of starting first-line antiretroviral therapy and are discussed in a previous paper [66]. Of the 210 (55%) deaths which occurred after week 48, 78 were on second-line antiretroviral therapy and 20 deaths occurred in patients who had received a structured treatment interruption. Therefore, there was a total of 112 late deaths over 10,582 person-years of follow-up on continuous first-line antiretroviral therapy after week 48, giving an overall mortality rate of 10.6 per 1,000 person years.

The baseline characteristics of patients who died after 48 weeks on first-line antiretroviral therapy are displayed in Table 19.

Table 19: Baseline characteristics of patients who died

Factor	Died after week 48 (n=112)	Alive (n=1,650)
Monitoring randomisation		
LCM	49 (44%)	833 (50%)
CDM	63 (56%)	817 (50%)
Gender		
Male	44 (39%)	543 (33%)
Female	68 (61%)	1,107 (67%)
Age (years) Median (IQR)	38 (32-44)	37 (32-43)
Baseline CD4 (Cells/mm^3) Median (IQR)	73 (23-123)	83 (32-137)
Baseline viral load (Copies/mL)[1]		
<30,000	13 (12%)	119 (10%)
30,000 - 100,000	11 (10%)	170 (14%)
100,000 - 300,000	36 (32%)	337 (28%)
300,000 – 700,000	32 (29%)	308 (25%)
>700,000	19 (17%)	265 (22%)
Initial ART		
TDF	91 (81%)	1,013 (61%)
NVP	11 (10%)	393 (24%)
ABC	10 (9%)	244 (14%)
Centre		
Entebbe, Uganda	34 (30%)	509 (31%)
Kampala, Uganda	45 (40%)	764 (46%)
Harare, Zimbabwe	33 (30%)	377 (23%)

Viral load measurements were available for 102/112 (91%) deaths taken a median (IQR) of 10 (6-13) weeks prior to death. In total 41/102 (40%) patients were virologically suppressed at the time of death. The mortality rate on first-line antiretroviral therapy, while virologically suppressed, was 5.0 per 1,000 person-years (Table 20). In contrast, the mortality rate on first-line antiretroviral therapy when patients have detectable viral load was 25.6 per 1,000 person-years.

Table 20: Death rate by person-years with virological status

Virological Status	Number of deaths	Person-years	Death rate (per 1,000 PYs)	95% CI
All	112	10,582	10.6	8.8-12.9
VL<200 copies/mL	41	8,197	5.0	3.8-7.1
VL≥200 copies/mL	61	2,387	25.6	19.2-33.3

[1] Available in n=111 amd 1,199 respectively

Reported causes of death were adjudicated by an independent endpoint review committee and are shown by virological failure status in Table 21. Patients who died while virologically suppressed were more likely to die due to a gastrointestinal event[2] or an HIV-related malignancy (e.g. cervical cancer; n=4 or Kaposi's sarcoma; n=1). No patients died from wasting, diarrhoea or other types of cancer with virological failure, although there was not sufficient evidence to show a difference by virological status. Patients who died due to Cryptococcus were more likely to die with virological failure. While more patients died due to either malaria, cholera, or lung events with virological failure, there no evidence for a difference by virological status. Approximately a quarter of patients could not have their cause of death determined; this did not differ by virological failure status.

Table 21: Cause of death by virological failure status

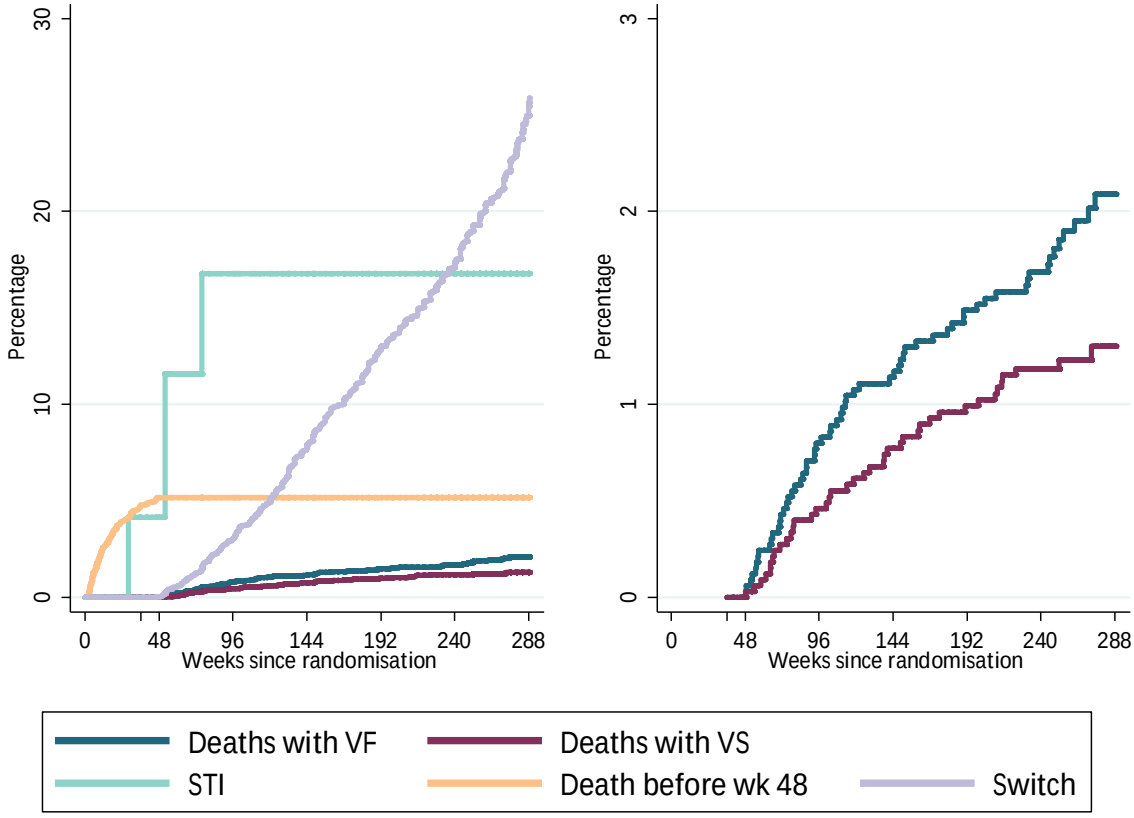
Cause of Death	Virological suppression	Virological failure	p-value	Total
Neurological Event	6 (15%)	4 (6%)	0.20	10 (9%)
TB	3 (7%)	7 (11%)	0.74	11 (10%)
Gastrointestinal	5 (12%)	1 (2%)	0.04	6 (5%)
HIV-related malignancy	4 (10%)	1 (2%)	0.16	7 (6%)
Septicaemia/Neutropenia	2 (5%)	4 (6%)	1.00	6 (5%)
Trauma/Suicide	1 (2%)	5 (8%)	0.40	6 (5%)
Cryptococcus	1 (2%)	4 (7%)	0.65	5 (5%)
Renal	2 (5%)	3 (5%)	1.00	5 (4%)
Cardiovascular disease	3 (7%)	2 (3%)	0.39	5 (4%)
Lung Event	0 (0%)	4 (6%)	0.15	5 (4%)
Lactic acidosis	1 (2%)	2 (3%)	1.00	5 (4%)
Malaria/Cholera	0 (0%)	3 (5%)	0.27	3 (3%)
Anae/Pan/Thromb	0 (0%)	1 (2%)	1.00	2 (2%)
Cancer (Other)	2 (5%)	0 (0%)	0.16	2 (2%)
Hepatic	0 (0%)	2 (3%)	0.51	2 (2%)
Wasting/Diarrhoea	2 (5%)	0 (0%)	0.16	2 (2%)
Unknown	9 (22%)	18 (29%)	0.49	30 (27%)
Total	41	61	-	112

Cumulative incidence curves (Figure 16) depict when deaths on continuous first-line antiretroviral therapy occurred and also when patients stopped

[2] Gastroduodenitis, acute gastrointestinal bleed, pancreatic, perforated gastric ulcer, paralytic ileus

continuous first-line antiretroviral therapy due to either a treatment switch or interruption. The median time of death with virological failure was 111 (IQR: 74-190) weeks. The median time of death with virological suppression was 122 (76-172) weeks.

Figure 16: Cumulative incidence of death and censoring events



3.3.2 CD4 cell counts prior to death

CD4 cell counts were available a median of 7 (5-11) weeks prior to death. A scatter plot (Figure 17) displays viral load and CD4 cell count for patients who died after 48 weeks of continuous first-line ART. CD4 cell count at the time of death was highly variable, although a greater proportion of patients who died with virological failure had fewer than 100 CD4 cells/mm^3 at the time of death compared to patients who died while virologically suppressed (65% versus 18%; p<0.01). Median CD4 cell count was lower in patients who died with virological failure than virological suppression (73 versus 238 cells/mm^3; p<0.01).

Figure 17: Scatter plot of viral load and CD4 at the time of death

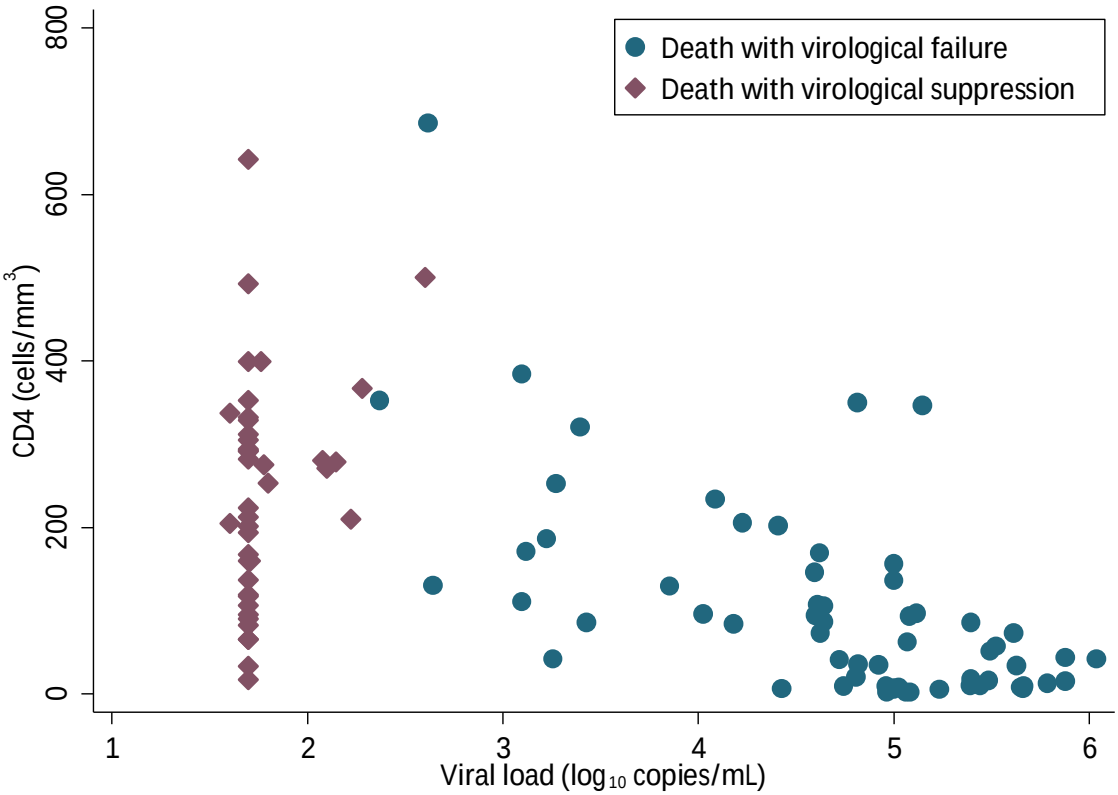


Figure 18 displays observed CD4 cell count trajectories in the 48 weeks prior to death by virological status for individual patients. In black are predicted marginal trajectories from a mixed-effects generalised linear model (Table 22) with a log link function. This mixed effect model was conducted including all patients who died after 48 weeks of continuous first-line ART. The predicted marginal CD4 trajectory declined by 25 CD4 cells/mm^3 (95% CI: -57 to 7; p=0.13) in patients with virological failure and increased by 32 CD4 cells/mm^3 (95% CI: -37 to 100; p=0.36) in patients with virological suppression. There was evidence of a decline in CD4 cell count in patients who died with virological failure (Coef=-0.83; 95% CI: -1.44 to -0.23; p=0.007). However, there was insufficient evidence to suggest that CD4 cell count changed among patients who died with virological suppression (Coef=-0.50; 95% CI: -1.11 to 0.11; p=0.11).

Figure 18: CD4 trajectory in the 48 weeks before death

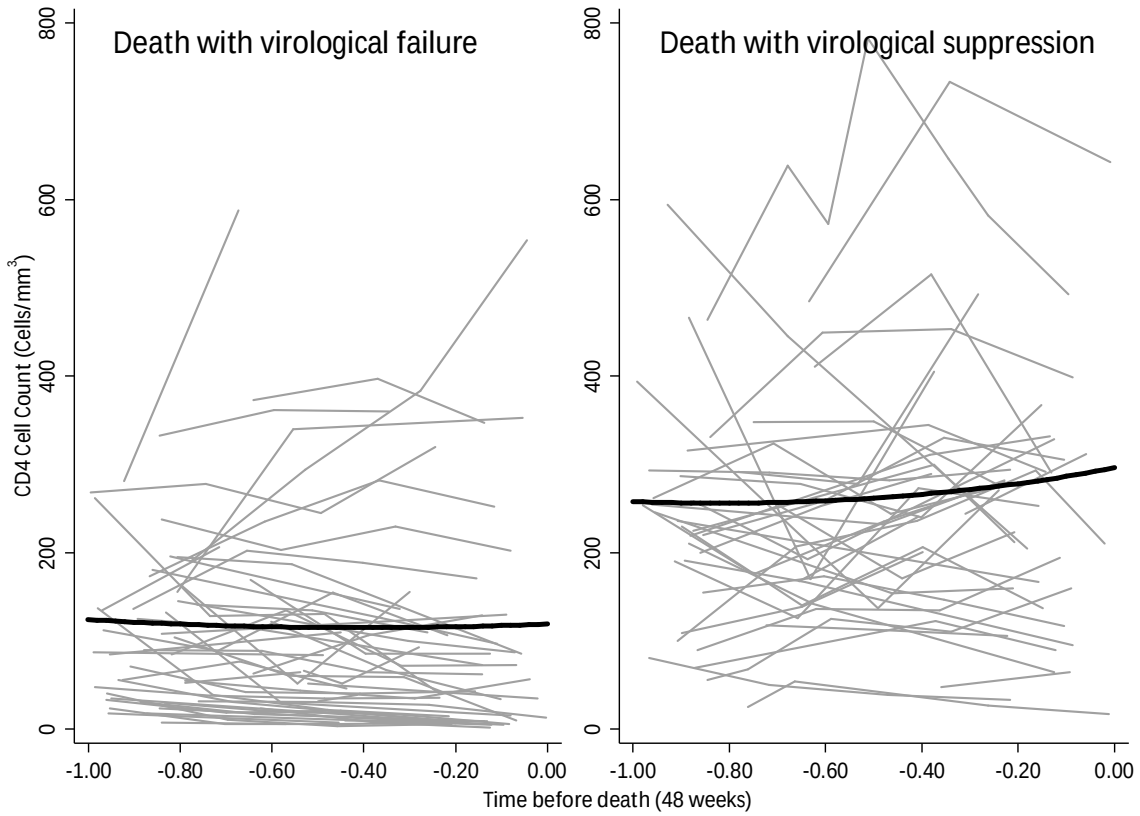


Table 22: Generalised mixed-effects model of CD4 trajectory

Variable	Coef	95% CI	p-value
Constant	4.57	4.18 to 4.97	<0.001
Monitoring randomisation			
LCM	0.00	-	-
CDM	-0.73	-1.16 to -0.31	0.001
Virological Status at death			
Virological failure	0.00	-	-
Virological suppression	0.98	0.54 to 1.41	<0.001
Time prior to death (48 weeks)	-0.83	-1.44 to -0.23	0.007
Monitoring randomisation # time before death (48 weeks)			
LCM	-	-	-
CDM	-0.45	-0.80 to -0.10	0.01
Age # time before death (48 weeks)	0.15	0.01 to 0.29	0.03
Virological status at death # time before death (48 weeks)			
Virological failure	0.00	-	-
Virological suppression	0.33	-0.04 to 0.71	0.08

3.3.3 Predictors of death by virological status

Throughout follow-up, 1,409/3,316 (42%) patients experienced a competing event (549 STI, 172 deaths before week 48, 689 treatment switches).

3.3.3.1 Death with virological suppression

Table 23 presents the results from univariate and multivariate cause-specific and subdistribution hazard models of death with virological suppression. The results from a joint model, which includes an interaction between trial monitoring randomisation and time-dependent CD4 cell count, are presented in Table 24.

Including time-dependent CD4 cell count, increased the aSHR for baseline CD4 cell count but did not have a substantive effect on other covariates. Interpretation of the predictors of death with virological suppression will focus on the joint model of Table 24. The hazard ratios in Table 24 for both multivariate cause-specific and subdistribution hazard are similar. There was no evidence that monitoring randomisation had an effect on the aSHR in either model through the interaction with time-dependent CD4 cell count (aSHR=0.97; 95% CI: 0.93-1.01; p=0.15). There was evidence of an association between death with virological suppression and time-dependent CD4 cell count in the cause-specific hazard model (aCSHR=0.92; 95% CI: 0.84-1.00; p=0.05). However, in the subdistribution hazard model there was a slightly smaller effect size and overall insufficient evidence to declare an association (aSHR=0.96; 95% CI: 0.89-1.03; p=0.24). This suggests that, when patients who died with virological failure are accounted for, there is no evidence that patients with higher time-dependent CD4 cell count have a reduced time to death with virological suppression.

Patients in Harare, Zimbabwe, had a lower cumulative incidence of death with virological suppression compared to patients in Entebbe, Uganda (aSHR=0.40; 95% CI: 0.15-1.02). There was no evidence that patients in

Kampala, Uganda, had a different cumulative incidence of death with virological suppression (aSHR=0.92; 95% CI: 0.46-1.81). The effects of baseline BMI and CD4 cell count were highly significant in both cause-specific and subdistribution hazard models. Each additional 100 CD4 cells/mm^3 at baseline led to a 95% increase (aSHR=1.95; 95% CI: 1.06-3.55; p=0.03) in the cumulative incidence of death with virological suppression; while a one kg/m^2 increase in BMI predicted a 12% reduction (aSHR=0.88; 95% CI: 0.80-0.98; p=0.02) and a ten-year increase in age predicted a 41% increase (aSHR=1.41; 95% CI: 0.97-2.06; p=0.07).

Table 23: Predictors of death with virological suppression

Variable	CSH	p-value	aCSH	95% CI	p-value	SH	p-value	aSH	95% CI	p-value
Monitoring randomisation										
LCM	1.00	0.09	1.00	-	0.08	1.00	0.11	1.00	-	0.11
CDM	0.57	-	0.57	0.30-1.07	-	0.60	-	0.60	0.32-1.13	-
Gender										
Male	1.00	0.49	-	-	-	1.00	0.50	-	-	-
Female	1.27	-	-	-	-	1.26	-	-	-	-
Initial ART										
TDF	1.00	0.51	-	-	-	1.00	0.40	-	-	-
NVP	1.04	-	-	-	-	1.04	-	-	-	-
ABC	1.74	-	-	-	-	1.93	-	-	-	-
TB at enrolment	1.11	0.76	-	-	-	1.11	0.77	-	-	-
Centre										
Entebbe	1.00	0.05	1.00	-	0.04	1.00	0.12	1.00	-	0.15
Kampala	0.79	-	0.89	0.46-1.74	-	0.87	-	0.97	0.50-1.90	-
Harare	0.34	-	0.37	0.14-0.95	-	0.38	-	0.42	0.16-1.08	-
Age (10 years)	1.39	0.07	1.44	0.99-2.09	0.05	1.41	0.05	1.45	1.00-2.09	0.05
Baseline BMI (kg/m^2)	0.90	0.09	0.88	0.77-1.01	0.06	0.91	0.11	0.89	0.79-1.01	0.08
WHO Stage										
2	1.00	0.60	-	-	-	1.00	0.69	-	-	-
3	1.51	-	-	-	-	1.44	-	-	-	-
4	1.58	-	-	-	-	1.44	-	-	-	-
Baseline CD4 cell count (100 $Cells/mm^3$)	1.58	0.09	1.68	0.97-2.89	0.06	1.47	0.12	1.56	0.93-2.62	0.09

Table 24: Joint model determining predictors of death with virological suppression

Variable	aCSH	95% CI	p-value	aSH	95% CI	p-value
Centre						
Entebbe	1.00	-	0.05	1.00	-	0.09
Kampala	0.82	0.41-1.61	-	0.92	0.46-1.81	-
Harare	0.34	0.13-0.88	-	0.40	0.15-1.02	-
Age (10 years)	1.36	0.93-2.00	0.12	1.41	0.97-2.06	0.07
Baseline BMI (kg/m^2)	0.87	0.78-0.97	0.01	0.88	0.80-0.98	0.02
Baseline CD4 cell count (100 $Cells/mm^3$)	2.18	1.20-3.98	0.01	1.95	1.06-3.55	0.03
Association with time-dependent CD4 ($\sqrt{Cells/mm^3}$)	0.92	0.84-1.00	0.05	0.96	0.89-1.03	0.24
Association with time-dependent CD4 # CDM ($\sqrt{Cells/mm^3}$)	0.97	0.93-1.01	0.12	0.97	0.93-1.01	0.15

3.3.3.2 Death with virological failure

Table 25 presents the cause-specific and subdistribution hazard ratios for death with virological failure from a model which did not include time-dependent CD4 cell count. Female patients had a lower cumulative incidence than male patients (aSHR=0.67; 95% CI: 0.41-1.11; p=0.12), but this effect was lost (aSHR=0.95; 95% CI: 0.57-1.58; p=0.83) after inclusion of time-dependent CD4 cell count. This implies that male patients may have poorer immunological recovery which mediated the gender effect.

Joint cause-specific and subdistribution hazard models produced similar results. Higher baseline CD4 cell count had a strong effect reducing the cumulative incidence of death with virological failure in models without time-dependent CD4 cell count (aSHR=0.48; 95% CI 0.30-0.77; p=0.002). However, after inclusion of time-dependent CD4 cell count, there was no evidence of an effect (aSHR=1.43; 95% CI: 0.84-2.43; p=0.18). This likely reflects strong correlation between baseline and time-dependent CD4 cell counts and is discussed further in Section 3.4. To allow for easier comparison, summaries of the results from the joint cause-specific and subdistribution hazard models for deaths with virological suppression and deaths with virological failure are available in Table 27.

Higher time-dependent CD4 cell count reduced the cumulative incidence of death with virological failure (aSHR=0.76; 95% CI: 0.72-0.82; p<0.001). There was no evidence of an interaction with trial monitoring randomisation (aSHR=1.02; 95% CI: 0.97-1.06; p=0.51). Patients in the clinically driven monitoring only arm were at an increased risk of death with virological failure (aSHR=2.26; 95% CI: 1.32-3.88; p=0.003).

Table 25: Predictors of death with virological failure

Variable	CSH	p-value	aCSH	95% CI	p-value	SH	p-value	aSH	95% CI	p-value
Monitoring randomisation										
LCM	1.00	0.005	1.00	-	0.006	1.00	0.003	1.00	-	0.003
CDM	2.18	-	2.12	1.25-3.62	-	2.28	-	2.26	1.32-3.88	-
Gender										
Male	1.00	0.06	1.00	-	0.13	1.00	0.06	1.00	-	0.12
Female	0.62	-	0.68	0.41-1.12	-	0.61	-	0.67	0.41-1.11	-
Initial ART										
TDF	1.00	0.20	-	-	-	1.00	0.22	-	-	-
NVP	0.49	-	-	-	-	0.49	-	-	-	-
ABC	0.67	-	-	-	-	0.73	-	-	-	-
TB at enrolment	0.93	0.80	-	-	-	0.92	0.78	-	-	-
Centre										
Entebbe	1.00	0.46	-	-	-	1.00	0.36	-	-	-
Kampala	0.97	-	-	-	-	1.05	-	-	-	-
Harare	1.36	-	-	-	-	1.50	-	-	-	-
Age (10 years)	0.90	0.54	-	-	-	0.92	0.63	-	-	-
BMI (kg/m^2)	0.97	0.40	-	-	-	0.97	0.48	-	-	-
WHO Stage										
2	1.00	0.92	-	-	-	1.00	0.98	-	-	-
3	1.10	-	-	-	-	1.05	-	-	-	-
4	1.18	-	-	-	-	1.07	-	-	-	-
Baseline CD4 cell count ($100\ Cells/mm^3$)	0.47	0.003	0.49	0.30-0.79	0.004	0.46	0.001	0.48	0.30-0.77	0.002

Table 26: Joint model determining predictors of death with virological failure

Variable	aCSH	95% CI	p-value	aSH	95% CI	p-value
Gender						
Male	1.00	-	0.99	1.00	-	0.83
Female	1.00	0.60-1.66	-	0.95	0.57-1.58	-
Baseline CD4 cell count (100 Cells/mm^3)	1.47	0.87-2.49	0.15	1.43	0.84-2.43	0.18
Association with time-dependent CD4 (√Cells/mm^3)	0.71	0.66-0.76	<0.001	0.76	0.72-0.82	<0.001
Association with time-dependent CD4 # CDM (√Cells/mm3)	0.99	0.94-1.04	0.71	1.02	0.97-1.06	0.51

Table 27: Comparison table of cause-specific and subdistribution hazard models

Variable	Death with virological suppression				Death with virological failure			
	aCSH	p-value	aSH	p-value	aCSH	p-value	aSH	p-value
Gender								
Male	-	-	-	-	1.00	0.99	1.00	0.83
Female	-	-	-	-	1.00	-	0.95	-
Centre								
Entebbe	1.00	0.05	1.00	0.09	-	-	-	-
Kampala	0.82	-	0.92	-	-	-	-	-
Harare	0.34	-	0.40	-	-	-	-	-
Age (10 years)	1.36	0.12	1.41	0.07				
Baseline BMI (kg/m^2)	0.87	0.01	0.88	0.02	-	-	-	-
Baseline CD4 cell count (100 Cells/mm^3)	2.18	0.01	1.95	0.03	1.47	0.15	1.43	0.18
Association with time-dependent CD4 (√Cells/mm^3)	0.92	0.05	0.96	0.24	0.71	<0.001	0.76	<0.001
Association with time-dependent CD4 # CDM (√Cells/mm^3)	0.97	0.12	0.97	0.15	0.99	0.71	1.02	0.51

3.3.4 Predicted cumulative incidence

Figure 19 displays differences in the predicted cumulative incidence of death with virological suppression for four illustrative patients after 48 weeks of antiretroviral therapy. The lower panel displays these patient's associated CD4 counts during the first 48 weeks. Shaded regions depict 95% confidence intervals. The scenario in the left panel displays two patients with different monitoring randomisations, but with an identical immunological response during the first 48 weeks. Similarly, the right panel shows two patients with a limited immunological response during the first 48 weeks. Despite substantial differences in CD4 cell count trajectory, there were no difference in the predicted cumulative incidence of death with virological suppression.

Figure 19: Predicted cumulative incidence of death with suppression

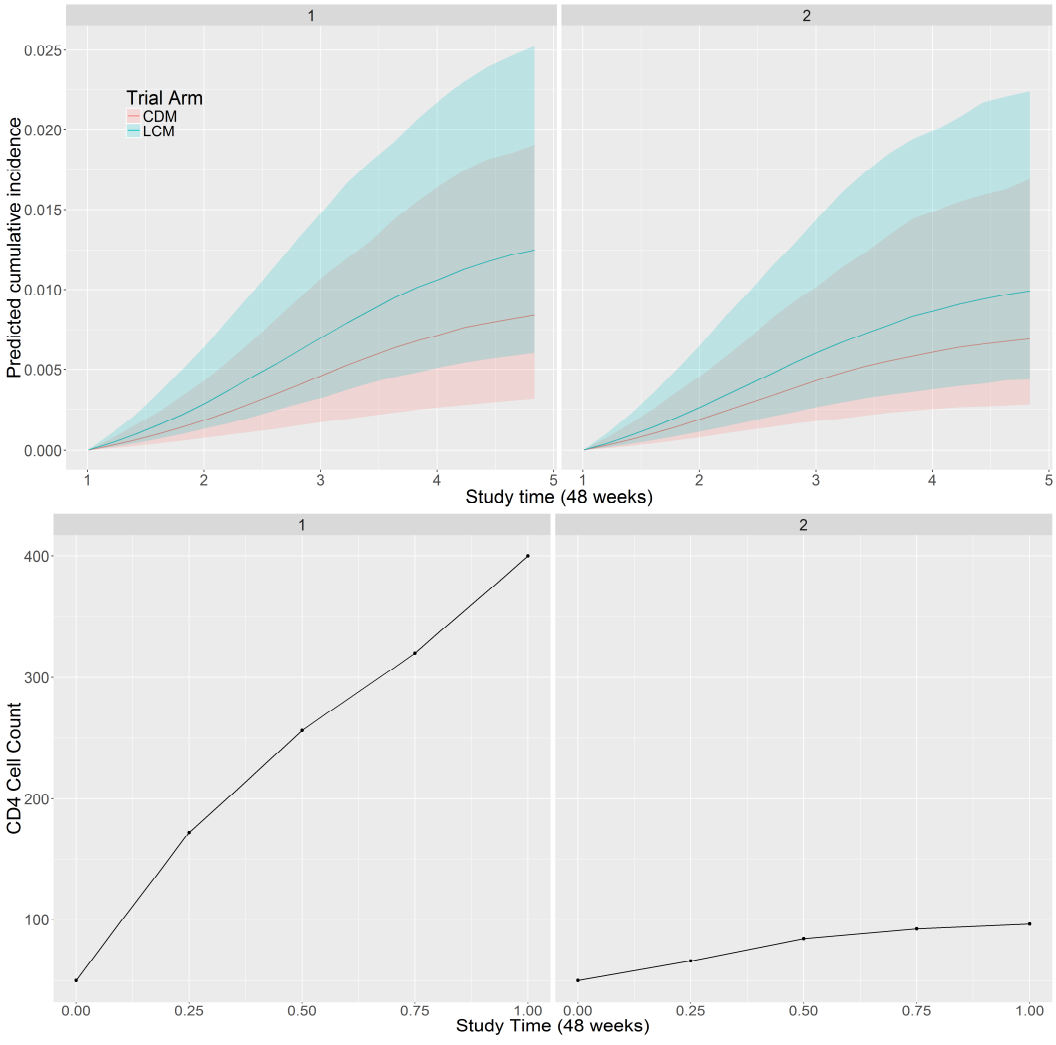
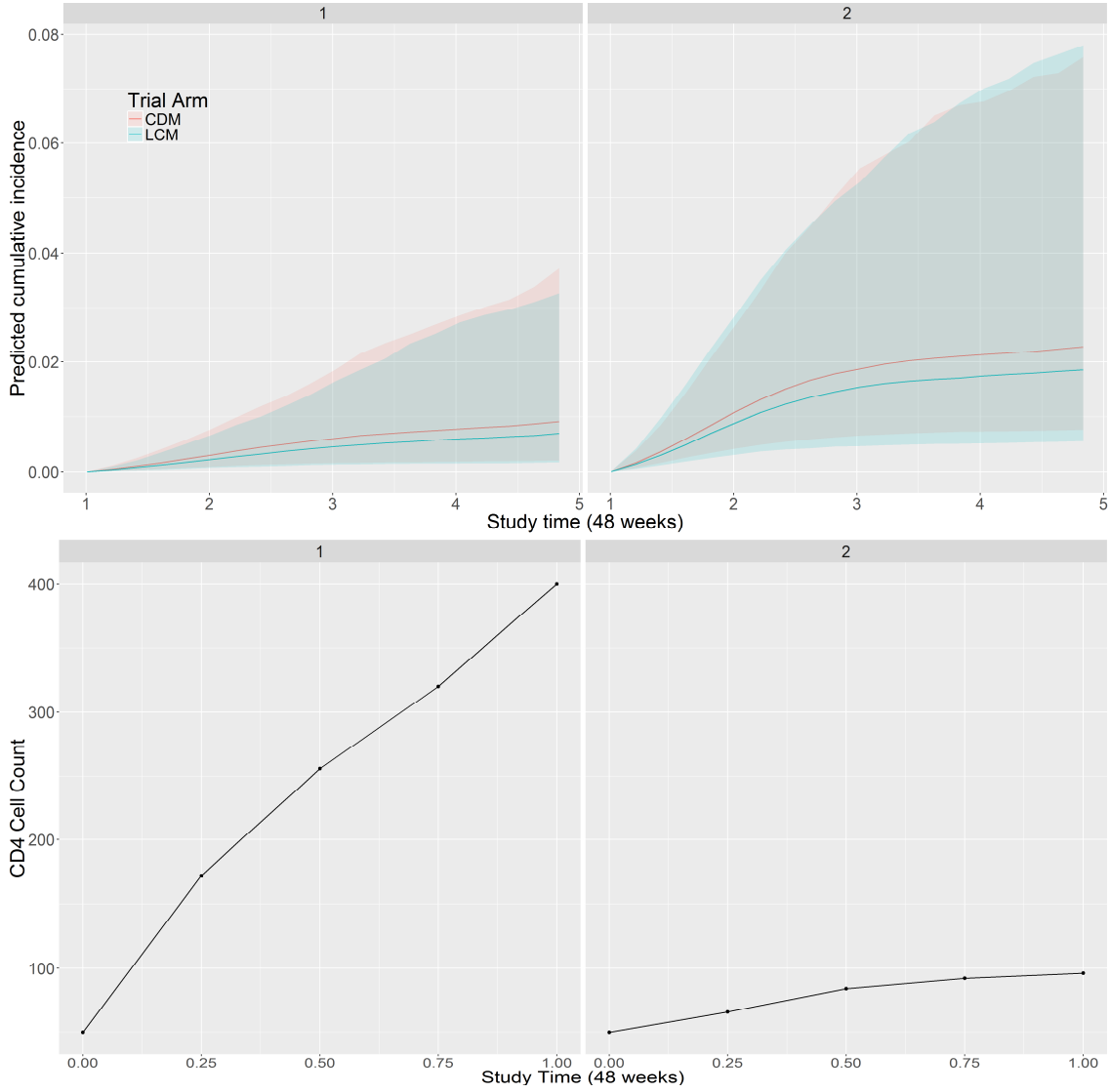


Figure 20 demonstrates the effect of lower time-dependent CD4 cell counts on the cumulative incidence of death with virological failure. For patients with a good immunological response during the first 48 weeks, there was a near identical predicted cumulative incidence of death with virological failure by monitoring randomisation. In contrast, patients in scenario 2 had a poorer CD4 response during the first 48 weeks and had a higher cumulative incidence of death with virological failure.

Figure 20: Predicted cumulative incidence of death with failure



3.3.5 Sensitivity Analyses

Two analytical sensitivity analyses were conducted to assess the robustness of the findings. Firstly, the Fine and Gray subdistribution hazard regression method (*stcrreg* command in Stata v14) was used, rather than manually specifying the survival times for competing risks using the JM package in R. The underlying assumptions for these two estimation methods appear to be identical although the numerical maximisation algorithms differ. As the *stcrreg* command does not appropriately accommodate non-external time-dependent covariates, the comparison was limited to the analysis of baseline covariates, i.e. the non-joint model. Second, sampling weights analogous to those used in Chapter 2 were applied, so that the results of analysis reflect the entire population enrolled in the DART trial rather than those who were included in the virology substudy. The results are shown in Table 28 and Table 29 and should be compared with Table 23 and Table 25 respectively.

The differences between the results of the analyses from the two programs are, in general, vanishingly small. For example, the adjusted SHR for baseline CD4 cell count in the model of deaths with virological suppression was 1.56 (95% CI: 0.93-2.62; p=0.09) under both the analysis in Stata and R. In conclusion, these sensitivity analyses confirmed that the main results of this chapter were robust to the statistical software used.

In the adjusted subdistribution hazard model for patients who died with virological suppression an adjusted SHR for CDM compared to LCM of 0.54 (95% CI: 0.28-1.03; p=0.06) was found using sampling weights compared to 0.60 (0.31-1.13; p=0.11) in the original approach. Both effects are similar in magnitude, but with the sampling weights model this would be interpreted as more clearly demonstrating that patients with CDM have a lower incidence of death with virological suppression. Higher baseline CD4 cell count was associated with a higher incidence of death with virological suppression in the sampling weights model compared to the

original analysis (aSHR=1.62; 95% CI=0.94-2.81 compared to aSHR=1.56; 95% CI: 0.93-2.62). This result was not expected but was robust to the analysis method used, so may reflect a chance finding due to the small number of patients analysed within this group (n=40).

In the adjusted subdistribution hazard model for patients who died with virological failure there were small differences in the adjusted SHR for monitoring randomisation and baseline CD4 cell count. There was a lower incidence of death with virological failure for female patients in the sampling weights approach (aSHR=0.60; 95% CI=0.36-1.01; p=0.05) compared to the original analysis (aSHR=0.67; 95% CI=0.41-1.11; p=0.12). In Chapter 2, female patients were observed to have a lower incidence of virological failure so this result is reasonable. However, differences in the analysis population may contribute to this difference. A greater proportion of male patients (n=71; 6% vs n=100; 5%) died before week 48. The original analysis method for this chapter accounted for this as a competing risk, whereas this difference is not adjusted for with the sampling weights approach.

Overall, these sensitivity analyses revealed that the main results of this chapter were robust to the use of statistical software package and analysis approach although there was minor variation in the exact estimated subdistribution hazard ratios.

Table 28: Sensitivity analyses of competing risks for deaths with virological suppression conducted in Stata

Variable	Analysis without sampling weights (n=3,316)						Analysis with sampling weights (n=1,741)					
	SHR	95% CI	p-value	aSHR	95% CI	p-value	SHR	95% CI	p-value	aSHR	95% CI	p-value
Monitoring randomisation												
LCM	1.00	-	0.11	1.00	-	0.11	1.00	-	0.11	1.00	-	0.06
CDM	0.60	0.31-1.13	-	0.60	0.31-1.13	-	0.59	0.31-1.12	-	0.54	0.28-1.03	-
Gender												
Male	1.00	-	0.49	-	-	-	1.00	-	0.68	-	-	-
Female	1.27	0.64-2.49	-	-	-	-	1.16	0.58-2.29	-	-	-	-
Initial ART												
TDF	1.00	-	0.37	-	-	-	1.00	-	0.34	-	-	-
NVP	1.01	0.42-2.43	-	-	-	-	0.98	0.40-2.37	-	-	-	-
ABC	1.87	0.78-4.52	-	-	-	-	1.93	0.79-4.72	-	-	-	-
TB at enrolment	1.11	0.56-2.22	0.76	-	-	-	1.11	0.55-2.24	0.77	-	-	-
Centre												
Entebbe	1.00	-	0.12	1.00	-	0.15	1.00	-	0.10	1.00	-	0.10
Kampala	0.88	0.45-1.71	-	0.98	0.50-1.91	-	0.91	0.46-1.80	-	0.99	0.50-1.93	-
Harare	0.38	0.15-0.97	-	0.42	0.16-1.07	-	0.37	0.14-0.94	-	0.37	0.14-0.98	-
Age (10 years)	1.41	1.00-1.99	0.05	1.45	1.00-2.09	0.05	1.38	0.97-1.96	0.07	1.42	0.97-2.08	0.07
BMI (kg/m^2)	0.91	0.81-1.02	0.11	0.89	0.79-1.01	0.08	0.91	0.81-1.04	0.16	0.89	0.77-1.02	0.09
WHO Stage												
2	1.00	-	0.71	-	-	-	1.00	-	0.68	-	-	-
3	1.44	0.59-3.53	-	-	-	-	1.47	0.59-3.65	-	-	-	-
4	1.45	0.53-3.97	-	-	-	-	1.52	0.54-4.24	-	-	-	-
Baseline CD4 cell count (100 Cells/mm^3)	1.48	0.90-2.42	0.12	1.56	0.93-2.62	0.09	1.53	0.91-2.58	0.11	1.62	0.94-2.81	0.09

Table 29: Sensitivity analyses of competing risks for deaths with virological failure conducted in Stata

Variable	Analysis without sampling weights (n=3,316)						Analysis with sampling weights (n=1,741)					
	SHR	95% CI	p-value	aSHR	95% CI	p-value	SHR	95% CI	p-value	aSHR	95% CI	p-value
Monitoring randomisation												
LCM	1.00	-	0.003	1.00	-	0.003	1.00		0.005	1.00	-	0.004
CDM	2.28	1.33-3.90	-	2.25	1.32-3.87	-	2.22		-	2.27	1.30-3.95	-
Gender												
Male	1.00	-	0.06	1.00	-	0.12	1.00		0.03	1.00	-	0.05
Female	0.62	0.37-1.01	-	0.67	0.41-1.11	-	0.56		-	0.60	0.36-1.01	-
Initial ART												
TDF	1.00	-	0.21	-	-	-	1.00		0.29	-	-	-
NVP	0.46	0.18-1.15	-	-	-	-	0.51		-	-	-	-
ABC	0.69	0.25-1.90	-	-	-	-	0.66		-	-	-	-
TB at enrolment	0.93	0.52-1.67	0.81	-	-	-	0.93		0.82			
Centre										-	-	-
Entebbe	1.00	-	0.35	-	-	-	1.00		0.41	-	-	-
Kampala	1.06	0.56-2.02	-	-	-	-	1.07		-	-	-	-
Harare	1.51	0.80-2.83	-	-	-	-	1.48		-	-	-	-
Age (10 years)	0.92	0.66-1.29	0.63	-	-	-	0.89		0.53	-	-	-
BMI (kg/m^2)	0.97	0.90-1.05	0.50	-	-	-	0.96		0.39	-	-	-
WHO Stage												
2	1.00	-	0.98	-	-	-	1.00		0.85	-	-	-
3	1.05	0.54-2.02	-	-	-	-	1.18		-	-	-	-
4	1.08	0.51-2.31	-	-	-	-	1.24		-	-	-	-
Baseline CD4 cell count (100 Cells/mm^3)	0.46	0.29-0.74	0.001	0.48	0.30-0.77	0.002	0.45		0.001	0.45	0.28-0.74	0.002

3.4 Discussion

3.4.1 Deaths with virological suppression

The mortality rate while virologically suppressed was 5.1 per 1,000 person-years, while the mortality rate during periods of viremia was 25.1 per 1,000 person-years. In comparison, mortality rates of 4.3 (95% CI: 3.6–5.1) and 3.6 (3.0–4.3) per 1,000 person-years were observed in men and women respectively in an HIV-negative population cohort in Masaka, Uganda [138]. A separate study estimated the mortality rate in Rakai, Uganda as 3.2 and 2.5 per 1,000 person-years in HIV-negative men and women respectively [139]. The observation that death rates during periods of virological suppression in DART were only marginally higher than observed in these two HIV-negative studies during the same period, suggests that non-HIV-related background mortality explains most deaths that occurred with virological suppression.

Patients with a low body mass index at ART initiation were at an increased risk of death with virological suppression. However, there was no clear excess of deaths due to diarrhoea or wasting disease among patients who died with virological suppression. Body mass index was not associated with the cumulative incidence of death with virological failure.

Masiira et al. [107] categorised baseline body mass index into three categories and found that patients with a body mass index of less than 17.5 and 17.5 to 18.5 had adjusted rate ratios for mortality of 6.11 (2.30-16.20) and 4.52 (1.54-13.32) respectively compared to those with a body mass index greater than or equal to 18.5. Brennan et al. [110] and Fregonese et al. [111] both concluded lower body mass index increased mortality when they dichotomized BMI with a cut-off of 18.5 kg/m^2. Converting continuous variables into categorical variables is widely criticised as both an unnecessary simplification for statistical analyses and statistically inefficient (approximately 65% efficiency compared to ungrouped analyses [136]). Our analysis expands upon these by using fractional polynomials for

body mass index, allowing for a non-linear relationship, and by providing a distinction between the virological status at mortality and the effect of baseline body mass index.

Hoffmann et al. [112] observed that 18% of deaths after one year of antiretroviral therapy in a South African cohort had a suppressed viral load at the time of the last measurement. However, their analysis used viral loads measured up to six months prior to death. It is likely that the actual proportion of patients with virological suppression at the time of death would be lower in this cohort. Brennan et al. [110] observed that 60% of deaths occurred with a suppressed viral load, although this viral load was measured within one year of death. While Hoffmann et al. [112] and Brennan et al. [110] both report the proportion of deaths which occurred while virological suppressed, the analysis presented in this chapter is the first to our knowledge to investigate whether predictors of death differ by virological status.

In current low-income populations, the proportion of deaths occurring with virological suppression is likely to be greater than those observed in the DART trial due to three considerations. Firstly, WHO guidelines now recommend patients initiate antiretroviral therapy with dual-class treatment regimens containing efavirenz. While not statistically significant in our findings, probably due to small numbers, NRTI-NNRTI regimens were observed to have a lower cumulative incidence of death with virological failure (SH=0.49) compared to triple-NRTI regimens containing tenofovir. The analyses in this chapter indicate that increased use of NRTI-NNRTI regimens would reduce the number of deaths with virological failure. Secondly, recent changes to treatment guidelines [140] recommend starting antiretroviral therapy irrespective of CD4 cell count. In analyses without time-dependent CD4 cell count, patients who initiated antiretroviral therapy at higher CD4 cell counts experienced lower mortality with virological failure although similar mortality with virological

suppression. However, the inclusion criteria in DART specified a baseline CD4 cell count less than 200 cells/mm^3, and our inferences may not apply to higher values. Finally, increasing access to CD4 cell count or viral load monitoring is likely to lead to higher CD4 cell count at time of death, as patients switch treatment at higher immunological thresholds. This is expected to result in a decrease in deaths with virological failure while having no impact on deaths with virological suppression.

3.4.2 The role of laboratory monitoring

There was not clear evidence that randomisation to CD4 cell count monitoring had an effect on the incidence of deaths with virological suppression in the non-joint model (aSHR: 0.60; 95% CI: 0.32-1.13; p=0.11). In the joint model, there was no evidence that time-dependent CD4 cell count had an effect on the cumulative incidence of death with virological suppression (aSHR=0.96; 95% CI: 0.89-1.03; p=0.24) nor that this differed by trial monitoring randomisation. A linear mixed model demonstrated that there was no evidence of a decline in CD4 cell count in the 48 weeks before death among patients who died with virological suppression. Only 19% of patients who died with virological suppression met the immunological criteria for treatment switch used in DART (<100 cells/mm^3) and, by definition, none had reached virological criteria. In conclusion, more intensive laboratory monitoring than was used in DART, both immunological or virological, would have had a limited impact in preventing the occurrence of deaths with virological suppression.

Patients in the CDM arm had a higher incidence of death with virological failure (aSHR: 2.26; p=0.003) in analyses without time-dependent CD4 cell count. A strong association with time-dependent CD4 cell count was shown in the joint model (aSHR=0.76; 95% CI: 0.72-0.82; p<0.001). There was no evidence that the effect of time-dependent CD4 cell count differed by trial monitoring randomisation (aSHR=1.02; 95% CI: 0.97-1.06; p=0.51). Patients who died with virological failure had a decline in CD4 cell count

before death. The inclusion of CD4 cell count monitoring would be expected to reduce the number of deaths with virological failure.

Other techniques to examine the impact of different laboratory monitoring strategies on mortality in low-income settings have been used. Ford et al. [141] used dynamic marginal structural models applied to DART CD4 cell count data, to determine the impact of different CD4 cell count monitoring strategies. Defining baseline as 48 weeks of antiretroviral therapy, the estimated survival probabilities at 240 weeks of antiretroviral therapy were 0.92 (95% CI: 0.91 – 0.94) with no CD4 cell count monitoring, 0.95 (95% CI: 0.93 – 0.96) with a single CD4 test at week 48 and 0.96 (95% CI: 0.94 – 0.97) with testing every twelve weeks. The findings from this chapter imply that this small improvement is likely achieved by preventing deaths with virological failure. More frequent testing had a limited impact on mortality since most patients who die with virological suppression would not have a treatment switch indicated, even with enhanced monitoring.

3.4.3 Conclusions

A surprisingly high proportion (40%) of deaths after one year of first-line ART in DART were observed among patients who were virologically suppressed. Separate analyses were conducted of predictors of deaths that occurred with and without virological failure using a competing risks framework. Predictors of death with virological failure were largely as expected, the most powerful individual predictor being time-dependent CD4 count. It is presumed that most deaths without virological failure were due to non-HIV related causes, backed by the fact that the incidence rates of such deaths were broadly similar to background mortality rates in these populations. Increased laboratory monitoring would likely have a limited impact on the incidence of such deaths. Two baseline factors were identified that were significantly associated with the risk of death with virological suppression: low BMI and high CD4 cell count. Whether providing nutritional counselling and food supplements in patients

initiation antiretroviral therapy with low CD4 cell count is one of the objectives of the REALITY trial. The finding that the risk of death with virological suppression increased with baseline CD4 cell count, in both cause-specific and subdistribution hazard models, is paradoxical and remains unexplained. However, the number of such deaths was small (n=40) and the possibility that this was a chance observation cannot be excluded.

4 HIV-1 drug resistance after persistent virological failure

4.1 Introduction

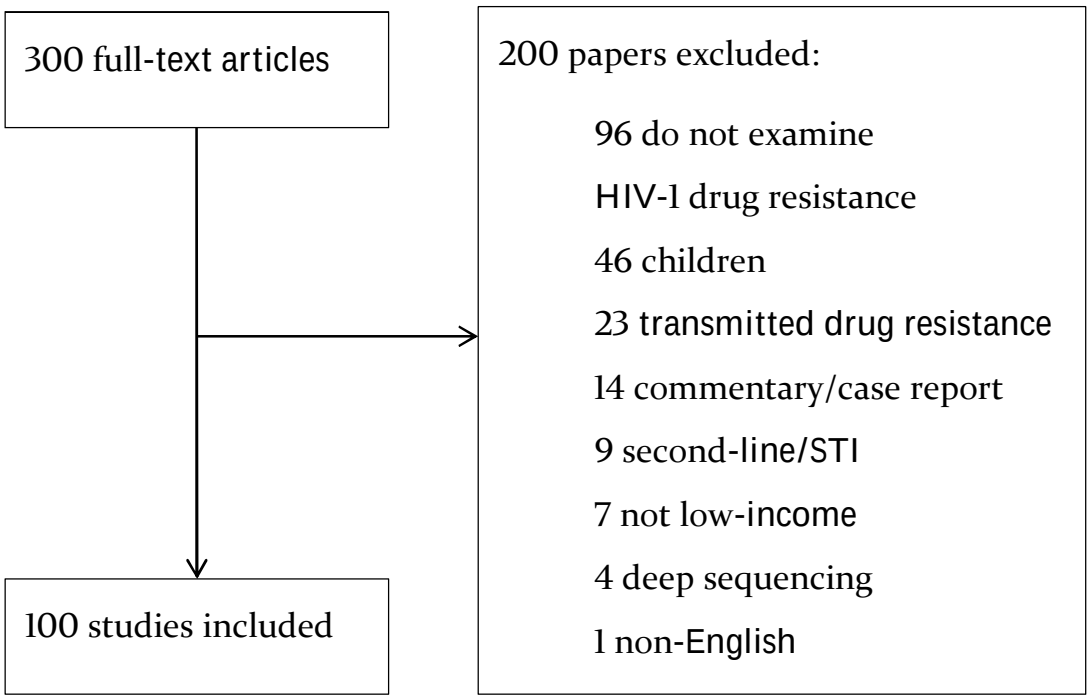
One of the principal concerns with clinical or CD4 cell monitoring is that patients may remain on a regimen with virological failure for longer compared to regular virological monitoring. This has the potential to lead to an accumulation of acquired HIV-1 drug resistance mutations which could compromise subsequent regimens. Furthermore, drug resistant HIV may be transmitted to others if a patient has high viral load.

Recent research [142] has estimated the prevalence of transmitted drug resistance to be 5.7% in Africa and 7.6% in Asia, with patients 1.7 times more like to have transmitted drug resistance in a country where antiretroviral therapy had been available for more than five years. Another study by Gupta et al. [143] found that drug resistance was increasing by 29% per year in East Africa, particularly NNRTI resistance [143].

4.1.1 Acquired HIV-1 Drug Resistance

A literature review was conducted on the 12th August 2016 using PubMed to find English language publications which investigated acquired HIV-1 drug resistance in low-income settings. Search terms for HIV, low-income settings (resource* OR “sub-Saharan” OR “low-income” OR Uganda OR Zimbabwe OR "South Africa"), resistance and first-line were used. The search identified 300 publications and 100 were found to be relevant (Figure 21). Notable results from these publications are discussed below, and the full list is available in Appendix E.

Figure 21: Search strategy for HIV-1 drug resistance publications



Sigaloff et al. [144] examined HIV-1 drug resistance in a cross-sectional analysis of 250 patients in multiple sub-Saharan African countries. These patients initiated treatment on a first-line regimen containing either nevirapine or efavirenz and were switching treatment at the time of testing. Patients switched treatment based on either clinic immunological criterion only (either a new WHO clinical stage 3 or 4 condition, a fall of CD4 cell count below pre-treatment value, a CD4 cell count decrease of greater than 50% or a persistent CD4 cell count less than 100 cells/mm^3) or with additional targeted viral load testing (local real-time HIV RNA test to confirm suspected treatment failure). Treatment was switched after a median of 28 months in the group monitored with clinic immunological criterion only and after 25 months in the group with targeted viral load testing. Extensive NRTI cross-resistance was observed in both groups. The M184I/V mutation was present in 82% of patients, often alongside thymidine analogue mutations (TAMs) (53%). Patients frequently had multiple TAMs, 38% of patients had two or more TAMs and 24% had three or more. Multiple TAMs were associated with an increased duration on antiretroviral therapy and a history of zidovudine use. NRTI cross-

resistance was associated with a higher viral load at the time of the resistance test, longer duration on antiretroviral therapy and either zidovudine or tenofovir use. The researchers noted that while their research was representative of the current clinical practice in Africa, it was limited by focusing on individuals who were switching treatment.

Hamers et al. [145] examined HIV-1 drug resistance in a retrospective cross-sectional analysis of 2,588 patients who had virologically failed (viral load greater than 1,000 copies/mL) a NRTI + NNRTI treatment after twelve months of antiretroviral therapy in six sub-Saharan African countries. They found that 70% of patients had one or more mutation; 58% had NRTI and 61% had NNRTI mutations. The M184V mutation was the most prevalent (58%) and, as a combination, was less frequently observed with a TAM (8%) but more often seen in conjunction with an NNRTI mutation (45%). TAMs were rarer in this study compared to Sigaloff et al. [144], with 13% of patients having one or more. In patients with exposure to nevirapine, 63% of those with virological failure had an NNRTI mutation and common mutations included K103N (25%) and Y181C (23%). Patients with drug resistance mutations had lower viral load at the time of resistance test ($p=0.04$), in particular, patients with the M184V mutation (4.21 versus 4.59 $\log_{10}$ copies/mL; $p=0.02$).

The findings of Hamers et al. differ to Sigaloff et al. [144] and are likely a result of lower adherence during the first twelve months of therapy, reflected in the prevalence of the M184V mutation. Sigaloff et al. [144] examined patients who had been on treatment for longer, who had met either clinical or immunological criteria for treatment switch and who frequently had HIV-1 drug resistance, so virological failure was less likely to be due to non-adherence.

The TenoRes Study Group [146] conducted a global study examining the prevalence of tenofovir resistance (defined as K65R/N or K70E/G/Q) after virological failure on regimens containing tenofovir with lamivudine or

emtricitabine and either nevirapine or efavirenz. The highest prevalence of tenofovir resistance was observed in sub-Saharan Africa (370 out of 654 patients; 57%). Tenofovir resistance was strongly associated with a CD4 cell count at antiretroviral therapy initiation of less than 100 cells/mm^3 (OR: 1.50; 95% CI: 1.27-1.77) but not associated with a higher viral load at antiretroviral therapy initiation. The use of lamivudine compared to emtricitabine (OR: 1.48; 95% CI: 1.20-1.82) and nevirapine compared to efavirenz (OR: 1.46; 1.27-1.67) were associated with higher prevalence of tenofovir resistance. Unlike the other studies discussed, there was no association between viral load at treatment failure and tenofovir resistance (p=0.63). This could be a result of other studies comparing viral load at treatment failure with any detectable resistance rather than these specific tenofovir resistance mutations.

Pinoges et al. [147] examined first-line drug resistance using a cross-sectional survey of patients without routine virological monitoring from three sub-Saharan African countries and Cambodia. In total, 151 out of 180 (84%) patients had major drug-resistance as defined using the Stanford interpretation algorithm, and 133 of 180 (74%) had resistance to NRTI and NNRTIs. Patients had a median of 3 mutations, and these were commonly M184V (74%), K103N (35%), Y181C (31%), V179I (30%), T215Y (13%) and M41L (11%), while 27% of patients had one or more TAMs. There was higher viral load at the time of failure in those with more mutations, but no relationship was observed with the duration of antiretroviral therapy.

Jiamsakul et al. [148] evaluated patients in the TREAT Asia cohort who had a resistance test in the six months before switching to second-line antiretroviral therapy. Of the 105 patients, 92% had drug resistance to any class, and 37% had multi-drug NRTI resistance (classified as either Q151M, the 69 insertion mutation, two or more TAMs or M184V and one or more TAM). TAMs were frequent, with 33% having one or more and 23% having two or more. The most common individual mutation was M184V (75%)

although NNRTI mutations were also common (Y181C; 35% and K103N; 33%).

4.1.2 Resistance by HIV-1 Subtype

Sigaloff et al. [144] found a univariate association between two or more TAMs and NRTI cross-resistance with a patient's HIV-1 subtype. In univariate logistic regression analyses there were increased odds of resistance for subtype D compared to subtype C (OR: 2.49; 95% CI: 1.03-5.97 for two or more TAMs and 2.29; 95% CI: 0.95-5.52 for NRTI cross-resistance). In addition, there was increased resistance for subtype A compared to subtype D. However, this association with HIV-1 subtype was no longer significant after accounting for the duration of antiretroviral therapy use and the NRTI backbone. The K65R mutation was associated with tenofovir use and higher HIV viral load but not with HIV-1 subtype.

In Hamers et al. [145] there was no association between subtype and the presence of one or more drug resistance mutations. Univariate analyses initially suggested that the K65R mutation may be more frequent in patients with subtype C (17%) than non-C (7%). However, after adjusting for the use of tenofovir and stavudine, there was no evidence of a difference in multivariate analyses (p=0.68). The K103N mutation was more frequent in subtype D than subtype A after adjusting for efavirenz and nevirapine use (OR for D versus non-D: 3.40; 95% CI: 1.21-9.58; p=0.014). V106M was exclusively found in patients with subtype C virus, but no other mutations were associated with HIV-1 subtype.

The TenoRes study group conducted an analysis within immigrant populations in western Europe and observed an association between subtype C and tenofovir resistance compared with non-C, non-B subtypes in unadjusted analyses (OR: 2.44; 95% CI: 1.66-3.59).

4.1.3 Objective

The purpose of this chapter is to determine the extent of HIV-1 drug resistance at the end of first-line antiretroviral therapy in patients observed to have experienced persistent virological failure (using the definition of Chapter 2). The proportion of patients with virological failure in whom HIV drug resistance develops will be evaluated by antiretroviral therapy regimen and HIV-1 subtype. The number and combinations of mutations will be quantified leading to recommendations about the antiretroviral drugs which retain the most susceptibility after a patient has potentially had prolonged periods with persistent viraemia.

4.2 Methods

Patients with virological failure (viral load greater than 200 copies/mL) had genotypic sequencing attempted at the last stored plasma sample on first-line antiretroviral therapy, i.e. the first sample tested as part of the walkback procedure described in Section 2.2.1. If a stored plasma sample could not be located, or if RNA could not be amplified, then the next available stored plasma sample was requested. At least two additional replacement samples were requested for each sequence which could not be obtained. Samples were sequenced using reverse transcriptase-polymerase chain reaction and sequencing of the *pol* gene using an in-house sequencing method. Sequencing was carried out at either the MRC/URVI Uganda Research Unit on AIDS, Entebbe, Uganda or the Joint Clinical Research Centre, Kampala, Uganda.

Sequences were processed using the Stanford Sierra HIVdb algorithm v7.0. Resistance to an HIV-1 drug class was defined as one or more major IAS-USA [149] mutations for that class. Susceptibility to individual antiretroviral drugs was determined using the Stanford drug resistance mutation penalty scores with categories of susceptible (0-9), potential low-level resistance (10-14), low-level resistance (15-29), intermediate resistance (30-59) and high-level resistance (≥60). Thymidine analogue mutations

(TAMs) were classified as M41L, D67N, K70R, L210W, T215Y, T215F, K219E and K219Q.

All sequences had HIV-1 subtype determined using the REGA v3 subtyping algorithm [20]. This tool uses a phylogenetic-based approach to define HIV-1 subtype. HIV-1 sub-subtypes, such as HIV-1 Subtype A1 and A2, were combined in analyses and treated as a pure HIV-1 subtype. Sequences identified as potential recombinants, such as "HIV-1 Subtype C-like", were dealt with as complex/recombinant subtypes in analyses alongside recognised CRFs (e.g. CRF01_AE).

4.2.1 Quality control

Retrieving and sequencing plasma samples, several years after the DART trial originally concluded, has been a lengthy and time-consuming process. During this period, there have been multiple occasions where samples from different patients could have been switched in error. A longitudinal analysis of the evolution of HIV-1 drug resistance within an individual (Chapter 5) would be severely compromised if a sequence from a different patient was used. Similarly, cross-sectional analyses in this chapter relating patient characteristics to the evolution of HIV-1 drug resistance could be misleading.

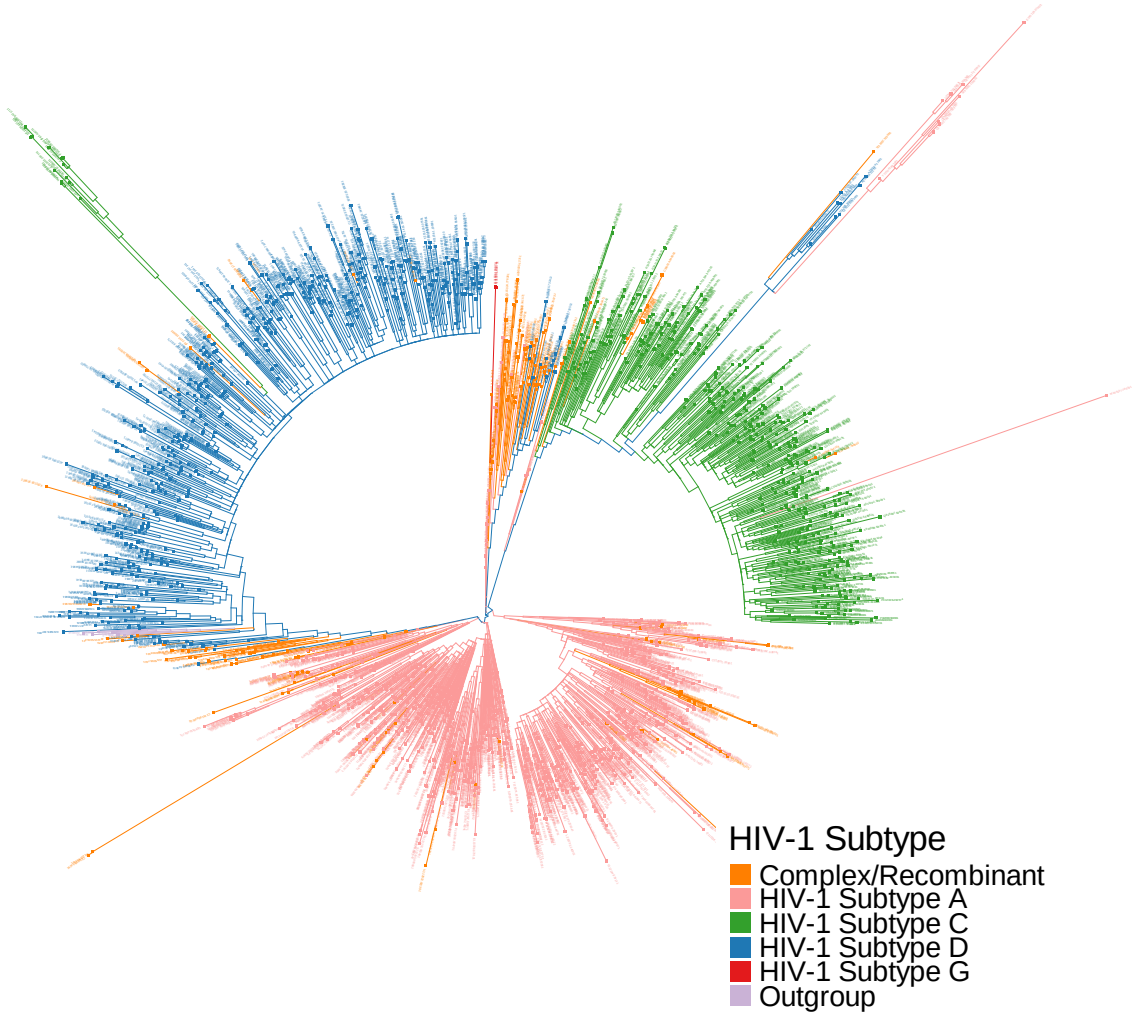
Phylogenetic analyses are typically used to provide inference about transmission networks, clustering patients whose transmission is likely linked. Phylogenetic analyses use the genetic distance between sequences and a model for HIV-1 evolution to construct a phylogenetic tree. These group sequences related together to form clusters, the length of each branch in the tree representing the genetic distance between a pair of sequences. Within DART, sequences from the same patient should appear to be more closely related to each other than those from a separate patient. When sequences appear highly dissimilar, this indicates that an error may have occurred and this sequence is removed from subsequent analyses.

All sequences in the DART database were used to investigate inter-patient sequence similarity, including sequences from patients not part of the DART Virology study. These sequences offer additional information for the structure of the tree and increase the potential for errors to be identified. A total of 2,362 sequences from 920 patients were included, and 614 patients had more than one sequence. The average length of the sequences included was 1,247 nucleotides. Clustal Omega [150] was used to align the sequences, including six additional sequences representing an "outgroup". These outgroup sequences were selected from the NCBI Genbank using HIV BLAST and were all *pol* regions of subtype B sequences from the UK HIV Drug Resistance Database. They were chosen as they were genetically distinct from HIV sequences in both Uganda and Zimbabwe, known apriori to be predominantly subtype A and C, while still being high-quality sequences from the same major HIV-1 group. All amino acid positions related to HIV-1 drug resistance (according to the IAS-USA 2013 mutation list [151]) were removed using BioEdit v7.2.5 from aligned sequences. Phylogenetic analyses were conducted using the Kimura 2-parameter model of genetic distance using the third codon position since this is better approximated by a neutral model of HIV-1 evolution [152]. Ambiguous positions were removed for each pair of sequences when genetic distance was calculated.

The phylogenetic tree (Figure 22) and pairwise distance calculations were conducted in MEGA6 [153]. The colours in the tree represent HIV-1 subtype and are predominantly clustered together. The top left and top right areas of the phylogenetic tree feature long branches which may be misclassified subtypes. As an example, towards the top right is a cluster of apparent subtype A, D and complex/recombinant sequences branching from the subtype C clade. This group is genetically distant from the subtype C clade, as indicated by the long length of this branch, so may be unidentified complex recombinants. Nonetheless, samples are consistently clustered by

patients within these groups, so this does not indicate errors were made with patient samples.

Figure 22: Phylogenetic tree of *pol* region data from DART colour coded by HIV-1 Subtype (plotted using the R [137] ape package [154])

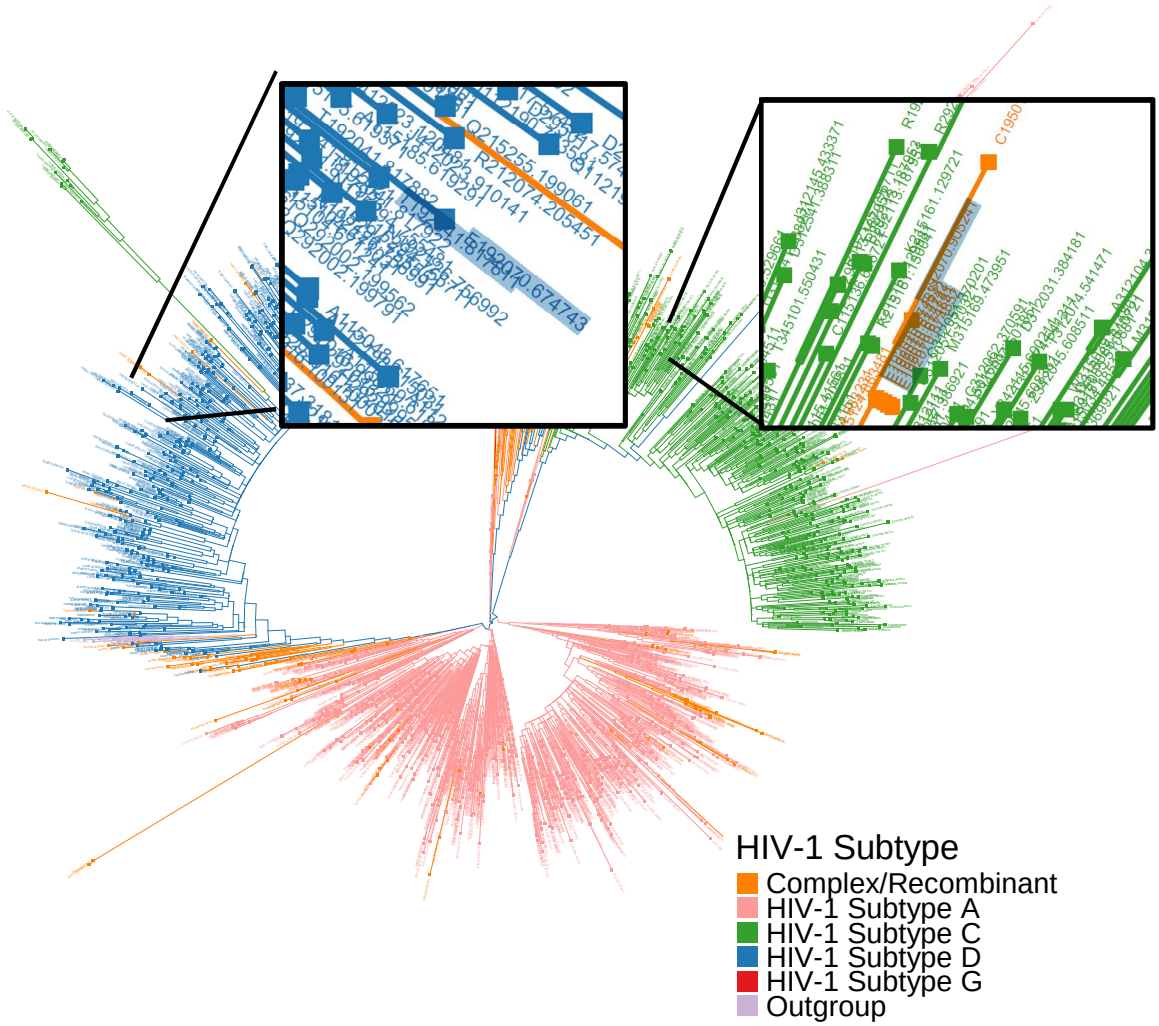


Within this phylogenetic tree, 1,839 sequences from the 609 individual DART Virology study patients were examined to determine if they were clustered. Figure 23 illustrates an example where all sequences are grouped, and there is no suspicion that a sequence has been mislabelled. If a sequence from one patient appeared to be more closely related to the sequence from another patient, then the pairwise genetics distance was examined. When the sequence was substantially closer to another patient's (a difference of more than 0.03 was used as a cut-off), then the sequence was dropped from subsequent analyses. For patients with just two sequences, both were dropped. Figure 24 illustrates a case where sequence

E192070.674743 is dissimilar to others from the same patient, with a distance of 0.45, whereas it is a distance of 0.04 from patient T192041's sequences. The subtypes of these sequences also differ, with the first assigned a pure subtype D virus and others, supposedly from the same patient, a complex subtype C recombinant. In total, 62 (3%) sequences from 46 (8%) patients were removed from further analyses based on this quality control procedure. Of these, 12 (2%) sequences were from the last time point and so were excluded from analyses in this chapter.

Figure 23: Sequences from patient B192073 are clustered

Figure 24: Sequences from patient E192070 are distantly related



4.3 Results

4.3.1 Resistance tests available

In total, resistance tests were available for 542 patients (89%) of the 609 patients with virological failure (Chapter 2) within 24 weeks of the last time point on first-line antiretroviral therapy. The median (IQR) viral load at this time was 35,688 (3,932-116,868) copies/mL. Resistance tests were missing due to either a plasma sample not being located (26; 39%), a failure to amplify the sample (29; 43%) or due to a sequence failing phylogenetic quality control (12; 18%). Resistance tests were more likely to be missing depending on the type of treatment failure observed (χ^2 test: p<0.01). Patients who virologically failed before death were the most likely to be missing a resistance test (11/62; 18%), followed by patients who were on first-line antiretroviral therapy at the end of the DART trial (41/311; 13%)

and patients who switched antiretroviral therapy (15/236; 6%). The higher proportion missing a resistance test among patients who died is a result of fewer plasma samples with virological failure being available for testing. Tests were more likely to fail in patients with lower viral load at the time of resistance test. Of the 73 patients with a viral load of 200-1,000 copies/mL, 30% were missing a resistance test due to difficulties amplifying stored plasma samples with low viral load levels. There was no difference in the proportion missing by any baseline characteristic (Table 30).

Table 30: Proportion missing by baseline and test characteristic

Variable	Number (%)	Missing (%)	χ^2 test p-value
Gender			
Male	240 (39%)	24 (10%)	0.52
Female	369 (61%)	43 (12%)	-
Monitoring randomisation			
LCM	294 (48%)	33 (11%)	0.87
CDM	315 (52%)	34 (11%)	-
Centre			
Entebbe	204 (34%)	20 (10%)	0.32
Kampala	231 (38%)	31 (13%)	-
Harare	174 (29%)	16 (9%)	-
First-line ART			
TDF	400 (67%)	47 (12%)	0.36
NVP	101 (17%)	7 (7%)	-
ABC	108 (18%)	13 (12%)	-
Baseline CD4 Cell Count			
0-49	265 (44%)	30 (11%)	0.85
50-99	145 (24%)	18 (12%)	-
100-149	111 (18%)	11 (10%)	-
150-199	88 (14%)	8 (9%)	-
Patient status at test			
Death	62 (10%)	11 (18%)	0.02
Switch	236 (39%)	15 (6%)	-
First-line	311 (51%)	41 (13%)	-
VL at target test (copies/mL)			
200-1,000	73 (12%)	23 (32%)	<0.001
1,000-10,000	143 (23%)	22 (15%)	-
10,000-100,000	200 (33%)	11(6%)	-
≥100,000	193 (32%)	11 (6%)	-

The subtype distribution within country (Table 31) revealed that the vast majority of patients in Zimbabwe had subtype C virus. The subtype

distribution within Uganda was more varied than in Zimbabwe with a mixture of subtype A and D. The existence of multiple pure subtypes within the region has led to the development of several complex/recombinant forms, although these are still a minority.

Table 31: HIV-1 Subtype by country

HIV-1 Subtype	Uganda	Zimbabwe	Overall
Subtype A	214 (56%)	0 (0%)	214 (39%)
Subtype C	6 (2%)	157 (99%)	163 (30%)
Subtype D	122 (32%)	0	122 (23%)
Subtype G	0 (0%)	1 (1%)	1 (0%)
Complex	42 (11%)	0 (0%)	42 (8%)

4.3.2 Treatment switches

Analyses in previous chapters have used an intention to treat approach and analysed patients according to the initial antiretroviral therapy regimen assigned at randomisation. In the DART trial protocol, a switch to a second-line regimen was defined as the inclusion of a protease inhibitor. Antiretroviral drugs could otherwise be substituted for adverse events, preferably within antiretroviral class, without this fulfilling the definition of a switch to second-line. Within this analysis, substitutions from an NRTI to an NNRTI are treated as a treatment switch and a separate regimen.

Of the 3,316 patients in the DART trial, a stavudine (D4T) substitution was made in 458 patients (14%), a switch to nevirapine was made in 128 (5%), a efavirenz substitution from nevirapine was made in 27 (5%) patients and a switch to efavirenz was made in 225 (8%) patients. A stavudine substitution was not associated with initial antiretroviral therapy regimen (χ^2 test p=0.526). A nevirapine switch was more likely in patients on abacavir than tenofovir (7% versus 4%; p=0.018). Switches to efavirenz were more likely in patients on either tenofovir or abacavir than patients who started on nevirapine (8% and 7% versus 5% respectively; p=0.027). There was no evidence of a difference in the time to stavudine substitution by initial antiretroviral therapy regimen (Nonparametric equality-of-median test p=0.40) which occurred after a median (IQR) of 20 (12-60)

weeks. A switch to nevirapine occurred earlier in patients on abacavir (n=22; Median=116; IQR=24-197 weeks) compared to those on tenofovir (n=106; Median=208; IQR=118-240 weeks) (Nonparametric equality-of-median test p=0.010). A substitution including efavirenz occurred earlier in patients on nevirapine (n=27; Median: 141; IQR=112-224 weeks) than either tenofovir (n=204; Median: 212; 156-248 weeks) or abacavir (n=21; Median: 203; IQR=139-231 weeks) (Nonparametric equality-of-median test p=0.049).

Switches from a triple-NRTI regimen to one containing an NNRTI were reported as being due to adverse events (286/323; 89%), starting a non-PI-containing second-line regimen (18/323; 6%, erroneously reported since this didn't fulfil the definition), a patient decision (2/323; 1%) or another reason (wrong dispensation (n=8), antiretrovirals from another source (n=4) or unknown (n=4)).

Within patients who were selected for HIV-1 drug resistance testing, 36 patients on tenofovir and 14 patients on abacavir switched to a regimen containing an NNRTI. Of these, patients who switched on tenofovir received the NNRTI for a median of 36 weeks (IQR=24-84 weeks) at the time of resistance test; 18% (IQR=10-37%) of the time spent on first-line antiretroviral therapy. Patients on abacavir who switched had received the NNRTI for a median of 59 weeks (IQR=36-117 weeks) at the time of resistance test; 29% (IQR=15-66%) of the time on first-line antiretroviral therapy before the HIV-1 drug resistance test.

To account for the complete individual drug history of patients in the following analyses is infeasible due to the limited sample size. However, a substitution to a regimen containing NNRTIs when analysing resistance by antiretroviral therapy regimen may influence findings, so this will be included alongside initial antiretroviral therapy regimen.

4.3.3 Resistance by drug class

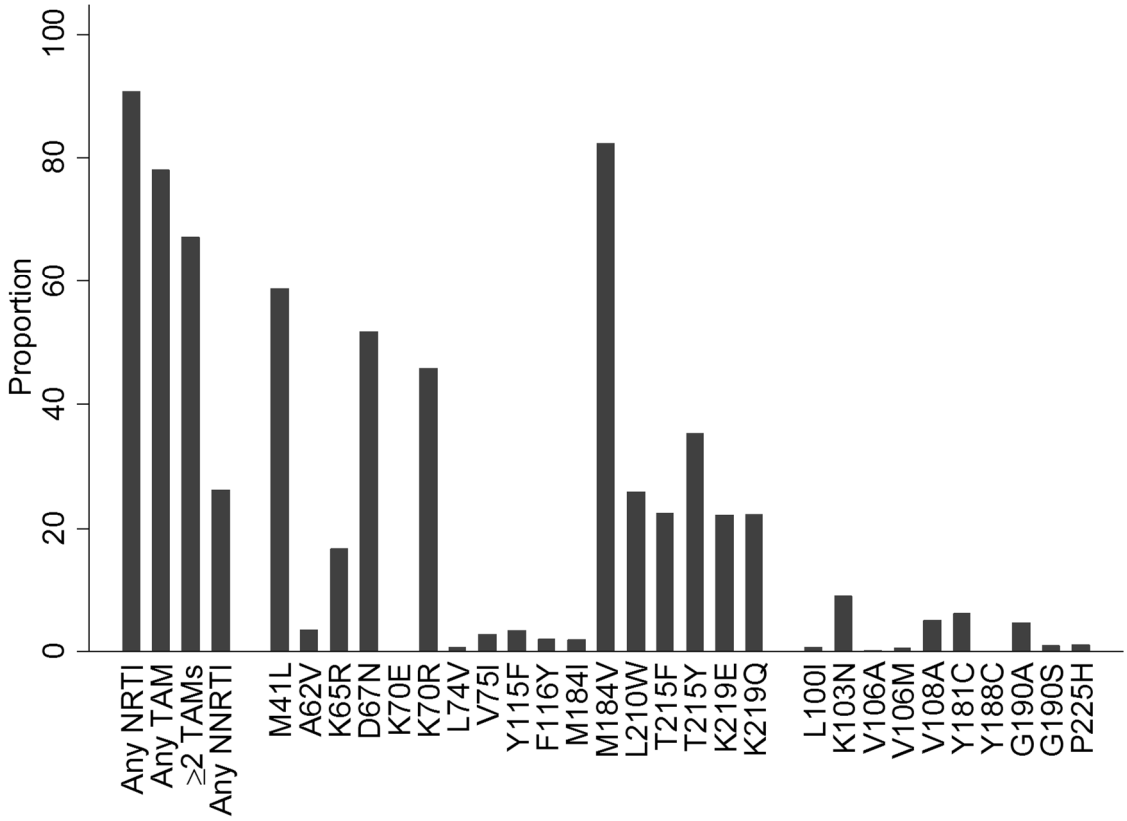
Resistance to a drug class was defined as one or more major IAS mutations [149] to that class (Table 32). NRTI resistance was high with 90% of patients with virological failure having one or more major NRTI mutations at the last time in the trial on first-line antiretroviral therapy. Major NNRTI resistance mutations were lower and observed in 66% of virologically failing patients who received first-line nevirapine. Both NRTI and NNRTI resistance mutations were highest among participants who switched to a regimen including an NNRTI, 97% and 100% of these participants who started on tenofovir and abacavir respectively had NRTI resistance while 72% and 86% had NNRTI resistance. Nonetheless, even in virologically failing patients with no reported NNRTI exposure, there remained 41/398 (10%) with NNRTI resistance. There was no evidence for a difference by gender in these patients (23 of 41 were female; 56%; p=0.69), suggesting that undocumented NNRTI use to prevent mother to child transmission was not a factor.

Table 32: Resistance to drug class by ARTs received

ARTs received (ZDV+3TC+)	NRTI Resistance	NNRTI Resistance
TDF	286/317 (90%)	31/317 (10%)
TDF & NNRTI	35/36 (97%)	26/36 (72%)
NVP	83/94 (88%)	62/94 (66%)
ABC	70/81 (86%)	10/81 (12%)
ABC & NNRTI	14/14 (100%)	12/14 (86%)
Total	488/542 (90%)	141/542 (26%)

Figure 25 displays the major IAS mutations observed among all patients with a HIV-1 drug resistance test. The prevalence of M184V mutations was high among those with NRTI resistance, 442 (82%) patients had this mutation. One or more TAMs were found in 419 (78%) patients and these were, in order of frequency, M41L (n=315; 59%), D67N (n=278; 52%), K70R (n=246; 46%), T215Y (n=190; 35%), L210W (n=139; 26%), T215F (n=121; 23%), K219Q (n=120; 22%) and K219E (n=119; 22%).

Figure 25: Mutations observed



Predictors of one or more major NRTI mutations were examined in Table 33 among all patients with a HIV-1 drug resistance test. Variables from univariate analyses with a p-value less than 0.2 were included in multivariate analyses. The patient status at the time of resistance test was a significant predictor in univariate analyses, with patients genotyped at the end of the trial or at the time of second line switch having more NRTI resistance than patients who died with virological failure. In multivariate analyses, this variable was no longer significant, likely due to correlation with the time since virological failure. Time since virological failure

significantly predicted NRTI resistance; each additional 48 weeks was associated with a 70% increase in the odds of NRTI resistance. Patients who started antiretroviral therapy with a higher CD4 cell count were less likely to develop NRTI resistance at virological failure (OR=0.28 per 100 cells/mm^3). Patients with higher viral load at the time virological failure was first detected were less likely to develop NRTI resistance (OR=0.57). This suggests that patients with very high viral loads at failure were non-adherent. Viral load at the time of test was no longer included in multivariate analyses due to strong correlation with the viral load at the time virological failure was first detected.

The logistic regression model results, shown in Table 34, expand upon these findings by excluding M184I/V and examining for the presence of any other major IAS NRTI mutation among all patients with a HIV-1 drug resistance test. Findings were similar to the overall analysis of NRTI resistance, except there was no evidence that either viral load at failure or gender predicted the presence of NRTI resistance in univariate analyses.

Time since virological failure was first detected and the baseline CD4 cell count were predictive of NNRTI resistance (Table 35). Each additional 48 weeks of antiretroviral therapy was associated with a 34% increase in the odds of NNRTI resistance. In contrast to NRTI resistance, there was no evidence that the viral load at the time of virological failure was predictive of NNRTI resistance in either univariate or multivariate analyses. Both age and baseline viral load were predictive of NNRTI resistance. Older patients had reduced odds of NNRTI resistance (5% lower per year) possibly due to improved adherence. Patients with a higher baseline viral load were less likely to develop NNRTI resistance (47% per $\log_{10}$ copies/mL), although this latter finding is not currently explained and could be due to chance.

Table 33: Logistic regression model of NRTI Resistance

Variable	N (% with NRTI Res)	Uni OR	p-value	Multi OR	95% CI	p-value
ARTs received						
TDF	286/317 (90%)	1.00	0.35	-	-	-
TDF & NNRTI	35/36 (97%)	3.79	-	-	-	-
NVP	83/94 (88%)	0.82	-	-	-	-
ABC	70/81 (86%)	0.69	-	-	-	-
ABC & NNRTI	14/14 (100%)	NA	-	-	-	-
Patient status at test						
First-line	246/270 (91%)	1.00	0.07	1.00	-	0.52
Death	42/52 (81%)	0.41	-	1.01	0.38-2.64	-
Switch	200/220 (91%)	0.98	-	1.48	0.70-3.12	-
Gender						
Male	190/216 (88%)	1.00	0.19	1.00	-	0.18
Female	298/326 (91%)	1.46	-	1.53	0.83-2.83	
Monitoring randomisation						
LCM	234/261 (90%)	1.00	0.78	-	-	-
CDM	254/281 (90%)	1.09	-	-	-	-
	Median (IQR)					
Age (years)	36 (31-42)	0.98	0.23	-	-	-
Time since failure (per 48 weeks)	2.25 (0.75-3.70)	1.63	<0.01	1.70	1.35-2.16	<0.01
Baseline CD4 Cell Count (per 100 cells/mm^3)	63 (22–120)	0.47	<0.01	0.28	0.16-0.49	<0.01
Baseline Viral Load (Log_{10} copies/mL)	5.51 (5.02–5.84)	0.95	0.78	-	-	-
Viral load at failure (Log_{10} copies/mL)	4.22 (3.32-4.90)	0.52	<0.01	0.57	0.36-0.90	0.02
Viral load at test (Log_{10} copies/mL)	4.70 (3.94-5.11)	0.69	0.03	0.85	0.53-1.33	0.47

Table 34: Logistic regression model of NRTI Resistance (excluding M184I/V)

Variable	N (% with NRTI Res excl M184I/V)	Uni OR	p-value	Multi OR	95% CI	p-value
ARTs received						
TDF	283/317 (89%)	1.00	<0.001	-	-	-
TDF & NNRTI	35/36 (97%)	4.20	-	-	-	-
NVP	69/94 (73%)	0.33	-	-	-	-
ABC	62/81 (77%)	0.39	-	-	-	-
ABC & NNRTI	14/14 (100%)	NA	-	-	-	-
Patient status at test						
First-line	230/270 (85%)	1.00	0.14	1.00	-	0.16
Death	40/52 (77%)	0.58	-	1.29	0.55-3.01	-
Switch	192/220 (88%)	1.24	-	1.82	0.98-3.36	-
Gender						
Male	184/216 (85%)	1.00	0.90	-	-	-
Female	279/326 (86%)	1.03	-	-	-	-
Monitoring randomisation						
LCM	221/261 (85%)	1.00	0.63	-	-	-
CDM	242/281 (86%)	1.12	-	-	-	-
	Median (IQR)			-	-	-
Age (years)	36 (31-42)	0.99	0.59	-	-	-
Time since failure (per 48 weeks)	2.25 (0.75-3.70)	1.57	<0.001	1.66	1.37-2.00	<0.001
Baseline CD4 Cell Count (per 100 cells/mm^3)	63 (22–120)	0.51	0.001	0.36	0.23-0.59	<0.001
Baseline Viral Load (Log_{10} copies/mL)	5.51 (5.02–5.84)	1.11	0.49	-	-	-
Viral load at failure (Log_{10} copies/mL)	4.22 (3.32-4.90)	0.66	0.004	0.63	0.46-0.86	0.004
Viral load at test (Log_{10} copies/mL)	4.70 (3.94-5.11)	0.86	0.27	-	-	-

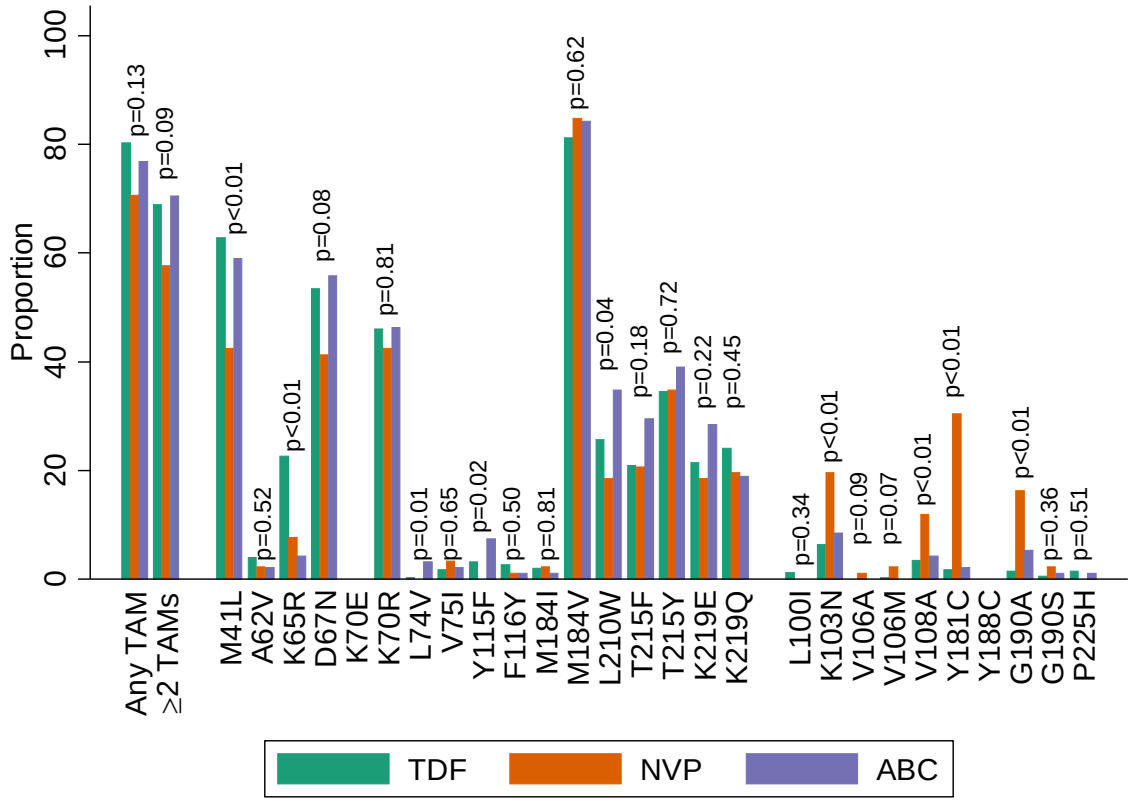
Table 35: Logistic regression model of NNRTI Resistance among patients who received an NNRTI

Variable	N (% with NNRTI Res)	Uni OR	p-value	Multi OR	95% CI	p-value
ARTs received						
NVP	62/94 (66%)	1.00	0.32	-	-	-
TDF & NNRTI	26/36 (72%)	1.34	-	-	-	-
ABC & NNRTI	12/14 (86%)	3.10	-	-	-	-
Patient status at test						
First-line	56/81 (69%)	1.00	0.97	-	-	-
Death	6/9 (67%)	0.89	-	-	-	-
Switch	38/54 (70%)	1.06	-	-	-	-
Gender						
Male	37/53 (70%)	1.00	0.94	-	-	-
Female	63/91 (69%)	0.97	-	-	-	-
Monitoring randomisation						
LCM	50/72 (69%)	1.00	1.00	-	-	-
CDM	50/72 (69%)	1.00	-	-	-	-
	Median (IQR)					
Age (years)	36 (31-42)	0.94	0.02	0.95	0.90-1.00	0.07
Time since failure (per 48 weeks)	2.25 (0.75-3.70)	1.26	0.06	1.34	1.03-1.75	0.03
Baseline CD4 Cell Count (per 100 cells/mm^3)	63 (22–120)	0.55	0.06	0.60	0.31-1.18	0.14
Baseline Viral Load (Log_{10} copies/mL)	5.51 (5.02–5.84)	0.61	0.11	0.53	0.27-1.02	0.06
Viral load at failure (Log_{10} copies/mL)	4.22 (3.32-4.90)	1.33	0.18	1.31	0.83-2.09	0.25
Viral load at test (Log_{10} copies/mL)	4.70 (3.94-5.11)	1.18	0.44	-	-	-

4.3.4 Resistance by antiretroviral therapy received

Figure 26 displays the number of NRTI and NNRTI mutations by the initial first-line antiretroviral therapy received among all patients with a HIV-1 drug resistance test. The p-values displayed are the results from a χ^2 test. Patients on nevirapine had fewer TAMs than patients who started on triple-NRTI regimens, particularly M41L, D67N and L210W. Patients who received a first-line antiretroviral therapy containing tenofovir had the most K65R mutations. This is not surprising because K65R is selected for most strongly by tenofovir. The M184V mutation was universally high, regardless of first-line antiretroviral therapy regimen.

Figure 26: Mutations observed by first-line ART received

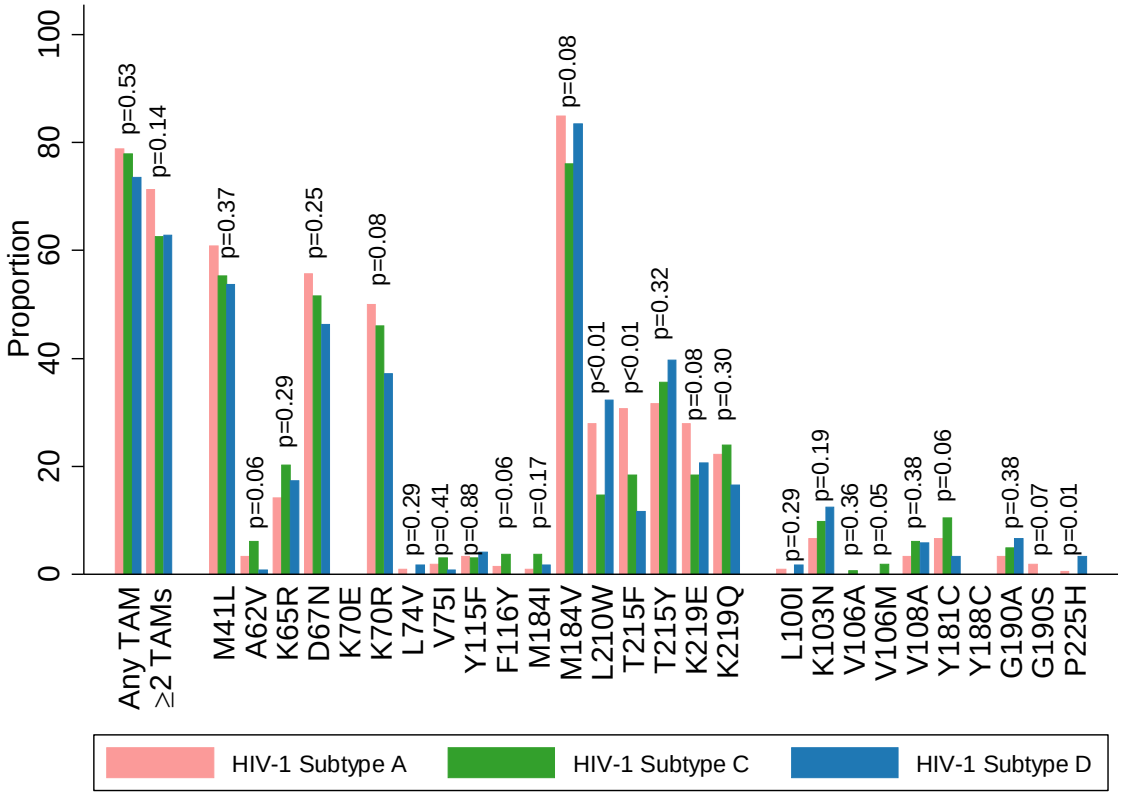


4.3.5 Resistance by HIV-1 subtype

Figure 27 displays the specific mutations observed by HIV-1 subtype among all patients with a HIV-1 drug resistance test. There was no apparent difference in the proportion of patients with K65R by HIV-1 subtype. The K70R and T215F mutations had the greatest prevalence among patients with subtype A virus and the lowest prevalence among patients with the

subtype D virus. The L210W mutation was infrequently observed in patients with subtype C compared to either subtype A or D. There was some evidence that A62V and F116Y were more likely to occur in patients with subtype C virus, although these were less frequently occurring mutations.

Figure 27: Mutations by HIV-1 subtype



For mutations where the χ^2 test suggested a difference by HIV-1 subtype ($p<0.1$) a multivariate logistic regression model (Table 36) was conducted with all patients with a HIV-1 drug resistance test (n=542) including covariates (time since virological failure, viral load at the time of resistance test and baseline CD4 cell count) which affected the multivariate odds of any NRTI resistance in Table 33. Furthermore, K65R was also analysed due to the strong *a priori* belief from other research that K65R's prevalence is influenced by HIV-1 subtype.

After adjustment, there was no evidence that HIV-1 subtype affected the proportion of patients with either the K65R or the F116Y mutations. Patients with subtype D virus had reduced odds of the K70R mutation

compared to patients with subtype A virus (OR=0.57; 95% CI: 0.36-0.91), although the global test in a multivariate logistic regression model showed no evidence of an overall effect of HIV-1 subtype (p=0.13). Patients with subtype C virus were less likely to have the M184V mutation compared to patients with subtype A (OR=0.46; 95% CI: 0.26-0.81). This is a surprising result not previously suggested in the literature and is probably a false positive. Since HIV-1 subtype is highly correlated with countries, this finding may indicate a country effect where patients in Zimbabwe develop less HIV-1 drug resistance mutations. Subtype C virus was also predictive of fewer L210W, T215F and K219E mutations compared to subtype A virus in multivariate analyses, although this may still reflect the effect of country. More robustly, there was evidence that patients with subtype D virus were less likely to develop the T215F mutation (OR=0.25; 95% CI: 0.13-0.48).

Table 36: Logistic regression model for selected NRTI mutations

Variables	K65R		K70R		F116Y		M184V	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Subtype								
A	1.00	0.36	1.00	0.13	1.00	0.44	1.00	0.01
C	1.49 (0.84-2.64)	-	0.85 (0.56-1.28)	-	2.50 (0.61-10.22)	-	0.46 (0.26-0.81)	-
D	0.95 (0.50-1.83)	-	0.57 (0.36-0.91)	-	-	-	0.55 (0.29-1.07)	-
Complex	0.74 (0.25-2.18)	-	0.78 (0.40-1.55)	-	1.64 (0.17-16.36)	-	1.93 (0.53-6.98)	-
Time since virological failure (48 weeks)	0.97 (0.82-1.14)	0.70	1.10 (0.98-1.23)	0.10	0.95 (0.62-1.46)	0.80	1.69 (1.41-2.02)	<0.001
Baseline CD4 Cell Count (per 100 cells/mm^3)	0.41 (0.26-0.66)	<0.001	0.94 (0.69-1.28)	0.70	0.51 (0.15-1.75)	0.29	0.44 (0.28-0.69)	<0.001
Viral load at virological failure (log_{10} copies/mL)	0.40 (0.30-0.54)	<0.001	1.10 (0.90-1.34)	0.35	0.92 (0.43-1.95)	0.82	0.62 (0.46-0.83)	0.001

Table 38 (continued): Logistic regression model for selected NRTI mutations

Variables	L210W		T215F		K219E	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Subtype						
A	1.00	<0.001	1.00	<0.001	1.00	0.08
C	0.45 (0.26-0.76)	-	0.49 (0.30-0.81)	-	0.60 (0.37-1.00)	-
D	1.22 (0.74-2.03)	-	0.25 (0.13-0.48)	-	0.69 (0.40-1.19)	-
Complex	2.06 (1.02-4.19)	-	0.77 (0.36-1.66)	-	0.37 (0.14-1.00	-
Time since virological failure (48 weeks)	1.21 (1.06-1.38)	0.01	1.27 (1.11-1.47)	<0.001	1.14 (0.99-1.30)	0.06
Baseline CD4 Cell Count (per 100 cells/mm^3)	0.82 (0.56-1.19)	0.30	0.69 (0.47-1.02)	0.06	1.17 (0.81-1.69)	0.41
Viral load at virological failure (log_{10} copies/mL)	1.57 (1.24-2.00)	<0.001	1.08 (0.85-1.38)	0.51	1.04 (0.82-1.32)	0.73

4.3.6 Number of mutations

Virologically failing patients typically had multiple NRTI mutations at their last time point on first-line antiretroviral therapy in the DART trial. These are shown by antiretroviral therapy received in Figure 28. In the 488 patients with one or more NRTI mutations, there was a median (IQR) of 5 (3-6) major NRTI mutations, as measured by the 2013 IAS-USA mutation list [149]. In contrast, for the 141 patients with one or more NNRTI mutations, there was a median (IQR) of 1 (1-2) major NNRTI mutations. Predictors for the number of NNRTI mutations were not examined further because there was insufficient variability in the number of NNRTI mutations (Figure 29) to distinguish this from the analysis of any NNRTI resistance.

Figure 28: Number of NRTI mutations by ARTs received

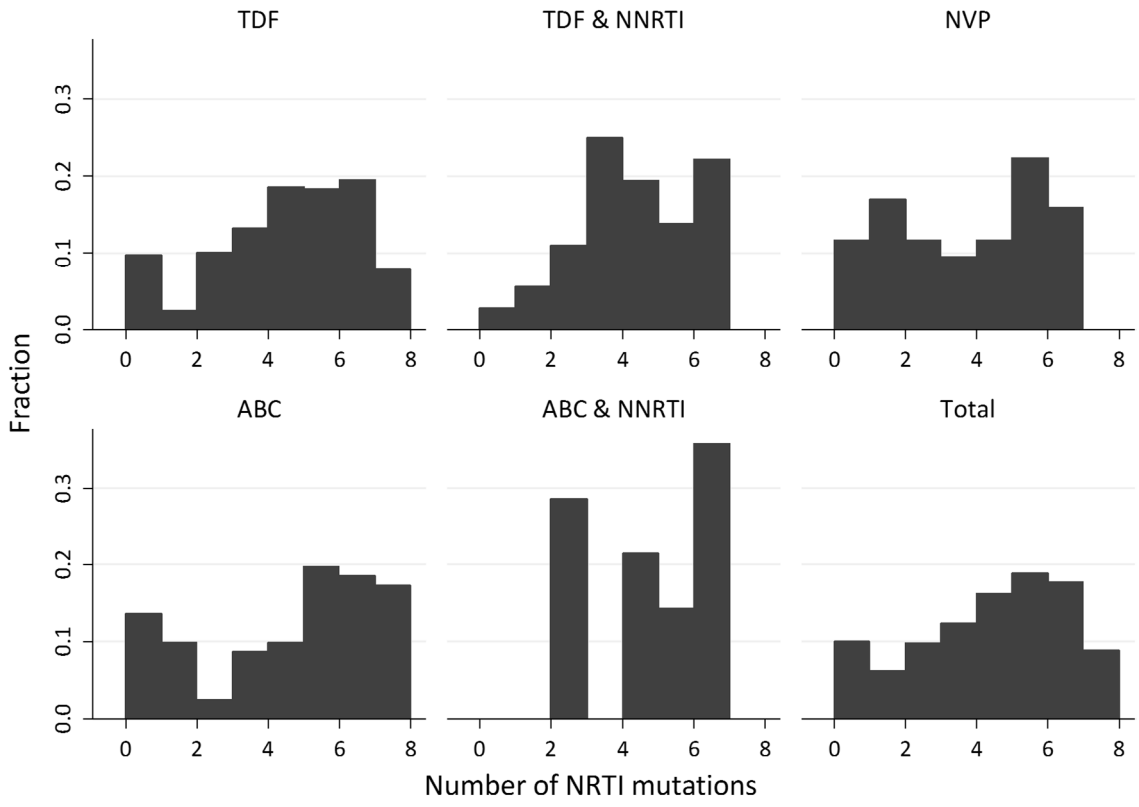
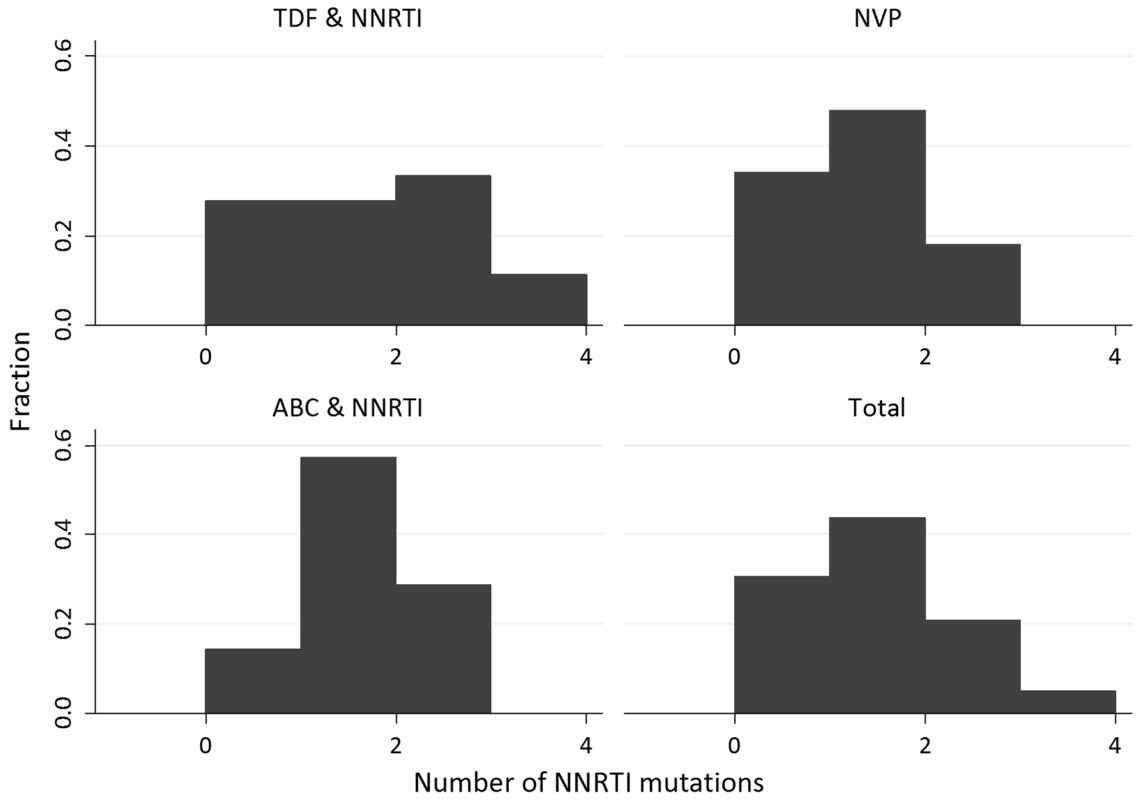


Figure 29: Number of NNRTI mutations by ARTs received



A Poisson model of the number of NRTI mutations demonstrated signs of overdispersion [155]. Therefore, a negative binomial model was used to investigate predictors of the number of NRTI mutations among all patients with a HIV-1 drug resistance test result (n=542). The results are reported as rate ratios (RR) which are applied in combination on the multiplicative scale (Table 37).

The multivariate analysis results showed that patients on nevirapine had a 16% decrease in the number of NRTI mutations compared to those on tenofovir. There was no evidence that the number of NRTI mutations differed in those who received abacavir. Patients who switched treatment at the time of resistance test had a 33% increase in the number of NRTI mutations compared to patients who remained alive on first-line antiretroviral therapy, even after adjusting for the time since virological failure. There was no evidence of a difference in the number of NRTI mutations for patients who died. Each additional 48 weeks since virological failure was first detected led to a 12% increase in the number of NRTI mutations. In the multivariate model, the viral load at the time of

resistance test did not influence the number of NRTI mutations. Each 100 cell/mm^3 increase in baseline CD4 cell count reduced the number of NRTI mutations by 8%.

Table 37: Negative binomial regression model for the number of NRTI mutation

Variable	Uni RR	p-value	Multi RR	95% CI	p-value
ARTs received					
TDF	1.00	0.04	1.00	-	0.02
TDF & NNRTI	0.96	-	0.95	0.79-1.13	-
NVP	0.82	-	0.84	0.74-0.95	-
ABC	1.03	-	1.08	0.95-1.22	-
ABC & NNRTI	1.10	-	1.12	0.87-1.45	-
Patient status at test					
First-line	1.00	<0.001	1.00	-	<0.001
Death	0.93	-	1.07	0.89-1.28	-
Switch	1.23	-	1.33	1.20-1.49	-
Gender					
Male	1.00	0.53	-	-	-
Female	0.97	-	-	-	-
Monitoring randomisation					
LCM	1.00	0.59	-	-	-
CDM	1.03	-	-	-	-
Age (10 years)	1.00	0.52	-	-	-
Time since failure (48 weeks)	1.08	<0.001	1.12	1.08-1.15	<0.001
Baseline CD4 Cell Count (100 cells/mm^3)	0.92	0.04	0.92	0.85-1.00	0.04
Viral load at failure (log_{10} copies/mL)	1.01	0.75	-	-	-
Viral load at test (log_{10} copies/mL)	1.08	0.01	1.03	0.97-1.08	0.35

4.3.7 Combinations of mutations

In total, 90% of patients had one or more NRTI mutations. The overlap between major NRTI mutations is plotted using Gephi v0.9 [156] and displayed in Figure 30. In this visualisation, the size of bubbles corresponds to the proportion of patients with this combination of mutations at the last time point. Patients can only appear in one group in the diagram. Arrows link groups of mutations which are subsets and were chosen using an ad

hoc basis, such that each group could have at most three parent groups. For example, patients with 41+67+70+184+215+219 are a subset of those with 67+70+184+215+219 and this group is a subset of 67+70+184+219. The hue of each circle corresponds to proximity (by the number of links) to patients with wild-type virus. This figure is clarified in Figure 31 by removing subsets of mutations occurring in less than five patients and dividing patients with these subsets of mutations equally between the parent nodes.

The largest set of mutation combinations observed at the last time-point was 41+67+70+184+215+219. In total, there were 77 (14%) patients with this combination of mutations exactly and 121 (22%) patients with at least these mutations. Specific combinations, such as K65R/E/N mutation, were less common, and there was a greater diversity of secondary mutations. In total, 92 (17%) patients had at least this mutation and the largest specific combination of mutations was 65+184 (n=23; 4%). Distinctive mutations from the 151 complex, which confer cross-resistance to all the main NRTIs apart from tenofovir, appeared alongside K65R/E/N and were otherwise infrequent. A62V appeared 17 times with K65R/E/N and 4 times without, Q151M appeared 9 times with K65R/E/N and 1 time without, Y115F appeared 13 times with K65R/E/N and 6 without and F116Y appeared 11 times with K65R/E/N and never without.

Mutations at codon 210 often appeared with mutations at codon 215 (n=138; 26%) and rarely without (n=3; 1%). The second most common combination of mutations for all patients contained 210+215 as 41+67+184+210+215 (n=44; 8%). Another common mutation pair was 215+219 (n=173; 32%) and these three mutations occurred together in 52 (10%) patients. Mutations at codons 210 and 219 seldom occurred without a mutation at codon 215 (n=2 times as a solo combination).

Figure 30: Overlap between NRTI mutations

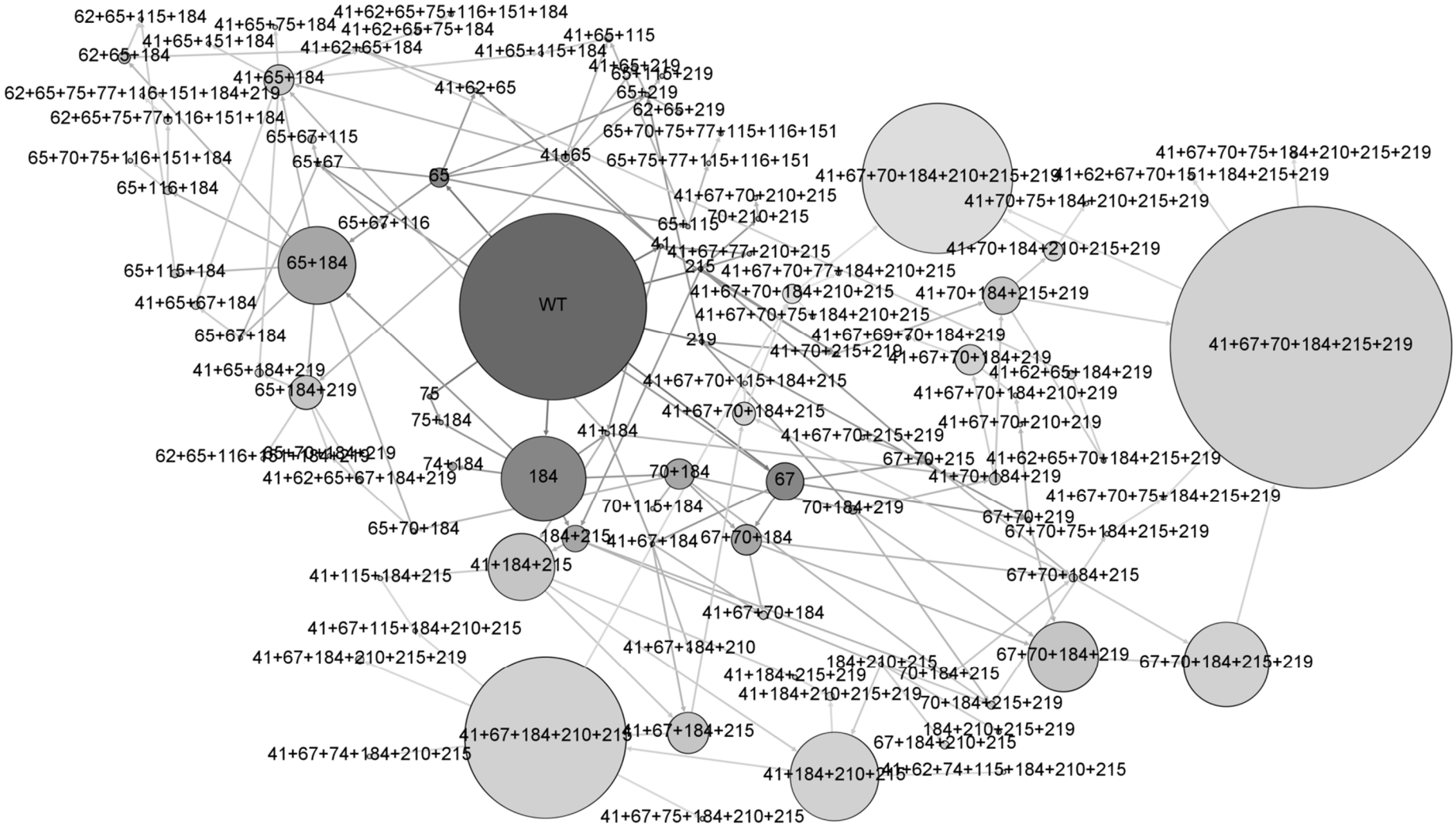


Figure 31: Simplified overlap between NRTI mutations

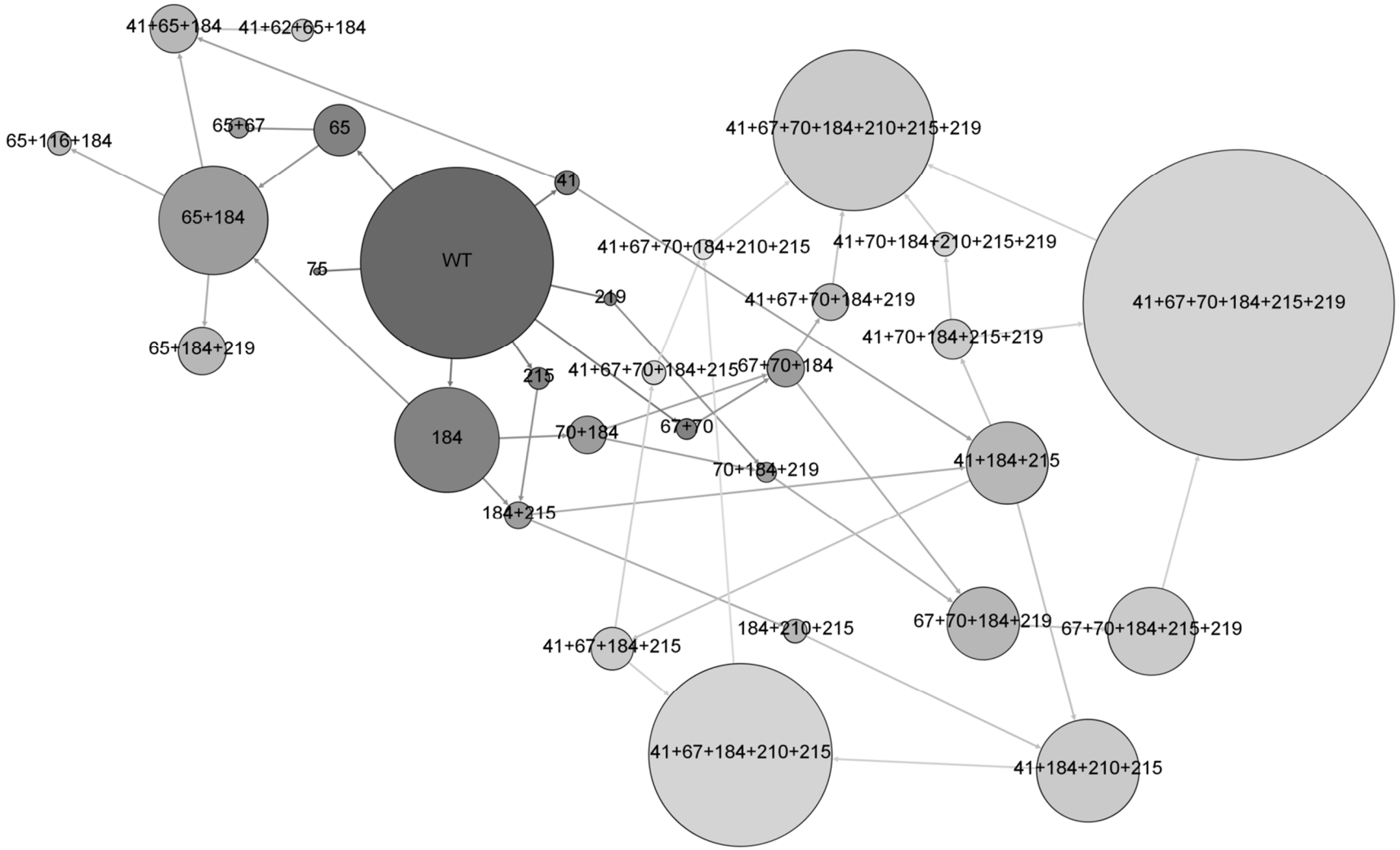
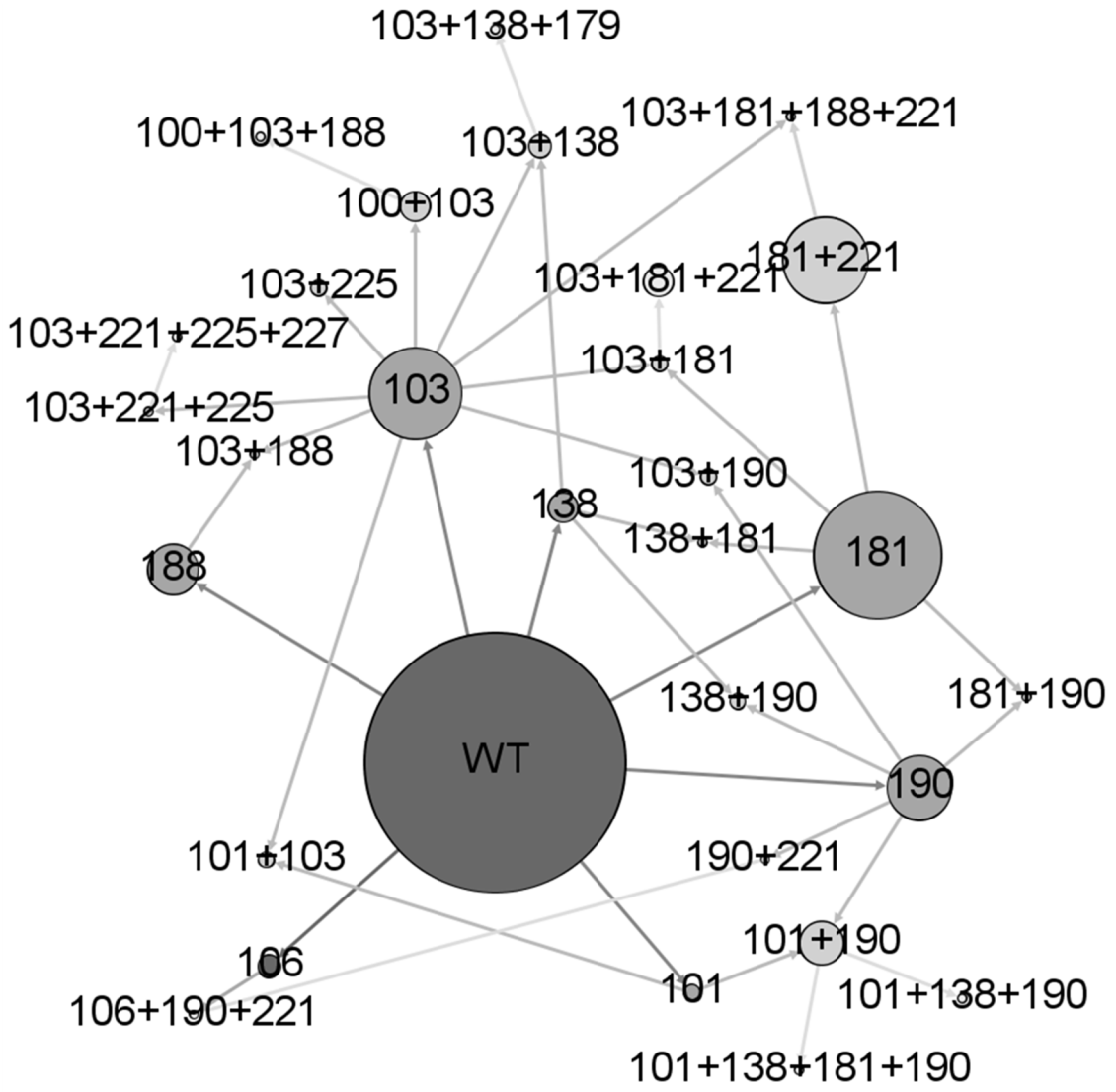


Figure 32: Overlap between NNRTI mutations



The overlap between NNRTI mutations is displayed in Figure 32 for patients who received an NNRTI during follow-up. There were substantially fewer combinations of mutations than for NRTIs. The most common mutation to appear in conjunction with others was K103N/S which appeared in 60% of patients with two or more NNRTI mutations. This was followed by Y181C/I/V which appeared in 45% of patients with two or more NNRTI mutations.

4.3.8 Predicted susceptibility

Susceptibility to NRTI and NNRTIs were defined using the Stanford algorithm for predicted drug susceptibility based on the individual mutations observed. The results for NRTI and NNRTIs are shown in Table Table 38 and Table 39 respectively and are displayed graphically in Figure 33. Possible low level and low resistance were combined as low resistance in the tables. There was less susceptible, low level and intermediate resistance to zidovudine compared to tenofovir (38% compared to 54%) although there was greater high-level resistance to zidovudine (62% compared to 46%). Remaining susceptibility to emtricitabine and lamivudine was low, just 12% had low-level resistance or less.

Table 38: Susceptibility to NRTIs (N=542)

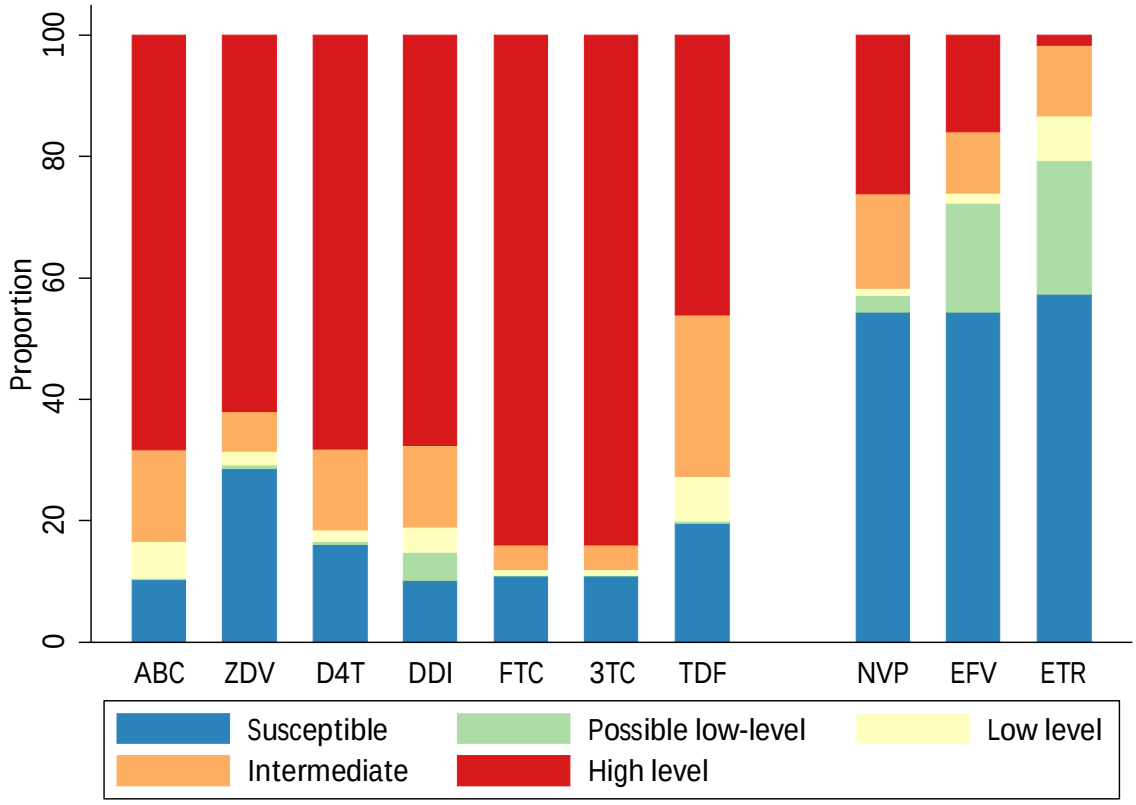
NRTI	Susceptible	Low	Intermediate	High
Abacavir	56 (10%)	34 (6%)	81 (15%)	371 (68%)
Didanosine	55 (10%)	48 (9%)	72 (13%)	367 (68%)
Emtricitabine	59 (11%)	6 (1%)	21 (4%)	456 (84%)
Stavudine	87 (16%)	13 (2%)	72 (13%)	370 (68%)
Tenofovir	106 (20%)	42 (8%)	144 (27%)	250 (46%)
Zidovudine	155 (29%)	15 (3%)	36 (7%)	336 (62%)

Table 39: Susceptibility to NNRTIs (N=542)

NNRTI	Susceptible	Low	Intermediate	High
Nevirapine	295 (54%)	20 (4%)	85 (16%)	142 (26%)
Efavirenz	295 (54%)	106 (20%)	54 (10%)	87 (16%)
Etravirine	310 (57%)	159 (29%)	64 (12%)	9 (2%)

NNRTI resistance was greatest for nevirapine; in total 42% of patients who experienced virological failure had intermediate or greater nevirapine resistance at the last time point. The NNRTI which retained the greatest activity was etravirine. This is unsurprising since there is limited crossover in resistance mutations between efavirenz, nevirapine and etravirine.

Figure 33: Overall susceptibility to NRTI and NNRTIs



Susceptibility was also examined by antiretroviral therapy received (Figure 34 to Figure 36 below). Patients who received either tenofovir or abacavir as a triple-NRTI first-line regimen had similar levels of high-level resistance. For both regimens, the NRTIs which retained the most susceptibility were zidovudine and tenofovir. Tenofovir had the lowest level of high-level drug resistance across the NRTIs and zidovudine had the greatest proportion of patients with virus susceptible to low-level drug resistance. Patients who received an NRTI-NNRTI regimen had greater susceptibility to NRTIs and reduced susceptibility to NNRTIs. Nevertheless, both tenofovir and zidovudine retained the greatest susceptibility.

Figure 34: Susceptibility in patients who received triple-NRTI first-line ART including tenofovir

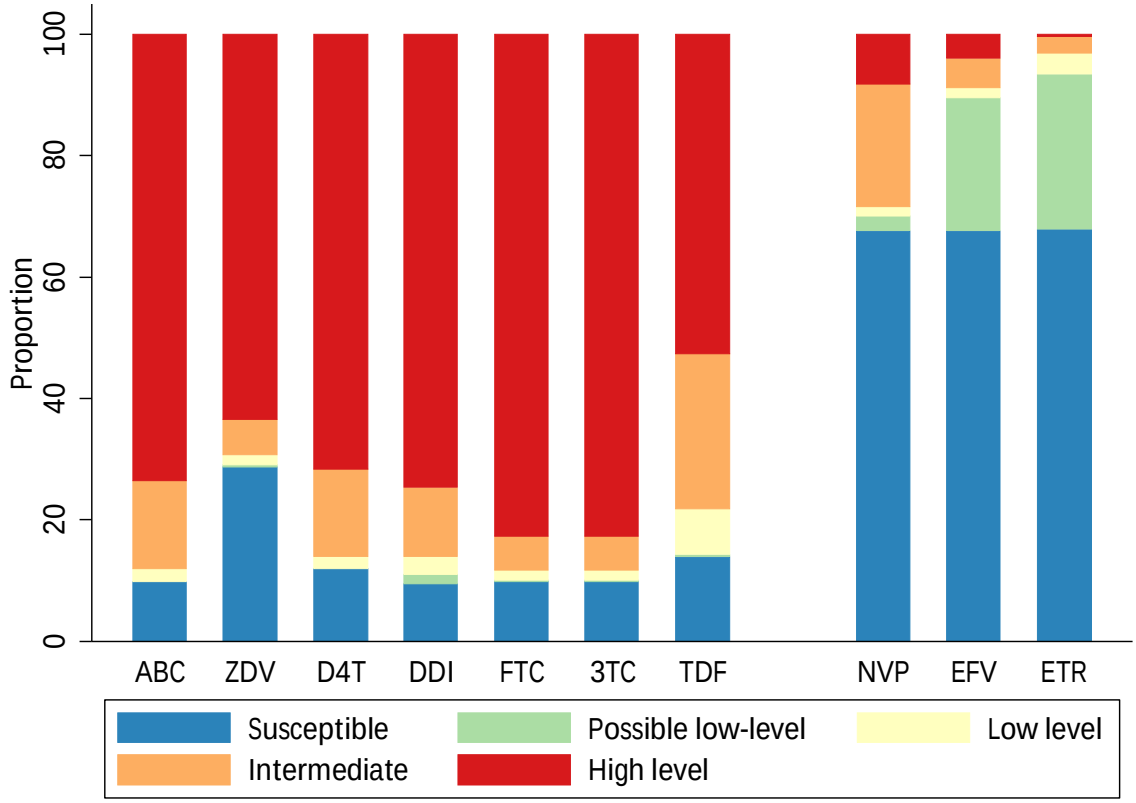


Figure 35: Susceptibility in patients who received triple-NRTI first-line ART including abacavir

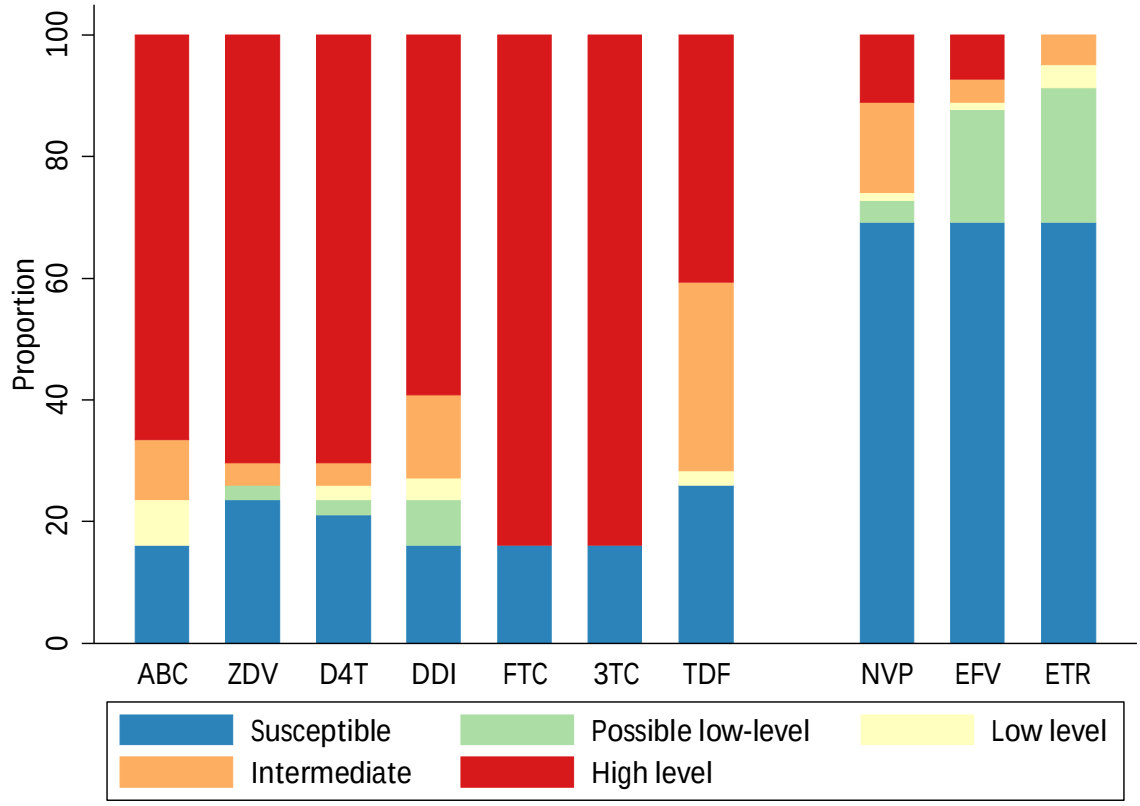
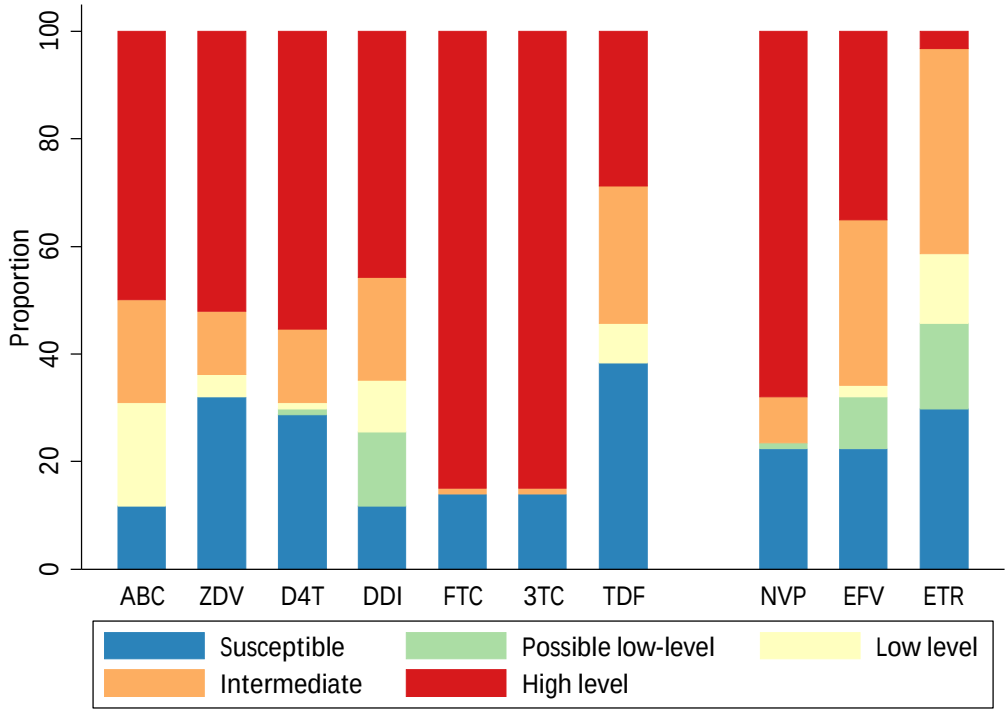


Figure 36: Susceptibility in patients who received dual-class first-line ART including nevirapine



4.3.9 HIV-1 Drug Resistance and Viral Load

A linear regression of viral load at the time of test and individual mutations was conducted to determine whether mutations increase or decrease viral replication fitness. Viral load was transformed using a zero-skewness log transformation (selected to be ln(rna+2180)) [157] to satisfy the linear regression model's assumption that errors are normally distributed. Models were fitted to patients who had one or more NRTI or NNRTI mutations, excluding patients who were potentially non-adherent. Models were stratified by the regimen received at the time the plasma sample was taken. The regimens examined were triple-NRTI including tenofovir (Table 40, allowing for stavudine substitution), triple-NRTI including abacavir (Table 41) and a dual-class regimen containing an NNRTI (Table 42, either efavirenz or nevirapine). Multivariate analyses were conducted with and without variables for patient status at test (gender, monitoring randomisation, age, time since virological failure and baseline CD4 cell count) to reduce confounding.

Table 40: Linear regression model for transformed viral load among patients on tenofovir-containing triple-NRTI regimens with any HIV-1 drug resistance

Variable	N=286	Multi Coef	95% CI	p-value	Multi Coef	95% CI	p-value
41	196 (69%)	0.24	-0.21 to 0.70	0.29	0.23	-0.20 to 0.67	0.30
62	15 (5%)	0.26	-0.73 to 1.24	0.61	0.29	-0.55 to 1.13	0.49
65	69 (24%)	-0.53	-1.25 to 0.20	0.16	-0.42	-1.12 to 0.27	0.23
67	169 (59%)	0.71	0.28 to 1.14	0.001	0.38	-0.03 to 0.80	0.07
70	148 (52%)	0.08	-0.55 to 0.71	0.80	-0.09	-0.66 to 0.48	0.76
115	10 (3%)	-1.22	-2.56 to 0.11	0.07	-0.55	-1.72 to 0.63	0.36
116	9 (3%)	0.10	-1.39 to 1.58	0.90	-0.27	-1.44 to 0.91	0.66
151	8 (3%)	2.16	0.70 to 3.61	0.004	1.59	0.25 to 2.94	0.02
184	260 (91%)	-0.91	-1.67 to -0.14	0.02	-0.41	-1.10 to 0.28	0.25
210	84 (29%)	0.31	-0.18 to 0.80	0.21	0.25	-0.17 to 0.68	0.24
215	177 (62%)	0.56	-0.02 to 1.14	0.06	0.28	-0.27 to 0.83	0.32
219	141 (49%)	-0.32	-0.80 to 0.16	0.19	-0.11	-0.55 to 0.33	0.62
Patient status at test							
First-line	136 (48%)	-	-	-	0.00	-	<0.001
Death	31 (11%)	-	-	-	1.54	0.98 to 2.10	-
Switch	119 (42%)	-	-	-	1.45	1.03 to 1.87	-
Gender							
Male	123 (43%)	-	-	-	0.00	-	0.30
Female	163 (57%)	-	-	-	0.16	-0.47 to 0.15	-
Monitoring randomisation							
LCM	128 (45%)	-	-	-	0.00	-	0.08
CDM	158 (55%)	-	-	-	0.29	-0.03 to 0.61	-
Age (10 years)	35 (31-42)	-	-	-	-0.00	-0.02 to 0.02	0.69
Time since failure (per 48 weeks)	2.4 (0.8-3.8)	-	-	-	0.05	-0.07 to 0.16	0.40
Baseline CD4 Cell Count (per 100 cells/mm^3)	60 (22-117)	-	-	-	0.14	-0.17 to 0.45	0.38
Constant	-	10.64	9.63 to 11.66		9.64	8.37 to 10.91	

Table 41: Linear regression model for transformed viral load among patients on abacavir-containing triple-NRTI regimens with any HIV-1 drug resistance

Variable	N=71	Multi Coef	95% CI	p-value	Multi Coef	95% CI	p-value
41	49 (69%)	-0.01	-1.23 to 1.20	0.98	-0.08	-1.45 to 1.28	0.90
62	2 (3%)	0.10	-1.27 to 1.46	0.89	1.20	-0.58 to 2.98	0.18
65	3 (4%)	-2.31	-3.44 to -1.19	<0.001	-2.51	-3.78 to -1.24	<0.001
67	44 (62%)	0.24	-0.75 to 1.23	0.63	0.14	-0.93 to 1.21	0.80
70	36 (51%)	-2.21	-4.06 to -0.36	0.02	-2.27	-4.26 to -0.27	0.03
115	7 (10%)	0.25	-1.06 to 1.55	0.71	0.01	-1.37 to 1.40	0.99
116[3]	1 (1%)	6.19	4.13 to 8.20	<0.001	5.98	3.10 to 8.86	<0.001
151	1 (1%)	-	-	-	-	-	-
184	68 (96%)	0.73	-1.61 to 3.07	0.54	0.87	-2.17 to 3.91	0.57
210	29 (41%)	0.55	-0.39 to 1.49	0.24	0.20	-0.84 to 1.23	0.70
215	55 (77%)	-0.48	-1.72 to 0.77	0.45	-0.36	-1.74 to 1.01	0.60
219	34 (48%)	1.88	-0.09 to 3.86	0.06	2.00	-0.16 to 4.15	0.07
Patient status at test							
First-line	35 (49%)	-	-	-	0.00	-	0.06
Death	3 (4%)	-	-	-	-1.52	-3.71 to 0.67	-
Switch	33 (46%)	-	-	-	0.79	-0.44 to 2.02	-
Gender							
Male	23 (32%)	-	-	-	0.00	-	0.44
Female	48 (68%)	-	-	-	-0.39	-1.41 to 0.62	-
Monitoring randomisation							
LCM	40 (56%)	-	-	-	0.00	-	0.76
CDM	31 (44%)	-	-	-	0.16	-0.89 to 1.21	-
Age (10 years)	39 (31-43)	-	-	-	-0.00	-0.06 to 0.05	0.88
Time since failure (per 48 weeks)	2.0 (0.9-2.9)	-	-	-	-0.06	-0.59 to 0.47	0.82
Baseline CD4 Cell Count (per 100 cells/mm^3)	82 (35-143)	-	-	-	0.14	-0.70 to 0.97	0.74
Constant	-	9.91	7.98 to 11.85		9.98	6.43 to 13.55	

[3] 151 not included because the model failed to converge for this variable due to an insufficient number of mutations.

Table 42: Linear regression model for transformed viral load among patients on NRTI-NNRTI regimens with any HIV-1 drug resistance

Variable	N=135	Multi Coef	95% CI	p-value	Multi Coef	95% CI	p-value
41	69 (51%)	-0.25	-0.90 to 0.40	0.45	-0.35	-1.00 to 0.31	0.30
62	4 (3%)	-0.30	-1.37 to 0.77	0.58	-0.37	-1.51 to 0.77	0.52
65	20 (15%)	0.59	-0.30 to 1.47	0.19	0.74	-0.15 to 1.64	0.10
67	64 (47%)	0.24	-0.58 to 1.05	0.57	0.00	-0.84 to 0.85	0.99
70	61 (45%)	-0.46	-1.22 to 0.30	0.23	-0.34	-1.10 to 0.42	0.38
103	38 (28%)	0.57	0.01 to 1.13	0.05	-0.59	0.08 to 1.11	0.03
108	18 (13%)	-0.51	-1.28 to 0.26	0.19	-0.68	-1.33 to -0.02	0.04
115	2 (1%)	-2.08	-2.94 to -1.22	<0.001	-1.96	-2.85 to -1.07	<0.001
116[4]	1 (1%)	2.09	1.07 to 3.11	<0.001	2.45	1.36 to 3.54	<0.001
181	40 (30%)	0.37	-0.22 to 0.97	0.21	0.38	-0.18 to 0.94	0.19
184	126 (93%)	-1.98	-2.75 to -1.21	<0.001	-1.26	-2.02 to -0.49	0.001
190	24 (18%)	0.45	-0.19 to 1.09	0.17	0.45	-0.20 to 1.11	0.17
210	28 (21%)	0.37	-0.38 to 1.12	0.34	0.15	-0.55 to 0.84	0.68
215	77 (57%)	1.13	0.40 to 1.86	0.003	1.00	0.28 to 1.73	0.007
219	59 (44%)	0.71	0.02 to 1.40	0.04	0.62	-0.02 to 1.26	0.06
Patient status at test							
First-line	76 (56%)	-	-	-	0.00	-	<0.001
Death	8 (6%)	-	-	-	1.84	1.07 to 2.60	-
Switch	51 (38%)	-	-	-	1.07	0.53 to 1.61	-
Gender							
Male	48 (36%)	-	-	-	0.00	-	0.47
Female	87 (64%)	-	-	-	0.20	-0.34 to 0.74	-
Monitoring randomisation							
LCM	68 (50%)	-	-	-	0.00	-	0.12
CDM	67 (50%)	-	-	-	0.36	-0.10 to 0.82	-
Age (10 years)	35 (31-41)	-	-	-	-0.02	-0.06 to 0.02	0.32
Time since failure (per 48 weeks)	2.3 (1.0-3.5)	-	-	-	0.01	-0.15 to 0.18	0.88
Baseline CD4 Cell Count (per 100 cells/mm^3)	53 (18-107)	-	-	-	-0.12	-0.55 to 0.31	0.58
Constant	-	11.39	10.60 to 12.18		10.93	9.13 to 12.73	

[4] 151 not included because the model failed to converge for this variable due to an insufficient number of mutations.

The mutation with the largest impact on viral load in multivariate analyses across all three regimen types was Q151M, which was predicted to increase viral load by 244,639 copies/mL (95% CI: -161,747 to 651,025 copies/mL; p=0.24) in patients on tenofovir. This mutation was observed in eight patients on tenofovir and is known to lead to high-level resistance to all NRTIs apart from tenofovir when combined with mutations at codons 62, 75, 77 and 116 [14]. The D67N mutation increased the viral load of patients on tenofovir by a predicted 23,571 copies/mL (95% CI: -1,313 to 48,456 copies/mL; p=0.06). There was no evidence that other mutations influenced viral load in patients on tenofovir. However, in multivariate analyses which did not adjust for patient variables, mutations at codons 115 and 184 reduced viral load and mutations at codon 215 increased viral load.

Among these five mutations (67, 115, 151, 184 and 215) the Stanford HIV drug resistance interpretation algorithm [14] suggests that mutations at 115+184 and 151+184 may have interaction effects for tenofovir and zidovudine respectively, increasing the levels of drug resistance when simultaneously observed. These were examined in separate multivariate models adjusting for other variables. No interaction effect was observed for 151+184 (Coef=-1.42, 95% CI: -3.89 to 1.04; p=0.26) but evidence for an interaction was observed for 115+184 (Coef=2.09; 95% CI: -0.07 to 4.25; p=0.06). There was some evidence that mutations at codons 115 and 184 individually reduced viral load by 82,621 copies/mL (95% CI: -170,362 to 5,119 copies/mL; p=0.07) and 56,655 copies/mL (95% CI: -138,057 to 24,746 copies/mL; p=0.17) respectively, but in combination there was no evidence for a change in viral load (49,202 copies/m; 95% CI:-272,436 to 370,840 copies/mL; p=0.76)

Few patients on abacavir were observed to have mutations at codons 65 (n=3) or 116 (n=1), so the large observed influence on viral load should be interpreted cautiously. Patients on abacavir with mutations at codon 70 had a predicted decrease in viral load of 186,984 copies/mL

(95% CI: -543,493 to 169,524 copies/mL; p=0.30), indicating a fitness decrease, while patients with mutations at codon 219 had an increase in viral load of 182,695 copies/mL (95% CI: -211,671 to 577,060 copies/mL; p=0.36). The Stanford HIV drug resistance interpretation algorithm [14] suggests that the combination of mutations at codons 70 and 219 will further decrease susceptibility to all NRTIs. Evidence for an interaction effect was observed in the multivariate model (Coef=-4.58; 95% CI: -7.49 to -1.69; p=0.003), but individual coefficients had a p-value larger than 0.05.

Patients on an NNRTI with mutations at codons 115 or 116 had evidence for a change in viral load, but these findings were based on few patients (n=2 and n=1 respectively). Mutations at codon 184 reduced the viral load by 157,331 copies/mL (95% CI: -313,288 to -1,373; p=0.05) and mutations at codon 215 increased the predicted viral load by 77,643 copies/mL (95% CI: 6,383 to 148,902; p=0.03). There was limited evidence that mutations at codon 219, increased viral load (p=0.06), and there was no evidence for an effect at codon 67 after adjusting for other variables (p=0.90). An interaction between mutations at codons 184 and 215 was not tested for because there was no prior evidence. NNRTI mutations K103N and V108I both influenced viral load. K103N increased viral load by 51,762 copies/mL (95% CI: -3,292 to 106,817; p=0.07) and V108I decreased viral load by 39,983 copies (95% CI: -73,973 to -5,993; p=0.02).

4.4 Discussion

4.4.1 Predictors of HIV-1 drug resistance

This analysis has shown that patients who remain on regimens which they are virologically failing, for a median of 108 weeks after virological failure was first detected, typically develop extensive HIV-1 drug resistance. NRTI resistance was nearly universal, and a lack of any HIV-1 drug resistance mutations (approximately 9% of patients) is likely to reflect a lack of adherence. There was no difference in the occurrence or extent of HIV-1 drug resistance by monitoring strategy, suggesting that routine CD4 cell

count monitoring offers no improvement over clinically driven monitoring for reducing HIV-1 drug resistance. However, there was evidence that reducing the time spent with virological failure decreased the number of mutations detected. This suggests that if virological monitoring lead to earlier treatment switches then HIV-1 drug resistance would be reduced.

4.4.1.1 Duration of virological failure

The time since virological failure was first detected was a predictive variable for the presence of NRTI (Multivariate OR=1.70 per 48 weeks) and NNRTI resistance (Multivariate OR=1.34 per 48 weeks) as well as the number of NRTI mutations (Multivariate RR=1.12 per 48 weeks).

Sigaloff et al. [144] found that two to three years of first-line antiretroviral therapy use increased the odds of two or more TAMs by 190% compared to less than two years of antiretroviral therapy. Similarly, the odds of NRTI cross-resistance were increased by 99%, while more than three years of antiretroviral therapy use increased the odds of two or more TAMs by 337% and the odds of NRTI cross-resistance by 295%. Jiamsakul et al. [148] demonstrated that patients with more than two years since antiretroviral therapy initiation had a multivariate odds ratio of 6.25 (95% CI: 2.39-16.36) for multi-NRTI resistance mutations. Sigaloff et al. [158] concluded that 0.07 TAMs accumulated per month in patients with continued virological failure. In contrast, Pinoges et al. [147] found that there was no association between HIV-1 drug resistance and the duration of antiretroviral therapy.

Previous studies have typically examined the duration of antiretroviral therapy use. This analysis was able to directly examine the time spent with virological failure in patients where virological status was unknown at the time. This analysis is limited by the 48-week viral load measurement intervals, so the duration of virological failure could be inaccurate. Nonetheless, monitoring patients immunologically or clinically compared to virologically is likely to increase the duration of virological failure and this analysis has shown that this leads to the presence of HIV-1 drug

resistance and the further accumulation of TAMs. Chapter 5 will further quantify the rate at which HIV-1 drug resistance mutations develop on a virologically failing regimen using paired HIV resistance test data.

4.4.1.2 Antiretroviral therapy regimen

The use of a triple-NRTI regimen was not a significant predictor of any NRTI or NNRTI resistance (p=0.35 and p=0.32 in univariate analyses respectively) compared to an NRTI-NNRTI regimen, although triple-NRTI did predict an increased number of NRTI mutations (Multivariate RR=0.84; 95% CI: 0.74-0.95). A third of patients on a dual-class regimen containing nevirapine had no NNRTI resistance and patients with NNRTI resistance typically had one or two NNRTI mutations.

Other studies have observed differences in the prevalence of resistance by antiretrovirals used. Sigaloff et al. [144] found that using zidovudine compared to stavudine increase the odds of two or more TAMs being detected at the time of treatment switch by 3.49 (95% CI: 1.46-8.32) and the odds of NRTI cross-resistance by 2.66 (95% CI: 1.12-6.28). Tenofovir use was shown to increase the odds of NRTI cross-resistance by 5.00 (95% CI: 1.67-14.94) although had no impact on the odds of two or more TAMs. Pinoges et al. [147] observed that more patients who had received zidovudine had at least one TAM (48%) compared to stavudine (21%).

In this analysis, 91% of patients on an NRTI-NNRTI first-line regimen had drug resistance, 87% had M184I/V, 72% had one or more TAMs, and 58% had more than one TAM. Sigaloff et al. [144] observed that 88% of patients with virological failure at treatment switch had drug resistance, 82% had M184I/V, 55% had one or more TAMs and 38% had more than one TAM. After 12 months of antiretroviral therapy, Hamers et al. [145] found that 70% of those with virological failure had drug resistance, 49% had NRTI and NNRTI resistance, 54% had M184V, and 9% had at least one TAM.

In conclusion, the level of HIV-1 drug resistance observed in DART is similar to the level found in these other studies, but more TAMs were observed. This is potentially a result of a longer period on virologically failing regimens and the use of a zidovudine-lamivudine backbone compared to a tenofovir or stavudine-containing NRTI backbone.

4.4.1.3 Pre-ART CD4 Cell Count

Patients with a higher pre-antiretroviral therapy CD4 cell count were less likely to develop NRTI resistance (Multivariate OR=0.29 per 100 cells/mm^3) and to have fewer NRTI mutations (Multivariate RR=0.92 per 100 cells/mm^3). There was no evidence of an association with NNRTI resistance in this analysis (OR=0.60; 95% CI: 0.31-1.18).

This provides further support for guidelines which suggest that antiretroviral therapy should be started at higher baseline CD4 cell counts. However, all patients in DART started antiretroviral therapy with a CD4 cell count less than 200 cells/mm^3, so these findings might not be generalisable at baseline counts higher than this. These results agree with those from the TenoRes study [146] where patients in Southern Africa with a baseline CD4 cell count less than 100 cells/mm^3 had an odds ratio of 1.39 (1.00-1.93) for tenofovir resistance. However, the TenoRes study also found that a baseline CD4 cell count less than 100 cells/mm^3 increased the odds of NNRTI resistance (OR=1.27; 95% CI: 1.09-1.48).

4.4.2 Active second-line antiretroviral therapy regimens

Patients who switched from a triple-NRTI regimen to one including an NNRTI had higher levels of resistance than patients who remained on triple-NRTI regimens. This suggests that NNRTIs should be used cautiously with potentially compromised NRTI backbones due to a lower genetic barrier to HIV-1 drug resistance.

The EARNEST trial [159] evaluated second-line antiretroviral therapy regimens in patients who had advanced treatment failure (42% had a viral

load greater than or equal to 100,000 copies/mL, and 62% had a CD4 cell count less than 100 cells/mm^3) in sub-Saharan Africa. Patients were randomised to receive either boosted PI (lopinavir/ritonavir) with NRTIs (two-thirds selected by clinicians), boosted PI with raltegravir (an integrase inhibitor) or a boosted PI as monotherapy (after a twelve week induction period with raltegravir). Despite extensive NRTI resistance (59% had no predicted active NRTIs, and 33% had one predicted active NRTI), the proportion of patients with virological suppression (less than 50 copies/mL) at week 144 was similar in the arm containing NRTIs (76% in those with zero or one active NRTIs) compared to the arm containing integrase inhibitors (72%; p=0.28) and superior to the monotherapy arm (44%; p<0.001). The EARNEST trial demonstrates that despite extensive NRTI drug resistance an NRTI-sparing second-line regimen may not offer improved virological durability. If NRTIs are used as part of a second-line regimen, then these results suggest that tenofovir and zidovudine are likely to be the NRTIs which retain the most susceptibility.

Patients who used first-line NRTI-NNRTI regimens were likely to develop NNRTI mutations (66%). Due to the cross-over in resistance profile between mutations which developed on nevirapine compared to efavirenz, any future NNRTI use should include etravirine.

4.4.3 Individual mutation prevalence

The K65R and K70R mutations were observed in 17% and 23% of patients in DART who received a triple-NRTI regimen including tenofovir. In comparison, 20% of patients in Europe and 50% of patients in sub-Saharan Africa in the TenoRes Study [146] were observed to have K65E/R or K70E/G/Q at virological failure and 12% had K65R in the analysis by Hamers et al. [145]. This discrepancy could be a result of the use of zidovudine and lamivudine alongside tenofovir. This may lead to the development of TAMs rather than K65R, which in this analysis typically occurred with few other mutations (Figure 31). Extended periods on

virologically failing regimens lead to an increased accumulation of additional TAMs as opposed to K65R. This is thought to be due to an antagonistic relationship between these mutations [160]. The order mutations develop in will be examined in Chapter 5.

4.4.4 Mutation prevalence by HIV-1 Subtype

In DART, there was no significant difference in the prevalence of K65R by subtype C compared to non-B, non-C subtypes (20% versus 15%; p-value=0.15). However, while patients received standardised care, this comparison is limited because subtype C virus was almost exclusively observed in Zimbabwe. It has been suggested that subtype C virus may be more likely to develop the K65R mutation due to a different template sequence in this region, which leads to greater transcription errors [161]. Other studies have found a difference; The TenoRes Study Group [146] evaluated the prevalence of K65R/N and K70E/G/Q by HIV-1 subtype, and tenofovir resistance was greater in patients with subtype C compared with non-C, non-B infections (OR=2.44; 95% CI: 1.66-3.59). The TenoRes analysis was restricted to patients in Western Europe, due to the consistency of treatment in this region. Hamers et al. [145] observed that K65R was detected more frequently in patients with subtype C virus compared to subtypes A or D (p=0.05), although evidence was limited after adjusting for differences in use of tenofovir (p=0.68).

Unlike Hamers et al. [145], we observed several other differences in the proportion of mutations by HIV-1 subtype. In agreement with some previous studies [162, 163], TAMs such as L210W and T215F were less likely to occur in patients with subtype C virus. Unlike other studies, the DART data also suggested that the M184V mutation was less common in patients with subtype C virus and K70R was less likely to occur in patients with subtype D virus. These findings are previously unreported and are likely to be either a false positive or due to the strong correlation between subtype C and Zimbabwe. M184V is an extensively studied major mutation, so it is

unlikely that a subtype effect could exist which has not been previously shown.

In this analysis, the V106M mutation was exclusively observed in three patients with subtype C virus. This is supported by previous studies where V106M has been identified as a subtype-C specific mutation which reduces susceptible to both nevirapine and efavirenz [164]. Subtype C patients typically have RNA GTG at codon 106, whereas subtypes A, B and D have RNA GTA. For a valine to methionine (ATG) mutation to occur, two transitions are required for subtype A, B and D compared to just one for subtype C.

4.4.5 Relationship between mutations and viral load

Patients receiving virological monitoring has confounded previous analyses [165-167] examining the relationship between specific mutations and viral load. Specifically, patients who are maintained on regimens which clinicians know are virologically failing and who have HIV-1 drug resistance may be highly selective compared to populations in low-income countries without virological monitoring. Nonetheless, these studies support the findings of Section 4.3.9 and are outlined below.

In general, an increased number of resistance mutations have been associated with a reduced viral load. Chin et al. [165] observed that the mean sum of the average resistance scores was lower in patients with a viral load greater than or equal to 100,000 copies/mL compared to less than 100,000 copies/mL ($p=0.03$), although there was no evidence of individual mutations having a detectable effect. De Mendoza et al. [167] concluded that patients with drug-resistant viruses had significantly lower median viral load values than those carrying wild-type virus ($p<0.0001$). Machouf et al. [166] demonstrated a more nuanced non-linear "U-shaped" relationship, where up to five mutations reduced viral load by 0.8 $\log_{10}$ copies/mL compared to wild-type, but where six or more mutations lead to an increase in viral load.

This analysis identified specific mutations association with viral load. M184V was associated with a reduced viral load in patients on NRTI-NNRTI regimens (Coef=-1.32; p=0.002) and had no influence on viral load in patients on triple-NRTI regimens containing tenofovir (Coef=-0.41; p=0.25) or abacavir (Coef=0.87; p=0.57). de Mendoza et al. [167] observed that patients with the M184V mutation had a median viral load of 3.8 $\log_{10}$ copies/mL compared to 4.3 $\log_{10}$ copies/mL in patients with wild-type virus (p<0.0001). Similarly, Machouf et al. [166] concluded that M184V/I was associated with a 0.35 $\log_{10}$ copies/mL lower viral load (p<0.001). Machouf et al. [166] also observed that K70R and V108I reduced viral load. This agrees with findings from this analysis for patients on triple-NRTI regimens containing abacavir and NRTI-NNRTI regimens respectively, although was not shown for triple-NRTI regimens containing tenofovir.

Unlike other analyses, an increase in viral load for mutations at codons 67 and 151 in patients on triple-NRTI regimens containing tenofovir, codon 219 in patients on triple-NRTI regimens containing abacavir and at codons 103 and 219 in patients on NRTI-NNRTI regimens was observed. These findings may be a result of the U-shaped relationship reported by Machouf et al. [166] where more heavily mutated virus overcomes viral fitness impairments. Further analyses in datasets without routine virological monitoring are required to rule out chance findings.

The results from Section 4.3.9 are the first to have been obtained from patients without routine virological monitoring. This analysis benefits from being conducted in patients with one or more mutation, so differences in viral load are unlikely to be a result of differences in adherence. Some of the differences in viral load by mutation are remarkable and may reflect the order in which mutations are acquired.

4.4.6 Conclusions

This analysis has demonstrated that patients may accumulate mutations on a virologically failing regimen, leading to extensive HIV-1 drug resistance in the absence of virological monitoring. The inclusion of CD4 cell count monitoring did not reduce the extent or prevalence of resistance in any of the analyses conducted. Time since virological failure was a strong predictor, suggesting that virological monitoring could reduce the prevalence and number of mutations occurring on first-line antiretroviral therapy. The use of a zidovudine and lamivudine backbone typically resulted in an accumulation of TAMs. K65R mutations were less frequent, even if tenofovir was included as part of a triple-NRTI regimen. For patients who received a first-line antiretroviral therapy regimen of zidovudine, lamivudine and nevirapine, a second-line antiretroviral therapy regimen consisting of tenofovir, lamivudine (or emtricitabine) and a ritonavir-boosted protease inhibitor will be the most potent, supporting WHO guidelines [27].

5 The development of HIV-1 drug resistance mutations

5.1 Introduction

Prolonged use of antiretroviral therapy with replicating viremia leads to an accumulation of HIV-1 drug resistance mutations. The specific mutations which develop are influenced by the combinations of antiretrovirals used, the order in which mutations occur, interactions between mutations, random genetic variation and HIV-1 subtype. Research on HIV-1 drug resistance has focused on data from high-income settings due to the availability of routinely conducted HIV-1 drug resistance tests. However, patients in high-income settings also receive regular virological monitoring; therefore virological failure is typically detected earlier than in low-income settings. Data is more limited from low-income settings, yet highly relevant for the future of antiretroviral therapy in these regions.

5.1.1 Literature search

A PubMed search was conducted on the 31st April 2016 to find studies examining how HIV-1 drug resistance develops. This search was not restricted to low-income settings and reviewed all English language research. The PubMed search used the terms (HIV AND (resistance OR mutation) AND (accumulation[Title] OR development[Title] OR pathway*[Title]) and found 699 publications. These publications were filtered using a relevance feedback tool called RefMed [168]. This tool learns from a user's rankings which publications from a search are the most relevant and orders PubMed search results. This was found to be necessary, since this PubMed search returned hundreds of articles which were not relevant. As an example, "Lipodystrophy in HIV-1-positive patients is associated with insulin resistance in multiple metabolic pathways" by van der Valk et al. [169]. This fulfilled the search criteria but is irrelevant for examining the development of HIV-1 drug resistance mutations.

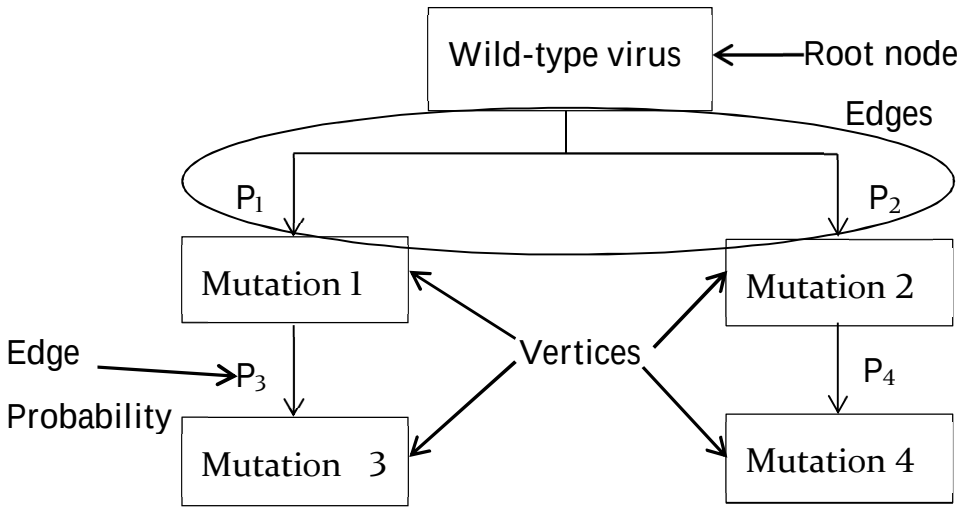
After filtering papers using RefMed, 49 relevant publications were identified (Appendix F) and described in the following sections.

5.1.2 Mutagenic Tree Methodology

5.1.2.1 Single mutagenic tree model

A HIV-1 drug resistance test gives a set of mutations, which occurred in an unknown order. The ordering is not random, since certain mutations are likely to occur directly from wild-type virus and increase the probability of subsequent mutations. Directed graphs were initially used in HIV research to represent the order in which mutations developed on zidovudine monotherapy [170]. Mutagenic trees formalise this approach with "vertices", or "nodes", representing binary events (Figure 37), such as a mutation occurring. "Edges" represent conditional events, where a mutation cannot occur unless the predecessor mutation has also taken place. Probabilities attached to each edge display the conditional probability of a mutation given the parent mutation has occurred.

Figure 37: Example mutagenic tree



Bayesian tree models were introduced by Desper et al. [171] to describe tumour progression in cross-sectional data. Mutagenic or Bayesian tree models are a specialised version of the more general Bayesian network models. The former have the added constraints that each mutation has only one entering edge, that mutations are irreversible and that there is a root node (wild-type) with no entering edges. These constaints ensure that the number of parameters in the model is equal to the number of mutations, whereas a Bayesian network model has an exponential relationship between the number of mutations and parameters.

Succinctly, trees are constructed by initially calculating weights for all possible combinations of pairs from the total, n, mutations:

$$w(j,k) = \log(Pr(j,k)) - \log(Pr(j) + Pr(k)) - \log(Pr(k))$$

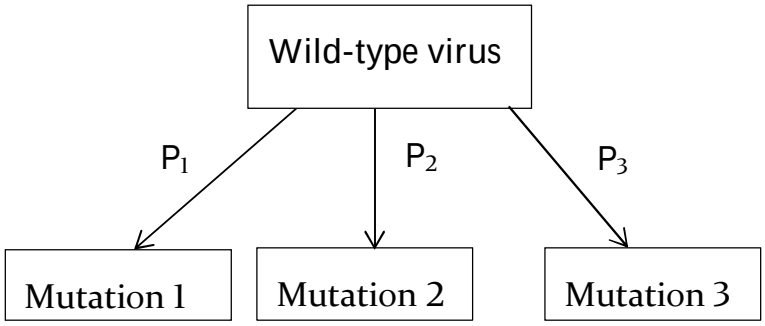
where Pr(j) is the marginal probability of mutation j, j=1...n, Pr(k) is the marginal probability of mutation k, k=1...n, and Pr(j,k) is the joint probability of events j and k, j=1...n, k=1...n, j≠k. This initial choice gives large weights for mutations j and k which frequently occur together, $\frac{Pr(j,k)}{Pr(j)\,Pr(k)}$, and a preference for an edge from j to k if Pr(j) > Pr(k) by including $\frac{Pr(j)}{Pr(j)+Pr(k)}$. Multiplied these give $\frac{Pr(j,k)}{(Pr(j)+Pr(k))Pr(k)}$ and the logarithm of this is a monotone increasing function. Desper et al. [171] show that by maximising the sum of the edge weights that this choice of initial weights finds the optimal tree. Despite the computational ease of constructing mutagenic trees there are limitations. For instance, only a proportion of the mutational patterns observed in a dataset can be represented by a single tree. This may lead to a lack of fit if the underlying biological mechanism is more complicated than the model or has more natural variation.

5.1.2.2 Mixture mutagenic tree models

To overcome this limitation, Beerenwinkel et al. [172] introduced mixture models. In these, there are multiple (K) trees with an associated probability

of each occurring. A star tree is also included, essentially a tree which assumes all mutations other than the root are independent (Figure 38). This ensures that all combinations of mutations are possible and occur with non-zero probability. Beerenwinkel et al. [172] discuss the methodology for fitting mixture models in detail. They are implemented through an expectation-maximisation (EM)-like algorithm which maximises the log-likelihood of the data. Beerenwinkel et al. developed the software mtreemix, run under Linux or Unix, to fit these models [173]. This method uses cross-validation to determine the number of trees which give the most parsimonious model, while still being within a sufficient margin of the maximum mean log-likelihood.

Figure 38: Example of a star tree



5.1.2.3 Longitudinal mutagenic tree models

Montazeri et al. [174] analyse cross-sectional data but incorporate the additional information of time on antiretroviral therapy to estimate the rate at which mutations occurred. However, while their Bayesian Network model controlled for the time a measurement was taken, it did not account for data where patients had multiple genotypes conducted at multiple time points. Cross-sectional approaches need to be adapted for longitudinal mutagenic data. Ignoring the correlation between genotypes performed on the same patient in a model would overestimate the probability of earlier mutations occurring.

Beerenwinkel and Drton [175] expanded upon the cross-sectional approach of mutagenic trees (described at the start of Section 5.1.2 above) by

utilising a hidden Markov model to analyse longitudinal clonal data. They also presented a Perl program, mtreehmm, for fitting these models. This method uses the additional data from longitudinal measurements to determine which ordering of mutations is the most likely. Firstly, the topology of the mutagenic tree is calculated treating the longitudinal data as cross-sectional and fitting a mutagenic tree. Secondly, the longitudinal element is fitted assuming that mutations occur as independent Poisson processes with parameter $\lambda_m>0$. The probability that a mutation, m, occurs providing that the parent mutation of m, pa(m), has already happened, during a time period of length Δt is:

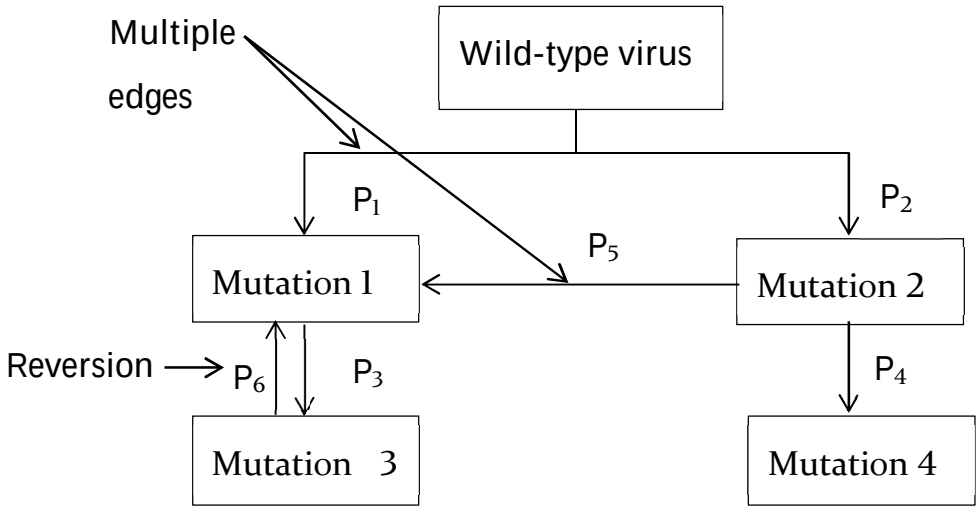
$$Pr\left(X_m = 1 \middle| X_{pa(m)} = 1\right) = 1 - e^{-\lambda_m \Delta t}$$

Beerenwinkel and Drton [175] applied this method to longitudinal clonal data to evaluate resistance to efavirenz in 163 patients with 3,350 clones at a median of three time points. They identified two distinct pathways, involving mutation K103N and G190S respectively, and calculated that K103N had an expected waiting time (defined as $1/\lambda_m$, the mean time of mutation m occurring) of 19 weeks compared to 478 weeks for G190S. The naïve cross-sectional approach consistently overestimated the progression rate of mutations.

5.1.2.4 Bayesian network models

Bayesian network models do not include the restrictions of mutagenic tree models. In these models, mutations are no longer considered permanent and may be lost. Furthermore, mutations may have more than one edge entering them, allowing for associations with multiple parent mutations. Bayesian network models are often built using the B-course software [176]. The lack of restrictions can make Bayesian network models challenging to interpret. As a case in point, the model presented by Theys et al. [177] includes 100 mutations and 230 edges, so key associations can be difficult to identify.

Figure 39: Example Bayesian network model



From the methods outlined in this section, the approach of Beerenwinkel and Drton [175] was felt to be the most suitable for the DART data. This method uses the multiple genotypes from within patients and the time after antiretroviral therapy initiation at which genotypes were conducted. Other approaches discussed were either not able to correctly account for the correlation between genotypes on the same patient or ignored the information provided by the time of measurement.

5.1.3 Development of HIV-1 drug resistance mutations

This section summarises key research from the literature review which analysed the development of resistance without using a mutagenic tree or a Bayesian network model.

Kuritzkes et al. [178] utilised data from 120 patients in the US ACTG 306 and 370 trials and compared the development of resistance on zidovudine + lamivudine, stavudine + lamivudine and stavudine monotherapy arms. HIV-1 genotyping was performed if HIV viral load was greater than 500 copies/mL at weeks 0, 24, 48 or 72. After 72 weeks of therapy, TAMS were detected at similar frequencies for both thymidine analogues (50% on zidovudine and 45% on stavudine; p=0.79). The K70R and T215Y

mutations were observed in a similar proportion of patients (25% and 12% respectively), but M41L was observed more frequently in patients treated with zidovudine (22% versus 6%; p=0.032). Despite having repeated measurements within patients, the authors did not calculate the rate of mutational development.

Barth et al. [179] investigated the accumulation of HIV-1 drug resistance mutations in a South African cohort of 836 patients on a dual-class regimen containing an NNRTI. Patients received biannual monitoring, and 145 of 642 (23%) were on a regimen they were virologically failing (viral load greater than 1,000 copies/mL) after previously achieving virological suppression. Of these patients, 58 (40%) remained on the same regimen with continued virological failure six to twelve months later. Genotyping was conducted at the time virological failure was first detected and at either six months (n=18) or twelve months (n=8) for 26 patients; 18 patients were excluded because no blood sample was available and 14 had a gap between measurements longer than twelve months. In general, there was a 54% increase in major IAS mutations and this was predominantly driven by an increase in NRTI resistance (88% increase) rather than NNRTI resistance (36% increase). The largest increase was in the number of TAMs (250% increase), and these were more frequently observed in patients who received zidovudine compared to stavudine (57% compared to 8%; p<0.01). The M184V and K103N mutations were the most frequently observed (73% and 65% at the second time point respectively) but the proportional increase in these mutations was lower (22% and 42% respectively). The proportion of patients on zidovudine and stavudine with TAMs differed to the previous study; this could be a result of using an NNRTI as part of the treatment regimen.

Cozzi-Lepri et al. [180] used data from 339 patients in the EuroSIDA study and investigated the accumulation of TAMs on predominantly NRTI-NNRTI regimens containing either stavudine or zidovudine. Pairs of

resistance tests (n=603) were analysed while patients remained virologically failing (viral load greater than 500 copies/mL) on the same regimen. Patients had virologically failed a median of four antiretrovirals before their first resistance test. There was a median (range) of 6 (1-89) months between HIV-1 drug resistance tests. 126 TAMs accumulated during 548 person-years of follow-up, an accumulation rate of 23.0 per year (95% CI: 19.5-26.7 per year). The calculated mutation accumulation rate assumed a constant rate over time. Mutations from the TAM-1 pathway (M41L, L210W and T215F/Y) accumulated approximately twofold faster than mutations from the TAM-2 pathway (D67N, 69 insertion, K70R and K219E/Q). The authors estimated that 13.6 (95% CI: 9.2-19.0) M41L mutations, 13.1 (8.8-18.5) T215Y mutations and 9.3 (6.5-12.9) L210W mutations accumulated per 100 person years. Mutations from the TAM-2 profile were predominantly D67N (7.6 per 100 person-year; 95% CI: 4.9-11.2) and T215F (4.9 per 100 person-years; 95% CI: 3.2-7.3). The authors note that this data may suffer from selection bias, since patients received routine laboratory monitoring yet were maintained on regimens with virological failure.

Cozzi-Lepri et al. [181] established the rate at which NNRTI mutations developed in 227 patients (467 pairs) on dual-class regimens containing either nevirapine or efavirenz from the EuroSIDA study. There was a median (range) of 6 months (1-74) between HIV-1 drug resistance tests. Major IAS NNRTI mutations [182] developed at a rate of 79.6 (65.6-89.8) per 100 person-years. The highest rate of accumulation for an individual mutation occurred for K103N (27.6 mutations per 100 person-years; 95% CI: 20.7-35.5), followed by Y181C (12.2; 95% CI: 8.0-17.7), G190A (9.4; 5.8-14.3) and V108I (6.7; 4.0-10.6). The authors found that mutations accumulated more rapidly during the first six months following virological failure and concluded that NNRTI mutations develop at an average rate at

least threefold higher than TAMs. In this study, just 6% of patients were antiretroviral therapy naïve at the time they initiated the NNRTI.

Lawyer et al. [183] utilised a Cox model and data from 1,495 patients who had 1,981 reverse-transcriptase based episodes of therapy where genotyping was conducted before initiating therapy and also before the end of therapy. The paper does not record whether genotyping guided the choice of antiretroviral therapy or why patients remained on failing regimens. The Cox proportional hazards model was formulated to allow individual pre-existing mutations to alter the hazard of a mutation developing. Mutations known to influence drug susceptibility and codons with mutations in more than five percent of patient at antiretroviral therapy initiation were investigated. Mutations from wild-type were treated as a single variable, irrespective of the specific amino acid change which had occurred. Lawyer et al. presented the results as mutational pathways. The TAM-1 pathway was observed with an association between mutations at codons 215 and 41 (HR=3.23; 95% CI: 2.15-4.85). The TAM-2 pathway was also noted with an association between mutations at codons 67 and 70 (HR=3.52; 95% CI: 2.08-5.98) and codons 70 and 219 (HR=2.68; 95% CI: 1.60-4.47). Similarly, the two TAM pathways were found to inhibit each other as mutations at codon 210 were associated with a tenfold reduction in the risk of mutation at codon 70. Several associations were found between mutations considered to be NRTI-associated and mutations considered to be NNRTI-associated (e.g. 41 →108, 67 →190, 74 →100 and 77 →103).

Boender et al. [184] utilised data from 63 adults and 56 children in sub-Saharan Africa to examine the accumulation of HIV-1 drug resistance mutations on an NNRTI-based first-line antiretroviral therapy. Plasma viral load testing was retrospectively performed and resistance tests were conducted if viral load was greater than 1,000 copies/mL. Samples were available after 12 and 24 months of therapy in all sites and after 36 months in 80% of locations. There was a median of 303 (IQR: 183-365) days

between resistance tests on the same patient. Analyses examined the change in predicted susceptibility between tests, and the rate individual mutations were accumulated per year. On average, 1.45 new drug resistance mutations (0.84 NRTI mutations and 0.62 NNRTI mutations) accumulated per 52 weeks.

5.1.4 Bayesian Network Models

This section summarises some of the key research found in the literature review, outlined in Section 5.1.1, which analysed the development of resistance using either mutagenic trees or Bayesian network models.

Deforche et al. [185] used a Bayesian network model to examine patients on NRTI-NNRTI regimens with a variety of HIV-1 subtypes (approximately a quarter of patients on nevirapine had subtype B virus) to determine the order in which mutations develop. The dataset included 3,837 antiretroviral-naïve patients, of which 462 were treated with an efavirenz-containing regimen and 533 patients were treated with a nevirapine-containing regimen. Unlike a previous analysis conducted on patients on protease inhibitors [186], very few interactions were found between subtype-dependent polymorphisms and NNRTI resistance mutations. Mutations E203K, L228H/R were associated with the TAMs K219E/Q. For resistance to nevirapine, a number of associations between known NNRTI mutations were observed. The K103N pathway was associated with mutations V90I, L100I, K101P, Y108I, Y181C, H221Y, P225H, K238T, and G190A. The G190E/S/A mutations were further associated with K101E/Q, K103N, Y181C, and H221Y. The authors concluded that Bayesian network learning provided useful insights into the simultaneous selection of HIV-1 drug resistance mutations to two separate classes of inhibitors as part of combination therapy.

Theys et al. [177] applied Bayesian network learning to a dataset consisting of 1,124 sequences to determine the order in which resistance develops in patients on a regimen containing zidovudine and lamivudine. They found

that M41L, K70R, M184V and T215F/Y were directly linked to vertices representing drug experience so were likely to be the first resistance mutations to occur. TAM pathways were not found to be exclusive, since a number of patients were observed with combinations of TAM pathway mutations T215Y (TAM-1) and K70R (TAM-2).

Hernandez-Leal et al. [187] used a temporal node Bayesian network model with data from 2,373 patients who had used a regimen containing a protease inhibitor. Ten protease mutations were analysed, and the method was able to confirm known associations between specific protease inhibitors and mutations as well as between mutations, particularly for resistance to lopinavir.

Buendia et al. [188] used a two-phase approach, combining a phylogenetic and a Markov model, to examine mutational pathways to HIV-1 drug resistance. By combining models, this approach was able to include longitudinal clonal sequence data. The model was applied to 120 patients from a phase II efavirenz study. The results were similar to those obtained from a mutagenic tree model but, by modelling the different combinations of mutations as states of a Markov model, the loss of mutations could also be determined. This revealed that sequences with K103N and a mutation other than P225H mutation were more likely to lose this additional mutation than if the patient developed both K103N and P225H. The loss of mutations cannot be determined using the mutagenic tree method.

5.1.5 Mathematics of antiretroviral agents

Soriano et al [189] describe the viral dynamics of HIV in comparison to hepatitis B and C virus and explain how HIV's very rapid viral dynamics and shorter half-life of intracellular virions leads to a faster selection of drug resistance in HIV compared to hepatitis B. Shen et al [190] suggest that the dose-response curve slope has substantial effects on antiviral activity and will generally correlate better with clinical outcomes. They show that different antiretroviral drug classes have characteristic dose-

response curve slopes with NRTIs typically having a slope of approximately 1, "characteristic of non-cooperative reactions", while NNRTIs had a slope of approximately 1.7 and PIs had a slope of 1.8 to 4.5 indicative of more potent antiviral activity.

Vaidya et al [191] use mathematical modelling to describe the relationship between HIV-1 drug resistance, viral load, CD4 cell count and clinical outcomes in patients treated with enfuvirtide, a fusion inhibitor. Mathematical modelling demonstrated that when treatment was interrupted it is outgrowth of drug-resistant virus by previously latent wild-type virus which results in the rapid loss in detectability of HIV-1 drug resistance rather than backwards mutation towards the wild-type strain. In this study, the authors describe how, despite drug resistance, the use of an antiretroviral can lead to increases in CD4 cell count. After a treatment interruption, re-administering the same antiretroviral to patients resulted in the resistant virus rapidly becoming the dominant detectable strain. However, patients who re-received a drug to which they had developed HIV-1 drug resistance were measured to have 35% higher CD4 cell counts after three months compared to patients who had not. This should lead to some clinical benefit and has been shown for a variety of HIV-1 mutations such as D30N, K65R and M184V [192-193].

Rong et al [194] use a mathematical pharmacokinetic model to study the emergence of drug-resistant HIV and support Vaidya et al's findings. In these models, perfect adherence can suppress wild-type virus but drug-resistant variants develop slowly, perhaps in areas of the body lacking adequate drug exposure. In contrast, intermediate adherence can lead to the dominance of drug-resistant HIV within several months. With low levels of adherence, wild-type virus will quickly emerge and there will be a relatively slow increase in drug-resistant viral load.

Mathematical modelling demonstrates how patients in DART with virological failure and extensive drug resistance may have continued to

derive benefit from their antiretroviral regimens. Similarly, they describe how the pharmacokinetics of different antiretroviral drug classes can influence the emergence of HIV-1 drug resistance.

5.1.6 Objective

The rate at which mutations accumulate will help to quantify how delayed diagnosis of virological failure may influence the susceptibility to second-line regimens. Furthermore, understanding the order in which mutations develop will guide the choice of antiretrovirals for first-line and subsequent regimens by maximising their potency. Finally, this work can inform cost-effectiveness analyses evaluating alternative types and frequencies of laboratory monitoring strategies.

The objective of this chapter is to determine the rate at which mutations develop by comparing genotypic testing conducted within patients experiencing persistent virological failure at repeated time points. This analysis will also explore the extent of HIV drug resistance at the time point where virological failure was first detected. Using this rich data, the order and favoured pathways of HIV-1 drug resistance mutations will be determined by antiretroviral regimen.

5.2 Methods

All patients who experienced virological failure greater than 200 copies/mL had HIV-1 drug resistance tests conducted at the time virological failure was first detected as well as the last time-point on first-line antiretroviral therapy. Genotypic testing methodology for selected plasma samples were conducted using the approach previously described in Section 4.2.

5.2.1 Analysis

The accumulation of mutations was examined in patients with two HIV-1 drug resistance tests performed on separate dates. Major NRTI and NNRTI mutations were classified using the IAS-USA 2013 list [149]. Due to the

extended period on a regimen with continued virological failure, there was the potential to examine the development of secondary compensatory mutations. These are mutations which do not directly impact the susceptibility of the virus to antiretrovirals but potentially restore replication fitness of the virus. Compensatory mutations examined due to an *a priori* interest included A62V [195], S68G [195], V75I [196], F77L [196], S162A [197], S163N [198] and Q207D [199]. V60I [197] was considered, but is a natural polymorphism for subtype D and extremely common among subtype A and C virus [200], so was excluded.

The rate of accumulation was calculated for each person as the number of new major mutations divided by the difference in time between tests. The loss of detectable mutations, observed in some patients, was ignored as mutations are assumed to be archived. Predictors for the number of mutations were examined using mixed effect generalised linear regression model with a Poisson link and included time since ART initiation, gender, age, trial monitoring randomisation, centre, trial regimen (either triple-NRTI or NRTI-NNRTI if any exposure to NNRTIs) and HIV-1 subtype as covariates. All variables were assessed for an interaction with study time. A random intercept term for each patient was included in the model.

Susceptibility to antiretrovirals was measured using Stanford HIV drug resistance database scores. Antiretrovirals with low level or intermediate resistance were given a score of 0.5, and where there was high-level resistance were given a score of 0. Antiretrovirals to which a virus was fully susceptible, or to which there was possible low-level resistance, were given a score of 1. The difference in genotypic sensitivity scores (GSSs) [201] summarised the changes in susceptibility between time points.

HIV-1 subtype was determined for each patient by REGA v3. If the classification between the paired tests disagreed, with one genotype classified as a pure HIV-1 subtype and a second as Complex/Recombinant, then a pure subtype determination was used since the virus was likely to be

genetically closest to this (19 patients). If there were different pure HIV-1 subtypes, then a Complex/Recombinant definition was used due to a lack of consensus (2 patients).

The model proposed by Beerenwinkel and Drton [175] (Section 5.1.2 above) was utilised to investigate the development of NRTI mutations among patients receiving triple-NRTI therapy and to determine if this differed by NRTI backbone used. Similarly, the models were used to describe the development of NRTI and NNRTI mutations on NRTI-NNRTI regimens.

Firstly, mutational data from both time points was grouped and used to calculate the structure of a single mutagenic tree using the mtreemix v1.3 software. Secondly, this structure was used with the mtreehmm Perl program to calculate the rate at which mutations occurred. One hundred bootstraps with replacements were applied to each tree to calculate 95% confidence intervals. This software was run on the UCL Legion high performance and high throughput computing cluster due to the large amount of CPU time required. For the abacavir and NRTI-NNRTI regimen mutagenic trees, the mtreehmm program was relatively quick, probably due to the smaller number of patients. A hundred bootstraps could be performed in approximately 24 hours of single core computing time on this cluster. In contrast, the tenofovir mutagenic tree was slow, and 100 bootstraps took approximately 300 days of single core computing time so bootstraps were run in parallel.

Mutations were included in a model if they occurred in more than 3% of patients. Analyses which included rarer mutations created problems during the bootstrap process, since they were sometimes not selected within a bootstrap, leading to undefined values for λ_m. This lead to the exclusion of mutations V75I, F77L, S162A, S163N and Q151M from the model for patients who received triple-NRTI regimens including tenofovir. A62V, K65R, S68G, V75I, F77L, Q151M and S163N were excluded for patients who received triple-NRTI regimens including abacavir. A62V, K65R, V75I,

F77L, Q151M and S163N were excluded for patients who received NRTI-NNRTI regimens.

5.3 Results

5.3.1 Missing tests

Paired HIV-1 resistance tests were available within 24 weeks of target dates for 414 of 609 (68%) patients. For 104 (17%) patients, the last time point on first-line antiretroviral therapy was identical to the first time point with virological failure. Among these were 42 patients who died at the time of virological failure, 27 who switched treatment and 35 who were alive on first-line antiretroviral therapy at the end of the DART trial. These patients were included in Section 5.3.7 but were otherwise not analysed. There were 91 (15%) patients who were missing one or more sequences.

Sequences were available only for the time virological failure was first detected in 24 patients and at the last time point on first-line antiretroviral therapy for 24 patients. Sequences were unavailable at both time points for 12 patients. For 31 patients the last time point on first-line antiretroviral therapy coincided with the first time point with virological failure and a sequence was not available. Overall, 103 genotypes were not available for analysis. The 36 additional missing resistance tests, not discussed in Section 4.3.1, were missing from the first time point due to either an amplification failure (n=32; 89%) or due to a sequence failing phylogenetic quality control (n=4; 11%).

5.3.2 Accumulation of mutations by class

The median (IQR) time between tests among the 414 patients was 132 (72-180) weeks. Patients on triple-NRTI regimens containing tenofovir and NRTI-NNRTI regimens had a median time between tests of 132 (73-194) and 132 (69-166) weeks respectively. Patients on triple-NRTI regimens containing abacavir had a shorter time difference between tests of 104 weeks (74-144; Nonparametric equality-of-medians test p-value=0.05). The

range was 9 to 260 weeks. The first resistance test was conducted after a median (IQR) of 48 (48-96) weeks of antiretroviral therapy. The time after initiating antiretroviral therapy for each pair of test is shown in Figure 40. Figure 41 displays a boxplot of the number of mutations at each test by antiretroviral therapy regimen.

Figure 40: Time of each resistance test

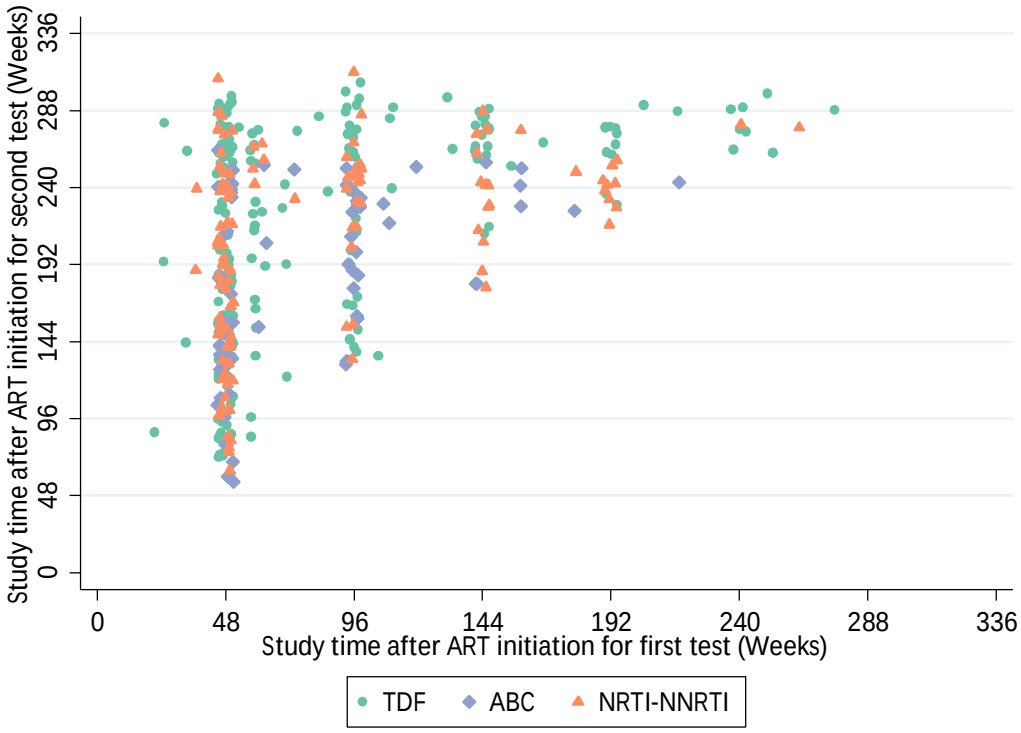
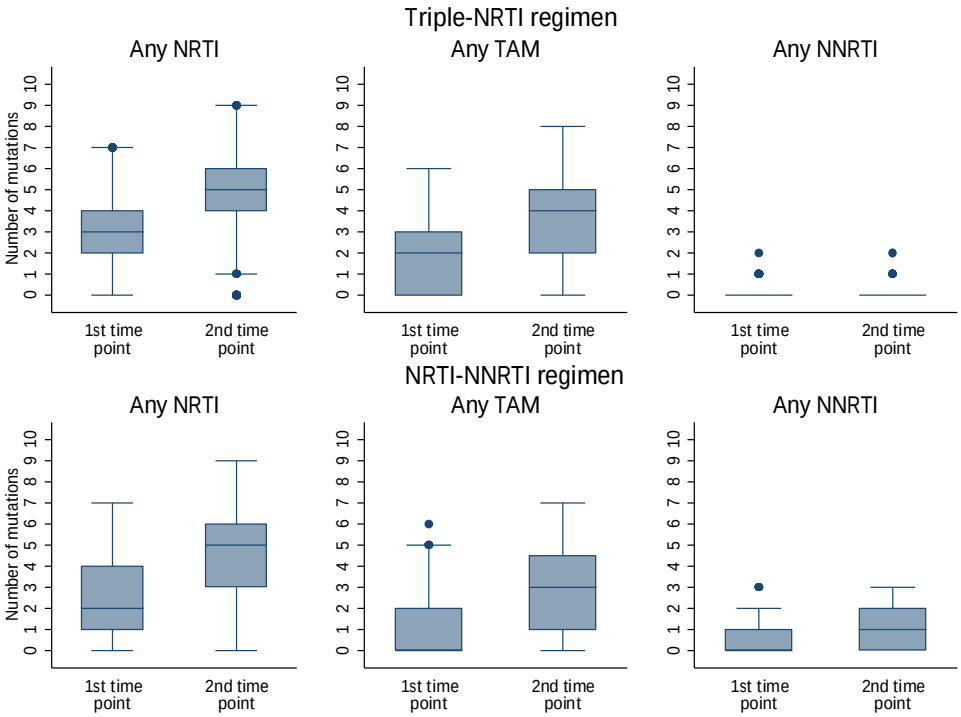


Figure 41: Boxplot of mutations by time point



Overall, NRTI mutations developed at a mean (Standard Deviation) rate of 0.92 (1.11) per 52 weeks and TAMs developed at a mean rate of 0.81 (1.06) per 52 weeks. There was a mean (SD) increase of 1.84 (1.61) NRTI mutations and 1.64 (1.54) TAMs. Among patients on triple-NRTI regimens, NRTI mutations developed at a rate of 0.90 (1.15) per 52 weeks and TAMs developed at a rate of 0.80 (1.09) per 52 weeks; there was a mean increase of 1.80 (1.60) NRTI mutations and 1.62 (1.50) TAMs. Among patients on NRTI-NNRTI regimens, NNRTI mutations developed at a rate of 0.21 (0.40) per 52 weeks; NRTI mutations developed at a rate of 0.96 (1.01) per 52 weeks and TAMs developed at a rate of 0.82 (0.99) per 52 weeks. There was no evidence of a difference in the rate of NRTI mutations and TAMs compared to patients on triple-NRTI regimens (t-test p-value=0.60 and 0.91 respectively). There was a mean increase of 1.93 (1.64) NRTI mutations, 0.44 (0.76) NNRTI mutations and 1.69 (1.64) TAMs.

Figure 42: Boxplot of mutations by time point and subtype

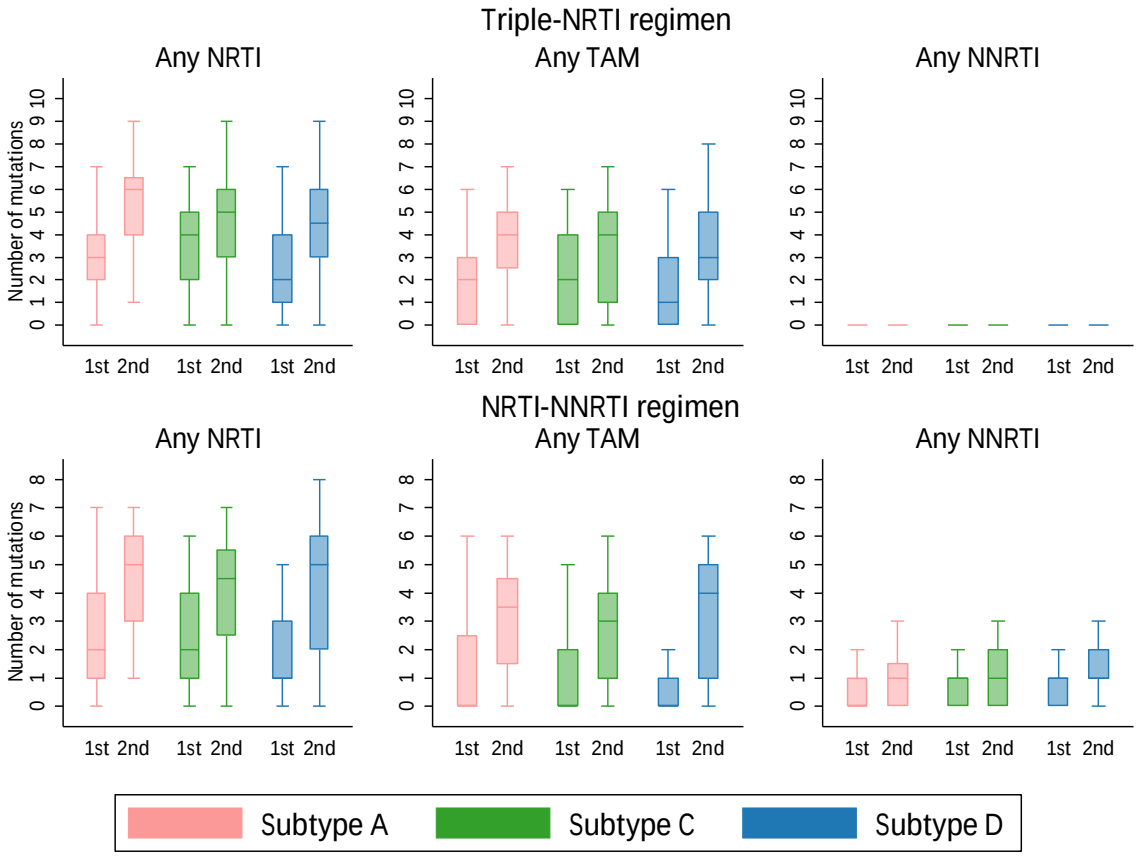


Figure 42 displays this information by HIV-1 subtype. There were 160 (39%), 116 (28%) and 95 (23%) patients with subtype A, C and D virus respectively. Not shown in this image were 42 (10%) patients with complex/recombinant subtypes and 1 (0%) patient with subtype G virus. There was little difference by subtype on the overall number, or the proportional growth, of mutations.

Predictors of the number of TAMs, NRTI and NNRTI mutations were examined using a Poisson mixed effect generalised linear models, and the results are displayed in Table 43. All patients with paired HIV-1 drug resistance tests were analysed. Time since antiretroviral therapy initiation was transformed so that week 48, the earliest date resistance tests were conducted, was the baseline. In these models, only treatment regimen, time since antiretroviral therapy initiation and their interaction had an impact on the number of TAMs, NRTI and NNRTI mutations.

Patients on NRTI-NNRTI regimens had fewer NRTI mutations at week 48 (Coef=-0.29) but a greater increase over time since 48 weeks of antiretroviral therapy compared to triple-NRTI regimens containing tenofovir (Coef=0.05). These results were similar for the number of TAMs. There was not sufficient evidence for a change in the number of NNRTI mutations between tests over time to be significant, probably a result of the smaller number of patients in this group. There was no evidence that HIV-1 subtype influenced the absolute number or change in the number of TAMs, NRTI or NNRTI mutations, supporting the conclusions drawn from Figure 42.

Table 43: Poisson mixed effect generalised linear models for number of mutations

Variable	Number of NRTI mutations (n=414)			Number of NNRTI mutations (n=414)			Number of TAMs (n=414)		
	Coef	95% CI	p-value	Coef	95% CI	p-value	Coef	95% CI	p-value
Fixed effects at week 48									
Constant	0.92	0.50 to 1.35	<0.001	-1.90	-3.45 to -0.36	0.016	0.30	-0.30 to 0.90	0.32
Time since ART (48 weeks)	0.16	0.03 to 0.29	0.016	0.18	-0.35 to 0.71	0.51	0.21	0.05 to 0.39	0.013
Regimen									
Triple-NRTI	0.00	-	0.001	0.00	-	0.51	0.00	-	0.003
NRTI-NNRTI	-0.29	-0.46 to -0.13	-	0.06	-0.13 to 0.26	-	-0.38	-0.63 to -0.13	-
Gender									
Male	0.00	-	0.37	0.00	-	0.18	0.00	-	0.18
Female	-0.07	-0.21 to 0.08	-	-0.37	-0.90 to 0.17	-	-0.14	-0.36 to 0.07	-
Age (10 years)	0.01	-0.00 to 0.01	0.29	-0.02	-0.05 to 0.02	0.29	0.01	-0.01 to 0.02	0.35
Trial Monitoring Randomisation									
LCM	0.00	-	0.83	0.00	-	0.26	0.00	-	0.99
CDM	0.02	-0.13 to 0.16	-	-0.30	-0.82 to 0.22	-	0.00	-0.20 to 0.21	-
HIV-1 Subtype									
A	0.00	-	0.33	0.00	-	0.98	0.00	-	0.22
C	-0.19	-1.62 to 1.24	-	0.46	-1.90 to 2.82	-	-0.58	-2.61 to 1.44	-
D	-0.12	-0.31 to 0.07	-	0.04	-0.64 to 0.71	-	-0.21	-0.51 to 0.09	-
G	-4.15	-15.52 to 7.21	-	-17.51	-384892 to 384857	-	-18.71	-81579 to 81542	-
Complex	0.14	-0.10 to 0.38	-	-0.26	-1.33 to 0.82	-	0.24	-0.10 to 0.59	-
Centre									
Entebbe	0.00	-	0.93	0.00	-	0.38	0.00	-	0.77
Kampala	0.01	-0.15 to 0.18	-	-0.45	-1.10 to 0.20	-	-0.06	-0.09 to 0.04	-
Harare	0.27	-1.16 to 1.70	-	-0.25	-2.59 to 2.09	-	0.61	-0.62 to 0.50	-

Interaction with time since week 48									
Regimen									
Triple-NRTI	0.00	-	0.001	0.00	-	0.51	0.00	-	0.11
NRTI-NNRTI	0.05	-0.00 to 0.10	-	0.06	-0.13 to 0.26	-	0.06	-0.01 to 0.13	-
Gender									
Male	0.00	-	0.77	0.00	-	0.71	0.00	-	0.62
Female	0.01	-0.04 to 0.05	-	0.03	-0.15 to 0.22	-	0.02	-0.04 to 0.08	-
Age (10 years)	-0.00	-0.00 to 0.00	0.62	-0.01	-0.02 to 0.01	0.33	-0.00	-0.00 to 0.00	0.84
Trial Monitoring Randomisation									
LCM	0.00	-	0.56	0.00	-	0.41	0.00	-	0.45
CDM	-0.01	-0.06 to 0.03	-	0.07	-0.10 to 0.24	-	-0.02	-0.08 to 0.04	-
HIV-1 Subtype									
A	0.00	-	0.88	0.00	-	0.80	0.00	-	0.80
C	-0.11	-0.48 to 0.26	-	-0.19	-0.97 to 0.59	-	-0.03	-0.59 to 0.53	-
D	0.01	-0.05 to 0.07	-	0.09	-0.13 to 0.31	-	0.02	-0.06 to 0.10	-
G	0.50	-1.92 to 2.92	-	-0.14	-85062 to 85061	-	-0.19	-18043 to 18043	-
Complex	-0.02	-0.10 to 0.05	-	0.08	-0.23 to 0.39	-	-0.04	-0.13 to 0.05	-
Centre									
Entebbe	0.00	-	0.75	0.00	-	0.79	0.00	-	0.79
Kampala	-0.02	-0.07 to 0.03	-	-0.02	-0.09 to 0.04	-	-0.02	-0.09 to 0.04	-
Harare	0.06	-0.31 to 0.42	-	-0.06	-0.62 to 0.50	-	-0.06	-0.62 to 0.50	-

5.3.3 Accumulation of individual mutations

Table 44 to Table 46 display the overall number and proportion of patients with each mutation at the first and second time point by antiretroviral regimen. The number and proportion of patients who had a mutation at the first time point in whom this was undetectable at the second time point ("lost") are shown, and this is discussed further in Section 5.3.4. The individual mean rate of each mutation per year and the standard deviation (SD) is calculated based on the number of new mutations gained, treating mutations no longer detectable as still present.

Table 44: Overall accumulation and persistence of mutations

Mutation	# at 1st time point	# who gained mutation	# who lost mutation[5]	# at 2nd time point	Accumulation rate (new muts/year) Mean	SD
NRTI Mutations						
M41L	132 (32%)	143 (34%)	9 (7%)	266 (64%)	0.17	0.39
A62V	11 (3%)	12 (3%)	6 (55%)	17 (4%)	0.01	0.09
K65R	80 (19%)	3 (1%)	25 (31%)	58 (14%)	0.00	0.04
D67N	173 (42%)	77 (19%)	18 (10%)	232 (56%)	0.08	0.22
K70E	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.00	0.00
K70R	166 (40%)	63 (15%)	24 (14%)	205 (49%)	0.08	0.24
L74V	1 (0%)	2 (0%)	0 (0%)	3 (1%)	0.00	0.04
V75I	3 (1%)	13 (3%)	1 (33%)	15 (4%)	0.03	0.30
Y115F	16 (4%)	2 (0%)	9 (56%)	9 (2%)	0.00	0.03
F116Y	2 (0%)	7 (2%)	0 (0%)	9 (2%)	0.01	0.09
Q151M	4 (1%)	4 (1%)	0 (0%)	8 (2%)	0.00	0.04
M184I	1 (0%)	4 (1%)	0 (0%)	5 (1%)	0.00	0.04
M184V	334 (80%)	35 (8%)	14 (4%)	365 (88%)	0.04	0.17
L210W	28 (7%)	98 (24%)	2 (7%)	124 (30%)	0.12	0.29
T215F	50 (12%)	79 (19%)	19 (38%)	110 (27%)	0.09	0.30
T215Y	82 (20%)	86 (21%)	6 (7%)	162 (39%)	0.11	0.34
K219E	35 (8%)	69 (17%)	5 (14%)	99 (24%)	0.08	0.23
K219Q	60 (17%)	66 (16%)	19 (32%)	107 (26%)	0.08	0.22
NNRTI Mutations						
L100I	0 (0%)	4 (1%)	0 (0%)	4 (1%)	0.00	0.04
K103N	24 (6%)	19 (5%)	4 (17%)	39 (9%)	0.02	0.13
V106A	4 (1%)	1 (0%)	3 (75%)	2 (0%)	0.01	0.28
V106M	4 (1%)	1 (0%)	2 (50%)	3 (1%)	0.00	0.01
V108I	7 (2%)	17 (4%)	2 (29%)	22 (5%)	0.03	0.18
Y181C	25 (6%)	5 (15)	3 (12%)	27 (7%)	0.00	0.04
Y188C	1 (0%)	0 (0%)	1 (100%)	0 (0%)	0.00	0.00
G190A	16 (4%)	10 (2%)	5 (31%)	21 (5%)	0.01	0.08
G190S	2 (0%)	4 (1%)	0 (0%)	6 (1%)	0.00	0.05
P225H	0 (0%)	4 (1%)	0 (0%)	4 (1%)	0.00	0.04

[5] Percentage is of those with a mutation at the first time point, other percentages are of total.

Table 45: Accumulation and persistence of mutations on triple-NRTI regimens

Mutation	# at 1st time point	# who gained mutation	# who lost mutation[6]	# at 2nd time point	Accumulation rate (new muts/year) Mean	SD
NRTI Mutations						
M41L	99 (33%)	113 (38%)	5 (5%)	207 (69%)	0.18	0.43
A62V	7 (2%)	9 (3%)	3 (43%)	13 (4%)	0.01	0.09
K65R	62 (21%)	0 (0%)	19 (31%)	43 (14%)	0.00	0.00
D67N	137 (46%)	51 (17%)	14 (10%)	174 (58%)	0.07	0.20
K70E	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.00	0.00
K70R	137 (46%)	34 (11%)	22 (16%)	149 (50%)	0.06	0.20
L74V	1 (0%)	0 (0%)	0 (0%)	1 (0%)	0.00	0.00
V75I	2 (1%)	10 (3%)	0 (0%)	12 (4%)	0.03	0.34
Y115F	13 (4%)	2 (1%)	8 (62%)	7 (2%)	0.00	0.03
F116Y	2 (1%)	6 (2%)	0 (0%)	8 (3%)	0.01	0.10
Q151M	3 (1%)	4 (1%)	0 (0%)	7 (2%)	0.01	0.05
M184I	1 (0%)	0 (0%)	0 (0%)	1 (0%)	0.00	0.00
M184V	246 (82%)	23 (8%)	11 (4%)	258 (86%)	0.03	0.12
L210W	23 (8%)	78 (26%)	1 (4%)	100 (33%)	0.14	0.31
T215F	41 (14%)	55 (18%)	18 (44%)	78 (26%)	0.09	0.33
T215Y	63 (21%)	65 (22%)	4 (6%)	124 (41%)	0.12	0.38
K219E	27 (9%)	46 (15%)	2 (7%)	71 (24%)	0.07	0.22
K219Q	51 (17%)	43 (14%)	15 (29%)	79 (26%)	0.07	0.19
NNRTI Mutations						
L100I	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.00	0.00
K103N	7 (2%)	1 (0%)	0 (0%)	8 (3%)	0.00	0.01
V106A	0 (0%)	1 (0%)	0 (0%)	1 (0%)	0.02	0.33
V106M	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.00	0.00
V108I	3 (1%)	7 (2%)	2 (67%)	8 (3%)	0.02	0.19
Y181C	1 (0%)	1 (0%)	1 (100%	1 (0%)	0.00	0.02
Y188C	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.00	0.00
G190A	3 (1%)	3 (1%)	0 (0%)	6 (2%)	0.00	0.04
G190S	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.00	0.00
P225H	0 (0%)	1 (0%)	0 (0%)	1 (0%)	0.00	0.02

[6] Percentage is of those with a mutation at the first time point, other percentages are of total.

Table 46: Accumulation and persistence of mutations on NRTI-NNRTI regimens

Mutation	# at 1st time point	# who gained mutation	# who lost mutation[7]	# at 2nd time point	Accumulation rate (new muts/year) Mean	SD
NRTI Mutations						
M41L	33 (28%)	30 (26%)	4 (12%)	59 (51%)	0.12	0.24
A62V	4 (3%)	3 (3%)	3 (75%)	4 (3%)	0.01	0.10
K65R	18 (16%)	3 (3%)	6 (33%)	15 (13%)	0.01	0.08
D67N	36 (31%)	26 (22%)	4 (11%)	58 (50%)	0.10	0.24
K70E	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.00	0.00
K70R	29 (25%)	29 (25%)	2 (7%)	56 (48%)	0.13	0.32
L74V	0 (0%)	2 (2%)	0 (0%)	2 (2%)	0.01	0.08
V75I	1 (1%)	3 (3%)	1 (100%)	3 (3%)	0.02	0.13
Y115F	3 (3%)	0 (0%)	1 (33%)	2 (2%)	0.00	0.00
F116Y	0 (0%)	1 (1%)	0 (0%)	1 (1%)	0.01	0.08
Q151M	1 (1%)	0 (0%)	0 (0%)	1 (1%)	0.00	0.00
M184I	0 (0%)	4 (3%)	0 (0%)	4 (3%)	0.01	0.07
M184V	98 (84%)	12 (10%)	3 (3%)	107 (92%)	0.07	0.24
L210W	5 (4%)	20 (17%)	1 (20%)	24 (21%)	0.09	0.23
T215F	9 (8%)	24 (21%)	1 (11%)	32 (28%)	0.09	0.19
T215Y	19 (16%)	21 (18%)	2 (11%)	38 (33%)	0.08	0.20
K219E	8 (7%)	23 (20%)	3 (38%)	28 (24%)	0.10	0.26
K219Q	9 (8%)	23 (20%)	4 (44%)	28 (24%)	0.10	0.27
NNRTI Mutations						
L100I	0 (0%)	4 (3%)	0 (0%)	4 (3%)	0.01	0.07
K103N	17 (15%)	18 (16%)	4 (24%)	31 (27%)	0.08	0.24
V106A	4 (3%)	0 (0%)	3 (75%)	1 (1%)	0.00	0.00
V106M	4 (3%)	1 (1%)	2 (50%)	3 (3%)	0.00	0.02
V108I	4 (3%)	10 (9%)	0 (0%)	14 (12%)	0.04	0.18
Y181C	24 (21%)	4 (3%)	2 (8%)	26 (22%)	0.01	0.06
Y188C	1 (1%)	0 (0%)	1 (100%)	0 (0%)	0.00	0.00
G190A	13 (11%)	7 (6%)	5 (38%)	15 (13%)	0.03	0.14
G190S	2 (2%)	4 (3%)	0 (0%)	6 (5%)	0.02	0.09
P225H	0 (0%)	3 (3%)	0 (0%)	3 (3%)	0.01	0.08

There was no increase in the proportion of patients with the K65R mutation among those on triple-NRTI regimens, with a similar number of patients gaining and losing this mutation. There were substantial increases in the proportion with M41L (38% of patients), L210W (26%), T215Y (22%), T215F (18%), D67N (17%), K219E (15%) and K219Q (14%). There were smaller increases in the proportion with K70R (11%) and M184V (8%),

[7] Percentage is of those with a mutation at the first time point, other percentages are of total.

although these mutations were the most prevalent at the time virological failure was first detected.

Among patients who received NRTI-NNRTI regimens, there were small increases in the proportion with the major NNRTI mutations, K103N (12%) and V108I (9%). For other major NNRTI mutations, such as Y181C (1%), there were smaller increases. There were comparable increases in the proportion of patients with D67N (22%), T215F (21%), K219E (20%), K219Q (20%), T215Y (18%), L210W (17%) and M184V (10%) compared to patients who received triple-NRTI regimens. There were smaller increases in the proportion of patients with M41L (26%).

5.3.4 Persistence of HIV-1 drug resistance mutations

The failure to detect previously identified HIV-1 drug resistance mutations is often the result of either a patient changing their antiretroviral therapy regimen or a lack of adherence, which could lead to virus reverting and regaining replication fitness. Even if mutations are no longer detected, they continue to exist as either a low-level undetectable population or archived within a patient's cells. Archived mutations could continue to affect response to subsequent antiretroviral therapy regimens. In this section, the reversion or loss of a mutation is defined as when a previously detected mutation is no longer detectable using population sequencing.

The mutations with the highest rate of reversion (Table 44) were T215F (19/50; 38%), K219Q (19/60; 32%), K65R (25/80; 31%), K70R (24/166; 14%) and D67N (18/173; 10%). These mutations reverted in 78 patients (65 with one mutation, 8 with two mutations and 5 with three mutations reverting). Patients in whom mutations reverted continued to have at least one HIV-1 drug resistance mutation (only 4/78 were wild-type; 5%). Despite the reversion, patients had a similar or greater numbers of HIV-1 drug resistance mutations than patients who had individual mutations detected at both time points (Mean 4.55 compared to 4.26; p=0.44).

Many of these mutational changes were due to the development of alternative mutations at the same codon. For example, 11 of 19 (58%) patients in whom the K219Q mutation was no longer detectable developed K219E. In addition, 17 of 19 (89%) patients in whom the T215F mutation was no longer detectable developed T215Y. There were a mean increase of 0.74 NRTI and 0.47 NRTI mutations respectively in patients where K219Q and T215F were lost respectively. Other mutational changes often coincided with the development of additional mutations at different codons, which may be antagonistic with the mutation no longer detected. Patients in whom the K65R mutation was lost gained a mean of 2.3 NRTI mutations (specifically TAMs) between tests and just 4 of 24 (17%) had fewer NRTI mutations. However, patients in whom either the K70R or D67N mutation was lost typically had a mean of 1.0 and 0.5 fewer NRTI mutations. The impact of this upon the susceptibility of HIV-1 to antiretrovirals is examined in the next section.

5.3.5 Changes in susceptibility

The change in predicted susceptibility for individual antiretrovirals (according to the Stanford susceptibility algorithm) between the two time points is displayed in Figure 43. There was little change in the predicted susceptibility to either emtricitabine or lamivudine. since most patients had intermediate or high-level resistance at the time point where virological failure was first detected. A similar proportion of patients had high-level resistance to abacavir, zidovudine, stavudine and didanosine at the first time point. The period on a regimen with virological failure had a similar impact for these four drugs, although zidovudine retained the most susceptibility. There was a decrease in the proportion of patients with HIV which retained susceptibility to tenofovir but in comparison to other NRTIs this was marginal. NNRTI susceptibility did not substantially change due to the small increase in the number of NNRTI mutations among patients on an NRTI-NNRTI regimen. At the time virological failure was

first detected the mean GSS for NRTIs was 2.6/7.0 and for NNRTIs was 2.6/3.0. At the last time point on first-line antiretroviral therapy the mean GSS for NRTIs was 1.3 and for NNRTIs was 2.3.

Figure 44 displays the change in predicted susceptibility for individual drugs by antiretroviral therapy regimen. HIV in patients on triple-NRTI regimens had a lower susceptibility to NRTIs at the time virological failure was first detected compared to NRTI-NNRTI regimens. The mean GSS for NRTIs at the time virological failure was first detected was 2.3 and 2.8 for triple-NRTI regimens containing tenofovir and abacavir respectively and 3.5 for NRTI-NNRTI regimens. The mean GSS for NNRTIs at the time virological failure was first detected was 2.9 and 2.8 for triple-NRTI regimens and 1.4 for NRTI-NNRTI regimens. The susceptibility of NRTIs remained lower at the last timepoint on first-line antiretroviral therapy among patients on triple-NRTI regimens compared to NRTI-NNRTI regimens. The mean GSS for NRTIs at this point was 1.2 (approximately 50% decrease) and 1.5 (approximately 45% decrease) for triple-NRTI regimens containing tenofovir and abacavir respectively and 1.6 (approximately 55% decrease) for NRTI-NNRTI regimens. The mean GSS for NNRTIs at the last time point was 2.7 (7% decrease) and 2.7 (4% decrease) for triple-NRTI regimens containing tenofovir and abacavir respectively and 1.1 (21% decrease) for NRTI-NNRTI regimens.

Figure 43: Change in predicted drug susceptibility

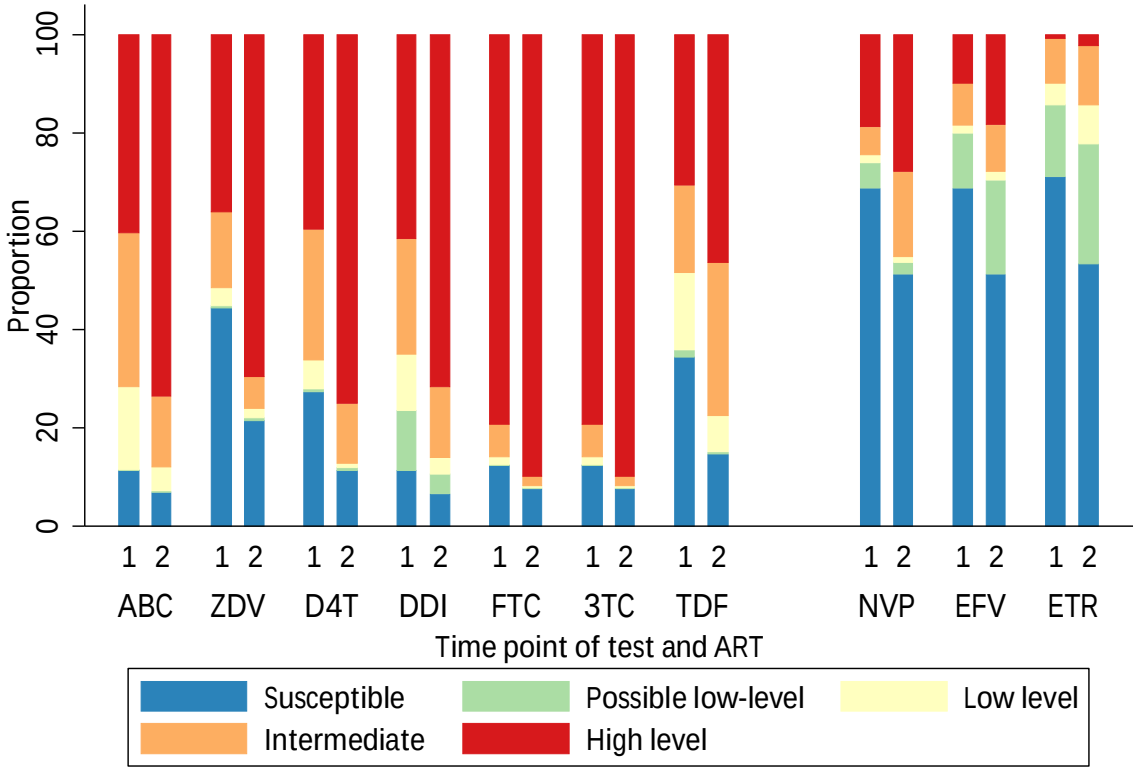
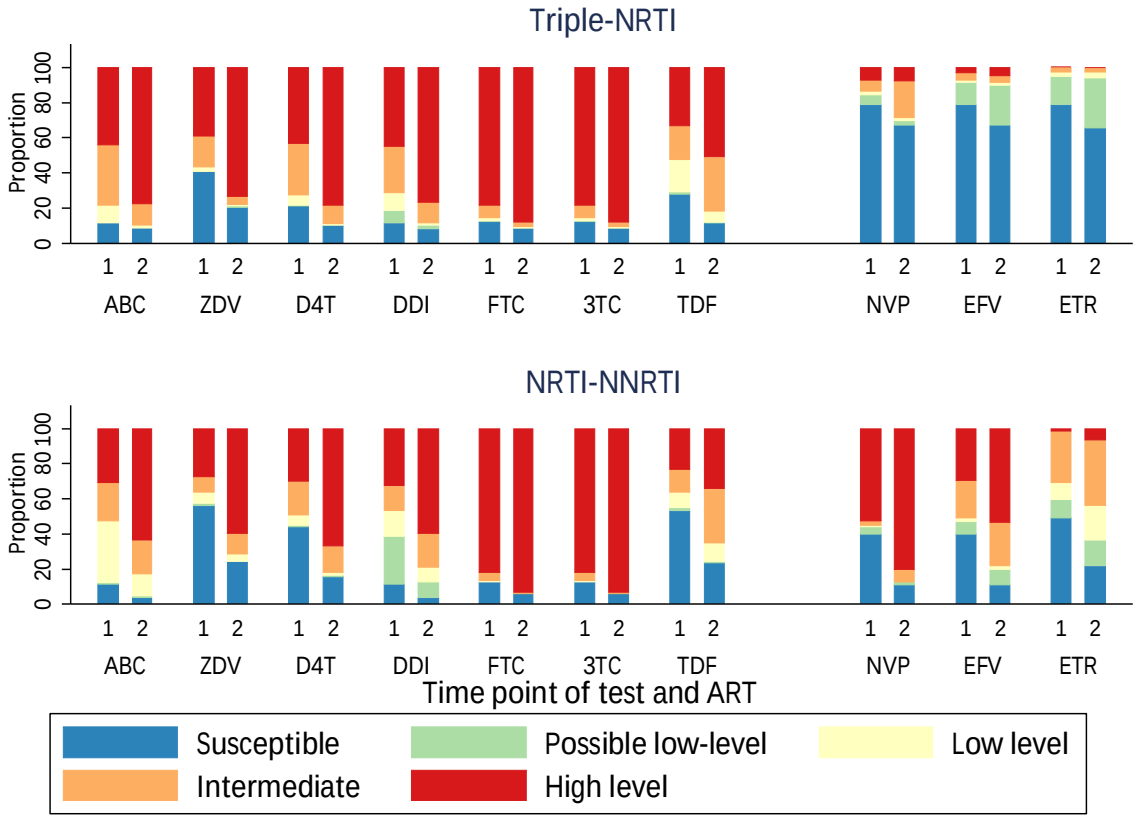


Figure 44: Change in predicted susceptibility by antiretroviral regimen



Among virus where K70R or D67N mutation reverted there was an increase in viral susceptibility to antiretrovirals. Where K70R reverted, there was a 29% increase in the proportion of patients who had virus susceptible to zidovudine at the second time point and an increase of 17% among patients where D67N reverted (Figure 45). Changes in susceptibility to tenofovir were conflicting (Figure 46), there was an increase in the proportion of patients with susceptible virus although there was also an increase in the proportion with high-level resistance. Patients in whom the K65R mutation reverted had a large increase in the proportion with virus resistant to thymidine analogues such as zidovudine, a result of gaining other mutations. As a direct consequence of K65R reverting, there was a decrease in the proportion resistant to tenofovir. Patients in whom either T215F or K219Q reverted saw changes in susceptibility similar to the overall population since these were mostly other resistant mutations developing at this codon.

Figure 45: Change in susceptibility to zidovudine among patients who lost mutations

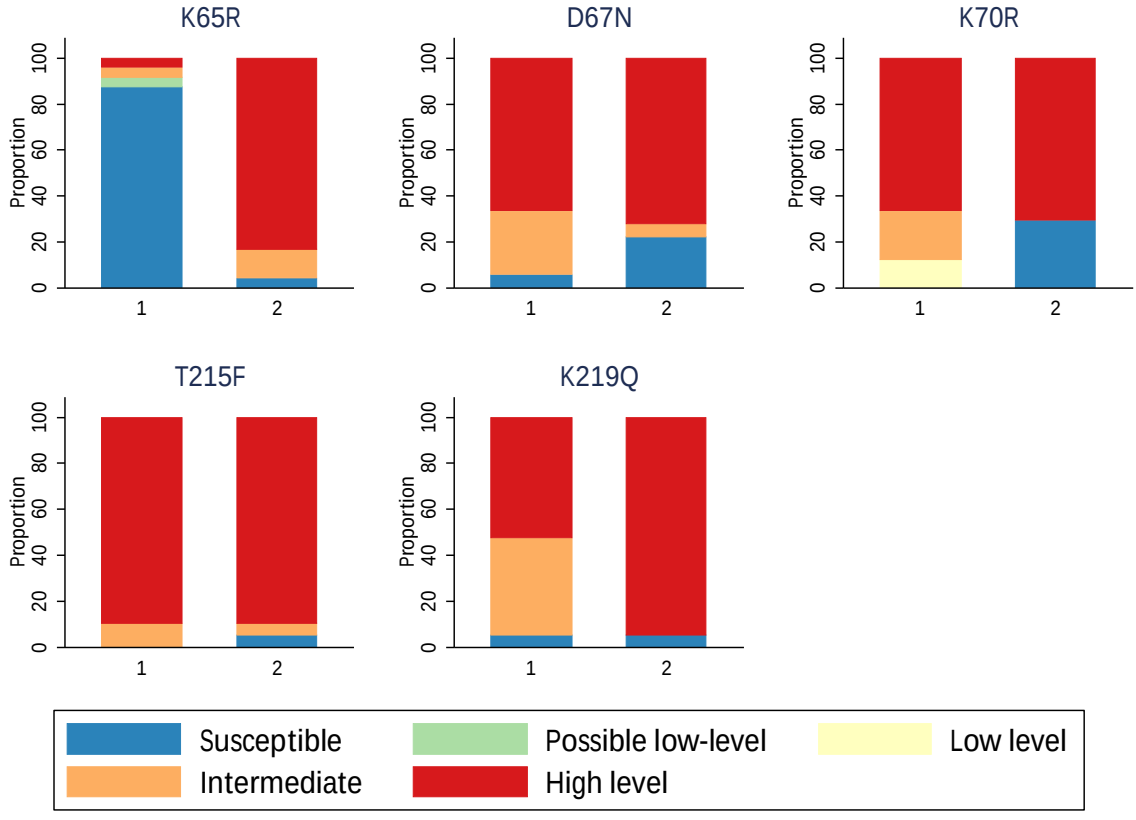
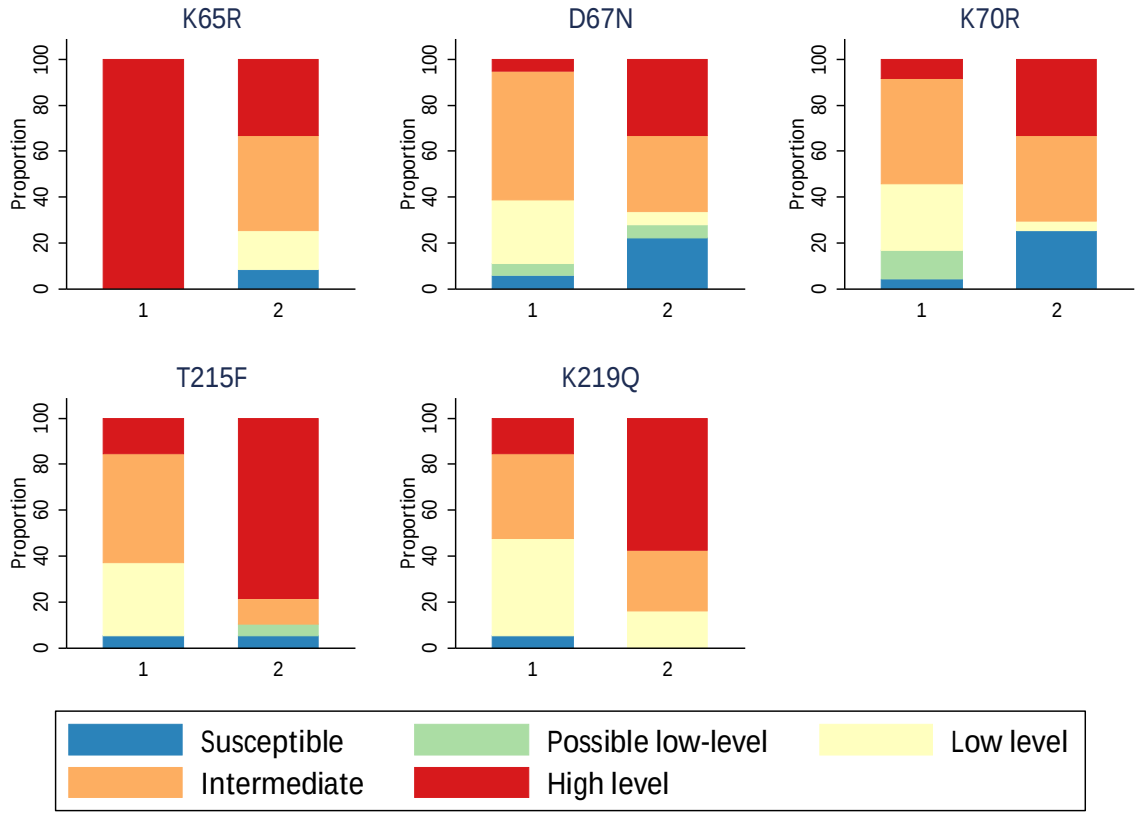


Figure 46: Change in susceptibility to tenofovir among patients who lost mutations



5.3.6 Compensatory mutations

Compensatory mutations have been defined as mutations which restore the replication capacity of virus but which do not impact the function of antiretrovirals themselves. In the DART data, there was no evidence that S162A was more likely to exist in patients with the M41L mutation (9 of 265; 3% compared to 5 of 149; 4%; χ^2 test p=0.98). Jeeninga et al. [198] found that the S163N mutation restored viral replication capacity in patients with both the M41L and K70R mutations. In the DART data, the S163N compensatory mutation was only observed to occur in one patient, despite M41L and K70R frequently co-occurring at the last observed time point (146/414).

Svarovskaia et al. [195] concluded that A62V and S68G restored some of the replication defects which resulted from the K65R mutation. There was strong evidence in the DART data that both were associated with K65R. The A62V mutation occurred in 14 of 58 (24%) patients with K65R

compared to 3 of 356 (1%) in patients without (p<0.001).The S68G mutation was extremely common in patients with K65R (28/58; 48% compared to 11/356; 3%; p<0.001).

The A62V, V75I, F77L and F116Y mutations were initially suggested by Maeda et al. [196] as compensatory mutations for the Q151M mutation in the presence of zidovudine or didanosine. While the Q151M mutation was rare, there was evidence that these mutations were all more likely to occur in DART patients with Q151M. A62V occurred in 6 of 8 (75%) with the Q151M mutation compared to 11 of 406 (3%; p<0.001). V75I occurred in 6 of 8 (75%) patients with Q151M compared to 9 of 406 (2%; p<0.001). F77L occurred in 4 of 8 (50%) with Q151M compared to 2 of 406 (0%; p<0.001) and F116Y occurred in 7 of 8 (88%) with the Q151M mutation compared to 2 of 406 (0%; p<0.001).

The Q207D mutation is thought to improve the fitness of zidovudine-resistant HIV-1 in the presence of TAMs. In this data, there was evidence that the mutation was more common in patients with the M41L mutation (30/265; 11% compared to 6/149 without; 4%; p=0.011). Mutations were less common in patients with the K70R mutation (12/204; 6% compared to 24/210 without; 11%; p=0.045).

5.3.7 Mutagenic trees

5.3.7.1 NRTI mutagenic trees by ART regimen

Figure 47: Mutagenic tree for patients receiving tenofovir

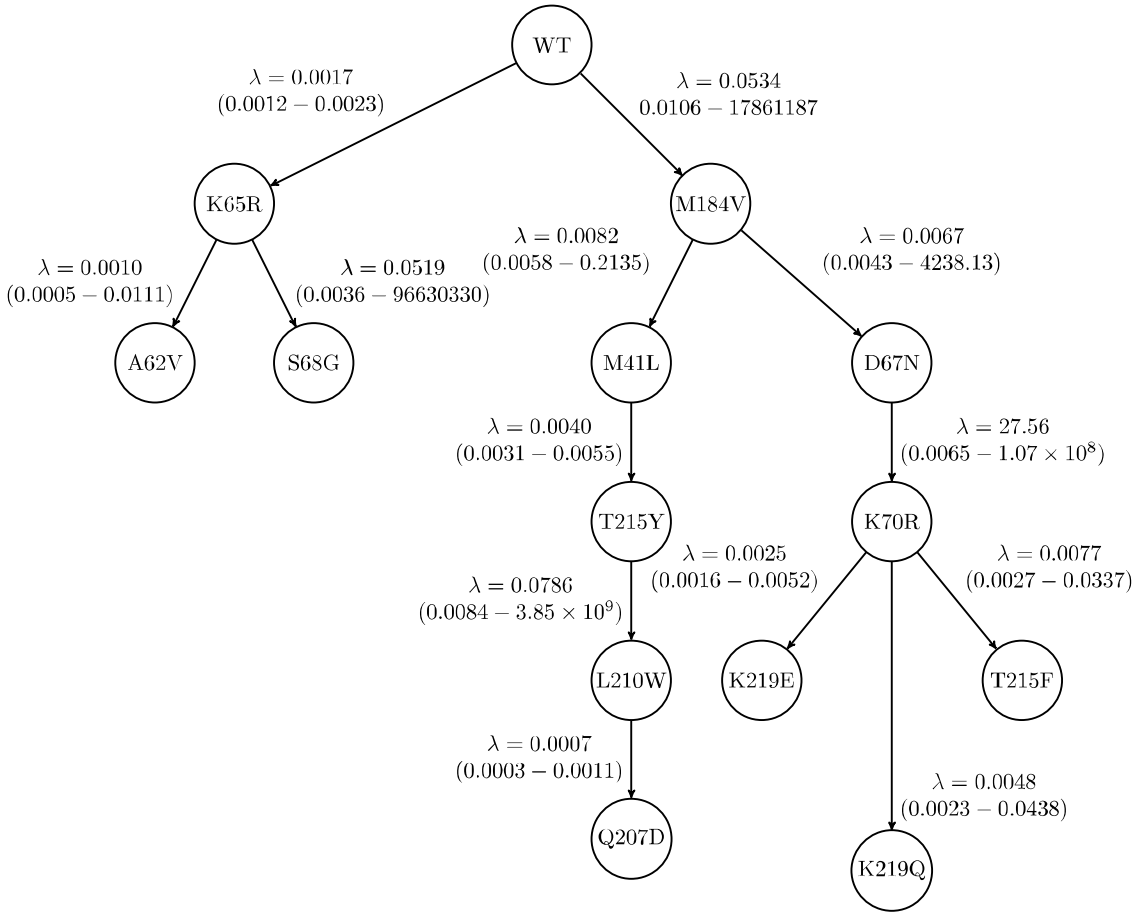


Figure 48: Mutagenic tree for patients receiving abacavir

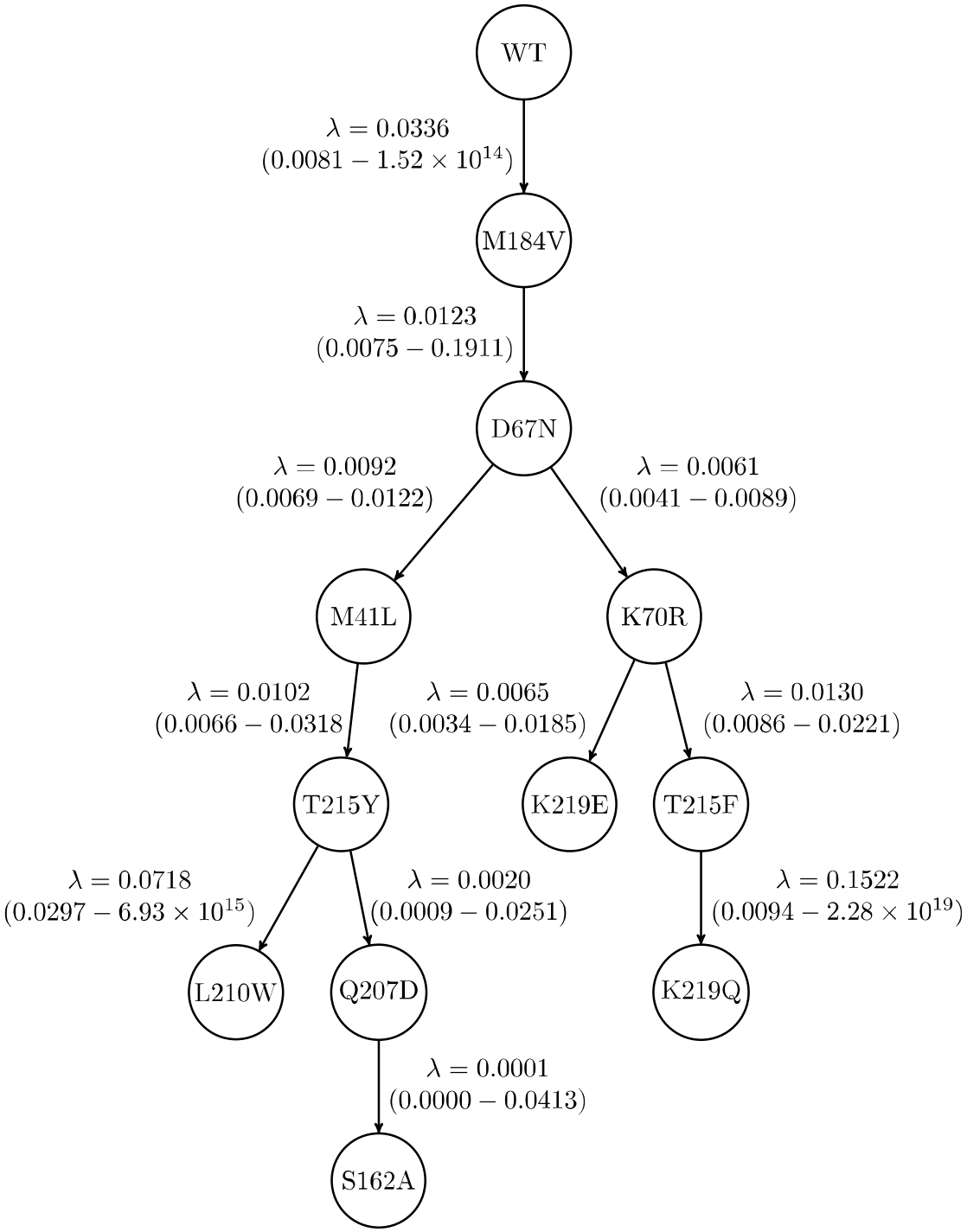


Figure 49: NRTI mutagenic tree for patients on NRTI-NNRTI regimens

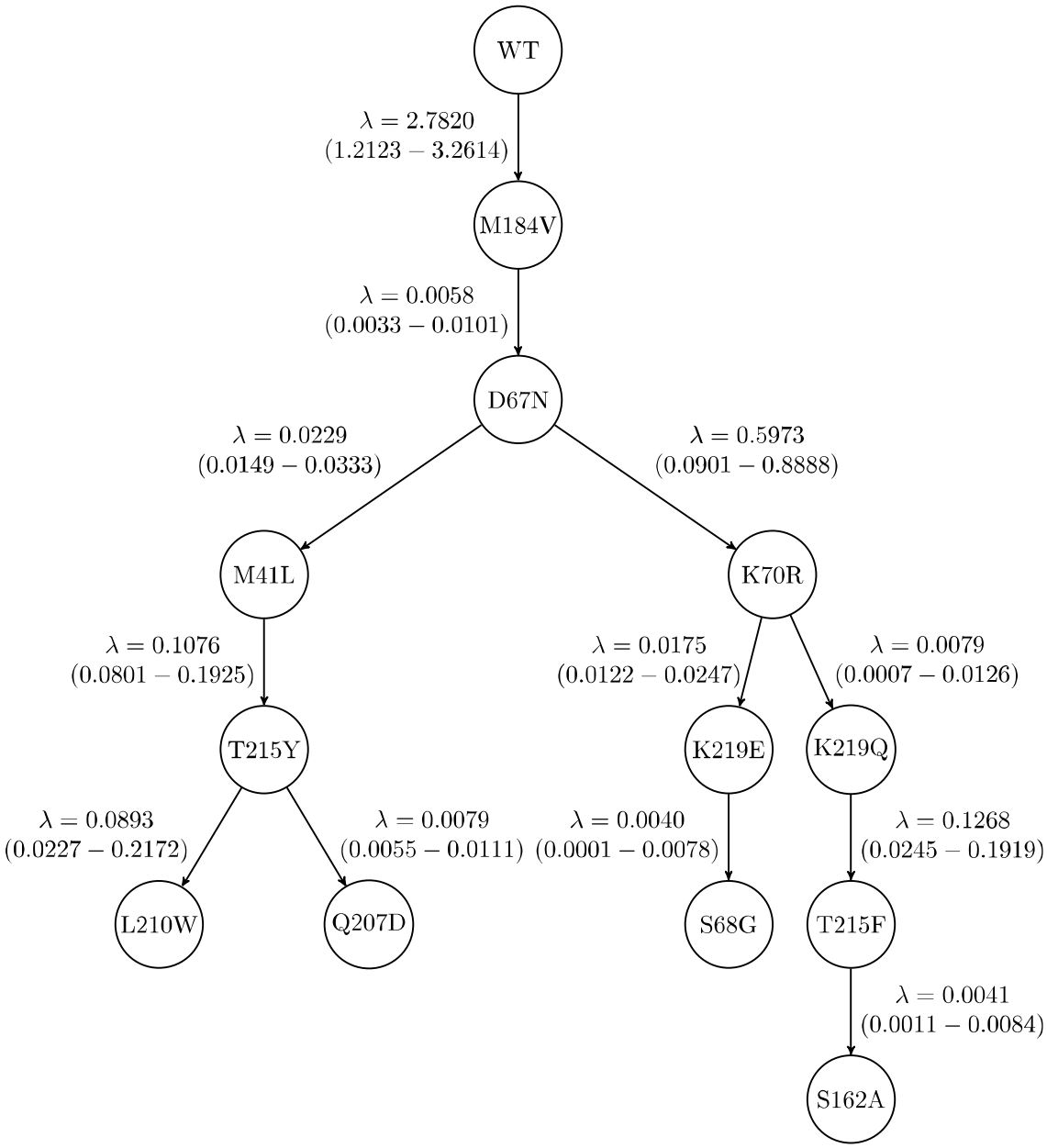


Figure 47, Figure 48 and Figure 49 show the results from three mutagenic models restricted to patients receiving triple-NRTI regimens containing tenofovir and abacavir and patients on NRTI-NNRTI regimens respectively. The mutagenic tree hidden Markov models examined major IAS NRTI and compensatory mutations in the 334 and 85 patients who received triple-NRTI therapy containing tenofovir and abacavir as well as the 147 patients who received an NRTI-NNRTI regimen. The mutation rate and 95% confidence intervals, based on 100 bootstraps, are displayed for each edge.

The mutagenic tree for each antiretroviral regimen had a separate structure which suggests an alternate order in which mutations are acquired. The tree structure represents mutations which are needed for other mutations to occur but these are not mutually exclusive linear pathways. For instance, for the mutagenic tree for NRTI-NNRTI regimens, the development of M41L does not exclude the development of K70R, which could occur before or after M41L. Similarly, a mutation at the bottom of the tree such as Q207D does not exclude the development of further mutations.

In all models, M184V occurs without any predecessor mutations. Other consistent aspects of the structure were T215Y occurring before L210W in all three trees. Finally, both K219E and K219Q only occurred following the development of K70R. This contradicts the findings of Cozzi-Lepri et al. [180] and Lawyer et al. [183], where mutations at codon 215 preceded the M41L mutation. Furthermore, Cozzi-Lepri et al. [180] found that K219E/Q occurred following K70R and before D67N. The Q207D compensatory mutation occurred similarly in all models following M41L and T215Y (and the L210W mutation for patients on tenofovir).

There were substantial differences in the order of appearance of the M41L, K70R and D67N mutations by antiretroviral therapy regimen. For patients who received tenofovir, M41L and D67N occurred following M184V as non-exclusive pathways and K70R occurred following D67N. For patients who received abacavir or were on NRTI-NNRTI regimens, M41L and K70R were also non-exclusive pathways but could only occur after the D67N mutation had developed. For patients who received tenofovir, the K65R mutation could occur directly from wild-type virus. For patients who received NRTI-NNRTI regimens, K65R was infrequent and not included in the model.

5.3.7.2 Tenofovir

For patients who received tenofovir, there were three major branches in the mutagenic tree. While the structure of a mutagenic tree ensures that child mutations cannot occur without parent mutations (e.g. M41L cannot occur without M184V) they do not exclude mutations developing on multiple branches (e.g. K65R and M184V can both occur). The first branch involved K65R-A62V-S68G and had the slowest rate of occurrence. After 52 weeks of antiretroviral therapy, the probability of K65R developing was 8%. The expected waiting time for K65R to occur is estimated to be 588 weeks. After 52 weeks with the K65R mutation, the probability of A62V developing was 5% and there was a 93% probability that S68G developed. The expected waiting time after K65R developed was 1,000 and 19 weeks respectively.

The M184V mutation developed rapidly, with an expected waiting time of 19 weeks. The other two branches for patients on tenofovir, following the development of the M184V mutation, support previous research suggesting the existence of two TAM pathways. M41L, T215Y and L210W formed a cluster of mutations which have previously been referred to as the TAM-1 pathway. The tree structure suggested that the M41L mutation was the first to develop after the M184V mutation, and had a 35% probability of doing so after 52 weeks with the M184V mutation. The expected waiting time was 122 weeks. Q207D appeared as a final mutation in this cluster, suggesting it may have a compensatory role. Q207D developed slowly, with a 4% probability of occurring in the 52 week period following the development of L210W.

The final major branch is broadly considered the TAM-2 pathway. However, the T215F mutation appears towards the end of this branch and is typically considered a TAM-1 mutation. In this analysis, the K70R mutation developed almost instantaneously after the D67N mutation, although there was considerable uncertainty in this estimate. This may be

a result of uncertainty in the tree structure and additional research with mixture tree models found that K70R could often precede D67N. The current structure concurs with Lawyer et al. [183] where mutations at codon 67 increased the incidence of mutations at codon 70 and these, in turn, increased the incidence of mutations at codon 219. The D67N mutation occurred following M184V with an expected waiting time of 149 weeks.

5.3.7.3 Abacavir

The mutagenic tree suggested that the M184V mutation and D67N mutations were required before other TAMs developed. The D67N mutation occurred with a probability of 45% in the 52 weeks following the development of the M184V mutation (expected waiting time of 81 weeks). Unlike patients on NRTI-NNRTI regimens, K70R and M41L developed at a more comparable rate. After 52 weeks with M184V and D67N, the probability of this occurring was 25% and 36% respectively (waiting time of 164 and 109 weeks respectively). Unlike patients who received tenofovir, the K219Q mutation required the T215F mutation before it developed. Compared to the models for tenofovir and NRTI-NNRTI regimens, there was a large uncertainty in the rate parameter for M184V, L210W and K219Q and this may be a result of this model including the fewest patients.

The Q207D mutation developed at a slower rate following T215Y compared to patients on NRTI-NNRTI regimens (λ=0.0020; 95% CI: 0.0009-0.0251 compared to 0.0079; 95% CI: 0.0055-0.0111). There was evidence that the S162A mutation occurred after Q207D and at the end of the M41L branch, suggesting that this may also be a compensatory mutation for the TAM-1 pathway. Nonetheless, S162A was a rare mutation for these mutations and occurred at a slow rate (λ=0.0001; 95% CI: 0.0000-0.0413).

5.3.7.4 NRTI-NNRTI regimens

The mutagenic tree structure for patients who received NRTI-NNRTI regimens demonstrated two major branches after the M184V mutation and the D67N mutation. There was evidence that the M184V occurred more rapidly on NRTI-NNRTI regimens than on triple-NRTI regimens, although all models suggested that M184V developed quickly.

Both branches featured the D67N mutation, which developed with a probability of 24% in the 52 weeks after the M184V mutation occurred (expected waiting time of 172 weeks). Following the development of D67N, the K70R developed more rapidly than M41L. K70R developed with a 100% probability in the 52 weeks after D67N occurred (expected waiting time of 2 weeks) compared to 67% for M41L (expected waiting time 44 weeks). However, mutations which occurred after M41L along the TAM-1 branch occurred more rapidly than further TAM-2 branch mutations. For example, T215Y had a rate parameter of 0.1076 after M41L, compared to a rate of 0.0175 for K219E.

S68G and S162A did not appear to act as compensatory mutations for the K65R or M41L mutations, since neither directly followed these mutations. Both occurred at low rates (λ=0.0040 and 0.0041 respectively, wait times 250 and 244 weeks) and there was evidence that S162A occurred before K65R. Q207D appeared to act as a compensatory mutation for mutations which occurred along the TAM-1 branch. Following the development of M41L and T215Y, this mutation occurred with a rate parameter of 0.0079 (32% probability of developing after 52 weeks with parent mutations, 127 weeks expected waiting time).

5.3.7.5 NNRTI mutagenic trees

Figure 50: NNRTI mutagenic tree for patients on NRTI-NNRTI regimens

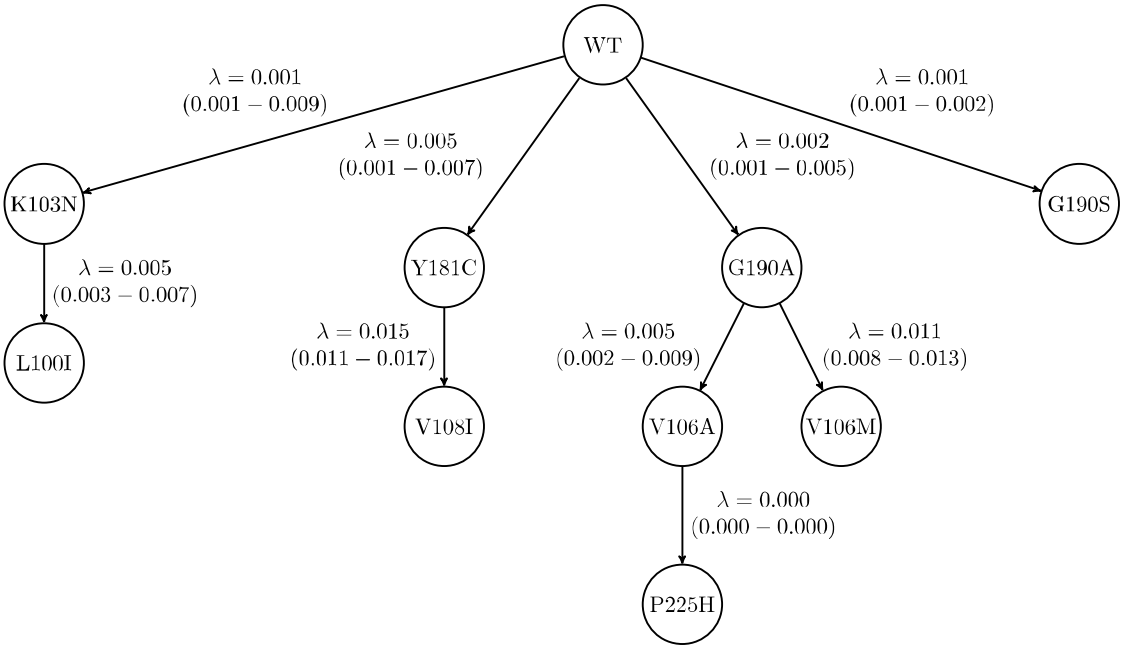


Figure 50 displays a mutagenic tree hidden Markov model examining major IAS NNRTI mutations in the 129 patients who received NRTI-NNRTI regimens. 95% confidence intervals were based on 100 bootstraps.

The mutagenic tree structure demonstrates that NNRTI mutations did not typically require predecessor mutations to occur. K103N, Y181C, G190A, and G190S could all develop directly from wild-type. The rate at which NNRTI mutations develop was slow, typically ten-fold slower than the rate at which NRTI mutations developed on either triple-NRTI regimen. After 48 weeks of therapy among patients with virological failure, the probability of K103N was estimated to be 5% and the most likely mutation was Y181C with a probability of 22%. L100I, V106A/M, and V108I were shown to be child mutations which developed at a similar rate to their parent mutations. These mutations may act as secondary mutations which improve the replication fitness of the parent mutation.

5.4 Discussion

In this chapter, the development of HIV-1 drug resistance mutations in patients with persistent virological failure without virological monitoring was analysed. Mutagenic trees demonstrated that NNRTI mutations accumulated at a slower rate than NRTI mutations in patients experiencing virological failure. The number of NRTI mutations which accumulated between paired tests was higher on NRTI-NNRTI regimens compared to triple-NRTI regimens; however patients on triple-NRTI regimens had a greater number of NRTI mutations at the time virological failure was first detected. The rate of development of individual mutations, calculated directly and using mutagenic tree models, helps quantify the expected loss in viral susceptibility to antiretrovirals among patients treated without routine laboratory monitoring.

5.4.1 Accumulation rates of mutations

Boender et al. [184] calculated that overall a mean (SD) of 1.45 (2.07) drug resistance mutations were accumulated per year with virological failure. This was comprised of 0.62 (1.11) NNRTI and 0.84 (1.38) NRTI mutations per year. The accumulation rate of NNRTI mutations was driven by K103N (mean of 0.11 per year), V108I (0.11 per year) and P225H (0.08 per year). NRTI mutations were mostly any TAM (0.26 per year), M184V mutation (0.20 per year) and K70R (0.13 per year). Cozzi-Lepri et al. [181] calculated a similar rate for NNRTI mutations where 0.61 (95% CI: 0.55-0.66) NNRTI mutations developed per year. The accumulation rate of TAMs in Cozzi-Lepri et al. [180] was 0.23 per year, matching Boender et al. [184]. These were mainly TAM 1 pathway mutations (0.16 per year) rather than TAM 2 pathway mutations (0.07 per year).

On NRTI-NNRTI regimens in this analysis, 0.96 NRTI, 0.21 NNRTI mutations and 0.82 TAMs developed per year. Despite the accumulation rate of NRTI mutations being only slightly higher than Boender et al. [184], there was a large difference in the reported accumulation rate of TAMs.

The reason for this is unclear, since there is also a large difference between the accumulation rate of NRTI mutations and TAMs within Boender et al's [184] study, despite the accumulation rate of non-TAMs other than M184V being low. It is likely that the accumulation rate of TAMs would be 0.58 per year if a similar method of analysis to this chapter were used. This would remain lower than our analysis and could be due to the shorter duration between tests or the greater proportion of patients who received an NRTI-NNRTI regimen containing efavirenz.

The accumulation rate of NNRTI mutations is lower in DART than these studies. This could be a result of the NRTI backbone used in this study or it could reflect the shorter timescale. If few NNRTI mutations develop, but all do so during the first year with virological failure, then this could lead to apparent lower rates if the same resulting combinations of mutations are examined over a longer follow-up period. As a whole, the lower accumulation rate reflects fewer major NNRTI mutations being required to reduce antiretroviral drug susceptibility and that most of these mutations were detectable at the time of virological failure.

5.4.2 Persistence of HIV-1 mutations

Previous analyses examining the persistence of HIV-1 drug resistance mutations have been conducted in patients with transmitted HIV-1 drug resistance before initiating antiretroviral therapy [202, 203].

Unlike these analyses, patients in this chapter were on antiretroviral therapy throughout and had developed mutations during therapy. NNRTI mutations were found to be persistent and were present at both time points. Individual NRTI mutations were frequently no longer detected at the last timepoint on first-line antiretroviral therapy, but for K65R, T215F and K219Q these often facilitated the gain of additional mutations which lead to an increase in high-level HIV-1 drug resistance. Patients who lost D67N or K70R had an overall reduction in the number of NRTI mutations and there was an increase in viral susceptibility to thymidine analogues.

10% of patients with D67N and 14% with K70R at the time virological failure was first detected did not have this mutation detectable at the end of the DART study. It is not apparent why these major NRTI mutations could no longer be detected despite patients remaining on the same antiretroviral regimen.

5.4.3 Changes in predicted antiretroviral susceptibility

Barth et al. [179] used genotypic sensitivity scores to determine how the number of treatment options declined during a prolonged period with virological failure. At the first time point the mean GSS was 5.1/7.0 for NRTIs and 1.0/4.0 for NNRTIs, at the second time point (six to twelve months later), the mean GSS was 4.0 for NRTIs and 0.7 for NNRTIs. In this chapter, greater changes in susceptibility were observed to NRTIs across all treatment regimens than found by Barth et al. [179]. This is likely to be a result of changes in susceptibility being observed over a longer period than Barth et al. Despite this, the GSS for NNRTIs was consistently lower in Barth et al. compared to this analysis, this may reflect differences in the populations examined or the NRTI backbones used.

Boender et al. [184] found that full susceptibility to nevirapine declined from 16% to 6% between first and last time points with virological failure. Full susceptibility to tenofovir fell from 89% to 70%, zidovudine from 92% to 66%, abacavir from 27% to 9% and lamivudine/emtricitabine from 27% to 10%. Patients on NRTI-NNRTI regimens in DART had full susceptibility change from 40% to 11% for nevirapine, 53% to 23% for tenofovir, 56% to 24% on zidovudine, 12% to 3% for abacavir and 13% to 6% for lamivudine and emtricitabine. In comparison, DART patients on NRTI-NNRTI regimens had greater susceptibility to NNRTIs at the time virological failure was first detected and lower susceptibility to NRTIs. A larger proportion of patients in Boender et al. [184] retained full susceptibility to tenofovir and zidovudine.

These findings suggest that the loss of susceptibility was already substantial at the time virological failure was first detected. Nonetheless, increased virological monitoring could avoid additional loss in susceptibility in patients with persistent virological failure.

5.4.4 Mutagenic pathways

In Lawyer et al. [183], no pathways between known NNRTI mutations were observed. This partially supports this chapter's findings that most NNRTI mutations did not have a predecessor and could occur directly from wild-type. However, it does contradict the possible accessory/compensatory mutations observed. Lawyer et al. [183] analysed data from a limited number of patients (457/1981; 23%) who received a dual-class regimen containing an NNRTI and there was a large number of patients on single-class regimens containing just one or two drugs. Analyses were not conducted within antiretroviral regimens, so this could explain why some pathways were not observed.

Many of the pathways found by Deforche et al. [185] are identical to those observed in this analysis. With a bootstrap support greater than 65%, drug exposure to nevirapine was directly associated to mutations K103N, G190A, and Y181C. Like this analysis, these were not exclusive pathways so could occur together and lead to greater declines in the susceptibility of antiretrovirals. Also shown in this analysis were secondary mutations between Y181C and V108I (bootstrap support>65%) and between G190A and V106M (bootstrap support>35%). Unlike this analysis, Deforche et al. [185] did not find a direct association between G190S and exposure to nevirapine but did observe a direct association between nevirapine and V106A. Similarly, they also found that mutation K103N and V108I were associated with bootstrap support greater than 35%.

The results from this chapter reinforce the results of Deforche et al. [185], showing the structure of the mutagenic tree for non-B HIV-1 subtypes. This chapter's findings extend previous research by using the additional data

provided by longitudinal pairs of samples from the same patient, allowing the accumulation rate of each of these mutations to be determined.

5.4.5 Compensatory mutations

This study demonstrated that S68G and Q207D acted as compensatory mutations. S68G was observed among patients who received tenofovir and NRTI-NNRTI regimens. There was strong evidence that S68G developed rapidly after the K65R mutation among patients on tenofovir and that the A62V mutation could develop alongside these mutations. This supports the results of Svarovskaia et al. [195] which showed, through phenotypic and viral growth competition analyses, that neither mutation influenced resistance but that both independently restored some replication deficit.

For all treatment regimens, Q207D appeared as a final mutation along the common TAM-1 pathway. Lu et al. [199] found through a site-directed mutagenesis study that the Q207D had no influence on zidovudine susceptibility in wild-type virus. However, the addition of Q207D into a viral strain with M41L, D67N, K70R, T215Y and K219Q increased zidovudine resistance 2.7 fold. The analyses in this chapter agree with these findings and suggest that strains with M41L, T215Y and L210W mutation are particularly likely to develop this compensatory mutation.

5.4.6 Methodological limitations

Mutagenic tree models are a simplification of Bayesian network models so have several limitations. Firstly, mutagenic tree models are not able to show antagonistic relationships between mutations. In the model, the probability of developing the K65R mutation does not change if a patient has multiple TAMs. This is biologically implausible since an antagonistic relationship between these mutations and NRTI's method of action has been previously demonstrated [160, 204].

Secondly, mutations at the same codon (e.g. T215Y and T215F) are modelled as independent events, whereas in reality developing one

mutation at this codon is likely to reduce the probability of the other. This limitation could have been overcome by modelling per codon mutations, but this would have been an oversimplification.

Finally, mutagenic trees are not able to account for mutations reverting. Biologically, this could be due to either a lack of adherence or an alternate clonal strain developing with higher replication fitness. A Bayesian network model could have included mutation reversion but these are slower to converge, and methodological developments are required to use longitudinal samples within a patient.

Mutational development rate was calculated using the observed data but ignored the interval censored observation points. This is likely to influence the rates calculated, particularly for mutations thought to develop quickly such as M184V. More sophisticated approaches, such as the observed time conjunctive Bayesian network developed by Montazeri et al. [174], account for the fact that a mutation could have developed at any point during this period. However, these approaches cannot yet account for multiple observations per patient so they were not applied to this analysis.

Finally, the mutagenic tree model only includes patients who experience virological failure. In each tree, the probability of the first mutation developing is the rate among those who experience virological failure and not the rate among all patients who received treatment. Nevertheless, the rate of subsequent child mutations should be accurate since these are conditional on parent mutations developing, and it is unlikely that a patient with a major IAS mutation would not have experienced virological failure.

5.4.7 Conclusions

Longitudinal resistance data from within patients are rare, and existing data are from highly selective groups of patients. This dataset is not biased since patients were not known to be virologically failing antiretroviral

therapy. This analysis has described the order in which mutations develop and the rate at which mutations are acquired by antiretroviral regimen. Virological monitoring has the greatest potential to reduce the loss in viral susceptibility to NRTIs on NRTI-NNRTI regimens.

6 Summary

This chapter describes the main conclusions from this thesis and the relationship with current HIV treatment guidelines. The general limitations of this thesis are outlined, and future research is described which could extend analyses of the DART data set.

6.1 Laboratory monitoring guidelines

Chapter 2 demonstrated that routine CD4 cell count monitoring did not impact the rate of virological failure. Initiating antiretroviral therapy at higher CD4 cell counts reduced the incidence of virological failure and could help to achieve UNAIDS 90-90-90 [29] long-term virological suppression targets. However, CD4 cell count monitoring would not be required to achieve this if ART is initiated at any CD4 cell count. CD4 cell count monitoring reduced mortality with virological failure, although had no impact on mortality with virological suppression (Chapter 3). Despite leading to earlier treatment switches; this did not reduce the extent or the prevalence of HIV-1 drug resistance (Chapter 4).

Ford et al. [141] suggest that offering a single CD4 cell count measurement after 48 weeks of antiretroviral therapy, and switching treatment regimen if CD4 cell count was below 100 cells/mm^3, would reduce mortality and achieve similar outcomes for patients as more frequent CD4 cell count monitoring strategies. Phillips et al. [205] created a simulated population for Zimbabwe and predicted that the WHO recommended CD4 count monitoring strategy (six monthly test and treatment switched if CD4 cell count less than 100 cells/mm^3 or pre-ART baseline) averts 540,000 disability-adjusted life years (DALY) over 20 years, with a cost of $500 million. This is likely to be above the threshold for a cost-effective use of resources in this region, thought to be around $500 per DALY. This thesis supports the established limitations of CD4 cell count monitoring for detecting virological failure and reducing HIV-1 drug resistance.

In this thesis, the strongest predictor for reducing the number of NRTI mutations was the time since virological failure; suggesting that more frequent viral load monitoring could reduce HIV-1 drug resistance and preserve second-line regimens. Cost-effectiveness studies [42] have calculated that routine viral load monitoring provides notable benefits in reduced disability adjusted life years, but that routine viral load monitoring is expensive and should not limit the enormous benefits of access to antiretroviral therapy for all patients.

Unlike CD4 cell count and routine viral load monitoring, Phillips et al. [205] found that a viral-load-informed differentiated care approach could be cost-effective in low-income settings delivering 1,200,000 DALYs over 20 years for $360 million. Viral-load-informed differentiated care was defined as viral load monitoring at six, twelve and then after every twelve months during therapy; if viral load is greater than 1,000 copies/mL then attempts to improve adherence are made and viral load is retested after three months. If viral-load-informed differentiated care reduced the time spent with virological failure, then the results of Chapter 4 show that this could have a substantial impact on the prevalence of HIV-1 drug resistance.

6.2 Limitations

One of the major limitations when interpreting results from the DART trial is the predominant use of older and superseded regimens; triple-NRTI regimens are no longer used, even in resource-limited settings. However, the data observed in the 404 patients who started an NRTI-NNRTI regimen represent a substantial contribution to the currently available literature. The systematic review by Boender et al. [44] on virological failure found no intention to treat data for NNRTI-based regimens beyond 48 months of antiretroviral therapy. Similarly, some of the best currently available data on the rate of mutation in patients without virological monitoring [184] is generated by just 63 adult patients. Additional data

from 94 patients on NRTI-NNRTI regimens is a major contribution to this research area.

The DART trial's inclusion criteria specified that patients had a CD4 cell count less than 200 cells/mm^3; enrolled patients had a median CD4 cell count of 86 cells/mm^3. Following the results of the START study [206], WHO guidelines [140] now recommend that antiretroviral therapy should be started irrespective of CD4 cell count. Findings from this thesis cannot necessarily be extended to patients who start ART with higher baseline CD4 cell counts and it is implausible that the effect of baseline CD4 cell count remains linear at cell counts greater than 200 cells/mm^3.

The walkback approach to viral load sampling meant that intermittent or early virological failure in patients who subsequently re-suppressed is not detected. The clinical consequences of intermittent viremia in low-income countries are complex but, with limited availability of second-line regimens in low-income countries, the priority of this thesis has been to investigate the effect of persistent virological failure on first-line regimens.

6.3 Future research

There are several directions in which analyses in this thesis could be extended. The primary outcome measures of the DART trial were mortality and the occurrence of new or recurrent WHO stage 4 events. In this thesis, mortality after virological failure was examined but other clinical events, such as the incidence of WHO stage 4 events, were not explored by virological status. Further analyses could examine the time between virological failure and the occurrence of opportunistic infections to help determine the optimal frequency of laboratory monitoring.

Secondly, this analysis did not investigate immunological changes around virological failure. Analyses of CD4 cell count are complicated by multiple issues, such as the structured treatment interruption substudy's CD4 threshold inclusion criteria, and the fact that patients who received CD4

cell count monitoring switched to second-line antiretroviral therapy when CD4 cell counts reached 100 cells/mm^3. Nonetheless, analyses could investigate the interaction between virological failure and immunological changes by determining the time between virological failure and immunological failure and whether the rate of immunological decline is influenced by the value of the viral load at virological failure.

Thirdly, the use of mutagenic tree models in Chapter 5 only began to explore the rate of individual drug resistance mutations. The mutagenic tree hidden Markov model generated interesting results but was restricted because only a single mutagenic tree can be fitted by current software. Mixture models of mutagenic trees with cross-sectional data provide better estimates of patterns of mutations and more plausible biological models for the occurrence of mutations. It is likely that expanding the statistical methodology and software implementations, to allow for mixture mutagenic tree hidden Markov models, could achieve a similar effect for longitudinal data. Additionally, it is unlikely that mutations develop at a linear rate over the long duration between tests, and further analyses could use a non-linear model.

This analysis could also be extended by developing a Bayesian network model parameterised to handle correlated observations. A Bayesian network model has the advantage that antagonistic relationships between mutations could be measured, and mutations could be linked to multiple predecessor mutations. Any future methodological development requires simple to use software implementations to allow their application by applied scientists.

References

1. Doitsh G, Galloway NL, Geng X, Yang Z, Monroe KM, Zepeda O, et al. Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. Nature 2014; 505:509-14.

2. Le Rouzic E, Benichou S. The Vpr protein from HIV-1: distinct roles along the viral life cycle. Retrovirology 2005; 2:11.

3. van Sighem AI, Gras LA, Reiss P, Brinkman K, de Wolf F. Life expectancy of recently diagnosed asymptomatic HIV-infected patients approaches that of uninfected individuals. Aids 2010; 24:1527-35.

4. Pantaleo G, Graziosi C, Fauci AS. New concepts in the immunopathogenesis of human immunodeficiency virus infection. The New England journal of medicine 1993; 328:327-35.

5. Grant RM, Lama JR, Anderson PL, McMahan V, Liu AY, Vargas L, et al. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. The New England journal of medicine 2010; 363:2587-99.

6. Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N, et al. Prevention of HIV-1 infection with early antiretroviral therapy. The New England journal of medicine 2011; 365:493-505.

7. Perelson AS, Neumann AU, Markowitz M, Leonard JM, Ho DD. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. Science 1996; 271:1582-6.

8. Mansky LM, Temin HM. Lower in vivo mutation rate of human immunodeficiency virus type 1 than that predicted from the fidelity of purified reverse transcriptase. Journal of Virology 1995; 69:5087-94.

9. Deeks SG, Wrin T, Liegler T, Hoh R, Hayden M, Barbour JD, et al. Virologic and immunologic consequences of discontinuing combination antiretroviral-drug therapy in HIV-infected patients with detectable viremia. The New England journal of medicine 2001; 344:472-80.

10. Williams I, Churchill D, Anderson J, Boffito M, Bower M, Cairns G, et al. British HIV Association guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy 2012. HIV medicine 2012; 13 Suppl 2:1-85.

11. Thompson MA, Aberg JA, Hoy JF, Telenti A, Benson C, Cahn P, et al. Antiretroviral treatment of adult HIV infection: 2012 recommendations of the International Antiviral Society-USA panel. JAMA : the journal of the American Medical Association 2012; 308:387-402.

12. Nijhuis M, Schuurman R, de Jong D, Erickson J, Gustchina E, Albert J, et al. Increased fitness of drug resistant HIV-1 protease as a result of acquisition of compensatory mutations during suboptimal therapy. Aids 1999; 13:2349-59.

13. Johnson VA, Calvez V, Gunthard HF, Paredes R, Pillay D, Shafer R, et al. 2011 update of the drug resistance mutations in HIV-1. Topics in antiviral medicine 2011; 19:156-64.

14. Liu TF, Shafer RW. Web resources for HIV type 1 genotypic-resistance test interpretation. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2006; 42:1608-18.

15. Robertson DL, Anderson JP, Bradac JA, Carr JK, Foley B, Funkhouser RK, et al. HIV-1 nomenclature proposal. Science 2000; 288:55-6.

16. Holguin A, Ramirez de Arellano E, Rivas P, Soriano V. Efficacy of antiretroviral therapy in individuals infected with HIV-1 non-B subtypes. AIDS reviews 2006; 8:98-107.

17. Pai NP, Shivkumar S, Cajas JM. Does Genetic Diversity of HIV-1 Non-B Subtypes Differentially Impact Disease Progression in Treatment-Naive HIV-1-Infected Individuals? A Systematic Review of Evidence: 1996-2010. Jaids 2012; 59:382-8.

18. Klein M, Odueyungbo A, Scherrer A, Ledergerber B, Fearnhill E, Sabin C, et al. Impact of Viral Subtype on Immunologic and Clinical Disease Progression in Antiretroviral-naïve HIV-infected Adults. 18th Conference on Retroviruses and Opportunistic Infections. Boston, USA, 2011.

19. Lodi S, Phillips A, Touloumi G, Geskus R, Meyer L, Thiebaut R, et al. Time from human immunodeficiency virus seroconversion to reaching CD4+ cell count thresholds <200, <350, and <500 Cells/mm(3): assessment of need following changes in treatment guidelines. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2011; 53:817-25.

20. Pineda-Pena AC, Faria NR, Imbrechts S, Libin P, Abecasis AB, Deforche K, et al. Automated subtyping of HIV-1 genetic sequences for clinical and surveillance purposes: performance evaluation of the new REGA version 3 and seven other tools. Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases 2013; 19:337-48.

21. Hemelaar J, Gouws E, Ghys PD, Osmanov S. Global trends in molecular epidemiology of HIV-1 during 2000-2007. AIDS 2011; 25:679-89.

22. Lihana RW, Ssemwanga D, Abimiku A, Ndembi N. Update on HIV-1 diversity in Africa: a decade in review. AIDS reviews 2012; 14:83-100.

23. UNAIDS. Global Report: UNAIDS report on the global aids epidemic. 2013.

24. World Health Organization. Antiretroviral Therapy for HIV Infection in Adults and Adolescents. Recommendations for a Public Health Approach (2010 revision). Geneva: World Health Organization, 2010: Geneva: WHO, 2011:1-359.

25. World Health Organization. Scaling up antiretroviral therapy in resource-limited settings: treatment guidelines for a public health approach, 2003 revision. Geneva: WHO 2004.

26. World Health Organization. Antiretroviral therapy for HIV infection in adults and adolescents: Recommendations for a public health approach, 2006 revision. Geneva: WHO 2006.

27. World Health Organization. Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a public health approach. Geneva: World Health Organization 2013.

28. Médecins Sans Frontières. Achieving undetectable: what questions remain in scaling-up HIV virologic treatment monitoring? 2014.

29. UNAIDS. 90-90-90: An ambitious treatment target to help end the AIDS epidemic. 2014.

30. DART Trial Team. Routine versus clinically driven laboratory monitoring of HIV antiretroviral therapy in Africa (DART): a randomised non-inferiority trial. The Lancet 2010; 375:123-31.

31. DART Trial Team. Twenty-four-week safety and tolerability of nevirapine vs. abacavir in combination with zidovudine/lamivudine as first-line antiretroviral therapy: a randomized double-blind trial (NORA). Tropical medicine & international health : TM & IH 2008; 13:6-16.

32. DART Trial Team. Fixed duration interruptions are inferior to continuous treatment in African adults starting therapy with CD4 cell counts < 200 cells/microl. Aids 2008; 22:237-47.

33. Gilks CF, Walker AS, Dunn DT, Gibb DM, Kikaire B, Reid A, et al. Lopinavir/ritonavir monotherapy after 24 weeks of second-line antiretroviral therapy in Africa: a randomized controlled trial (SARA). Antiviral therapy 2012; 17:1363-73.

34. Ivan M, Walker AS, Read A, Ssali F, Munderi P, Nalumenya R, et al. Second-line boosted protease containing therapy: assessing the impact of maintainiing 3TC versus switching to ddI in addition to 2 drugs from new classes in a randomised comparison. Abstracts of the Eighteenth Conference on Retroviruses and Opportunistic Infections, Boston, MA, USA, 2011 Abstract 541 Foundation for Retrovirology and Human Health, Alexandria, VA, USA 2011.

35. DART Virology Team. Virological response to a triple nucleoside/nucleotide analogue regimen over 48 weeks in HIV-1-infected adults in Africa. Aids 2006; 20:1391-9.

36. Ndembi N, Goodall RL, Dunn DT, McCormick A, Burke A, Lyagoba F, et al. Viral rebound and emergence of drug resistance in the absence of viral load testing: a randomized comparison between zidovudine-

lamivudine plus Nevirapine and zidovudine-lamivudine plus Abacavir. The Journal of infectious diseases 2010; 201:106-13.

37. Munderi P, Walker AS, Kityo C, Babiker AG, Ssali F, Reid A, et al. Nevirapine/zidovudine/lamivudine has superior immunological and virological responses not reflected in clinical outcomes in a 48-week randomized comparison with abacavir/zidovudine/lamivudine in HIV-infected Ugandan adults with low CD4 cell counts. HIV medicine 2010; 11:334-44.

38. Gupta RK, Goodall RL, Ranopa M, Kityo C, Munderi P, Lyagoba F, et al. High rate of HIV resuppression after viral failure on first-line antiretroviral therapy in the absence of switch to second-line therapy. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2014; 58:1023-6.

39. Kityo C, Gibb DM, Gilks CF, Goodall RL, Mambule I, Kaleebu P, et al. High level of viral suppression and low switch rate to second-line antiretroviral therapy among HIV-infected adult patients followed over five years: retrospective analysis of the DART trial. PloS one 2014; 9:e90772.

40. McCormick AL, Goodall RL, Joyce A, Ndembi N, Chirara M, Katundu P, et al. Lack of minority K65R-resistant viral populations detected after repeated treatment interruptions of tenofovir/zidovudine and lamivudine in a resource-limited setting. J Acquir Immune Defic Syndr 2010; 54:215-6.

41. Mee P, Fielding KL, Charalambous S, Churchyard GJ, Grant AD. Evaluation of the WHO criteria for antiretroviral treatment failure among adults in South Africa. Aids 2008; 22:1971-7.

42. Keebler D, Revill P, Braithwaite S, Phillips A, Blaser N, Borquez A, et al. Cost-effectiveness of different strategies to monitor adults on antiretroviral treatment: a combined analysis of three mathematical models. The Lancet Global Health 2013.

43. Barth RE, van der Loeff MFS, Schuurman R, Hoepelman AIM, Wensing AMJ. Virological follow-up of adult patients in antiretroviral treatment programmes in sub-Saharan Africa: a systematic review. Lancet Infect Dis 2010; 10:155-66.

44. Boender TS, Sigaloff KC, McMahon JH, Kiertiburanakul S, Jordan MR, Barcarolo J, et al. Long-term Virological Outcomes of First-Line Antiretroviral Therapy for HIV-1 in Low- and Middle-Income Countries: A Systematic Review and Meta-analysis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2015; 61:1453-61.

45. Mermin J, Ekwaru JP, Were W, Degerman R, Bunnell R, Kaharuza F, et al. Utility of routine viral load, CD4 cell count, and clinical monitoring among adults with HIV receiving antiretroviral therapy in Uganda: randomised trial. Bmj 2011; 343:d6792.

46. Haas AD, Keiser O, Balestre E, Brown S, Bissagnene E, Chimbetete C, et al. Monitoring and switching of first-line antiretroviral therapy in adult treatment cohorts in sub-Saharan Africa: collaborative analysis. The Lancet HIV 2015.

47. Boulle A, Bock P, Osler M, Cohen K, Channing L, Hilderbrand K, et al. Antiretroviral therapy and early mortality in South Africa. Bulletin of the World Health Organization 2008; 86:678-87.

48. Boulle A, Van Cutsem G, Hilderbrand K, Cragg C, Abrahams M, Mathee S, et al. Seven-year experience of a primary care antiretroviral treatment programme in Khayelitsha, South Africa. AIDS 2010; 24:563-72.

49. Bussmann H, Wester CW, Ndwapi N, Grundmann N, Gaolathe T, Puvimanasinghe J, et al. Five-year outcomes of initial patients treated in Botswana's National Antiretroviral Treatment Program. Aids 2008; 22:2303-11.

50. Duong T, Jourdain G, Ngo-Giang-Huong N, Le Cœur S, Kantipong P, Buranabanjasatean S, et al. Laboratory and Clinical Predictors of Disease Progression following Initiation of Combination Therapy in HIV-Infected Adults in Thailand. PloS one 2012; 7:e43375.

51. Fatti G, Meintjes G, Shea J, Eley B, Grimwood A. Improved survival and antiretroviral treatment outcomes in adults receiving community-based adherence support: 5-year results from a multicentre cohort study in South Africa. J Acquir Immune Defic Syndr 2012; 61:e50-8.

52. Hamers RL, Oyomopito R, Kityo C, Phanuphak P, Siwale M, Sungkanuparph S, et al. Cohort profile: The PharmAccess African (PASER-M) and the TREAT Asia (TASER-M) monitoring studies to evaluate resistance--HIV drug resistance in sub-Saharan Africa and the Asia-Pacific. International journal of epidemiology 2012; 41:43-54.

53. He M, Zheng Y-H, Zhou H-Y, Mamadou D, Chen Z, Chen X, et al. Prospective Observation for Seven-Year's Highly Active Antiretroviral Therapy in Chinese HIV-1 Infected Patients. Current HIV Research 2011; 9:160-5.

54. Manosuthi W, Tantanathip P, Chimsuntorn S, Eampokarap B, Thongyen S, Nilkamhang S, et al. Treatment outcomes of patients co-infected with HIV and tuberculosis who received a nevirapine-based antiretroviral regimen: a four-year prospective study. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases 2010; 14:e1013-7.

55. Soe AN, Tansuphasawadikul S, Phonrat B, Boonpok L, Tepsupa S, Japrasert C. Early viral suppression predicting long-term treatment success among HIV patients commencing NNRTI-based antiretroviral therapy. Journal of Antivirals & Antiretrovirals 2012; 2010.

56. Castelnuovo B, Kiragga A, Musaazi J, Sempa J, Mubiru F, Wanyama J, et al. Outcomes in a Cohort of Patients Started on Antiretroviral Treatment and Followed up for a Decade in an Urban Clinic in Uganda. PloS one 2015; 10:e0142722.

57. Sax PE, Tierney C, Collier AC, Daar ES, Mollan K, Budhathoki C, et al. Abacavir/lamivudine versus tenofovir DF/emtricitabine as part of combination regimens for initial treatment of HIV: final results. The Journal of infectious diseases 2011; 204:1191-201.

58. Martínez E, Arranz J, Podzamczer D, Lonca M, Sanz J, Barragán P, et al. Long-term outcomes of switching to fixed-dose abacavir/lamivudine (ABC/3TC) or tenofovir/emtricitabine (TDF/FTC): 3-year results of the BICOMBO study. J Int AIDS Soc 2010; 13:P43.

59. Smith KY, Patel P, Fine D, Bellos N, Sloan L, Lackey P, et al. Randomized, double-blind, placebo-matched, multicenter trial of abacavir/lamivudine or tenofovir/emtricitabine with lopinavir/ritonavir for initial HIV treatment. Aids 2009; 23:1547-56.

60. Post FA, Moyle GJ, Stellbrink HJ, Domingo P, Podzamczer D, Fisher M, et al. Randomized comparison of renal effects, efficacy, and safety with once-daily abacavir/lamivudine versus tenofovir/emtricitabine, administered with efavirenz, in antiretroviral-naive, HIV-1-infected adults: 48-week results from the ASSERT study. J Acquir Immune Defic Syndr 2010; 55:49-57.

61. Hill A, Sawyer W. Effects of nucleoside reverse transcriptase inhibitor backbone on the efficacy of first-line boosted highly active antiretroviral therapy based on protease inhibitors: meta-regression analysis of 12 clinical trials in 5168 patients. HIV medicine 2009; 10:527-35.

62. Gulick RM, Ribaudo HJ, Shikuma CM, Lustgarten S, Squires KE, Meyer WA, 3rd, et al. Triple-nucleoside regimens versus efavirenz-containing regimens for the initial treatment of HIV-1 infection. The New England journal of medicine 2004; 350:1850-61.

63. Kumar PN, Salvato P, Lamarca A, Dejesus E, Patel P, McClernon D, et al. A randomized, controlled trial of initial anti-retroviral therapy with abacavir/lamivudine/zidovudine twice-daily compared to atazanavir once-daily with lamivudine/zidovudine twice-daily in HIV-infected patients over 48 weeks (ESS100327, the ACTION Study). AIDS research and therapy 2009; 6:3.

64. Gallant JE, Rodriguez AE, Weinberg WG, Young B, Berger DS, Lim ML, et al. Early virologic nonresponse to tenofovir, abacavir, and lamivudine in HIV-infected antiretroviral-naive subjects. The Journal of infectious diseases 2005; 192:1921-30.

65. Mugavero MJ, Napravnik S, Cole SR, Eron JJ, Lau B, Crane HM, et al. Viremia copy-years predicts mortality among treatment-naive HIV-

infected patients initiating antiretroviral therapy. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2011; 53:927-35.

66. Walker AS, Prendergast AJ, Mugyenyi P, Munderi P, Hakim J, Kekitiinwa A, et al. Mortality in the year following antiretroviral therapy initiation in HIV-infected adults and children in Uganda and Zimbabwe. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2012; 55:1707-18.

67. Do T, Duncan J, Butcher A, Liegler T. Comparative frequencies of HIV low-level viremia between real-time viral load assays at clinically relevant thresholds. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology 2011; 52 Suppl 1:S83-9.

68. Seaman SR, White IR. Review of inverse probability weighting for dealing with missing data. Statistical methods in medical research 2013; 22:278-95.

69. Xie J, Liu C. Adjusted Kaplan-Meier estimator and log-rank test with inverse probability of treatment weighting for survival data. Statistics in medicine 2005; 24:3089-110.

70. Cole SR, Hernan MA. Adjusted survival curves with inverse probability weights. Computer methods and programs in biomedicine 2004; 75:45-9.

71. Breslow NE, Wellner JA. Weighted Likelihood for Semiparametric Models and Two-phase Stratified Samples, with Application to Cox Regression. Scandinavian Journal of Statistics 2007; 34:86-102.

72. Kiwuwa-Muyingo S, Walker AS, Oja H, Levin J, Miiro G, Katabira E, et al. The impact of first year adherence to antiretroviral therapy on long-term clinical and immunological outcomes in the DART trial in Uganda and Zimbabwe. Tropical medicine & international health : TM & IH 2012; 17:584-94.

73. White IR, Royston P, Wood AM. Multiple imputation using chained equations: Issues and guidance for practice. Statistics in medicine 2011; 30:377-99.

74. Rubin DB. Multiple Imputation for Nonresponse in Surveys: John Wiley & Sons, Inc., 2008.

75. Morris TP, White IR, Carpenter JR, Stanworth SJ, Royston P. Combining fractional polynomial model building with multiple imputation. Statistics in medicine 2015.

76. StataCorp. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP. 2013.

77. Gilks CF, Walker AS, Munderi P, Kityo C, Reid A, Katabira E, et al. A single CD4 test with 250 cells/mm3 threshold predicts viral suppression in

HIV-infected adults failing first-line therapy by clinical criteria. PloS one 2013; 8:e57580.

78. Avila D, Althoff KN, Mugglin C, Wools-Kaloustian K, Koller M, Dabis F, et al. Immunodeficiency at the start of combination antiretroviral therapy in low-, middle-, and high-income countries. J Acquir Immune Defic Syndr 2014; 65:e8-16.

79. Phillips AN, Pillay D, Miners AH, Bennett DE, Gilks CF, Lundgren JD. Outcomes from monitoring of patients on antiretroviral therapy in resource-limited settings with viral load, CD4 cell count, or clinical observation alone: a computer simulation model. The Lancet 2008; 371:1443-51.

80. Braithwaite RS, Nucifora KA, Yiannoutsos CT, Musick B, Kimaiyo S, Diero L, et al. Alternative antiretroviral monitoring strategies for HIV-infected patients in east Africa: opportunities to save more lives? J Int AIDS Soc 2011; 14:38.

81. Estill J, Egger M, Blaser N, Vizcaya LS, Garone D, Wood R, et al. Cost-effectiveness of point-of-care viral load monitoring of antiretroviral therapy in resource-limited settings: mathematical modelling study. Aids 2013; 27:1483-92.

82. Cruciani M, Mengoli C, Malena M, Serpelloni G, Parisi SG, Moyle G, et al. Virological efficacy of abacavir: systematic review and meta-analysis. Journal of Antimicrobial Chemotherapy 2014; 69:3169-80.

83. Williams I, Churchill D, Anderson J, Boffito M, Bower M, Cairns G, et al. British HIV Association guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy 2012. HIV medicine 2014; 15 Suppl 1:1-85.

84. Pirrone V, Libon DJ, Sell C, Lerner CA, Nonnemacher MR, Wigdahl B. Impact of age on markers of HIV-1 disease. Future virology 2013; 8:81-101.

85. Sabin CA, Smith CJ, d'Arminio Monforte A, Battegay M, Gabiano C, Galli L, et al. Response to combination antiretroviral therapy: variation by age. Aids 2008; 22:1463-73.

86. Paredes R, Mocroft A, Kirk O, Lazzarin A, Barton SE, van Lunzen J, et al. Predictors of virological success and ensuing failure in HIV-positive patients starting highly active antiretroviral therapy in Europe - Results from the EuroSIDA Study. Archives of Internal Medicine 2000; 160:1123-32.

87. Ledergerber B, Egger M, Opravil M, Telenti A, Hirschel B, Battegay M, et al. Clinical progression and virological failure on highly active antiretroviral therapy in HIV-1 patients: a prospective cohort study. Lancet 1999; 353:863-8.

88. Szadkowski L, Tseng A, Walmsley SL, Salit I, Raboud JM. Short communication: effects of age on virologic suppression and CD4 cell

response in HIV-positive patients initiating combination antiretroviral therapy. AIDS research and human retroviruses 2012; 28:1579-83.

89. Carrieri MP, Leport C, Protopopescu C, Cassuto JP, Bouvet E, Peyramond D, et al. Factors associated with nonadherence to highly active antiretroviral therapy: a 5-year follow-up analysis with correction for the bias induced by missing data in the treatment maintenance phase. J Acquir Immune Defic Syndr 2006; 41:477-85.

90. Goodkin K, Shapshak P, Asthana D, Zheng WL, Concha M, Wilkie FL, et al. Older age and plasma viral load in HIV-1 infection. Aids 2004; 18:S87-S98.

91. Chesney MA. The elusive gold standard. Future perspectives for HIV adherence assessment and intervention. J Acquir Immune Defic Syndr 2006; 43 Suppl 1:S149-55.

92. Castilho JL, Melekhin VV, Sterling TR. Sex differences in HIV outcomes in the highly active antiretroviral therapy era: a systematic review. AIDS research and human retroviruses 2014; 30:446-56.

93. Skowron G, Street JC, Obee EM. Baseline CD4(+) cell count, not viral load, correlates with virologic suppression induced by potent antiretroviral therapy. J Acquir Immune Defic Syndr 2001; 28:313-9.

94. Turnbull BW. The empirical distribution function with arbitrarily grouped, censored and truncated data. Journal of the Royal Statistical Society Series B (Methodological) 1976:290-5.

95. Dorey FJ, Little RJ, Schenker N. Multiple imputation for threshold-crossing data with interval censoring. Statistics in medicine 1993; 12:1589-603.

96. Walker A, Babiker A, Kityo C, Hakim J, Munderi P, Ssali F, et al. Risk of WHO 4 events and death by current CD4 on ART in the DART trial: the impact of CD4-dependent reporting bias. CROI. San Francisco, 2010.

97. UNAIDS. The Gap Report, 2014.

98. Granich R, Gupta S, Hersh B, Williams B, Montaner J, Young B, et al. Trends in AIDS Deaths, New Infections and ART Coverage in the Top 30 Countries with the Highest AIDS Mortality Burden; 1990-2013. PloS one 2015; 10:e0131353.

99. May MT, Gompels M, Delpech V, Porter K, Orkin C, Kegg S, et al. Impact on life expectancy of HIV-1 positive individuals of CD4+ cell count and viral load response to antiretroviral therapy. AIDS 2014; 28:1193-202.

100. Samji H, Cescon A, Hogg RS, Modur SP, Althoff KN, Buchacz K, et al. Closing the gap: increases in life expectancy among treated HIV-positive individuals in the United States and Canada. PloS one 2013; 8:e81355.

101. The Antiretroviral Therapy in Lower Income Countries (ART-LINC) Collaboration and the ART Cohort Collaboration (ART-CC). Mortality of HIV-1-infected patients in the first year of antiretroviral therapy: comparison between low-income and high-income countries. The Lancet 2006; 367:817-24.

102. Helleberg M, Kronborg G, Larsen CS, Pedersen G, Pedersen C, Obel N, et al. CD4 decline is associated with increased risk of cardiovascular disease, cancer, and death in virally suppressed patients with HIV. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2013; 57:314-21.

103. Marin B, Thiebaut R, Bucher HC, Rondeau V, Costagliola D, Dorrucci M, et al. Non-AIDS-defining deaths and immunodeficiency in the era of combination antiretroviral therapy. AIDS 2009; 23:1743-53.

104. Phillips AN, Gazzard B, Gilson R, Easterbrook P, Johnson M, Walsh J, et al. Rate of AIDS diseases or death in HIV-infected antiretroviral therapy-naive individuals with high CD4 cell count. AIDS 2007; 21:1717-21.

105. Hogg RS. Rates of Disease Progression by Baseline CD4 Cell Count and Viral Load After Initiating Triple-Drug Therapy. JAMA : the journal of the American Medical Association 2001; 286:2568.

106. The antiretroviral therapy cohort collaboration (ART-CC), Lanoy E, May M, Mocroft A, Phillip A, Justice A, et al. Prognosis of patients treated with cART from 36 months after initiation, according to current and previous CD4 cell count and plasma HIV-1 RNA measurements. AIDS 2009; 23:2199-208.

107. Masiira B, Baisley K, Mayanja BN, Kazooba P, Maher D, Kaleebu P. Mortality and its predictors among antiretroviral therapy naive HIV-infected individuals with CD4 cell count >/=350 cells/mm(3) compared to the general population: data from a population-based prospective HIV cohort in Uganda. Global health action 2014; 7:21843.

108. Mutevedzi PC, Lessells RJ, Rodger AJ, Newell ML. Association of age with mortality and virological and immunological response to antiretroviral therapy in rural South African adults. PloS one 2011; 6:e21795.

109. Petersen ML, Tran L, Geng EH, Reynolds SJ, Kambugu A, Wood R, et al. Delayed switch of antiretroviral therapy after virologic failure associated with elevated mortality among HIV-infected adults in Africa. AIDS 2014; 28:2097-107.

110. Brennan AT, Maskew M, Sanne I, Fox MP. The interplay between CD4 cell count, viral load suppression and duration of antiretroviral therapy on mortality in a resource-limited setting. Tropical medicine & international health : TM & IH 2013; 18:619-31.

111. Fregonese F, Collins IJ, Jourdain G, Lecoeur S, Cressey TR, Ngo-Giang-Houng N, et al. Predictors of 5-year mortality in HIV-infected adults starting highly active antiretroviral therapy in Thailand. J Acquir Immune Defic Syndr 2012; 60:91-8.

112. Hoffmann CJ, Fielding KL, Johnston V, Charalambous S, Innes C, Moore RD, et al. Changing predictors of mortality over time from cART start: implications for care. J Acquir Immune Defic Syndr 2011; 58:269-76.

113. Lawn SD, Little F, Bekker LG, Kaplan R, Campbel E, Orrell C, et al. Changing mortality risk associated with CD4 cell response to antiretroviral therapy in South Africa. AIDS 2009; 23:335-42.

114. May M, Boulle A, Phiri S, Messou E, Myer L, Wood R, et al. Prognosis of patients with HIV-1 infection starting antiretroviral therapy in sub-Saharan Africa: a collaborative analysis of scale-up programmes. The Lancet 2010; 376:449-57.

115. Keiser O, Chi BH, Gsponer T, Boulle A, Orrell C, Phiri S, et al. Outcomes of antiretroviral treatment in programmes with and without routine viral load monitoring in Southern Africa. AIDS 2011; 25:1761-9.

116. Antiretroviral Therapy Cohort C. Causes of death in HIV-1-infected patients treated with antiretroviral therapy, 1996-2006: collaborative analysis of 13 HIV cohort studies. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2010; 50:1387-96.

117. Etard JF, Ndiaye I, Thierry-Mieg M, Gueye NF, Gueye PM, Laniece I, et al. Mortality and causes of death in adults receiving highly active antiretroviral therapy in Senegal: a 7-year cohort study. AIDS 2006; 20:1181-9.

118. Castelnuovo B, Manabe YC, Kiragga A, Kamya M, Easterbrook P, Kambugu A. Cause-specific mortality and the contribution of immune reconstitution inflammatory syndrome in the first 3 years after antiretroviral therapy initiation in an urban African cohort. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2009; 49:965-72.

119. Moore DM, Yiannoutsos CT, Musick BS, Tappero J, Degerman R, Campbell J, et al. Determinants of early and late mortality among HIV-infected individuals receiving home-based antiretroviral therapy in rural Uganda. J Acquir Immune Defic Syndr 2011; 58:289-96.

120. Dolling D, Kaleebu P, Nkurunziza P, Spyer M, Gilks CF, Pillay D, et al. High rate of viral suppression in late mortality on first-line ART in Uganda/Zimbabwe. Conference on Retroviruses and Opportunistic Infections (CROI). Boston, USA, 2016:abstract 1027.

121. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. Journal of the American Statistical Association 1999; 94:496-509.

122. Latouche A, Allignol A, Beyersmann J, Labopin M, Fine JP. A competing risks analysis should report results on all cause-specific hazards and cumulative incidence functions. J Clin Epidemiol 2013.

123. Lau B, Cole SR, Gange SJ. Competing risk regression models for epidemiologic data. American journal of epidemiology 2009; 170:244-56.

124. Bakoyannis G, Touloumi G. Practical methods for competing risks data: a review. Statistical methods in medical research 2012; 21:257-72.

125. Dignam JJ, Zhang Q, Kocherginsky M. The use and interpretation of competing risks regression models. Clinical cancer research : an official journal of the American Association for Cancer Research 2012; 18:2301-8.

126. Kalbfleisch JD, Prentice RL. The Statistical Analysis of Failure Time Data, Second Edition. . 2002:Section 6.2.

127. Rizopoulos D. JM: An R Package for the Joint Modelling of Longitudinal and Time-to-Event Data. Journal of Statistical Software 2010; 35.

128. Tsiatis AA, Davidian M. Joint modeling of longitudinal and time-to-event data: An overview. Stat Sin 2004; 14:809-34.

129. Rizopoulos D. Joint models for longitudinal and time-to-event data: With applications in R. CRC Press, 2012.

130. Picat MQ, Lewis J, Musiime V, Prendergast A, Nathoo K, Kekitiinwa A, et al. Predicting patterns of long-term CD4 reconstitution in HIV-infected children starting antiretroviral therapy in sub-Saharan Africa: a cohort-based modelling study. PLoS medicine 2013; 10:e1001542.

131. Williamson PR, Kolamunnage-Dona R, Philipson P, Marson AG. Joint modelling of longitudinal and competing risks data. Statistics in medicine 2008; 27:6426-38.

132. Hu W, Li G, Li N. A Bayesian approach to joint analysis of longitudinal measurements and competing risks failure time data. Statistics in medicine 2009; 28:1601-19.

133. Deslandes E, Chevret S. Joint modeling of multivariate longitudinal data and the dropout process in a competing risk setting: application to ICU data. BMC medical research methodology 2010; 10:69.

134. Kurland BF, Johnson LL, Egleston BL, Diehr PH. Longitudinal Data with Follow-up Truncated by Death: Match the Analysis Method to Research Aims. Statistical science : a review journal of the Institute of Mathematical Statistics 2009; 24:211.

135. Royston P, Sauerbrei W. Wiley Series in Probability and Statistics, in Multivariable Model-Building: A pragmatic approach to regression analysis based on fractional polynomials for modelling continuous variables. John Wiley & Sons, Ltd, Chichester, UK, 2008.

136. Royston P, Altman DG, Sauerbrei W. Dichotomizing continuous predictors in multiple regression: a bad idea. Statistics in medicine 2006; 25:127-41.

137. R Development Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria, 2015.

138. Kasamba I, Baisley K, Mayanja BN, Maher D, Grosskurth H. The impact of antiretroviral treatment on mortality trends of HIV-positive adults in rural Uganda: a longitudinal population-based study, 1999-2009. Tropical Medicine & International Health 2012; 17:e66-e73.

139. Slaymaker E, Todd J, Marston M, Calvert C, Michael D, Nakiyingi-Miiro J, et al. How have ART treatment programmes changed the patterns of excess mortality in people living with HIV? Estimates from four countries in East and Southern Africa. Global health action 2014; 7:22789.

140. World Health Organization. Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV. 2015.

141. Ford D, Robins JM, Petersen ML, Gibb DM, Gilks CF, Mugyenyi P, et al. The Impact of Different CD4 Cell-Count Monitoring and Switching Strategies on Mortality in HIV-Infected African Adults on Antiretroviral Therapy: An Application of Dynamic Marginal Structural Models. American journal of epidemiology 2015; 182:633-43.

142. Stadeli KM, Richman DD. Rates of emergence of HIV drug resistance in resource-limited settings: a systematic review. Antiviral therapy 2013; 18:115-23.

143. 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. The Lancet 2012; 380:1250-8.

144. Sigaloff KC, Hamers RL, Wallis CL, Kityo C, Siwale M, Ive P, et al. Unnecessary antiretroviral treatment switches and accumulation of HIV resistance mutations; two arguments for viral load monitoring in Africa. J Acquir Immune Defic Syndr 2011; 58:23-31.

145. Hamers RL, Sigaloff KC, Wensing AM, Wallis CL, Kityo C, Siwale M, et al. Patterns of HIV-1 drug resistance after first-line antiretroviral therapy (ART) failure in 6 sub-Saharan African countries: implications for second-line ART strategies. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2012; 54:1660-9.

146. The TenoRes Study Group. Global epidemiology of drug resistance after failure of WHO recommended first-line regimens for adult HIV-1 infection: a multicentre retrospective cohort study. The Lancet Infectious Diseases 2016.

147. Pinoges L, Schramm B, Poulet E, Balkan S, Szumilin E, Ferreyra C, et al. Risk factors and mortality associated with resistance to first-line antiretroviral therapy: multicentric cross-sectional and longitudinal analyses. J Acquir Immune Defic Syndr 2015; 68:527-35.

148. Jiamsakul A, Sungkanuparph S, Law M, Kantor R, Praparattanapan J, Li PC, et al. HIV multi-drug resistance at first-line antiretroviral failure and subsequent virological response in Asia. J Int AIDS Soc 2014; 17:19053.

149. Wensing AM, Calvez V, Gunthard HF, Johnson VA, Paredes R, Pillay D, et al. 2015 Update of the Drug Resistance Mutations in HIV-1. Topics in antiviral medicine 2015; 23:132-41.

150. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Molecular systems biology 2011; 7:539.

151. Johnson VA, Calvez V, Gunthard HF, Paredes R, Pillay D, Shafer RW, et al. Update of the drug resistance mutations in HIV-1: March 2013. Topics in antiviral medicine 2013; 21:6-14.

152. Lemey P, Salemi M, Vandamme A-M. The phylogenetic handbook: a practical approach to phylogenetic analysis and hypothesis testing. Cambridge University Press, 2009.

153. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular biology and evolution 2013; 30:2725-9.

154. Paradis E, Claude J, Strimmer K. APE: Analyses of Phylogenetics and Evolution in R language. Bioinformatics 2004; 20:289-90.

155. Cameron AC, Trivedi PK. Regression-based tests for overdispersion in the Poisson model. Journal of Econometrics 1990; 46:347-64.

156. Bastian M, Heymann S, Jacomy M. Gephi: An Open Source Software for Exploring and Manipulating Networks. 2009 2009).

157. Box GEP, Cox DR. An Analysis of Transformations. Journal of the Royal Statistical Society Series B (Methodological) 1964; 26:211-52.

158. Sigaloff KC, Ramatsebe T, Viana R, de Wit TF, Wallis CL, Stevens WS. Accumulation of HIV drug resistance mutations in patients failing first-line antiretroviral treatment in South Africa. AIDS research and human retroviruses 2012; 28:171-5.

159. Paton N, Kityo C, Thompson J, Bagenda L, Hakim J, van Oosterhout JJ, et al. 119: Impact of NRTI Cross-Resistance on Second-Line PI + NRTI Therapy Outcomes in Africa. Conference on Retroviruses and Opportunistic Infections (CROI). Seattle, Washington, 2015.

160. 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. Journal of Virology 2006; 80:4971-7.

161. Li JF, Lipscomb JT, Wei X, Martinson NA, Morris L, Heneine W, et al. Detection of low-level K65R variants in nucleoside reverse transcriptase inhibitor-naive chronic and acute HIV-1 subtype C infections. The Journal of infectious diseases 2011; 203:798-802.

162. van de Vijver DA, Wensing AM, Angarano G, Asjo B, Balotta C, Boeri E, et al. The calculated genetic barrier for antiretroviral drug resistance substitutions is largely similar for different HIV-1 subtypes. J Acquir Immune Defic Syndr 2006; 41:352-60.

163. Cunha RD, Abreu CM, Gonzalez LM, Nijhuis M, de Jong D, Aguiar RS, et al. Differential in vitro kinetics of drug resistance mutation acquisition in HIV-1 RT of subtypes B and C. PloS one 2012; 7:e46622.

164. Brenner B, Turner D, Oliveira M, Moisi D, Detorio M, Carobene M, et al. A V106M mutation in HIV-1 clade C viruses exposed to efavirenz confers cross-resistance to non-nucleoside reverse transcriptase inhibitors. AIDS 2003; 17:F1-5.

165. Chin BS, Choi J, Nam JG, Kee MK, Suh SD, Choi JY, et al. Inverse relationship between viral load and genotypic resistance mutations in Korean patients with primary HIV type 1 infections. AIDS research and human retroviruses 2006; 22:1142-7.

166. Machouf N, Thomas R, Nguyen VK, Trottier B, Boulassel MR, Wainberg MA, et al. Effects of drug resistance on viral load in patients failing antiretroviral therapy. Journal of medical virology 2006; 78:608-13.

167. de Mendoza C, Martin-Carbonero L, Gallego O, Corral A, Gonzalez-Lahoz J, Soriano V. Relationship between drug resistance mutations, plasma viremia, and CD4+ T-cell counts in patients with chronic HIV infection. Journal of medical virology 2005; 76:1-6.

168. Yu H, Kim T, Oh J, Ko I, Kim S, Han W-S. Enabling multi-level relevance feedback on PubMed by integrating rank learning into DBMS. BMC Bioinformatics 2010; 11:S6-S.

169. van der Valk M, Bisschop PH, Romijn JA, Ackermans MT, Lange JM, Endert E, et al. Lipodystrophy in HIV-1-positive patients is associated with insulin resistance in multiple metabolic pathways. Aids 2001; 15:2093-100.

170. Boucher CAB, O'Sullivan E, Mulder JW, Ramautarsing C, Kellam P, Darby G, et al. Ordered Appearance of Zidovudine Resistance Mutations during Treatment of 18 Human Immunodeficiency Virus-Positive Subjects. Journal of Infectious Diseases 1992; 165:105-10.

171. Desper R, Jiang F, Kallioniemi OP, Moch H, Papadimitriou CH, Schaffer AA. Inferring tree models for oncogenesis from comparative

genome hybridization data. Journal of computational biology : a journal of computational molecular cell biology 1999; 6:37-51.

172. Beerenwinkel N, Rahnenfuhrer J, Daumer M, Hoffmann D, Kaiser R, Selbig J, et al. Learning multiple evolutionary pathways from cross-sectional data. Journal of computational biology : a journal of computational molecular cell biology 2005; 12:584-98.

173. Beerenwinkel N, Rahnenfuhrer J, Kaiser R, Hoffmann D, Selbig J, Lengauer T. Mtreemix: a software package for learning and using mixture models of mutagenetic trees. Bioinformatics 2005; 21:2106-7.

174. Montazeri H, Gunthard HF, Yang WL, Kouyos R, Beerenwinkel N, Swiss HIVCS. Estimating the dynamics and dependencies of accumulating mutations with applications to HIV drug resistance. Biostatistics 2015.

175. Beerenwinkel N, Drton M. A mutagenetic tree hidden Markov model for longitudinal clonal HIV sequence data. Biostatistics 2007; 8:53-71.

176. Myllymäki P, Silander T, Tirri H, Uronen P. B-course: A web-based tool for Bayesian and causal data analysis. International Journal on Artificial Intelligence Tools 2002; 11:369-87.

177. Theys K, Deforche K, Libin P, Camacho RJ, Van Laethem K, Vandamme AM. Resistance pathways of human immunodeficiency virus type 1 against the combination of zidovudine and lamivudine. The Journal of general virology 2010; 91:1898-908.

178. Kuritzkes DR, Bassett RL, Hazelwood JD, Barrett H, Rhodes RA, Young RK, et al. Rate of thymidine analogue resistance mutation accumulation with zidovudine- or stavudine-based regimens. J Acquir Immune Defic Syndr 2004; 36:600-3.

179. Barth RE, Aitken SC, Tempelman H, Geelen SP, van Bussel EM, Hoepelman AI, et al. Accumulation of drug resistance and loss of therapeutic options precede commonly used criteria for treatment failure in HIV-1 subtype-C-infected patients. Antiviral therapy 2012; 17:377-86.

180. Cozzi-Lepri A, Phillips AN, Martinez-Picado J, Monforte A, Katlama C, Eg Hansen AB, et al. Rate of accumulation of thymidine analogue mutations in patients continuing to receive virologically failing regimens containing zidovudine or stavudine: implications for antiretroviral therapy programs in resource-limited settings. The Journal of infectious diseases 2009; 200:687-97.

181. Cozzi-Lepri A, Paredes, Phillips AN, Clotet B, Kjaer J, Von Wyl V, et al. The rate of accumulation of nonnucleoside reverse transcriptase inhibitor (NNRTI) resistance in patients kept on a virologically failing regimen containing an NNRTI*. HIV medicine 2012; 13:62-72.

182. Johnson VA, Brun-Vezinet F, Bonaventura C, Günthard HF, Kuritzkes D, Pillay D, et al. Update of the Drug Resistance Mutations in HIV-1: December 2010. Topics in HIV Medicine 2010; 18:156-63.

183. Lawyer G, Altmann A, Thielen A, Zazzi M, Sonnerborg A, Lengauer T. HIV-1 mutational pathways under multidrug therapy. AIDS research and therapy 2011; 8:26.

184. Boender TS, Kityo CM, Boerma RS, Hamers RL, Ondoa P, Wellington M, et al. Accumulation of HIV-1 drug resistance after continued virological failure on first-line ART in adults and children in sub-Saharan Africa. The Journal of antimicrobial chemotherapy 2016.

185. Deforche K, Camacho RJ, Grossman Z, Soares MA, Van Laethem K, Katzenstein DA, et al. Bayesian network analyses of resistance pathways against efavirenz and nevirapine. AIDS 2008; 22:2107-15.

186. Deforche K, Silander T, Camacho R, Grossman Z, Soares MA, Van Laethem K, et al. Analysis of HIV-1 pol sequences using Bayesian Networks: implications for drug resistance. Bioinformatics 2006; 22:2975-9.

187. Hernandez-Leal P, Rios-Flores A, Avila-Rios S, Reyes-Teran G, Gonzalez JA, Fiedler-Cameras L, et al. Discovering human immunodeficiency virus mutational pathways using temporal Bayesian networks. Artificial intelligence in medicine 2013; 57:185-95.

188. Buendia P, Cadwallader B, DeGruttola V. A phylogenetic and Markov model approach for the reconstruction of mutational pathways of drug resistance. Bioinformatics 2009; 25:2522-9.

189 Soriano V, Perelson AS, Zoulim F. Why are there different dynamics in the selection of drug resistance in HIV and hepatitis B and C viruses? Journal of Antimicrobial Chemotherapy 2008; 62: 1-4.

190 Shen L, Perelson S, Sedaghat AR, McMahon MA, Callender M, Zhang H, Zhou Y, Pitt E, Anderson KS, Acosta EP, Siliciano RF. Dose-response curve slope sets class-specific limits on inhibitory potential of anti-HIV drugs. Nature Medicine 2008; 14(7): 762-766.

191. Vaidya NK, Rong L, Marconi VC, Kuritzkes DR, Deeks SG, Perelon AS. Treatment-Mediated Alterations in HIV Fitness Preserve CD4+ T Cell Counts but Have Minimal Effects on Viral Load. PLoS Computational Biology 2010; 6(11): e1001012.

192. Miller MD. K65R, RAMS and tenofovir. AIDS Review 2004; 6(1):22-23.

193. Nicastri E, Sarmati L, d'Ettorre G, Parisi SG, Palmisano L, Galluzzo C, Montano M, Uccella I, Amici R, Gatti F, Vullo V, Concia E, Vella S, Andreoni M. High prevalence of M184 mutation among patients with viroimmunologic discordant responses to highly active antiretroviral therapy and outcomes after change of therapy guided by genotypic analysis. Journal of Clinical Microbiology 2003; 41(7):3007-3012.

194 Rong L, Feng Z, Perelson AS. Emergence of HIV-1 Drug Resistance During Antiretroviral Treatment. Bulletin of Mathematical Biology 2007; 69: 2027-2060.

195. Svarovskaia E, Feng J, Margot N, Myrick F, Goodman D, Ly J, et al. The A62V and S68G mutations in HIV-1 reverse transcriptase partially restore the replication defect associated with the K65R mutation (Abstract 591). In: The 14th Conference on Retroviruses and Opportunistic Infections. (Los Angeles, CA, USA).

196. Maeda Y, Venzon David J, Mitsuya H. Altered Drug Sensitivity, Fitness, and Evolution of Human Immunodeficiency Virus Type 1 withpolGene Mutations Conferring Multi-Dideoxynucleoside Resistance. The Journal of infectious diseases 1998; 177:1207-13.

197. Huigen MC, Albert J, Lindstrom A, Ohlis A, Bratt G, de Graaf L, et al. Compensatory fixation explains long term persistence of the M41L in HIV-1 reverse transcriptase in a large transmission cluster. Antiviral therapy 2006; 11:S113.

198. Jeeninga RE, Keulen W, Boucher C, Sanders RW, Berkhout B. Evolution of AZT resistance in HIV-1: the 41-70 intermediate that is not observed in vivo has a replication defect. Virology 2001; 283:294-305.

199. Lu J, Whitcomb J, Kuritzkes DR. Effect of the Q207D Mutation in HIV Type 1 Reverse Transcriptase on Zidovudine Susceptibility and Replicative Fitness. JAIDS Journal of Acquired Immune Deficiency Syndromes 2005; 40:20-3.

200. Kantor R, Zijenah LS, Shafer RW, Mutetwa S, Johnston E, Lloyd R, et al. HIV-1 subtype C reverse transcriptase and protease genotypes in Zimbabwean patients failing antiretroviral therapy. AIDS research and human retroviruses 2002; 18:1407-13.

201. DeGruttola V, Dix L, D'Aquila R, Holder D, Phillips A, Ait-Khaled M, et al. The relation between baseline HIV drug resistance and response to antiretroviral therapy: re-analysis of retrospective and prospective studies using a standardized data analysis plan. Antiviral therapy 2000; 5:41-8.

202. Castro H, Pillay D, Cane P, Asboe D, Cambiano V, Phillips A, et al. Persistence of HIV-1 transmitted drug resistance mutations. The Journal of infectious diseases 2013; 208:1459-63.

203. Yang WL, Kouyos RD, Boni J, Yerly S, Klimkait T, Aubert V, et al. Persistence of transmitted HIV-1 drug resistance mutations associated with fitness costs and viral genetic backgrounds. PLoS pathogens 2015; 11:e1004722.

204. Parikh UM, Zelina S, Sluis-Cremer N, Mellors JW. Molecular mechanisms of bidirectional antagonism between K65R and thymidine analog mutations in HIV-1 reverse transcriptase. AIDS 2007; 21:1405-14.

205. Phillips A, Shroufi A, Vojnov L, Cohn J, Roberts T, Ellman T, et al. Sustainable HIV treatment in Africa through viral-load-informed differentiated care. Nature 2015; 528:S68-76.

206. Insight Start Study Group, Lundgren JD, Babiker AG, Gordin F, Emery S, Grund B, et al. Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection. The New England journal of medicine 2015; 373:795-807.

Appendix A. Acknowledgements

We thank all the patients and staff from all the centres participating in the DART trial.

DART Virology Group: P. Kaleebu (Co-Chair), D. Pillay (Co-Chair), P. Awio , M. Chirara, D. Dunn, D. M. Gibb, C. Gilks, R. Goodall, A. Kapaata, M. Katuramur, F. Lyagoba, B. Magambo, K. Mataruka, L. Mugarura , T. Musunga, M. Nabankkema, J. Nkalubo, P. Nkurunziza, C. Parry, V. Robertson, M. Spyer, D. Mulima, D.E. Williams, I. Nankya, S. Nassimbwa, E. Ndashimye, E. Nabulime, M. Phiri, K. Mutasa, S. Mukasa.

MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda: H. Grosskurth, P. Munderi, G. Kabuye, D. Nsibambi, R. Kasirye, E. Zalwango, M. Nakazibwe, B. Kikaire, G. Nassuna, R. Massa, K Fadhiru, M. Namyalo, A. Zalwango, L. Generous, P. Khauka, N. Rutikarayo, W. Nakahima, A. Mugisha, J. Todd, J. Levin, S. Muyingo, A. Ruberantwari, P. Kaleebu, D. Yirrell, N. Ndembi, F. Lyagoba, P. Hughes, M. Aber, A. Medina Lara, S. Foster, J. Amurwon and B. Nyanzi Wakholi.

Joint Clinical Research Centre, Kampala, Uganda: P. Mugyenyi, C. Kityo, F. Ssali, D. Tumukunde, T. Otim, J. Kabanda, H. Musana, J. Akao, H. Kyomugisha, A. Byamukama, J. Sabiiti, J Komugyena, P. Wavamunno, S. Mukiibi, A. Drasiku, R. Byaruhanga, O. Labeja, P. Katundu, S. Tugume, P. Awio, A. Namazzi, G. T. Bakeinyaga, H. Katabira, D. Abaine, J Tukamushaba, W. Anywar, W. Ojiambo, E. Angweng, S. Murungi, W. Haguma, S. Atwiine and J. Kigozi.

University of Zimbabwe, Harare, Zimbabwe: A. Latif, J. Hakim, V. Robertson, A. Reid, E. Chidziva, R. Bulaya-Tembo, G. Musoro, F. Taziwa, C. Chimbetete, L. Chakonza, A. Mawora, C. Muvirimi, G. Tinago, P. Svovanapasis, M. Simango, O. Chirema, J. Machingura, S. Mutsai, M. Phiri, T. Bafana, M. Chirara, L. Muchabaiwa and M. Muzambi.

Infectious Diseases Institute (formerly the Academic Alliance) Makerere University, Mulago, Uganda: E. Katabira, A. Ronald, A. Kambungu, F. Lutwama, A. Nanfuka, J. Walusimbi, E. Nabankema, R. Nalumenya, T. Namuli, R. Kulume, I. Namata, L. Nyachwo, A. Florence, A. Kusiima, E. Lubwama, R. Nairuba, F. Oketta, E. Buluma, R. Waita, H. Ojiambo, F. Sadik, J. Wanyama and P. Nabongo.

The AIDS Support Organisation (TASO), Uganda: R Ochai and D Muhweezi.

Imperial College, London, UK: C. Gilks, K. Boocock, C. Puddephatt, D. Winogron and J. Bohannon.

MRC Clinical Trials Unit at UCL, London, UK: J. Darbyshire, D. M. Gibb, A. Burke, D. Bray, A. Babiker, A. S. Walker, H. Wilkes, M. Rauchenberger, S. Sheehan, L. Peto, K. Taylor, M. Spyer, A. Ferrier, B. Naidoo, D. Dunn and R. Goodall.

Independent DART Trial Monitors: R Nanfuka, C Mufuka-Kapuya.

Trial Steering Committee: I Weller (Chair), A Babiker (Trial Statistician), S Bahendeka, M Bassett, A Chogo Wapakhabulo, J Darbyshire, B Gazzard, C Gilks, H Grosskurth, J Hakim, A Latif, C Mapuchere, O Mugurungi, P Mugyenyi; Observers: C Burke, M Distel, S Jones, E Loeliger, P Naidoo, C Newland, G Pearce, S Rahim, J Rooney, M Smith, W Snowden, J-M Steens, M Ait-Khaled.

Data and Safety Monitoring Committee: A Breckenridge (Chair), A McLaren (Chair-deceased), C Hill, J Matenga, A Pozniak, D Serwadda**.**

Endpoint Review Committee: T Peto (Chair), A Palfreeman, M Borok, E Katabira.

Funding: DART was funded by the **UK Medical Research Council** (grant number G0600344) (including additional funding for viral load assays), the **UK Department for International Development (DFID)**, and the **Rockefeller Foundation**. **GlaxoSmithKline/ViiV Healthcare, Gilead** and **Boehringer-Ingelheim** donated first-line drugs for DART.

Appendix B. Chapter 2 literature Search

Search URL: goo.gl/FFCJUM

Year	Authors	Title	Journal	PubMed
2007	Kamya MR, Mayanja-Kizza H, Kambugu A, et al.	Predictors of long-term viral failure among Ugandan children and adults treated with antiretroviral therapy.	J Acquir Immune Defic Syndr.	www.ncbi.nlm.nih.gov/pubmed/17693883
2007	Orrell C, Harling G, Lawn SD, et al.	Conservation of first-line antiretroviral treatment regimen where therapeutic options are limited.	Antivir Ther.	www.ncbi.nlm.nih.gov/pubmed/17503751
2008	Mee P, Fielding KL, Charalambous S, et al.	Evaluation of the WHO criteria for antiretroviral treatment failure among adults in South Africa.	AIDS.	www.ncbi.nlm.nih.gov/pubmed/18784460
2009	Ahoua L, Guenther G, Pinoges L, et al.	Risk factors for virological failure and subtherapeutic antiretroviral drug concentrations in HIV-positive adults treated in rural northwestern Uganda.	BMC Infect Dis.	www.ncbi.nlm.nih.gov/pubmed/19493344
2009	Sanne IM, Westreich D, Macphail AP, et al.	Long term outcomes of antiretroviral therapy in a large HIV/AIDS care clinic in urban South Africa: a prospective cohort study.	J Int AIDS Soc	www.ncbi.nlm.nih.gov/pubmed/20017918
2010	Barth RE, van der Loeff MF, Schuurman R, et al.	Virological follow-up of adult patients in antiretroviral treatment programmes in sub-Saharan Africa: a systematic review.	Lancet Infect Dis.	www.ncbi.nlm.nih.gov/pubmed/20185094
2010	Boulle A, Van Cutsem G, Hilderbrand K, et al.	Seven-year experience of a primary care antiretroviral treatment programme in Khayelitsha, South Africa.	AIDS.	www.ncbi.nlm.nih.gov/pubmed/20057311
2010	El-Khatib Z, Ekstrom AM, Ledwaba J, et al.	Viremia and drug resistance among HIV-1 patients on antiretroviral treatment: a cross-sectional study in Soweto, South Africa.	AIDS.	www.ncbi.nlm.nih.gov/pubmed/20453629
2010	Lynen L, Van Griensven J, Elliott J.	Monitoring for treatment failure in patients on first-line antiretroviral treatment in resource-constrained settings.	Curr Opin HIV AIDS.	www.ncbi.nlm.nih.gov/pubmed/20046141
2010	Zolfo M, Schapiro JM, Phan V, et al.	Genotypic impact of prolonged detectable HIV type 1 RNA viral load after HAART failure in a CRF01_AE-infected cohort.	AIDS Res Hum Retroviruses.	www.ncbi.nlm.nih.gov/pubmed/20854169
2010	Charpentier C, Talla F, Nguepi E, et al.	Virological failure and HIV type 1 drug resistance profiles among patients followed-up in private sector, Douala, Cameroon.	AIDS Res Hum Retroviruses.	www.ncbi.nlm.nih.gov/pubmed/20977359
2010	Laurent C, Tchatchueng Mbougua JB, Ngom Guèye NF, et al.	Long-term effectiveness and safety of didanosine combined with lamivudine and efavirenz or nevirapine in antiretroviral-naive patients: a 9-year cohort study in Senegal.	Trop Med Int Health.	www.ncbi.nlm.nih.gov/pubmed/21087377

2010	Segeral O, Madec Y, Ban B, et al.	Simplified Assessment of Antiretroviral Adherence and Prediction of Virological Efficacy in HIV-Infected Patients in Cambodia.	AIDS Res Treat.	www.ncbi.nlm.nih.gov/pubmed/21490902
2011	Hamers RL, Schuurman R, Sigaloff KC, 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.	www.ncbi.nlm.nih.gov/pubmed/22036233
2011	Mermin J, Ekwaru JP, Were W, et al.	Utility of routine viral load, CD4 cell count, and clinical monitoring among adults with HIV receiving antiretroviral therapy in Uganda: randomised trial.	BMJ	www.ncbi.nlm.nih.gov/pubmed/22074711
2011	Ncaca LN, Kranzer K, Orrell C.	Treatment interruption and variation in tablet taking behaviour result in viral failure: a case-control study from Cape Town, South Africa.	PLoS One	www.ncbi.nlm.nih.gov/pubmed/21858001
2011	Orrell C, Kaplan R, Wood R, Bekker LG.	Virological breakthrough: a risk factor for loss to followup in a large community-based cohort on antiretroviral therapy.	AIDS Res Treat.	www.ncbi.nlm.nih.gov/pubmed/21716724
2011	Staehelin C, Keiser O, Calmy A, et al.	Longer term clinical and virological outcome of sub-Saharan African participants on antiretroviral treatment in the Swiss HIV Cohort Study.	J Acquir Immune Defic Syndr.	www.ncbi.nlm.nih.gov/pubmed/21937923
2012	Alvarez-Uria G, Naik PK, Pakam R, et al.	Early HIV viral load determination after initiating first-line antiretroviral therapy for indentifying patients with high risk of developing virological failure: data from a cohort study in a resource-limited setting.	Trop Med Int Health.	www.ncbi.nlm.nih.gov/pubmed/22487689
2012	Amoroso A, Etienne-Mesubi M, Edozien A, et al.	Treatment outcomes of recommended first-line antiretroviral regimens in resource-limited clinics.	J Acquir Immune Defic Syndr.	www.ncbi.nlm.nih.gov/pubmed/22343178
2012	Arnedo M, Alonso E, Eisenberg N, et al.	Monitoring HIV viral load in resource limited settings: still a matter of debate?	PLoS One.	www.ncbi.nlm.nih.gov/pubmed/23236346
2012	Bastard M, Pinoges L, Balkan S, et al.	Timeliness of clinic attendance is a good predictor of virological response and resistance to antiretroviral drugs in HIV-infected patients.	PLoS One.	www.ncbi.nlm.nih.gov/pubmed/23145079
2012	De Beaudrap P, Thiam M, Diouf A, et al.	Risk of virological failure and drug resistance during first and second-line antiretroviral therapy in a 10-year cohort in Senegal: results from the ANRS 1215 cohort.	J Acquir Immune Defic Syndr.	www.ncbi.nlm.nih.gov/pubmed/23117504
2012	Ferreyra C, Yun O, Eisenberg N, et al.	Evaluation of clinical and immunological markers for predicting virological failure in a HIV/AIDS treatment cohort in Busia, Kenya.	PLoS One.	www.ncbi.nlm.nih.gov/pubmed/23185450

2012	Johnston V, Fielding KL, Charalambous S, et al.	Outcomes following virological failure and predictors of switching to second-line antiretroviral therapy in a South African treatment program.	J Acquir Immune Defic Syndr.	www.ncbi.nlm.nih.gov/pubmed/22820803
2012	Liégeois F, Vella C, Eymard-Duvernay S, et al.	Virological failure rates and HIV-1 drug resistance patterns in patients on first-line antiretroviral treatment in semirural and rural Gabon.	J Int AIDS Soc.	www.ncbi.nlm.nih.gov/pubmed/23199801
2013	Aghokeng AF, Kouanfack C, Eymard-Duvernay S, et al.	Virological outcome and patterns of HIV-1 drug resistance in patients with 36 months' antiretroviral therapy experience in Cameroon.	J Int AIDS Soc.	www.ncbi.nlm.nih.gov/pubmed/23374858
2013	Evans D, Menezes C, Mahomed K, et al.	Treatment outcomes of HIV-infected adolescents attending public-sector HIV clinics across Gauteng and Mpumalanga, South Africa.	AIDS Res Hum Retroviruses.	www.ncbi.nlm.nih.gov/pubmed/23373540
2013	Fibriani A, Wisaksana R, Indrati A, et al.	Virological failure and drug resistance during first line anti-retroviral treatment in Indonesia.	J Med Virol.	www.ncbi.nlm.nih.gov/pubmed/23722251
2013	Gilks CF, Walker AS, Munderi P, et al.	A single CD4 test with 250 cells/mm3 threshold predicts viral suppression in HIV-infected adults failing first-line therapy by clinical criteria.	PLoS One.	www.ncbi.nlm.nih.gov/pubmed/23437399
2013	Ingole N, Mehta P, Pazare A, et al.	Performance of immunological response in predicting virological failure.	AIDS Res Hum Retroviruses.	www.ncbi.nlm.nih.gov/pubmed/2313729
2013	Neogi U, Heylen E, Shet A, et al.	Long-term efficacy of first line antiretroviral therapy in Indian HIV-1 infected patients: a longitudinal cohort study.	PLoS One.	www.ncbi.nlm.nih.gov/pubmed/23383185
2013	Njuguna C, Orrell C, Kaplan R, et al.	Rates of switching antiretroviral drugs in a primary care service in South Africa before and after introduction of tenofovir.	PLoS One.	www.ncbi.nlm.nih.gov/pubmed/23717451
2013	Oyomopito RA, Li PC, Sungkanuparph S, et al.	Evaluating immunologic response and clinical deterioration in treatment-naive patients initiating first-line therapies infected with HIV-1 CRF01_AE and subtype B.	J Acquir Immune Defic Syndr.	www.ncbi.nlm.nih.gov/pubmed/23138836
2013	Rusine J, Asiimwe-Kateera B, van de Wijgert J, et al.	Low primary and secondary HIV drug-resistance after 12 months of antiretroviral therapy in human immune-deficiency virus type 1 (HIV-1)-infected individuals from Kigali, Rwanda.	PLoS One.	www.ncbi.nlm.nih.gov/pubmed/23950859
2014	Crawford KW, Wakabi S, Magala F, et al.	Evaluation of treatment outcomes for patients on first-line regimens in US President's Emergency Plan for AIDS Relief (PEPFAR) clinics in Uganda: predictors of virological and immunological response from RV288 analyses.	HIV Med.	www.ncbi.nlm.nih.gov/pubmed/25124078
2014	Goodall RL, Dunn DT, Pattery T	Phenotypic and genotypic analyses to guide selection of reverse transcriptase inhibitors in second-line HIV therapy following extended virological failure in Uganda.	J Antimicrob Chemother.	www.ncbi.nlm.nih.gov/pubmed/24633208

2014	Kityo C, Gibb DM, Gilks CF, et al.	High level of viral suppression and low switch rate to second-line antiretroviral therapy among HIV-infected adult patients followed over five years: retrospective analysis of the DART trial.	PLoS One.	www.ncbi.nlm.nih.gov/pubmed/24625508
2014	Reepalu A, Balcha TT, Skogmar S, et al.	High rates of viral suppression in a cohort of HIV-positive adults receiving ART in Ethiopian health centers irrespective of concomitant tuberculosis.	J Int AIDS Soc	www.ncbi.nlm.nih.gov/pubmed/25394116
2014	Reepalu A, Balcha TT, Skogmar S, et al.	High rates of virological suppression in a cohort of human immunodeficiency virus-positive adults receiving antiretroviral therapy in ethiopian health centers irrespective of concomitant tuberculosis.	Open Forum Infect Dis.	www.ncbi.nlm.nih.gov/pubmed/25734107
2015	Boender TS, Sigaloff KC, McMahon JH, et al.	Long-term Virological Outcomes of First-Line Antiretroviral Therapy for HIV-1 in Low- and Middle-Income Countries: A Systematic Review and Meta-analysis.	Clin Infect Dis	www.ncbi.nlm.nih.gov/pubmed/26157050
2015	Castelnuovo B, Kiragga A, Musaazi J, et al.	Outcomes in a Cohort of Patients Started on Antiretroviral Treatment and Followed up for a Decade in an Urban Clinic in Uganda.	PLoS One	www.ncbi.nlm.nih.gov/pubmed/26642214
2015	Haas AD, Keiser O, Balestre E, et al.	Monitoring and switching of first-line antiretroviral therapy in adult treatment cohorts in sub-Saharan Africa: collaborative analysis.	Lancet HIV	www.ncbi.nlm.nih.gov/pubmed/26423252
2015	Konou AA, Dagnra AY, Vidal N, et al.	Alarming rates of virological failure and drug resistance in patients on long-term antiretroviral treatment in routine HIV clinics in Togo.	AIDS	www.ncbi.nlm.nih.gov/pubmed/26558549
2015	Konou AA, Salou M, Vidal N, et al.	Virological outcome among HIV-1 infected patients on first-line antiretroviral treatment in semi-rural HIV clinics in Togo.	AIDS Res Ther	www.ncbi.nlm.nih.gov/pubmed/26617663
2015	Orrell C, Cohen K, Mauff K, et al.	A Randomized Controlled Trial of Real-Time Electronic Adherence Monitoring With Text Message Dosing Reminders in People Starting First-Line Antiretroviral.	J Acquir Immune Defic Syndr	www.ncbi.nlm.nih.gov/pubmed/26218411
2015	Pinoges L, Schramm B, Poulet E, et al.	Risk factors and mortality associated with resistance to first-line antiretroviral therapy: multicentric cross-sectional and longitudinal analyses.	J Acquir Immune Defic Syndr	www.ncbi.nlm.nih.gov/pubmed/25585301
2015	Rupérez M, Pou C, Maculuve S, et al.	Determinants of virological failure and antiretroviral drug resistance in Mozambique.	J Antimicrob Chemother	www.ncbi.nlm.nih.gov/pubmed/26084302
2015	Shet A, Neogi U, Kumarasamy N, et al.	Virological efficacy with first-line antiretroviral treatment in India: predictors of viral failure and evidence of viral resuppression.	Trop Med Int Health	www.ncbi.nlm.nih.gov/pubmed/26146863

2016	Mekuria LA, Nieuwkerk PT, Yalew AW, et al.	High level of virological suppression among HIV-infected adults receiving combination antiretroviral therapy in Addis Ababa, Ethiopia.	Antivir Ther	www.ncbi.nlm.nih.gov/pubmed/26731316
2016	Moyo F, Chasela C, Brennan AT, et al.	Treatment outcomes of HIV-positive patients on first-line antiretroviral therapy in private versus public HIV clinics in Johannesburg, South Africa.	Clin Epidemiol	www.ncbi.nlm.nih.gov/pubmed/27051316
2016	Ndahimana Jd, Riedel DJ, Mwumvaneza M, et al.	Drug resistance mutations after the first 12 months on antiretroviral therapy and determinants of virological failure in Rwanda.	Trop Med Int Health	www.ncbi.nlm.nih.gov/pubmed/27125473

Appendix C. Chapter 3 literature Search

Search URL: goo.gl/wMbmDT

Year	Authors	Title	Journal	PubMed
2008	Nakanjako D, Kiragga A, Ibrahim F, et al.	Sub-optimal CD4 reconstitution despite viral suppression in an urban cohort on antiretroviral therapy (ART) in sub-Saharan Africa: frequency and clinical significance.	AIDS Res Ther.	www.ncbi.nlm.nih.gov/pubmed/18957083
2008	Petersen ML, van der Laan MJ, et al.	Long-term consequences of the delay between virologic failure of highly active antiretroviral therapy and regimen modification.	AIDS.	www.ncbi.nlm.nih.gov/pubmed/18832873
2009	Kantor R, Diero L, Delong A, et al.	Misclassification of first-line antiretroviral treatment failure based on immunological monitoring of HIV infection in resource-limited settings.	Clin Infect Dis.	www.ncbi.nlm.nih.gov/pubmed/19569972
2009	Keiser O, Tweya H, Braitstein P, et al.	Mortality after failure of antiretroviral therapy in sub-Saharan Africa.	Trop Med Int Health.	www.ncbi.nlm.nih.gov/pubmed/20003034
2009	Palombi L, Marazzi MC, Guidotti G, et al.	Incidence and predictors of death, retention, and switch to second-line regimens in antiretroviral- treated patients in sub-Saharan African Sites with comprehensive monitoring availability.	Clin Infect Dis.	www.ncbi.nlm.nih.gov/pubmed/20380075
2010	Boulle A, Van Cutsem G, Hilderbrand K, et al.	Seven-year experience of a primary care antiretroviral treatment programme in Khayelitsha, South Africa.	AIDS.	www.ncbi.nlm.nih.gov/pubmed/20057311
2010	Fox MP, Sanne IM, Conradie F, et al.	Initiating patients on antiretroviral therapy at CD4 cell counts above 200 cells/microl is associated with improved treatment outcomes in South Africa.	AIDS.	www.ncbi.nlm.nih.gov/pubmed/20613459
2010	Murphy RA, Sunpath H, Lu Z, et al.	Outcomes after virologic failure of first-line ART in South Africa.	AIDS.	www.ncbi.nlm.nih.gov/pubmed/20397305
2011	Hoffmann CJ, Fielding KL, Johnston V, et al.	Changing predictors of mortality over time from cART start: implications for care.	J Acquir Immune Defic Syndr.	www.ncbi.nlm.nih.gov/pubmed/21876447
2011	Keiser O, Chi BH, Gsponer T, et al.	Outcomes of antiretroviral treatment in programmes with and without routine viral load monitoring in Southern Africa.	AIDS.	www.ncbi.nlm.nih.gov/pubmed/21681057
2011	Laurent C, Kouanfack C, Laborde-Balen G, et al.	Monitoring of HIV viral loads, CD4 cell counts, and clinical assessments versus clinical monitoring alone for antiretroviral therapy in rural district hospitals in Cameroon (Stratall ANRS 12110/ESTHER): a randomised non-inferiority trial.	Lancet Infect Dis.	www.ncbi.nlm.nih.gov/pubmed/21831714

2011	Mermin J, Ekwaru JP, Were W, et al.	Utility of routine viral load, CD4 cell count, and clinical monitoring among adults with HIV receiving antiretroviral therapy in Uganda: randomised trial.	BMJ.	www.ncbi.nlm.nih.gov/pubmed/22074711
2011	Mutevedzi PC, Lessells RJ, Rodger AJ, et al.	Association of age with mortality and virological and immunological response to antiretroviral therapy in rural South African adults.	PLoS One.	www.ncbi.nlm.nih.gov/pubmed/21747959
2011	Orrell C, Kaplan R, Wood R, et al.	Virological breakthrough: a risk factor for loss to followup in a large community-based cohort on antiretroviral therapy.	AIDS Res Treat.	www.ncbi.nlm.nih.gov/pubmed/21716724
2011	Steele KT, Steenhoff AP, Newcomb CW, et al.	Early mortality and AIDS progression despite high initial antiretroviral therapy adherence and virologic suppression in Botswana.	PLoS One.	www.ncbi.nlm.nih.gov/pubmed/21698283
2012	Cornell M, Schomaker M, Garone DB, et al.	Gender differences in survival among adult patients starting antiretroviral therapy in South Africa: a multicentre cohort study.	PLoS Med.	www.ncbi.nlm.nih.gov/pubmed/22973181
2012	Johnston V, Fielding KL, Charalambous S, et al.	Outcomes following virological failure and predictors of switching to second-line antiretroviral therapy in a South African treatment program.	J Acquir Immune Defic Syndr.	www.ncbi.nlm.nih.gov/pubmed/22820803
2012	Karstaedt AS.	Profile of cause of death assigned to adults on antiretroviral therapy in Soweto.	S Afr Med J.	www.ncbi.nlm.nih.gov/pubmed/22831946
2012	Labhardt ND, Lejone T, Setoko M, et al.	A clinical prediction score in addition to WHO criteria for anti-retroviral treatment failure in resource-limited settings--experience from Lesotho.	PLoS One.	www.ncbi.nlm.nih.gov/pubmed/23118910
2012	Lockman S, Hughes M, Sawe F, et al.	Nevirapine- versus lopinavir/ritonavir-based initial therapy for HIV-1 infection among women in Africa: a randomized trial.	PLoS Med.	www.ncbi.nlm.nih.gov/pubmed/22719231
2012	Nglazi MD, Kranzer K, Holele P, et al.	Treatment outcomes in HIV-infected adolescents attending a community-based antiretroviral therapy clinic in South Africa.	BMC Infect Dis.	www.ncbi.nlm.nih.gov/pubmed/22273267
2013	Brennan AT, Maskew M, Sanne I, et al.	The interplay between CD4 cell count, viral load suppression and duration of antiretroviral therapy on mortality in a resource-limited setting.	Trop Med Int Health.	www.ncbi.nlm.nih.gov/pubmed/23419157
2014	Byakwaga H, Boum Y 2nd, Huang Y, et al.	The kynurenine pathway of tryptophan catabolism, CD4+ T-cell recovery, and mortality among HIV-infected Ugandans initiating antiretroviral therapy.	J Infect Dis.	www.ncbi.nlm.nih.gov/pubmed/24585899
2014	Havers F, Smeaton L, Gupte N, et al.	25-Hydroxyvitamin D insufficiency and deficiency is associated with HIV disease progression and virological failure post-antiretroviral therapy initiation in diverse multinational settings.	J Infect Dis.	www.ncbi.nlm.nih.gov/pubmed/24799602

2014	Petersen ML, Tran L, Geng EH, et al.	Delayed switch of antiretroviral therapy after virologic failure associated with elevated mortality among HIV-infected adults in Africa.	AIDS.	www.ncbi.nlm.nih.gov/pubmed/24977440
2014	Pettersen PS, Brox IK, Naman E, et al.	Antiretroviral treatment failure predicts mortality in rural Tanzania.	Int J STD AIDS.	www.ncbi.nlm.nih.gov/pubmed/25122578
2014	Takuva S, Maskew M, Brennan AT, et al.	Poor CD4 recovery and risk of subsequent progression to AIDS or death despite viral suppression in a South African cohort.	J Int AIDS Soc.	www.ncbi.nlm.nih.gov/pubmed/24594114
2015	Mingbunjerdsuk P, Asdamongkol N, Sungkanuparph S.	Factors Associated with Immunological Discordance in HIV-Infected Patients Receiving Antiretroviral Therapy with Complete Viral Suppression in a Resource-Limited Setting.	Jpn J Infect Dis.	www.ncbi.nlm.nih.gov/pubmed/25720640
2015	Pinoges L, Schramm B, Poulet E, et al.	Risk factors and mortality associated with resistance to first-line antiretroviral therapy: multicentric cross-sectional and longitudinal analyses.	J Acquir Immune Defic Syndr.	www.ncbi.nlm.nih.gov/pubmed/25585301
2015	Yotebieng M, Maskew M, Van Rie A.	CD4+ gain percentile curves for monitoring response to antiretroviral therapy in HIV-infected adults.	AIDS.	www.ncbi.nlm.nih.gov/pubmed/26125140
2016	Moyo F, Chasela C, Brennan AT, et al.	Treatment outcomes of HIV-positive patients on first-line antiretroviral therapy in private versus public HIV clinics in Johannesburg, South Africa.	Clin Epidemiol.	www.ncbi.nlm.nih.gov/pubmed/27051316

Appendix D. CROI abstract

Title: **High rate of viral suppression in late mortality on first-line ART in Uganda/Zimbabwe**

Author list: David Dolling, Pontiano Kaleebu, Peter Nkurunziza, Moira Spyer, Charles Gilks, Deenan Pillay, Ruth Goodall on behalf of the DART Virology Group

Category: Other Complications of HIV Infection and Antiretroviral therapy
Keywords: resource-limited, mortality, virological suppression, viral load

Background
Early mortality (<48 wks) after ART initiation in resource-limited settings is well recognised, but less well understood are the causes of later mortality, which are widely assumed to be due to virological failure (VF) or non-adherence. We investigated HIV viral load (VL) in patients who died after 48 weeks of continuous first-line ART in the DART study.

Methods
The DART trial randomised 3,316 Ugandan and Zimbabwean patients to laboratory monitoring (LCM; CD4 cell count every 12 weeks) or clinically driven monitoring (CDM). Prospective VL testing was not undertaken. Previous analyses found that low pre-ART CD4 cell count was strongly associated with higher mortality during the first year of ART, which was predominantly from infectious causes. All late mortalities had stored plasma samples from the closest visit to the date of death retrospectively tested for VL. Logistic regression models were used to determine predictors of mortality with virological suppression (VS) status (VL<200 copies/mL) in patients who died. Fractional polynomials were used for continuous variables, but non-linear risk was not found.

Results
210/382 (55%) deaths during the DART study occurred after week 48; 112 were on continuous first-line and 78 on second-line ART. The late mortality rate was low (10.7/1,000 PY). VL data were available for 105/112 (94%) mortalities at a median (IQR) of 10 (6-14) weeks before death. 43/105 (41%) patients were virologically suppressed (VS) at the time of death. VF deaths were more often due to opportunistic infections (26% vs 12%;p=0.09). CD4 cell count was significantly lower at the time of death in patients with VF than VS (Median: 62 vs 238 cells/mm^3; p<0.001) and a greater proportion had CD4<100 cells/mm^3 (66% vs 19%). In multivariate logistic regression analyses (Table), patients in the CDM arm had reduced

odds of 0.28 (95% CI: 0.11 – 0.68) death with VS, with no evidence of a change over time. The odds of death with VS were almost 4 times higher for each additional 100 cells/mm^3 increase in baseline CD4 count. Gender, age, initial ART regimen, CD4 cell count at week 48, baseline VL and opportunistic infections were not associated with VS at death in multivariate analyses.

Conclusions

40% of late deaths on ART occurred without VL criteria for treatment switch being fulfilled. There were significantly more deaths with VS among patients who received CD4 cell count monitoring. Further research is required to elucidate the cause of deaths in those without VF.

Variable	N (%)	N (%) with virological suppression	Uni OR (95% CI)	Multi OR (95% CI)	p-value
Monitoring randomisation					
LCM	45 (43%)	26 (58%)	1.00	1.00	<0.01
CDM	60 (57%)	17 (28%)	0.29 (0.13-0.65)	0.28 (0.11-0.68)	-
Centre					
Entebbe, Uganda	33 (31%)	17 (52%)	1.00	1.00	0.06
Kampala, Uganda	40 (38%)	18 (45%)	0.77 (0.31-1.94)	1.07 (0.37-3.04)	-
Harare, Zimbabwe	32 (30%)	8 (25%)	0.31 (0.11-0.90)	0.30 (0.09-0.98)	-
Death with opportunistic infection	21 (20%)	5 (24%)	0.38 (0.13-1.13)	-	-
	Median (IQR)	**Median with VS (IQR)**			
Baseline CD4 Count (per 100 Cells/mm^3 increase)	0.72 (0.25 – 1.22)	1.07 (0.56 – 1.42)	3.42 (1.65-7.10)	3.83 (1.69-8.69)	<0.01
Week 48 CD4 Count (per 100 Cells/mm^3 increase)	1.61 (0.89 – 2.14)	1.99 (1.23 – 2.67)	1.57 (1.07-2.30)	-	-
Baseline Viral Load (per Log_{10} copies/mL increase)	5.44 (5.00 – 5.77)	5.47 (5.01 – 5.66)	3.42 (1.65-7.10)	-	-

Appendix E. Chapter 4 literature search

Search URL: goo.gl/bJL5kF

Year	Authors	Title	Journal	PubMed
2004	Laurent C, Kouanfack C, Koulla-Shiro S, et al.	Effectiveness and safety of a generic fixed-dose combination of nevirapine, stavudine, and lamivudine in HIV-1-infected adults in Cameroon: open-label multicentre trial.	Lancet	www.ncbi.nlm.nih.gov/pubmed/15234853
2007	Marcelin AG, Jarrousse B, Derache A, et al.	HIV drug resistance after the use of generic fixed-dose combination stavudine/lamivudine/nevirapine as standard first-line regimen.	AIDS	www.ncbi.nlm.nih.gov/pubmed/18090283
2008	Gupta R, Hill A, Sawyer AW, et al.	Emergence of drug resistance in HIV type 1-infected patients after receipt of first-line highly active antiretroviral therapy: a systematic review of clinical trials.	Clin Infect Dis	www.ncbi.nlm.nih.gov/pubmed/18662137
2008	Marconi VC, Sunpath H, Lu Z, et al.	Prevalence of HIV-1 drug resistance after failure of a first highly active antiretroviral therapy regimen in KwaZulu Natal, South Africa.	Clin Infect Dis	www.ncbi.nlm.nih.gov/pubmed/18419495
2009	Adlington R, Richens J, Shahmanesh M.	First-line antiretroviral therapy in resource-limited settings: time to reconsider?	J Infect Dis	www.ncbi.nlm.nih.gov/pubmed/19358673
2009	Gupta RK, Hill A, Sawyer AW, 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	www.ncbi.nlm.nih.gov/pubmed/19555900
2009	Hoffmann CJ, Charalambous S, Sim J, et al.	Viremia, resuppression, and time to resistance in human immunodeficiency virus (HIV) subtype C during first-line antiretroviral therapy in South Africa.	Clin Infect Dis	www.ncbi.nlm.nih.gov/pubmed/19911963
2009	Kumarasamy N, Madhavan V, Venkatesh KK, et al.	High frequency of clinically significant mutations after first-line generic highly active antiretroviral therapy failure: implications for second-line options in resource-limited settings.	Clin Infect Dis	www.ncbi.nlm.nih.gov/pubmed/19522657
2009	Reynolds SJ, Kityo C, Mbamanya F, et al.	Evolution of drug resistance after virological failure of a first-line highly active antiretroviral therapy regimen in Uganda.	Antivir Ther	www.ncbi.nlm.nih.gov/pubmed/19430104
2009	Riedel DJ, Amoroso A.	Perspectives on treatment failure with thymidine analogue-based first-line regimens in resource-limited settings.	Clin Infect Dis	www.ncbi.nlm.nih.gov/pubmed/19929390

2009	Soria A, Porten K, Fampou-Toundji JC, et al.	Resistance profiles after different periods of exposure to a first-line antiretroviral regimen in a Cameroonian cohort of HIV type-1-infected patients.	Antivir Ther	www.ncbi.nlm.nih.gov/pubmed/19474468
2009	Vidya M, Saravanan S, Uma S, et al.	Genotypic HIV type-1 drug resistance among patients with immunological failure to first-line antiretroviral therapy in south India.	Antivir Ther	www.ncbi.nlm.nih.gov/pubmed/19918105
2009	Wallis CL, Mellors JW, Venter WD, et al.	Varied patterns of HIV-1 drug resistance on failing first-line antiretroviral therapy in South Africa.	J Acquir Immune Defic Syndr	www.ncbi.nlm.nih.gov/pubmed/19801944
2010	Barth RE, van der Loeff MF, Schuurman R, et al.	Virological follow-up of adult patients in antiretroviral treatment programmes in sub-Saharan Africa: a systematic review.	Lancet Infect Dis	www.ncbi.nlm.nih.gov/pubmed/20185094
2010	Chaplin B, Eisen G, Idoko J, et al.	Impact of HIV type 1 subtype on drug resistance mutations in Nigerian patients failing first-line therapy.	AIDS Res Hum Retroviruses	www.ncbi.nlm.nih.gov/pubmed/20964479
2010	El-Khatib Z, Ekstrom AM, Ledwaba J, et al.	Viremia and drug resistance among HIV-1 patients on antiretroviral treatment: a cross-sectional study in Soweto, South Africa.	AIDS	www.ncbi.nlm.nih.gov/pubmed/20453629
2010	Landman R, Diallo MB, Gueye NF, et al.	Efficacy and safety of unboosted atazanavir in combination with lamivudine and didanosine in naive HIV type 1 patients in Senegal.	AIDS Res Hum Retroviruses	www.ncbi.nlm.nih.gov/pubmed/20455760
2010	Lyagoba F, Dunn DT, Pillay D, et al.	Evolution of drug resistance during 48 weeks of zidovudine/lamivudine/tenofovir in the absence of real-time viral load monitoring.	J Acquir Immune Defic Syndr	www.ncbi.nlm.nih.gov/pubmed/20686411
2010	Murphy RA, Sunpath H, Lu Z, et al.	Outcomes after virologic failure of first-line ART in South Africa.	AIDS	www.ncbi.nlm.nih.gov/pubmed/20397305
2010	Wallis CL, Mellors JW, Venter WD, et al.	Varied patterns of HIV-1 drug resistance on failing first-line antiretroviral therapy in South Africa.	J Acquir Immune Defic Syndr	www.ncbi.nlm.nih.gov/pubmed/19801944
2010	Zolfo M, Schapiro JM, Phan V, et al.	Genotypic impact of prolonged detectable HIV type 1 RNA viral load after HAART failure in a CRF01_AE-infected cohort.	AIDS Res Hum Retroviruses	www.ncbi.nlm.nih.gov/pubmed/20854169
2011	Charpentier C, Talla F, Nguepi E, et al.	Virological failure and HIV type 1 drug resistance profiles among patients followed-up in private sector, Douala, Cameroon.	AIDS Res Hum Retroviruses	www.ncbi.nlm.nih.gov/pubmed/20977359
2011	El-Khatib Z, Delong AK, Katzenstein D, et al.	Drug resistance patterns and virus re-suppression among HIV-1 subtype C infected patients receiving non-nucleoside reverse transcriptase inhibitors in South Africa.	J AIDS Clin Res	www.ncbi.nlm.nih.gov/pubmed/21927716
2011	Hamers RL, Wensing AM, Back NK, et al.	Multi-nucleoside reverse transcriptase inhibitor resistant HIV type-1 in a patient from Sierra Leone failing stavudine, lamivudine and nevirapine.	Antivir Ther	www.ncbi.nlm.nih.gov/pubmed/21311115

2011	Muwonga J, Edidi S, Butel C, Vidal N, et al.	Resistance to antiretroviral drugs in treated and drug-naive patients in the Democratic Republic of Congo.	J Acquir Immune Defic Syndr	www.ncbi.nlm.nih.gov/pubmed/21857282
2011	Sigaloff KC, Ramatsebe T, Viana R, et al.	Accumulation of HIV drug resistance mutations in patients failing first-line antiretroviral treatment in South Africa.	AIDS Res Hum Retroviruses	www.ncbi.nlm.nih.gov/pubmed/21819219
2011	van Zyl GU, van der Merwe L, Claassen M, et al.	Antiretroviral resistance patterns and factors associated with resistance in adult patients failing NNRTI-based regimens in the Western Cape, South Africa.	J Med Virol	www.ncbi.nlm.nih.gov/pubmed/21837793
2012	Billong SC, Fokam J, Nkwescheu AS, et al.	Early warning indicators for HIV drug resistance in Cameroon during the year 2010.	PLoS One	www.ncbi.nlm.nih.gov/pubmed/22615810
2012	Brehm JH, Koontz DL, Wallis CL, et al.	Frequent emergence of N348I in HIV-1 subtype C reverse transcriptase with failure of initial therapy reduces susceptibility to reverse-transcriptase inhibitors.	Clin Infect Dis	www.ncbi.nlm.nih.gov/pubmed/22618567
2012	De Beaudrap P, Thiam M, Diouf A, et al.	Risk of virological failure and drug resistance during first and second-line antiretroviral therapy in a 10-year cohort in Senegal: results from the ANRS 1215 cohort.	J Acquir Immune Defic Syndr	www.ncbi.nlm.nih.gov/pubmed/23117504
2012	Etiebet MA, Shepherd J, Nowak RG, et al.	Tenofovir-based regimens associated with less drug resistance in HIV-1-infected Nigerians failing first-line antiretroviral therapy.	AIDS	www.ncbi.nlm.nih.gov/pubmed/23079810
2012	Fox MP, Cutsem GV, Giddy J, et al.	Rates and predictors of failure of first-line antiretroviral therapy and switch to second-line ART in South Africa.	J Acquir Immune Defic Syndr	www.ncbi.nlm.nih.gov/pubmed/22433846
2012	Hamers RL, Sigaloff KC, Kityo C, et al.	Emerging HIV-1 drug resistance after roll-out of antiretroviral therapy in sub-Saharan Africa.	Curr Opin HIV AIDS	www.ncbi.nlm.nih.gov/pubmed/23143140
2012	Hamers RL, Sigaloff KC, Wensing AM, et al.	Patterns of HIV-1 drug resistance after first-line antiretroviral therapy (ART) failure in 6 sub-Saharan African countries: implications for second-line ART strategies.	Clin Infect Dis	www.ncbi.nlm.nih.gov/pubmed/22474222
2012	Johnston V, Fielding KL, Charalambous S, et al.	Outcomes following virological failure and predictors of switching to second-line antiretroviral therapy in a South African treatment program.	J Acquir Immune Defic Syndr	www.ncbi.nlm.nih.gov/pubmed/22820803
2012	Levison JH, Wood R, Scott CA, et al.	The clinical and economic impact of genotype testing at first-line antiretroviral therapy failure for HIV-infected patients in South Africa.	Clin Infect Dis	www.ncbi.nlm.nih.gov/pubmed/23087386
2012	Liégeois F, Vella C, Eymard-Duvernay S, et al.	Virological failure rates and HIV-1 drug resistance patterns in patients on first-line antiretroviral treatment in semirural and rural Gabon.	J Int AIDS Soc	www.ncbi.nlm.nih.gov/pubmed/23199801
2012	Manasa J, Katzenstein D, Cassol S, et al.	Primary drug resistance in South Africa: data from 10 years of surveys.	AIDS Res Hum Retroviruses	www.ncbi.nlm.nih.gov/pubmed/22251009

2012	Praparattanapan J, Kotarathitithum W, Chaiwarith R, et al.	Impact of HIV-1 viral load on genotypic characteristics among patients failing non-nucleoside reverse trancriptase inhibitor-based first-line regimens in Northern Thailand.	Southeast Asian J Trop Med Public Health	www.ncbi.nlm.nih.gov/pubmed/22299468
2012	Reynolds SJ, Laeyendecker O, Nakigozi G, et al.	Antiretroviral drug susceptibility among HIV-infected adults failing antiretroviral therapy in Rakai, Uganda.	AIDS Res Hum Retroviruses	www.ncbi.nlm.nih.gov/pubmed/22443282
2012	Reynolds SJ, Sendagire H, Newell K, et al.	Virologic versus immunologic monitoring and the rate of accumulated genotypic resistance to first-line antiretroviral drugs in Uganda.	BMC Infect Dis	www.ncbi.nlm.nih.gov/pubmed/23270482
2012	Sungkanuparph S, Win MM, Kiertiburanakul S, et al.	HIV-1 drug resistance at virological failure versus immunological failure among patients failing first-line antiretroviral therapy in a resource-limited setting.	Int J STD AIDS	www.ncbi.nlm.nih.gov/pubmed/22648883
2012	Sunpath H, Wu B, Gordon M, et al.	High rate of K65R for antiretroviral therapy-naive patients with subtype C HIV infection failing a tenofovir-containing first-line regimen.	AIDS	www.ncbi.nlm.nih.gov/pubmed/22739389
2012	Thiam M, Diop-Ndiaye H, Diouf AD, et al.	HIV-1 genetic diversity and drug resistance among Senegalese patients in the public health system.	J Clin Microbiol	www.ncbi.nlm.nih.gov/pubmed/23241378
2012	von Wyl V, Cambiano V, Jordan MR, et al.	Cost-effectiveness of tenofovir instead of zidovudine for use in first-line antiretroviral therapy in settings without virological monitoring.	PLoS One	www.ncbi.nlm.nih.gov/pubmed/22905175
2012	Wallis CL, Papathanasopolous MA, Fox M, et al	Low rates of nucleoside reverse transcriptase inhibitor resistance in a well-monitored cohort in South Africa on antiretroviral therapy.	Antivir Ther	www.ncbi.nlm.nih.gov/pubmed/22293461
2013	Aghokeng AF, Kouanfack C, Eymard-Duvernay S, et al.	Virological outcome and patterns of HIV-1 drug resistance in patients with 36 months' antiretroviral therapy experience in Cameroon.	J Int AIDS Soc	www.ncbi.nlm.nih.gov/pubmed/23374858
2013	Billong SC, Fokam J, Aghokeng AF, et al.	Population-based monitoring of emerging HIV-1 drug resistance on antiretroviral therapy and associated factors in a sentinel site in Cameroon: low levels of resistance but poor programmatic performance.	PLoS One	www.ncbi.nlm.nih.gov/pubmed/23991142
2013	De Luca A, Hamers RL, Schapiro JM.	Antiretroviral treatment sequencing strategies to overcome HIV type 1 drug resistance in adolescents and adults in low-middle-income countries.	J Infect Dis	www.ncbi.nlm.nih.gov/pubmed/23687291
2013	Hill A, McBride A, Sawyer AW, et al.	Resistance at virological failure using boosted protease inhibitors versus nonnucleoside reverse transcriptase inhibitors as first-line antiretroviral therapy--implications for sustained efficacy of ART in resource-limited settings.	J Infect Dis	www.ncbi.nlm.nih.gov/pubmed/23687293
2013	Hosseinipour MC, Gupta RK, Van Zyl G, et al.	Emergence of HIV drug resistance during first- and second-line antiretroviral therapy in resource-limited settings.	J Infect Dis	www.ncbi.nlm.nih.gov/pubmed/23687289

2013	Landman R, Koulla-Shiro S, Sow PS, et al.	Evaluation of four tenofovir-containing regimens as first-line treatments in Cameroon and Senegal: the ANRS 12115 DAYANA Trial.	Antivir Ther	www.ncbi.nlm.nih.gov/pubmed/23970206
2013	Manasa J, Lessells RJ, Skingsley A, et al.	High-levels of acquired drug resistance in adult patients failing first-line antiretroviral therapy in a rural HIV treatment programme in KwaZulu-Natal, South Africa.	PLoS One	www.ncbi.nlm.nih.gov/pubmed/23991055
2013	Neogi U, Heylen E, Shet A, et al.	Long-term efficacy of first line antiretroviral therapy in Indian HIV-1 infected patients: a longitudinal cohort study.	PLoS One	www.ncbi.nlm.nih.gov/pubmed/23383185
2013	Nouhin J, Madec Y, Ngo-Giang-Huong N, et al.	Increased risk of Q151M and K65R mutations in patients failing stavudine-containing first-line antiretroviral therapy in Cambodia.	PLoS One	www.ncbi.nlm.nih.gov/pubmed/24015311
2013	Pillay D, Albert J, Bertagnolio S, et al.	Implications of HIV drug resistance on first- and second-line therapies in resource-limited settings: report from a workshop organized by the Collaborative HIV and Anti-HIV Drug Resistance Network.	Antivir Ther	www.ncbi.nlm.nih.gov/pubmed/23740717
2013	Tang MW, Rhee SY, Bertagnolio S, et al.	Nucleoside reverse transcriptase inhibitor resistance mutations associated with first-line stavudine-containing antiretroviral therapy: programmatic implications for countries phasing out stavudine.	J Infect Dis	www.ncbi.nlm.nih.gov/pubmed/23687292
2013	Vallabhaneni S, Chandy S, Heylen E, et al.	Evaluation of WHO immunologic criteria for treatment failure: implications for detection of virologic failure, evolution of drug resistance and choice of second-line therapy in India.	J Int AIDS Soc	www.ncbi.nlm.nih.gov/pubmed/23735817
2013	Van Zyl GU, Liu TF, Claassen M, et al.	Trends in Genotypic HIV-1 Antiretroviral Resistance between 2006 and 2012 in South African Patients Receiving First- and Second-Line Antiretroviral Treatment Regimens.	PLoS One	www.ncbi.nlm.nih.gov/pubmed/23840622
2014	Abdissa A, Yilma D, Fonager J, et al.	Drug resistance in HIV patients with virological failure or slow virological response to antiretroviral therapy in Ethiopia.	BMC Infect Dis	www.ncbi.nlm.nih.gov/pubmed/24708645
2014	Chimukangara B, Gwanzura L, Mitchell R, et al.	Drug resistance mutations from whole blood proviral DNA among patients on antiretroviral drugs in Zimbabwe.	Curr HIV Res	www.ncbi.nlm.nih.gov/pubmed/25323793
2014	Clumeck N, Mwamba C, Kabeya K, et al.	First-line antiretroviral therapy with nevirapine versus lopinavir-ritonavir based regimens in a resource-limited setting.	AIDS	www.ncbi.nlm.nih.gov/pubmed/25028911
2014	Crawford KW, Wakabi S, Kibuuka H, et al.	Short communication: east meets west: a description of HIV-1 drug resistance mutation patterns of patients failing first line therapy in PEPFAR clinics from Uganda and Nigeria.	AIDS Res Hum Retroviruses	www.ncbi.nlm.nih.gov/pubmed/24798614
2014	Fofana DB, Soulié C, Baldé A, et al.	High level of HIV-1 resistance in patients failing long-term first-line antiretroviral therapy in Mali.	J Antimicrob Chemother	www.ncbi.nlm.nih.gov/pubmed/24855120

2014	Goodall RL, Dunn DT, Pattery T, et al.	Phenotypic and genotypic analyses to guide selection of reverse transcriptase inhibitors in second-line HIV therapy following extended virological failure in Uganda.	J Antimicrob Chemother	www.ncbi.nlm.nih.gov/pubmed/24633208
2014	Hassan AS, Nabwera HM, Mwaringa SM, et al.	HIV-1 virologic failure and acquired drug resistance among first-line antiretroviral experienced adults at a rural HIV clinic in coastal Kenya: a cross-sectional study.	AIDS Res Ther	www.ncbi.nlm.nih.gov/pubmed/24456757
2014	Jiamsakul A, Sungkanuparph S, Law M, et al.	HIV multi-drug resistance at first-line antiretroviral failure and subsequent virological response in Asia.	J Int AIDS Soc	www.ncbi.nlm.nih.gov/pubmed/25141905
2014	Kantor R, DeLong A, Balamane M, et al.	HIV diversity and drug resistance from plasma and non-plasma analytes in a large treatment programme in western Kenya.	J Int AIDS Soc	www.ncbi.nlm.nih.gov/pubmed/25413893
2014	Koigi P, Ngayo MO, Khamadi S, et al.	HIV type 1 drug resistance patterns among patients failing first and second line antiretroviral therapy in Nairobi, Kenya.	BMC Res Notes	www.ncbi.nlm.nih.gov/pubmed/25487529
2014	Lessells RJ, Stott KE, Manasa J, et al.	Implementing antiretroviral resistance testing in a primary health care HIV treatment programme in rural KwaZulu-Natal, South Africa: early experiences, achievements and challenges.	BMC Health Serv Res	www.ncbi.nlm.nih.gov/pubmed/24606875
2014	Phillips A, Cambiano V, Nakagawa F, et al.	Cost-effectiveness of HIV drug resistance testing to inform switching to second line antiretroviral therapy in low income settings.	PLoS One	www.ncbi.nlm.nih.gov/pubmed/25290340
2014	Sluis-Cremer N.	The emerging profile of cross-resistance among the nonnucleoside HIV-1 reverse transcriptase inhibitors.	Viruses	www.ncbi.nlm.nih.gov/pubmed/25089538
2014	Wallis CL, Aga E, Ribaudo H, et al.	Drug susceptibility and resistance mutations after first-line failure in resource limited settings.	Clin Infect Dis	www.ncbi.nlm.nih.gov/pubmed/24795328
2014	Zoufaly A, Jochum J, Hammerl R, et al.	Determinants of HIV-1 drug resistance in treatment-naÃ¯ve patients and its clinical implications in an antiretroviral treatment program in Cameroon.	J Int AIDS Soc	www.ncbi.nlm.nih.gov/pubmed/25394119
2015	Boyd MA, Moore CL, Molina JM, Wood R, et al.	Baseline HIV-1 resistance, virological outcomes, and emergent resistance in the SECOND-LINE trial: an exploratory analysis.	Lancet HIV	www.ncbi.nlm.nih.gov/pubmed/26424460
2015	Derache A, Wallis CL, Vardhanabhuti S, et al.	Phenotype, Genotype, and Drug Resistance in Subtype C HIV-1 Infection.	J Infect Dis	www.ncbi.nlm.nih.gov/pubmed/26175454
2015	Hoffmann CJ, Maritz J, van Zyl GU.	CD4 count-based failure criteria combined with viral load monitoring may trigger worse switch decisions than viral load monitoring alone.	Trop Med Int Health	www.ncbi.nlm.nih.gov/pubmed/26584666
2015	Hong SY, Jonas A, DeKlerk M, et al.	Population-based surveillance of HIV drug resistance emerging on treatment and associated factors at sentinel antiretroviral therapy sites in Namibia.	J Acquir Immune Defic Syndr	www.ncbi.nlm.nih.gov/pubmed/25564107

2015	Kaleebu P, Kirungi W, Watera C, et al.	Virological Response and Antiretroviral Drug Resistance Emerging during Antiretroviral Therapy at Three Treatment Centers in Uganda.	PLoS One	www.ncbi.nlm.nih.gov/pubmed/26700639
2015	Konou AA, Dagnra AY, Vidal N, et al.	Alarming rates of virological failure and drug resistance in patients on long-term antiretroviral treatment in routine HIV clinics in Togo.	AIDS	www.ncbi.nlm.nih.gov/pubmed/26558549
2015	Konou AA, Salou M, Vidal N, et al.	Virological outcome among HIV-1 infected patients on first-line antiretroviral treatment in semi-rural HIV clinics in Togo.	AIDS Res Ther	www.ncbi.nlm.nih.gov/pubmed/26617663
2015	Pinoges L, Schramm B, Poulet E, et al.	Risk factors and mortality associated with resistance to first-line antiretroviral therapy: multicentric cross-sectional and longitudinal analyses.	J Acquir Immune Defic Syndr	www.ncbi.nlm.nih.gov/pubmed/25585301
2015	Rhee SY, Jordan MR, Raizes E, et al.	HIV-1 Drug Resistance Mutations: Potential Applications for Point-of-Care Genotypic Resistance Testing.	PLoS One	www.ncbi.nlm.nih.gov/pubmed/26717411
2015	Rupérez M, Pou C, Maculuve S, et al.	Determinants of virological failure and antiretroviral drug resistance in Mozambique.	J Antimicrob Chemother	www.ncbi.nlm.nih.gov/pubmed/26084302
2015	Seu L, Mulenga LB, Siwingwa M, et al.	Characterization of HIV drug resistance mutations among patients failing first-line antiretroviral therapy from a tertiary referral center in Lusaka, Zambia.	J Med Virol	www.ncbi.nlm.nih.gov/pubmed/25754408
2015	Skhosana L, Steegen K, Bronze M, et al.	High prevalence of the K65R mutation in HIV-1 subtype C infected patients failing tenofovir-based first-line regimens in South Africa.	PLoS One	www.ncbi.nlm.nih.gov/pubmed/25659108
2015	Sluis-Cremer N, Wainberg MA, Schinazi RF.	Resistance to reverse transcriptase inhibitors used in the treatment and prevention of HIV-1 infection.	Future Microbiol	www.ncbi.nlm.nih.gov/pubmed/26517190
2015	Sutherland KA, Goodall RL, McCormick A, et al.	Gag-Protease Sequence Evolution Following Protease Inhibitor Monotherapy Treatment Failure in HIV-1 Viruses Circulating in East Africa.	J Med Case Rep	www.ncbi.nlm.nih.gov/pubmed/26258548
2015	Xing H, Ruan Y, Hsi JH, et al.	Reductions in virological failure and drug resistance in Chinese antiretroviral-treated patients due to lamivudine-based regimens, 2003-12.	AIDS Res Hum Retroviruses	www.ncbi.nlm.nih.gov/pubmed/25855758
2016	Brooks K, Diero L, DeLong A, et al.	Treatment failure and drug resistance in HIV-positive patients on tenofovir-based first-line antiretroviral therapy in western Kenya.	J Int AIDS Soc	www.ncbi.nlm.nih.gov/pubmed/27231099
2016	Boender TS, Kityo CM, Boerma RS, et al.	Accumulation of HIV-1 drug resistance after continued virological failure on first-line ART in adults and children in sub-Saharan Africa.	J Antimicrob Chemother	www.ncbi.nlm.nih.gov/pubmed/27342546
2016	Casadellá M, Noguera-Julian M, Sunpath H, et al.	Treatment options after virological failure of first-line tenofovir-based regimens in South Africa: an analysis by deep sequencing.	J Antimicrob Chemother	www.ncbi.nlm.nih.gov/pubmed/26807968
2016	de Truchis P, Lê MP, Daou M, et al.	High efficacy of first-line ART in a West African cohort, assessed by dried blood spot virological and pharmacological measurements.	J Antimicrob Chemother	www.ncbi.nlm.nih.gov/pubmed/27439522

2016	Guichet E, Aghokeng A, Serrano L, et al.	High Viral Load and Multidrug Resistance Due to Late Switch to Second-Line Regimens Could Be a Major Obstacle to Reach the 90-90-90 UNAIDS Objectives in Sub-Saharan Africa.	AIDS Res Hum Retroviruses	www.ncbi.nlm.nih.gov/pubmed/27342228
2016	Jenabian MA, Talla F, Talla P, et al.	Pitfalls of antiretroviral drug resistance genotyping of HIV-1 Group M and Group N from Cameroon by sequenced-based assays.	AIDS	www.ncbi.nlm.nih.gov/pubmed/26903701
2016	Mungati M, Mhangara M, Gonese E, et al.	Pre-treatment drug resistance among patients initiating antiretroviral therapy (ART) in Zimbabwe: 2008-2010.	BMC Res Notes	www.ncbi.nlm.nih.gov/pubmed/27287672
2016	Rossouw TM, Nieuwoudt M, Manasa J, 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	www.ncbi.nlm.nih.gov/pubmed/27353262
2016	Rutstein SE, Hosseinipour MC, Weinberger M, 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	www.ncbi.nlm.nih.gov/pubmed/27296625
2016	Teeranaipong P, Sirivichayakul S, Mekprasan S, et al.	Role of Rilpivirine and Etravirine in Efavirenz and Nevirapine-Based Regimens Failure in a Resource-Limited Country: A Cross- Sectional Study.	Niger Med J	www.ncbi.nlm.nih.gov/pubmed/27120449
2016	TenoRes Study Group.	Global epidemiology of drug resistance after failure of WHO recommended first-line regimens for adult HIV-1 infection: a multicentre retrospective cohort study.	PLoS One	www.ncbi.nlm.nih.gov/pubmed/26831472

Appendix F. RefMed literature search

Year	Author	Title	Journal	PubMed
1996	de Clercq, E	Non-nucleoside reverse transcriptase inhibitors (NNRTIs) for the treatment of human immunodeficiency virus type 1 (HIV-1) infections: strategies to overcome drug resistance development	Med Res Rev	www.ncbi.nlm.nih.gov/pubmed/8656777
1998	Jonckheere, H., et al	Lamivudine Resistance of HIV Type 1 Does Not Delay Development of Resistance to Nonnucleoside HIV Type 1-Specific Reverse Transcriptase Inhibitors as Compared with Wild-Type HIV Type 1	AIDS Research and Human Retroviruses	www.ncbi.nlm.nih.gov/pubmed/9491915
1999	Catucci, M., et al	Development and significance of the HIV-1 reverse transcriptase M184V mutation during combination therapy with lamivudine, zidovudine, and protease inhibitors	J Acquir Immune Defic Syndr	www.ncbi.nlm.nih.gov/pubmed/10421243
2001	Fantini, J., et al	Secondary structure predictions of HIV-1 reverse transcriptase provide new insights into the development of drug-resistance genotypes	AIDS	www.ncbi.nlm.nih.gov/pubmed/11416728
2001	Tsuchiya, K., et al	Accumulation of lopinavir resistance-associated mutations over 3 years follow-up of patients on highly active antiretroviral therapy: implication in salvage therapy	AIDS	www.ncbi.nlm.nih.gov/pubmed/11416723
2002	Kuritzkes, D	Drug resistance. Navigating resistance pathways	AIDS Read	www.ncbi.nlm.nih.gov/pubmed/12402803
2002	Loemba, H., et al	Genetic divergence of human immunodeficiency virus type 1 Ethiopian clade C reverse transcriptase (RT) and rapid development of resistance against nonnucleoside inhibitors of RT	Antimicrob Agents Chemother	www.ncbi.nlm.nih.gov/pubmed/12069959
2002	Ray, A. S., et al	Mechanistic studies to understand the progressive development of resistance in human immunodeficiency virus type 1 reverse transcriptase to abacavir	J Biol Chem	www.ncbi.nlm.nih.gov/pubmed/12176989
2002	Re, M. C., et al	Development of drug resistance in HIV-1 patients receiving a combination of stavudine, lamivudine and efavirenz	Int J Antimicrob Agents	www.ncbi.nlm.nih.gov/pubmed/12385703
2002	Rusconi, S	Accumulation of drug-related mutations in HIV-1 genome and virus replicative capacity in multi-drug failure subjects	J of Antimicrobial Chemotherapy	www.ncbi.nlm.nih.gov/pubmed/11815597
2003	Diallo, K., et al	The M184V substitution in human immunodeficiency virus type 1 reverse transcriptase delays the development of resistance to amprenavir and efavirenz in subtype B and C clinical isolates	Antimicrob Agents Chemother	www.ncbi.nlm.nih.gov/pubmed/12821504
2003	Garcia-Lerma, J. G., et al	A novel genetic pathway of human immunodeficiency virus type 1 resistance to stavudine mediated by the K65R mutation	J Virol	www.ncbi.nlm.nih.gov/pubmed/12719561
2004	Kuritzkes, D. R., et al	Rate of thymidine analogue resistance mutation accumulation with zidovudine- or stavudine-based regimens	J Acquir Immune Defic Synd	www.ncbi.nlm.nih.gov/pubmed/15097303
2005	Beerenwinkel, N., et al	Estimating HIV evolutionary pathways and the genetic barrier to drug resistance	J Infect Dis	www.ncbi.nlm.nih.gov/pubmed/15871130

2005	Beerenwinkel, N., et al	Learning multiple evolutionary pathways from cross-sectional data	J Comput Biol	www.ncbi.nlm.nih.gov/pubmed/16108705
2006	Boyer, P. L., et al	Why do HIV-1 and HIV-2 use different pathways to develop AZT resistance?	PLoS Pathog	www.ncbi.nlm.nih.gov/pubmed/16485036
2006	Deforche, K., et al	Analysis of HIV-1 pol sequences using Bayesian Networks: implications for drug resistance	Bioinformatics	www.ncbi.nlm.nih.gov/pubmed/17021157
2006	Margot, N. A., et al	Resistance development over 144 weeks in treatment-naive patients receiving tenofovir disoproxil fumarate or stavudine with lamivudine and efavirenz in Study 903	HIV Med	www.ncbi.nlm.nih.gov/pubmed/16925730
2007	Beerenwinkel, N. and M. Drton	A mutagenetic tree hidden Markov model for longitudinal clonal HIV sequence data	Biostatistics	www.ncbi.nlm.nih.gov/pubmed/16569743
2007	Healy, B., et al	Combining cross-sectional and prospective data methods to improve transition parameter estimation for characterizing the accumulation of HIV-1 drug resistance mutations	Biometrics	www.ncbi.nlm.nih.gov/pubmed/17403101
2007	Novitsky, V., et al	The reverse transcriptase 67N 70R 215Y genotype is the predominant TAM pathway associated with virologic failure among HIV type 1C-infected adults treated with ZDV/ddI-containing HAART in southern Africa	AIDS Res Hum Retroviruses	www.ncbi.nlm.nih.gov/pubmed/17678469
2008	Bogojeska, J., et al	Rtreemix: an R package for estimating evolutionary pathways and genetic progression scores	Bioinformatics	www.ncbi.nlm.nih.gov/pubmed/18718947
2008	Deforche, K., et al	Estimation of an in vivo fitness landscape experienced by HIV-1 under drug selective pressure useful for prediction of drug resistance evolution during treatment	Bioinformatics	www.ncbi.nlm.nih.gov/pubmed/18024973
2008	Deforche, K., et al	Bayesian network analyses of resistance pathways against efavirenz and nevirapine	AIDS	www.ncbi.nlm.nih.gov/pubmed/18832874
2008	Kaye, M., et al	Phylogenetic investigation of transmission pathways of drug-resistant HIV-1 utilizing pol sequences derived from resistance genotyping	J Acquir Immune Defic Syndr	www.ncbi.nlm.nih.gov/pubmed/18667928
2008	Raffa, J. D., et al	Intermediate highly active antiretroviral therapy adherence thresholds and empirical models for the development of drug resistance mutations	J Acquir Immune Defic Syndr	www.ncbi.nlm.nih.gov/pubmed/18398974
2008	Waleria-Aleixo, A., et al	Drug resistance mutation profile and accumulation kinetics in human immunodeficiency virus-positive individuals infected with subtypes B and F failing highly active antiretroviral therapy are influenced by different viral codon usage patterns	Antimicrob Agents Chemother	www.ncbi.nlm.nih.gov/pubmed/18838582
2009	Buendia, P., et al	A phylogenetic and Markov model approach for the reconstruction of mutational pathways of drug resistance	Bioinformatics	www.ncbi.nlm.nih.gov/pubmed/19654117
2009	Cozzi-Lepri, A., et al	Rate of accumulation of thymidine analogue mutations in patients continuing to receive virologically failing regimens containing zidovudine or stavudine: implications for antiretroviral therapy programs in resource-limited settings	J Infect Dis	www.ncbi.nlm.nih.gov/pubmed/19604043

2009	Margot, N. A., et al	Development of HIV-1 drug resistance through 144 weeks in antiretroviral-naive subjects on emtricitabine, tenofovir disoproxil fumarate, and efavirenz compared with lamivudine/zidovudine and efavirenz in study GS-01-934	J Acquir Immune Defic Syndr	www.ncbi.nlm.nih.gov/pubmed/19644384
2009	Steegen, K., et al	Effectiveness of antiretroviral therapy and development of drug resistance in HIV-1 infected patients in Mombasa, Kenya	AIDS Res Ther	www.ncbi.nlm.nih.gov/pubmed/19531211
2009	Stevens, W. S	Slow accumulation of HIV resistance mutations: implications for resource-limited settings?	J Infect Dis	www.ncbi.nlm.nih.gov/pubmed/19604045
2010	Coutsinos, D., et al	Factors affecting template usage in the development of K65R resistance in subtype C variants of HIV type-1	Antivir Chem Chemother	www.ncbi.nlm.nih.gov/pubmed/20054099
2010	Radzio, J., et al	N348I in reverse transcriptase provides a genetic pathway for HIV-1 to select thymidine analogue mutations and mutations antagonistic to thymidine analogue mutations	AIDS	www.ncbi.nlm.nih.gov/pubmed/20160634
2011	Champenois, K., et al	Lopinavir/ritonavir resistance in patients infected with HIV-1: two divergent resistance pathways?	J Med Virol	www.ncbi.nlm.nih.gov/pubmed/21755502
2011	Paolucci, S., et al	Accumulation of defective HIV-1 variants in a patient with slow disease progression	Curr HIV Res	www.ncbi.nlm.nih.gov/pubmed/21198434
2011	Pingen, M., et al	Evolutionary pathways of transmitted drug-resistant HIV-1	J Antimicrob Chemother	www.ncbi.nlm.nih.gov/pubmed/21502281
2011	Sigaloff, K. C., et al	Unnecessary antiretroviral treatment switches and accumulation of HIV resistance mutations; two arguments for viral load monitoring in Africa	J Acquir Immune Defic Syndr	www.ncbi.nlm.nih.gov/pubmed/21694603
2011	Wainberg, M. A., et al	Development of antiretroviral drug resistance	N Engl J Med	www.ncbi.nlm.nih.gov/pubmed/21848464
2012	Barth, R. E., et al	Accumulation of drug resistance and loss of therapeutic options precede commonly used criteria for treatment failure in HIV-1 subtype-C-infected patients	Antivir Ther	www.ncbi.nlm.nih.gov/pubmed/22297391
2012	Cozzi-Lepri, A., et al	The rate of accumulation of nonnucleoside reverse transcriptase inhibitor (NNRTI) resistance in patients kept on a virologically failing regimen containing an NNRTI	HIV Med	www.ncbi.nlm.nih.gov/pubmed/21848790
2012	Sigaloff, K. C., et al	Accumulation of HIV drug resistance mutations in patients failing first-line antiretroviral treatment in South Africa	AIDS Res Hum Retroviruses	www.ncbi.nlm.nih.gov/pubmed/21819219
2013	Borchani, H., et al	Predicting human immunodeficiency virus inhibitors using multi-dimensional Bayesian network classifiers	Artif Intell Med	www.ncbi.nlm.nih.gov/pubmed/23375464
2013	Hernandez-Leal, P., et al	Discovering human immunodeficiency virus mutational pathways using temporal Bayesian networks	Artif Intell Med	www.ncbi.nlm.nih.gov/pubmed/23561488
2013	Invernizzi, C. F., et al	The preferential selection of K65R in HIV-1 subtype C is attenuated by nucleotide polymorphisms at thymidine analogue mutation sites	J Antimicrob Chemother	www.ncbi.nlm.nih.gov/pubmed/23749954

2016	Boender, TS, et al.	Accumulation of HIV-1 drug resistance after continued virological failure on first-line ART in adults and children in sub-Saharan Africa.	J Antimicrob Chemother.	www.ncbi.nlm.nih.gov/pubmed/27342546
2016	Jose, S, et al.	Virological failure and development of new resistance mutations according to CD4 count at combination antiretroviral therapy initiation	HIV Med	www.ncbi.nlm.nih.gov/pubmed/26306942
2016	Rustein, SE, 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	www.ncbi.nlm.nih.gov/pubmed/27296625
2016	Sivamalar, S, et al.	Accumulation of HIV-1 drug resistance mutations after first-line immunological failure to evaluate the options of recycling NRTI drugs in second-line treatment: A Study from South India.	AIDS Res Hum Retroviruses.	www.ncbi.nlm.nih.gov/pubmed/27460519