Will earlier treatment lead to drug resistance of the form and prevalence likely to compromise future elimination of HIV?

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I, Collins Chika Iwuji, 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

South Africa has the biggest HIV epidemic in the world. Early antiretroviral therapy (ART) is recognised as an effective HIV prevention approach and was introduced to control transmission as well as to delay HIV progression. However, it is unknown whether early treatment will result in suboptimal adherence, poor virological outcomes and emergence of drug resistance, which would hinder HIV elimination.

To address this knowledge gap, I undertook a cohort analysis nested within the ANRS-sponsored TasP trial, in which ART was initiated early, to examine adherence in individuals initiating ART at high CD4 counts and quantify virological suppression in those who were ART-naïve at trial entry. I also estimated virological suppression at trial entry amongst individuals already ART-experienced at their first trial clinic visit. I examined acquired resistance mutations in individuals with virological failure and estimated prevalence of pre-treatment drug resistance (PDR) in ART-naïve individuals and investigated impact of PDR on virological suppression.

I found no evidence of a relationship between CD4 count at ART initiation and adherence, but virological suppression was significantly better in individuals who initiated ART at higher CD4 counts, even at the same level of adherence. Most individuals with virological failure had emergent drug resistance, predominantly the M184V mutation associated with lamivudine or emtricitabine resistance. Prevalence of PDR was moderate at nearly 10%, but doubled when low frequency variants were accounted for. This was predominantly the K103N/S mutation which causes high-level resistance to efavirenz and nevirapine. However, PDR was not significantly associated with decreased virological suppression.

My results are encouraging over the 12 months' duration of ART investigated in this study with positive effects amongst patients who started ART at high CD4 counts. However, long-term follow up is required to evaluate the impact of HIV drug resistance on HIV prevention efforts.

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List of Abbreviations

AHRI	Africa Health Research Institute
AIDS	Acquired immune-deficiency virus
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
ART	Antiretroviral therapy
BREC	Biomedical Research Ethics Committee
BSID	Bounded structure identification number
CCMDD	Central chronic medicine dispensing and distribution programme
D4T	Stavudine
EWI	Early warning indicators
FTC	Emtricitabine
Hb	Haemoglobin
HCT	HIV Counselling and Testing
HIV	Human Immunodeficiency Virus
MEMS	Medication Event Monitoring System
MSM	Men who have Sex with Men
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NNRTI	Non Nucleoside Reverse Transcriptase Inhibitor
PC	Pill Count
PDR	Pre-treatment Drug Resistance
PHQ-4	The Patient Health Questionnaire for Depression and Anxiety
PI	Protease Inhibitor

PIPSA	Population Intervention Platform Study Area
PLHIV	People L:iving with HIV
PMTCT	Prevention of Mother-to-Child transmission
PrEP	Pre-Exposure Prophylaxis
QC	Quality control
PWID	People who inject drug
SSA	sub-Saharan Africa
STI	Sexually Transmitted Infection
TAM	Thymidine Analogue Mutation
TasP	Treatment as Prevention
TDF	Tenofovir
TDR	Transmitted Drug Resistance
UKZN	University of KwaZulu-Natal
UNAIDS	The Joint United Nations Programme on HIV/AIDS
VAS	Visual Analogue Scale
VL	Viral load
WGS	Whole Genome Sequencing
WHO	World Health Organisation
ZDV	Zidovudine
3TC	Lamivudine

Chapter 1 Introduction

1.1 Global HIV epidemic

The human immunodeficiency virus type 1 (HIV-1), a retrovirus (1, 2) was named in 1983 as the causative agent for the Acquired Immune Deficiency Syndrome (AIDS) identified in homosexual men in 1981 (3). Since its discovery, HIV has infected over 78 million people and caused over 35 million deaths (4), making it one of the most devastating pandemic of recent times (5).

As the HIV epidemic approaches its fourth decade, effective prevention remains elusive in the communities most affected by the virus. An estimated 36.7 million people were living with HIV globally by end 2015 (6), of whom 69% in sub-Saharan Africa. In 2015, an estimated 2.1 million people acquired HIV infection; 65% of these new infections and 73% of all HIV-related deaths occurred in sub-Saharan Africa, a region disproportionately affected by the epidemic. Remarkable strides have been made recently towards combating the epidemic and increasing ART coverage with considerable reduction in mortality and morbidity as a consequence (6) . Approximately 17 million individuals were estimated to be on ART as of the end of 2015, amounting to just under 50% of people living with HIV. There have been modest reductions in the number of new HIV infections occurring in many parts of sub-Saharan Africa. However, this is insufficient to bring about a reversal in the epidemic. The number of individuals requiring ART continues to grow, making universal coverage and the recent UNAIDS target of 90:90:90 (90% of people living with HIV aware of their HIV status, 90% of people diagnosed HIV-positive on ART, 90% of people on ART virologically suppressed) logistically difficult to reach in 2020 (7). This increase requires huge investments in ART programmes which is not sustainable in the long-term with current approaches (8).

1.2 The state of the HIV epidemic in South Africa

South Africa is home to the highest number of people living with HIV in the world, estimated at 7 million at the end of 2015. The HIV treatment programme in South Africa is also the largest globally with 3.4 million people on ART as of 2015. The adult (15-49 years) HIV prevalence in 2015 was 19.2% with very wide geographical variation. This heterogeneity is not limited to geography but also exists amongst different risk groups including sex workers, men who have sex with men (MSM) and people who inject drugs (PWID) (Table 1.1). HIV prevalence in pregnant women, from the national antenatal sentinel survey in 2013, varies from 17.5% in the Northern Cape province to 40.1% in KwaZulu-Natal (Figure 1.1) (9).

The South African government has made huge investments in the prevention of HIV and clinical care of people living with HIV (10). These investments are now beginning to bear fruits with the number of new infections and HIV-related deaths in South Africa declining. However, despite the declining number of new infections nationally, there are still areas of high transmission in which incidence has remained stubbornly static (11). Table 1.1 shows the evolution of the HIV epidemic in South Africa between 2010 and 2015 (6, 12). The HIV prevalence has remained virtually unchanged since 2010. There were 340,000 new HIV infections in 2013, with young women accounting for a quarter. At the current rate of new infections, coupled with the growing gap between funding requirements and funds earmarked for HIV, it is imperative that more needs to be done to reduce HIV transmission. Prevention efforts will need to be targeted to high transmission clusters and people at most risk of infection (13). Rural Kwazulu-Natal with a very high prevalence and incidence is an area of high transmission, and even within KwaZulu-Natal, there are micro-epidemics with prevalence higher in communities closer to the highway than communities in deep rural areas (14).

The ART coverage has increased by over 70% since 2010 and stood at 48% (6) just before the CD4 threshold for initiating ART was removed in Sept 2016. ART

Table 1.1 HIV epdemic indicators 2010-2015, South Africa (Data not available where there are gaps in table)

	2010	2013	2015
People living with HIV	6,100,000 (5,800,000-6,300,000)	6,300,000 (6,000,000-6,500,000)	7,000,000 (6,700,000-7,400,000)
HIV prevalence (adults 15-49 years)	18.9% (18.0-19.7)	19.1% (18.1-19.9)	19.2% (18.4-20.0)
HIV prevalence; women 15-24 years	14.8% (13.4-18.0)	13.1% (11.9-16.1)	-
HIV prevalence; men 15-24 years	4.4% (2.7-6.6)	4.0% (2.5-5.9)	-
HIV incidence (adults 15-49 years)	1.79% (1.70-1.88)	1.36% (1.26-1.45)	-
New HIV infections	440,000 (410,000-470,000)	340,000 (310,000-370,000)	380,000
Adult 15+ new HIV infections	420,000 (400,000-460,000)	330,000 (300,00-360,000)	-
New HIV infections; women 15-24 years	120,000 (110,000-130,000)	90,000 (81,000-100,00)	-
New HIV infections; men 15-24 years	50,000 (38,000-60,000)	36,000 (28,000-44,000)	-
AIDS-related deaths	410,000 (380,000-440,000)	200,000 (170,000-220,000)	180,000 (150,000-220,000)
Antiretroviral therapy coverage	729,312 (13%)	2,466,565 (42%)	3,400,000 (48%)
Number of women receiving PMTCT	253,468	232,854	-
Number of women needing PMTCT	270,000 (250,000-290,900)	260,000 (230,000-280,000)	-
PMTCT coverage	94% (86->95%)	90% (83->95%)	-
HIV prevalence: Sex workers	-	59.6% (26-60%)	-
HIV prevalence: Men who have sex with men	-	~10.4%-34.5%	-
HIV prevalence: People who inject drugs	-	19.4%	-

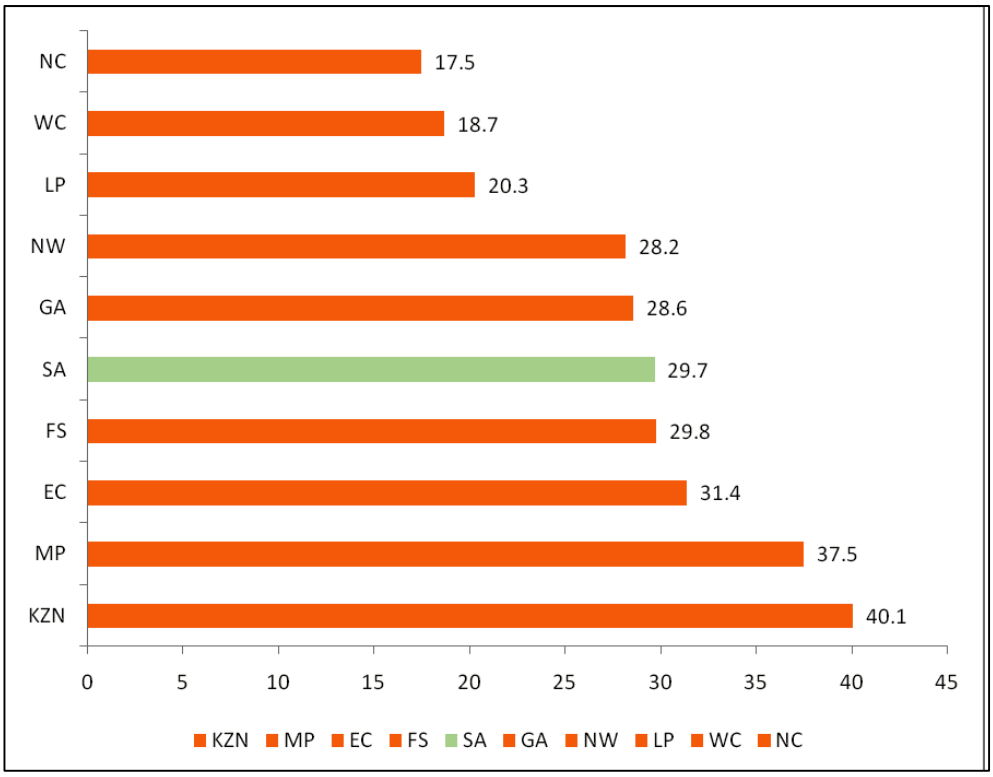


Figure 1.1. Antenatal HIV prevalence by province, South Africa, 2013 (Source, National Department of Health, South Africa**)**
green bar represents national estimate. KZN KwaZulu-Natal; MP Mpumalanga; EC Eastern Cape; FS Free State; SA South Africa, GA Gauteng; NW North West; LP Limpopo; WC Western Cape; NC Northern Cape

coverage is likely to increase substantially with the most recent South African HIV treatment.guidelines recommending immediate offer of ART in individuals diagnosed HIV-positive regardless of CD4 counts(15). It is expected that this will result in even further declines in HIV-related morbidity and mortality (16, 17) and a decrease in HIV transmission (14). However mathematical modelling has suggested that to bring about a sustained reduction in HIV transmission, treating people living with HIV alone will not suffice (PLHIV), combination prevention in the form of ART, pre-exposure prophylaxis with good adherence, medical male circumcision and consistent condom use will be required (18).

1.3 Background and context of MD research (October 2012)

It is increasingly recognised that prevention approaches need to be combined to accelerate the effective prevention of HIV acquisition and transmissions (19). HIV programme planning needs to move from the implementation of single preventive methods to combination context-specific prevention approaches, for which evidence of effectiveness exists.

One approach is to use ART to prevent both acquisition and transmission of HIV. It is an established fact that HIV transmission is primarily determined by plasma viral load in the HIV-infected individual and it is therefore likely that the risk of transmission can be substantially decreased by effective ART, which lowers viral load in all body compartments (20). This was shown to be the case in the HPTN 052 trial in stable serodiscordant partnerships (21). This trial demonstrated a 96% reduction in HIV transmission from the HIV-positive partner to the uninfected partner if the HIV-positive partner was on ART. It has been hypothesized that high ART coverage levels could contribute significantly to reducing HIV incidence at population-level (14).

However, for this strategy to be successful, HIV-infected individuals will need to be diagnosed early in the course of infection, which would require frequent point of care HIV tests that are easy to access, prompt linkage to care in those testing positive and a willingness to accept ART at high CD4 cell counts despite experiencing no symptoms of ill-health and a lack of trial evidence that there is benefit to their health. Furthermore, these individuals must be retained in care and be adherent to therapy life-long.

In the Hlabisa sub-district, the setting of this research, ART roll-out started in late 2004. The South African (SA) ART eligibility criteria for adults from 2004 to April 2010 were based on CD4 ≤ 200 cells/mm^3 or WHO stage 4 irrespective of CD4 count (22). In April 2010 this was expanded to CD4 ≤ 350 cells/mm^3 for pregnant women and in those with tuberculosis (23), and in August 2011, this CD4 threshold became applicable to all (24). In April 2012, a directive from the National Department of Health recommended ART for all TB patients regardless of CD4 count. This was as far as the South African ART guidelines had evolved at the time this research was conceptualised in October 2012.

In March 2012, a two-arm HIV Treatment as Prevention (TasP) cluster-randomised trial was implemented within the Hlabisa sub-district with participants in the intervention arm offered ART regardless of CD4 count and those in the control arm offered ART according to SA guidelines (25). The primary objective of the study was to investigate the effect of population ART on HIV incidence. Individuals already ART-experienced within the randomised communities had the option of transferring their HIV care to the trial. Individuals seroconverting within the trial setting were identified through six-monthly home-based HIV testing.

A study from the same sub-district using data from the public ART programme between August 2004 and October 2009, showed that amongst 3809 patients with 12 months' post-ART initiation viral loads available, 15% had detectable viral load (viral load >400 copies/mL) (26). Another study from this same cohort showed that amongst 222 individuals with virological failure on first-line ART (viral load > 1000

copies/mL), 86% had at least one drug resistant mutation (27). The concern is that with increasing exposure of a population to longer duration of ART, individuals developing resistance to ART could potentially transmit drug resistant virus to their sexual partners, leading to an increasing number of new infections with resistant virus (28, 29).

The question of when to start ART for the optimum management of an individual was still a matter of debate worldwide and being investigated in two randomised trials: START (clinicaltrials.govidentifier NCT00867048) and TEMPRANO (clinicaltrials.govidentifier NCT00495651). The START trial differed from the TEMPRANO trial in Ivory Coast in being multi-country, including high income countries and no upper CD4 count eligibility limit in those recruited into the study. The TEMPRANO trial compared individuals initiating ART at CD4 ≤ 800 cells/mm^3 with WHO-guided ART initiation criteria.

In two trials of pre-exposure prophylaxis in which HIV prevention was the focus (30, 31), poor adherence to the study drug with low drug levels was observed which was at variance with the high self-reported adherence in these trials. This could suggest that an individual's perception of taking ART for prevention may be different when taking ART for their own clinical benefit.

It is plausible that if infected individuals believe they are being treated primarily to prevent transmission to others, they may not maintain the same high motivation for adherence as they would if being treated for their own benefit and this could result in virological failure and the development and transmission of drug resistant viruses. The overarching aim of this research was to test the hypothesis that sub-optimal adherence, virological failure and drug resistance in individuals starting ART at high CD4 count are likely barriers to the elimination of HIV.

1.4 Aims and objectives

The research draws on my experience from the TasP trial with the overarching aim of evaluating if earlier ART initiation is likely to result in drug resistance of the form and prevalence that would hinder the elimination of HIV.

Objectives were:

- To examine the association between CD4 count at ART initiation and adherence
- To examine virological response by CD4 at ART initiation and extent of acquired resistance following virological failure
- To assess the level of pre-treatment drug resistance (PDR) in individuals seroconverting during the study period and the response to first-line ART.

1.5 Outline of Chapters

My research is nested within the TasP trial and evaluates adherence, virological response on ART, acquired and pre-treatment drug resistance in mainly asymptomatic HIV-positive individuals treated regardless of their CD4 counts.

Chapter 2 presents an overview of empirical studies on methods of HIV prevention including the use of ART. Strengths and weaknesses of each prevention approach are explored and the potential barriers to the elimination of HIV presented.

Chapter 3 presents the overall methods and design employed in the parent study and how my study is nested within the overall trial. The study procedures and trial setting are also discussed in detail

Chapter 4 describes the overall trial cohort and the selection of the clinical research cohort used for my MD research.

Chapter 5 summarises a review of the published literature on ART adherence in individuals initiating ART at CD4 counts >350 cells/mm^3 in the African setting. I examine the association between CD4 count at ART initiation and adherence using two different measures to estimate adherence. I present the results of the

correlation of these two adherence measures with each other and with virological suppression. The adherence measure with the best correlation for virological suppression was retained as the main adherence measure for subsequent analyses, whilst the other adherence measure was used for sensitivity analyses.

Chapter 6 first summarises a review of the published literature on virological suppression in individuals initiating ART at CD4 >350 cells/mm^3 in the African setting. I then examine the impact of CD4 count at initiation and adherence on virological suppression. I also examine acquired drug resistance in individuals with virological failure and the likely impact on second-line ART based on the public health approach.

Chapter 7 begins by summarising a review of the published literature on the prevalence of pre-treatment drug resistance and impact on virological outcomes in the African setting. Subsequently, I estimate the prevalence of pre-treatment drug resistance in individuals who were ART naïve at enrolment in the trial clinics including impact on virological response to ART.

Finally, Chapter 8 summarises the results of my MD thesis, the implications of my findings and contribution to knowledge. The outcome of each vital step examined, (adherence, virological response and drug resistance) is compared to targets set by the WHO as indication of a successful ART programme using a public health approach (32). I make recommendations based on my findings and suggest areas of future research to improve ART programme outcomes in order to eliminate HIV infection.

Chapter 2 Overview of Biomedical HIV prevention

2.1 Background

This chapter presents an overview of biomedical HIV prevention strategies to reduce exposure, transmission and or acquisition (33). I highlight the strengths and weaknesses of different approaches and draw attention to the current and emerging tools for prevention as well as the barriers to HIV elimination.

The following HIV prevention sub-topics are discussed:

- treatment of sexually transmitted infections,
- male circumcision,
- HIV vaccines,
- ART (including use for oral and topical pre-exposure prophylaxis)

Barriers to HIV elimination:

- sub-optimal HIV care cascade (HIV testing, linkage to care, retention),
- poor adherence,
- drug resistance
- risk compensation

These have been the subject of previous systematic reviews. It is outside the scope of my research to conduct a systematic review of each subject, rather I have taken advantage of previous reviews and updated them with currently published studies where available.

2.2 Biomedical HIV prevention

2.2.1 Treatment of sexually transmitted infections

Substantial evidence exists from observational studies suggesting an increased risk of HIV acquisition with curable STIs and genital herpes (34, 35). STIs have also been associated with increased HIV transmission and acquisition, although this has not

been quantified directly (36). HIV-STI co-infection appears more likely to result in HIV transmission than infection with HIV alone (37, 38)

However, nine randomised trials to date (four cluster randomised trials, two individual randomised trials on treating curable STIs, and three individual randomised trials on Herpes suppressive therapy) have together failed to confirm the hypothesis that STI treatment would reduce HIV transmission and acquisition (36)**.** Of the four cluster-randomised trials examining the impact of STI treatment on HIV incidence, only the Mwanza trial in Tanzania showed syndromic treatment of STIs to be associated with a 40% significant reduction in HIV incidence (39). Various factors may explain the differences in effect between trials, including differences in the HIV epidemic phase, enhanced interventions in the control group and higher prevalence of STIs in the Mwanza trial compared to the other sites (36). Two individual randomised trials involving female sex workers in Nairobi and Abidjan to evaluate the impact of STI management on HIV acquisition in HIV negative females also did not show any significant difference between the control and intervention arm (40, 41).

Syndromic treatment of STIs focusses on patients presenting with symptoms, but provision of inadequate treatment and poor adherence could result in low effectiveness of syndromic treatment, which was estimated to be only 13% for curable STIs in rural KwaZulu-Natal (42). Further, a significant proportion of STIs are asymptomatic (43) and the large pool of untreated individuals with asymptomatic STIs will continue to be a potential source of HIV transmission. This situation coupled with poor uptake of partner notification, could result in significant rates of STI re-infections, and will likely impact HIV transmission and acquisition rates.

The effect of herpes simplex virus (HSV) suppressive therapy on HIV incidence has been evaluated in two randomised trials; the first one in high-risk HSV-2 positive, HIV negative women in Tanzania (44) and the second involving women from three sites in Africa (Harare, Lusaka, Johannesburg) and MSM from Peru and the USA (45).In these trials, treating HIV negative, HSV-2 positive individuals with aciclovir did not result in reduced risk of HIV acquisition. A third randomised trial investigated the

impact of HSV-2 suppressive therapy in HIV positive individuals on the risk of HIV transmission. This involved serodiscordant couples from seven countries in Eastern and Southern Africa in which the HIV-HSV-2 co-infected partner was randomised to receive either aciclovir or placebo. Suppressive therapy with aciclovir reduced HIV plasma viral load by about 0.25 $\log_{10}$ and genital ulcers due to HSV-2 by 73%, but there was no significant effect on HIV transmission (RR 0.92, 95% CI 0.60-1.41).

Although these results are disappointing, compelling biological and epidemiological evidence that STIs are co-factors for HIV acquisition and transmission (46) remains and any HIV treatment and prevention programme should incorporate the treatment of STIs.

2.2.2 Male circumcision

A meta-analysis of 27 published observational studies on male circumcision in sub-Saharan Africa (47) provided evidence that male circumcision protects against HIV acquisition. This association was confirmed in three randomised controlled trials in South Africa, Uganda and Kenya, in which male circumcision was shown to be protective against HIV acquisition (48-50); in pooled analysis the combined incidence risk ratio (IRR) was 0.50 (95% CI 0.34-0.72) at 12 months and 0.46 (95% CI 0.34-0.62) at 24 months (51) – Figure 2.1

These observations in heterosexual HIV acquisition raised the question of whether this protection would also be observed in MSM. However, an observational analysis of data from a randomised controlled trial of HSV-2 suppressive therapy to prevent HIV acquisition found no evidence that circumcision was associated with reduced HIV incidence in MSM who practised predominantly insertive sex (RR 0.31, 95% CI: 0.06-1.51) (52).

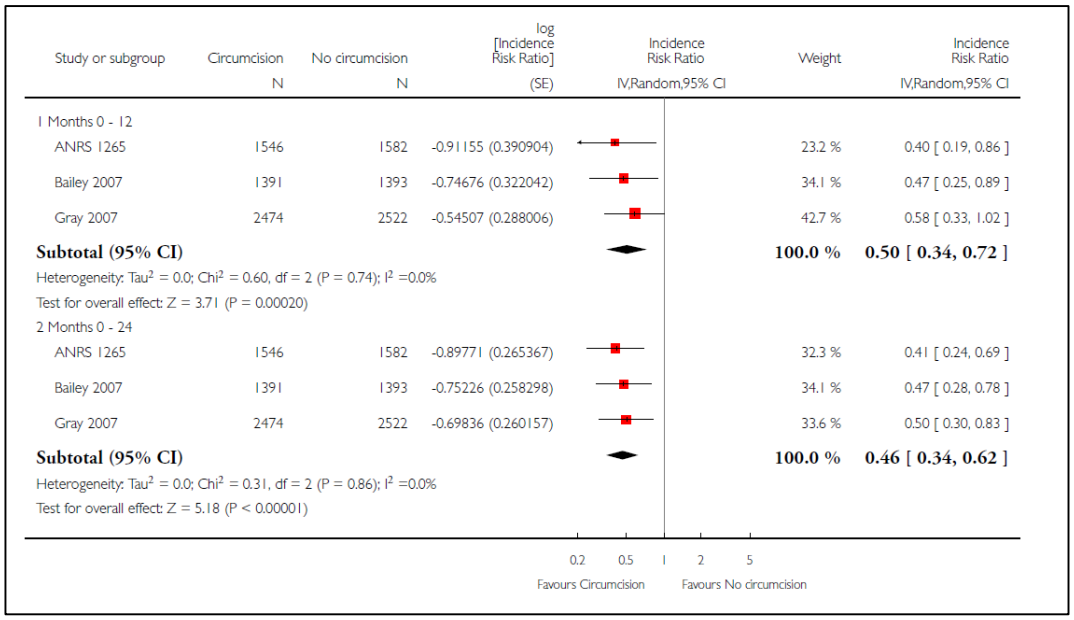


Figure 2.1 meta-analysis of the three randomised trial of male medical circumcision on HIV incidence *(Source: Siegfried et al; The Cochrane Library 2009, Issue 2)*

2.2.3 HIV vaccines

Recent HIV vaccine research has focused on antibody-based strategies following isolation of potent highly broadly neutralising monoclonal antibodies from infected individuals (53). However, both arms of the adaptive immune system have important roles to play against HIV infection and/or disease (53, 54). Neutralising antibody response aim to prevent acquisition of HIV infection, while cytotoxic T lymphocytes (CTL) response, which only recognises infected host cells, could play a role in controlling viral replication and disease progression. It is unclear if robust CTL response alone can eradicate HIV infection in humans (53).

The VAX004 (North America and the Netherlands) and VAX003 (Thailand) were protein-subunit trials using rgp120 monomers as immunogens aiming to elicit neutralising antibodies (Table 2.1). Both failed to show significant protection against HIV acquisition (55, 56).

Another vaccine approach is based on recombinant viral vectors engineered to express the gene of interest. The recombinant adenovirus serotype 5 was used as the vector for the Step (North and South America, the Caribbean and Australia) and Phambili (South Africa) trials (57, 58). These trials assessed the ability of these vaccines to stimulate the cellular immune responses. The Step trial was terminated early, on the grounds of futility and lack of control of early viraemia in those who became infected. Enrolment in the Phambili trial was stopped because of the results observed in the Step trial.

The HVTN 505 (USA), was a phase 11b DNA vaccine trial that evaluated a DNA prime expressing Gag, Pol, Nef and Env with a recombinant adenovirus serotype 5 boost expressing Gag, Pol and Env. This trial was also halted prematurely for futility (59).

The RV144 vaccine trial in Thailand employed a combination of vaccine approaches (60), comprising a canary pox viral vector prime expressing Env, Gag and Pol followed by a protein subunit vaccine boost (AIDSVAX B/E). The vaccine

Table 2.1Summary of HIV vaccine trials

Author	Vaccine trial (randomised-placebo controlled	Vaccine type	Sample size	Population	Phase	Intended immune response	Results
Flynn et al; 2005 (55)	VAX004	Protein: rgp120	5400	Mostly high-risk MSM	III	Antibodies, CD4+ T cells	VE: 6% (-17 to 24)
Pitisuttithum et al; 2006 (56)	VAX003	Protein: rgp120	2500	Injection drug users	III	Antibodies, CD4+ T cells	VE 0.1% (-30.8 to 23.8)
Rerks-Ngarm et al; 2009 (60)	RV144	Pox/protein: ALVAC/grp120	16,403	Low risk heterosexuals	III	Antibodies, CD4+ & CD8 T cells	VE 31% (1.1-52.1)
Buchbinder, SP et al; 2008 (57)	HVTN 502/Merck 023 (STEP)	Adenovirus type 5 (Ad5) gag/pol/nef	3000	High risk MSM, heterosexual men and women	IIb	CD8+ & CD4+ T cells	HR 1.2 (0.6-2.2)
Gray et al; 2011b (58)	HVTN 503 (Phambili)	Ad5 gag/pol/nef	801; original target of 3000	Heterosexual men and women	IIb	CD8+ & CD4+ T cells	HR 1·25 (0·76-2·05)
Hammer, S 2013 (61)	HVTN 505	DNA-Ad5 gag/pol/nef/env	2504	High risk MSM	IIb	Antibodies, CD4+ & CD8+ T cells	-25% (-121.2 to 29.3)

VE Vaccine efficacy

efficacy was 31% (95% CI, 1.1 to 52.1) after 3.5 years. To date, this remains the only vaccine trial to demonstrate some protection against HIV acquisition.

Two phase 2b vaccine trials are currently enrolling participants. They are both double blind placebo-controlled trials evaluating the safety and efficacy of VRCO1 broadly neutralizing monoclonal antibody in reducing HIV infection in women (HVTN 703/HPTN 081) **(**ClinicalTrials.gov Identifier**:**NCT02568215) or men who have sex with men/transgender women (HVTN 704/HPTN 085) (ClinicalTrials.gov Identifier:NCT02716675).

2.2.4 Antiretroviral treatment

The efficacy of antiretroviral therapy at preventing HIV transmission has been demonstrated in a variety of clinical scenarios such as in the prevention of mother-to-child (62, 63), and of heterosexual transmission (21), which led to the Swiss declaration that an HIV infected individual who is on ART and has undetectable viral loads for at least six months with no STIs is sexually non-infectious (64). Other uses include post-exposure prophylaxis in HIV-negative individuals after occupational or sexual exposure to body fluids from known or suspected HIV-positive individuals (65-68).

The use of ART to prevent HIV transmission gained renewed interest following the statistical modelling work of Granich and colleagues which boldly suggested that it was possible to eliminate HIV through universal HIV testing and immediate treatment of HIV infected people coupled with other prevention strategies (18). This model was criticised for making overoptimistic assumptions. A more cautious and recent model using STDSIM, calibrated to the actual data in the sub-district in which my MD research was done, showed that it would be possible to reduce prevalence from a peak of 24% in 2015 to 14% in 2040 and incidence from 2.6/100 person years in 2010 to 1.5/100 person years in 2040 if ART is started at CD4 count ≤ 350 cells/mm^3 (69).

2.2.4.1 Oral and topical ART-based pre-exposure prophylaxis

More recently, studies have shown that ART could also be used by HIV-negative individuals prior to exposure to HIV to prevent HIV acquisition, known as pre-exposure prophylaxis (PrEP).

Table 2.2 summarises the 13 trials on pre-exposure prophylaxis using ART completed to date.

The first trial evaluating the effectiveness of once daily oral tenofovir for pre-exposure prophylaxis was conducted in three sites in Ghana, Cameroon and Nigeria among high risk HIV-negative women aged 18-35 years (70). The Nigeria and Cameroun sites were closed prematurely . In Cameroun this was because of a lack of agreement between the Ministry of health and trial investigators concerning the standard of post-trial care for seroconverters whilst in the Nigerian site, closure was due to repeated protocol violations. As a result, the overall trial lacked statistical power because of the small number of HIV seroconversions observed.

In the CAPRISA 004, a proof-of-concept phase II trial including 889 HIV negative women, 1% tenofovir vaginal gel compared to placebo was shown to significantly decrease HIV acquisition, (RR 0.61, 95% CI 0.40-0.94) (71). However, the results of two further similar trials have been disappointing. The first, the FEM-PrEP study was a multicentre randomised double blind placebo-controlled trial evaluating the effectiveness of oral daily tenofovir (TDF) and emtricitabine (FTC) in preventing HIV acquisition in HIV negative women (30). This trial was stopped early due to lack of efficacy, almost certainly driven by poor adherence as evidenced by low or undetectable plasma drug levels, suggesting the high self-reported adherence by participants may have been an overestimate. The other trial, VOICE, was a phase IIB, multicentre randomized, double-blind, placebo-controlled, five-arm trial of daily oral TDF and FTC/TDF and daily 1% vaginal TDF gel as PrEP for the prevention of HIV acquisition by HIV negative women (72).

Table 2.2 ART-based oral and topical pre-exposure prophylaxis

Author	Study setting	Sample size contributing data	Study Population	Intervention/Control	Follow-up time (Person years)	HIV seroconversions	Impact on HIV incidence (95% CI)
Peterson, L 2007(70)	Ghana, Cameroun, Nigeria	936	18-35 year old high risk HIV negative women	Intervention: Oral daily tenofovir (TDF) Control: Placebo	476	Intervention: 2 Control: 6	Rate ratio (RR) 0.35 (0.03-1.93)
Abdool Karim, Q 2010(71) (CAPRISA 004)	South Africa	889	18-40 year old HIV-negative women	Intervention: coitally administered 1% vaginal gel formulation of TDF Control: Placebo	1341	Intervention:38 Control:60	RR 0.61(0.40-0.94)
Grant RM, 2010(73) (iPrEX study)	Peru, Ecuador, South Africa, Brazil, Thailand, USA	2499	>18 years, HIV negative MSM or transgender	Intervention: Oral daily tenofovir/emtricitabine (TDF-FTC) Control: Placebo	3324	Intervention: 36 Control: 64	44% reduction (15-63)
Thigpen MC, 2012 (74) (TDF2 Study)	Botswana	1219	18-39 years, HIV negative men and women	Intervention: Oral daily TDF-FTC Control: Placebo	1563	Intervention:9 Control:24	62.2% reduction (21.5-83.4)

Author	Study setting	Sample size contributing data	Study Population	Intervention/Control	Follow-up time (Person years)	HIV seroconversions	Impact on HIV incidence (95% CI)
Baeten J.M, 2012(75) Partners PrEP Study	Kenya, Uganda	4747	Heterosexual HIV serodiscordant couples	Interventions: i) Once daily oral TDF ii) Once daily TDF-FTC Control: Placebo	7830	Interventions: TDF 17 TDF/FTC: 13 Control: 52	67% reduction due to TDF (44-81) 75% reduction due to TDF/FTC (55-87)
Van Damme L, 2012(30) FEM-PrEP Study	Kenya, South Africa, Tanzania	2056	18-35 years, HIV negative women	Intervention: Oral daily TDF-FTC Control: Placebo	1407	Interventions: 33 Control: 35	Hazard ratio (HR) 0.94 (0.59-1.52)
Marrazzo J, 2013(31) VOICE Study	South Africa, Zimbabwe, Uganda	5029	Mean age 25.3 years, HIV negative women	Intervention: i) Oral daily TDF ii)Oral daily TDF/FTC iii) 1% TDF vaginal gel Control: i) Oral placebo ii) Placebo vaginal gel	5509	Interventions: i) oral TDF 52 ii) oral TDF-FTC 61 iii) Vaginal TDF gel: 61 Control i) Placebo for oral TDF: 35 ii) Placebo for oral TDF/FTC: 60	HR for Oral TDF 1.49 (0.97-2.3) HR for oral TDF/FTC 1.04 (0.7-1.5) HR for vaginal TDF gel 0.85 (0.6-1.2)

Author	Study setting	Sample size contributing data	Study Population	Intervention/Control	Follow-up time (Person years)	HIV seroconversions	Impact on HIV incidence (95% CI)
						iii) Placebo for vaginal gel: 70	
Choopanya K, 2013 (76) Bangkok Tenofovir study	Bangkok, Thailand	2413	20-60 years, HIV negative and reported injecting drug use within the past year	Intervention: Oral tenofovir Control: Placebo	9665	Intervention: 17 Placebo: 33	Efficacy of tenofovir 48.9% (9.6-72.2)
Rees H, 2015 FACTS 001 (77)	South Africa	2029	HIV negative women 18-30 years	Intervention: Pericoital 1% vaginal gel formulation of Tenofovir Control: Placebo	3036	Intervention: 61 Control:62	IRR 1.0 (0.7-1.4)
Molina JM 2015 IPERGAY (78)	France, Canada	400	HIV negative adult MSM	Intervention: On demand TDF/FTC Control: Placebo	9.3 months (IQR 4.9-20.6)	Intervention: 2 Control: 14	86% reduction (40-98)

Author	Study setting	Sample size contributing data	Study Population	Intervention/Control	Follow-up time (Person years)	HIV seroconversions	Impact on HIV incidence (95% CI)
McCormack S, 2015 PROUD (79)	England	544	HIV negative MSM ≥ 18 years	Immediate: oral daily TDF/ FTC Deferred: Oral daily TDF/ FTC after 12 months	465	Immediate: 3 Deferred: 20	86% reduction (64-96)
Baeten JM, 2016 ASPIRE (80)	Malawi, South Africa, Uganda and Zimbabwe	2629	HIV negative women aged 18-45 years	Intervention: 4 weekly Dapivirine vaginal ring Control: Placebo	4280	Intervention: 71 Control: 97	27% reduction (1-46)
Nel A, 2016 The Ring study (81)	South Africa, Uganda	1959	HIV negative women aged 18-45 years	Intervention: 4 weekly Dapivirine vaginal ring Control: Placebo	2805	Intervention: 77 Control: 56	HR 0.69 (0.49-0.99)

The oral TDF and the 1% vaginal TDF arm were stopped early within about 12 months of each other due to poor efficacy. The TDF/FTC arm continued to completion but was not efficacious. Again, lack of efficacy was likely to have been driven by poor adherence as TDF was detected in only 25-30% of individuals in the active arm. This was again at variance with adherence measured through pill/application counts and self-reports which suggested about 90% of participants were adherent to treatment.

The FACTS 001 study was a phase III licensure study aimed at confirming the findings of CAPRISA 004. It was a randomised placebo-controlled trial assessing the effectiveness and safety of 1% tenofovir vaginal gel administered peri-coitally in 2059 women. The study showed no protective effect of 1% tenofovir vaginal gel over placebo (77).

In MSM, two other PreP trials - IPERGAY and PROUD - were terminated early because of marked reduction in HIV acquisition in the intervention arm compared to the placebo or deferred arm respectively (78, 79). The IPERGAY study (78) was a phase III multicentre randomised double-blind, placebo-controlled two-arm trial investigating the effectiveness of "on demand" antiretroviral pre-exposure with Truvada versus placebo, against HIV acquisition in HIV negative MSM while PROUD (79) was a multi-centre, open label randomised design to immediate or deferred inclusion of pre-exposure prophylaxis as part of the package of HIV risk reduction interventions.

Two further PreP trials in high-risk women evaluating vaginal rings impregnated with the non-nucleoside reverse transcriptase inhibitor, dapivirine, showed modest reduction in HIV incidence (80, 81). The low efficacy was attributed to poor adherence to the use of the vaginal ring. Overcoming poor adherence remains the biggest challenge to the success of PrEP.

2.2.4.2 ART in HIV-discordant partnerships

Table 2.3 summarises the ten observational studies (82-91) and one randomised trial (21) evaluating the effectiveness of ART in preventing HIV transmission from the index to the HIV-uninfected partner. A Cochrane review and meta-analysis (92) that included nine of the observational studies identified 2112 HIV transmissions: 1,016 among ART-treated couples and 1096 in those not taking ART. The combined rate ratio (ART-treated couples vs. No ART) for the nine observational studies was 0.58 (95% CI: 0.17-0.75). The remaining observational study was conducted after the meta-analysis and estimated that ART was 77% effective in preventing transmission in heterosexual couple from an HIV-positive individual to their HIV-negative sexual partner (91).

The only randomised trial, which was multicentre (HPTN 052) (21) and recruited 1763 stable serodiscordant couples from nine countries (Table 2.3) reported initial findings in 2011. HIV infected individuals with CD4 counts between 350-550 cells/mm^3 and in a stable relationship with an uninfected partner were randomly allocated to receive ART immediately (early therapy) or delayed until CD4 count decreased below 250 cells/mm^3 or development of clinical symptoms (deferred therapy). The result of this study was released early because of clear efficacy of ART in preventing transmission in the early therapy arm. There were 39 HIV transmissions in total of which 28 were virologically linked to the infected partner. Of the linked transmissions, 27 occurred in the deferred and one in the early therapy group (HR 0.04, 95% CI: 0.01-0.27). The updated results published in 2016 showed that the efficacy of ART in preventing transmission to sexual partners was durable. Out of a total of 46 linked seroconversions, three occurred in the early therapy group after a median follow up of 5.5 years amounting to a risk reduction of 93%; HR = 0.07 (95% CI 0.02-0.22).

An earlier meta-analysis (93) reviewed observational studies of HIV transmission involving individuals on and not on ART from 11 cohorts comprising 5021

Table 2.3 ART for preventing HIV transmission in HIV discordant partnerships

Author	Study setting	No of couples	Study population	Study design/ intervention	Follow-up duration in person years	ART status of index case &sero-conversions (n)	Effect estimate (95% CI)
Musicco, M 1994 (82)	Italy	436	Female sexual partners of HIV-infected males majority of whom were injecting drug user	Observational/ Zidovudine (ZDV) monotherapy	740	Partners of men not on ZDV:21 Partners of men on ZDV: 6	Risk lower in partners of treated Men RR 0.50 (0.1-0.9)
Melo MG, 2008 (83)	Brazil	93	Female index case: 67 Male index case: 26	Observational/ 41 on triple ART 52 not on ART	Not stated	Partner on ART: 0 Partner not on ART : 6	Risk lower if partner on ART RR 0.10 (0.01-1.67)
Sullivan P, 2009 (84)	Rwanda, Zambia	2993	HIV discordant couples	Observational	5609	Partner on ART: 4 Partner not on ART: 171	Risk lower if partner on ART RR 0.21 (0.08-0.59)
Del Romero, J 2010 (85)	Spain	424	Stable sexual couples	Observational *144 couples with index partner on triple ART	1355	Partner on ART: 0 Partner not on ART: 5	Risk lower if partner on ART RR 0.21 (0.01-3.75)

Author	Study setting	No of couples	Study population	Study design/ intervention	Follow-up duration in person years	ART status of index case &sero-conversions (n)	Effect estimate (95% CI)
				*47 couples on mono/dual ART *341 couples with index partner not on ART			
Donnell D, 2010 (86)	Botswana, Kenya, Rwanda, South Africa, Tanzania, Uganda, Zambia	3381	HIV serodiscordant partners	Prospective cohort 349 initiated ART 3082 did not initiate ART	4831	Partner on ART: 1 Partner not on ART: 102	Risk lower if partner on ART 0.08 (0.00-0.57
Lu W, 2010 (87)	China	1927	HIV serodiscordant couples Male index 1092 Female index 835	Prospective cohort 1369 on ART 558 not on ART	4918	Partner on ART: 66 Partner not on ART: 18	RR 1.44 (0.85-2.44)
Reynolds SJ, 2011 (88)	Uganda	250	HIV discordant couples Male index: 145	Prospective cohort 32 initiated ART	513	Partner on ART: 0 Partner not on ART: 42	RR 0.10 (0.01-1.64)

Author	Study setting	No of couples	Study population	Study design/ intervention	Follow-up duration in person years	ART status of index case &sero-conversions (n)	Effect estimate (95% CI)
			Female index: 155	218 not on ART			
Cohen MS, 2011(21)	Botswana, Kenya, Malawi, South Africa, Zimbabwe, Brazil, India, Thailand, USA	1763	Stable HIV-discordant 97% heterosexual	Randomised Immediate versus deferred ART	Median follow up: 5.5 years (0.0-9.9)	Early therapy: 3 Deferred therapy: 43	HR 0.07 (0.02-0.22)
Birungi J, 2012 (89)	Uganda	586	Serodiscordant couples	Prospective 348 ART-eligible couples initiated 238 not eligible for ART	Median follow up of 1.3 years	ART group : 9 Non-ART group: 8	RR 0.91 (0.38-2.20)
Jia Z, 2012 (90)	China	38,862	Serodiscordant couples	Retrospective cohort 24057 ART-treated 14,805 non-ART group	101,295	ART-group: 935 ART-naïve: 696	RR 0.74 (0.65-0.84)

Author	Study setting	No of couples	Study population	Study design/ intervention	Follow-up duration in person years	ART status of index case &sero-conversions (n)	Effect estimate (95% CI)
Oldenburg C, 2016 (91)	South Africa	196	Serodiscordant couples	Longitudinal cohort 76 on ART 120 not on ART	707	Partner on ART: 4 Partner not on ART: 23	HR 0.23 (0.07-0.80)

heterosexual couples and 461 HIV transmission events. In the five studies that included heterosexual couples in which the HIV-positive partner was on ART, the overall risk of HIV transmission irrespective of viral load, was 0.46 (95% CI: 0.19-1.09) based on five transmissions and 1098 person-years of follow up. When this meta-analysis was restricted to the two studies in which individuals had undetectable viral load, no transmission was recorded in 291 person-years of follow up with an upper confidence limit of 1.27 per 100 person-years. It is now established that ART is effective at preventing transmission in stable heterosexual couples, but it remains unknown whether ART will be similarly effective at preventing HIV transmission at the population level. An observational study from rural KwaZulu-Natal suggests this may be the case (14); and this question is currently being addressed by four randomised trials (25, 94, 95), one of which recently reported its findings. The ANRS 12249 HIV Treatment as Prevention trial was a cluster randomised trial investigating the impact of home-based HIV testing and immediate ART on HIV incidence. Immediate ART compared to standard of care did not demonstrate a reduction in HIV incidence, adjusted relative risk 0.95 (95% CI 0.82-1.10) (96)

2.2.4.3 Population ART to prevent HIV transmission

A cohort study undertaken at the Africa Health Research Institute used routine data from the Hlabisa ART programme and HIV seroconversions estimated from the annual HIV surveillance in the sub-district to estimate the association between ART coverage and HIV acquisition. It was estimated that an HIV-uninfected individual living in a community with an ART coverage of 30 to 40% was 38% less likely to acquire HIV than someone living in a community where ART coverage was <10% (14) This is the only study to have shown an association between an increase in ART coverage and a decrease in HIV transmission in real-life setting at the population level. The ART eligibility criteria in this population at the time of the study was CD4 count ≤200 cells/mm^3 or WHO clinical stage 4 disease but this was increased to CD4 count ≤350 cells/mm^3 for pregnant women and tuberculosis patients in April

2010. Prior to this study, existing evidence had been based on ecological studies (97-99). However, in the Hlabisa study, whilst the exposure (ART) was ecological, the outcome, which was HIV acquisition was estimated at the individual level differentiating it from typical ecological studies. Ecological studies are prone to ecological fallacy as is often not possible to link exposure to outcome. It is also not possible to control for potential confounders. Apart from the ANRS TasP trial which has reported its findings, three cluster randomised trials are in progress in South Africa, Zambia, Botswana, Uganda and Kenya investigating the effect of population ART on HIV incidence. Even as these trials are still underway, the WHO guidelines recommend initiating treatment at CD4 ≤500 cells/mm^3 regardless of WHO clinical stage (25, 94, 95).

This recommendation was hinged on the premise that untreated HIV may be associated with the development of non-AIDS-defining conditions (100-102) and that initiating ART earlier reduces such events and improves survival as well as the results from the HPTN 052 trial demonstrating the effectiveness of ART in reducing HIV transmission in stable serodiscordant couples (21). A further supporting argument was that currently available regimens have become less toxic and easier to take and the cost of providing ART is becoming cheaper. Since the formulation of the objectives of my research, two randomised trials, TEMPRANO and START, have tipped the argument in favour of earlier initiation with the individual health benefits clearly demonstrated in these trials (103, 104). This informed the 2015 WHO ART guidelines which recommend treatment for HIV regardless of CD4 count (105), which became policy in South Africa on 1 September 2016 (15).

These trial results are from individuals enrolled in clinical trials with very close monitoring, it remains to be seen whether treating all HIV-positive individuals regardless of CD4 count or symptoms is logistically feasible, especially in sub-Saharan Africa where the burden of infection is huge.

It is also not known whether in real life settings, individuals treated at high CD4 count, who are mostly asymptomatic, would be motivated to adhere to ART consistently. If adherence is sub-optimal, this could result in virological failure and the

emergence and transmission of drug resistance. A further concern involves risk compensation/behavioural dis-inhibition from a false security of being on ART which could result in high risk sexual behaviour, thereby attenuating any benefits of ART in preventing transmission. These challenges, collectively, could hinder HIV elimination.

2.3 Barriers to HIV elimination

2.3.1 Sub-optimal HIV cascade of care

The term HIV 'cascade of care' was first used in 2009 to describe the HIV epidemic in Washington D.C (106) and has gained acceptance as an important tool to assess programmatic performance (107). This term refers to the sequential steps that take individuals from HIV infection to diagnosis, linkage to care, retention in care, timely initiation of ART, adherence to treatment and viral suppression (108). Dropout or leakage can occur at any of these steps so that the final number of beneficial outcomes may be small compared to the number of people at the beginning of the cascade. The cascade is typically presented as a linear process, but in reality, it is cyclical. This cyclical movement of individuals engaging, re-engaging and exiting clinical care at any given point in time is termed "churn", a terminology first used in this context by Gill and Krentz (109, 110). Hallet et al, described individuals who initiate ART in advanced stages of disease without prior knowledge of infection status or those who re-engage with care after dropping out of care as entering through a "side door" (111).

The cascade has been used to describe the performance of ART programmes in different countries with leakages demonstrated at different steps of the cascade. Where the greatest losses occur differs from country to country and even within the same country, leaks can be heterogeneous with respect to the demographic profile of the population (112-114). Understanding these differences is important for planning effective interventions to improve diagnosis, linkage and retention in care

as well as virological suppression so that the individual and public health benefits of ART can be maximised.

Figures 2.2 & 2.3 show the HIV cascade of care in South Africa which has the highest number of HIV positive individuals in the world and the largest HIV treatment programme (115, 116). In the South African cascade, the number of individuals who remain undiagnosed with HIV amongst those estimated to be HIV infected was not described. The disaggregation by sex showed that males performed worse in all the metrics measured and interventions to improve linkage, retention and treatment would need to target men. The very low percentage of individuals with virological suppression means there are large numbers of individuals within the community who can potentially transmit the virus and this has implications for test and treat programmes aimed at reducing HIV incidence.

2.3.2 HIV testing

HIV testing is necessary for linkage to HIV care and treatment. For HIV elimination, large numbers of individuals have to be willing to test for HIV regularly and those testing positive need to be linked to care and started on ART.

However, despite the availability of effective treatment for HIV, an estimated 36% of individuals in sub-Saharan Africa (SSA) have never been tested for HIV (117). It should be noted that HIV testing is not synonymous with awareness of HIV infection status. In a South African study, 45% of all HIV-infected persons were unaware of their HIV-positive status, despite 71% reporting a previous HIV test (116). In the USA, an estimated 21% of HIV-infected individuals are unaware of their HIV-positive status (118). Since awareness of infection status is a pre-requisite to engaging in care, these individuals would remain out of care with consequent increased morbidity and mortality and would be more likely to transmit the virus to sexual partners as they would not be on ART coupled with reported increased risk in sexual behaviour (119).

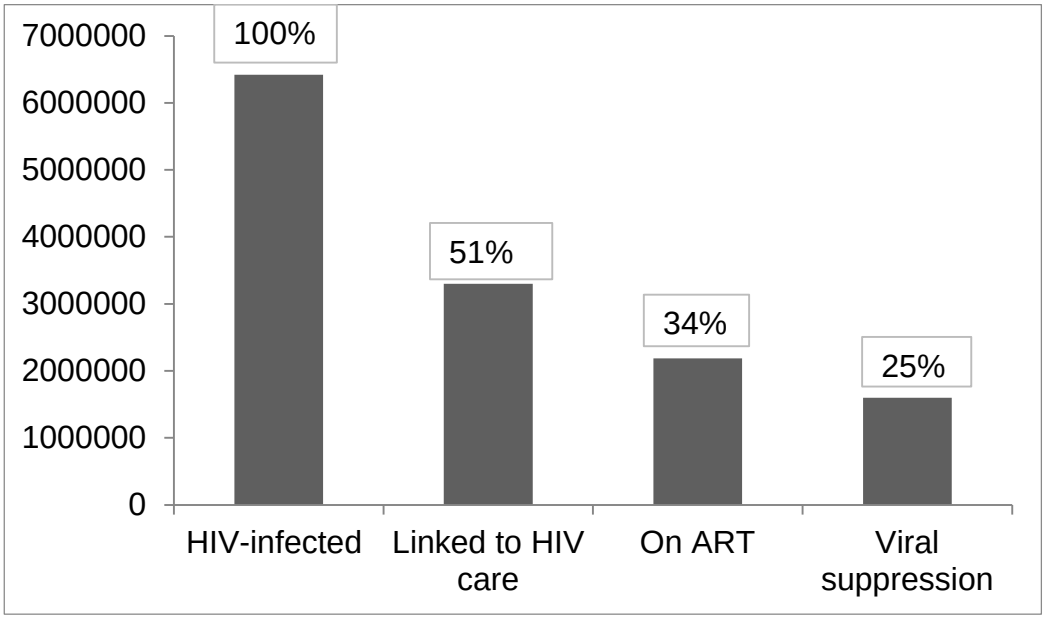


Figure 2.2 HIV treatment cascade in South Africa, 2012 (Source: CROI 2015, Seattle)

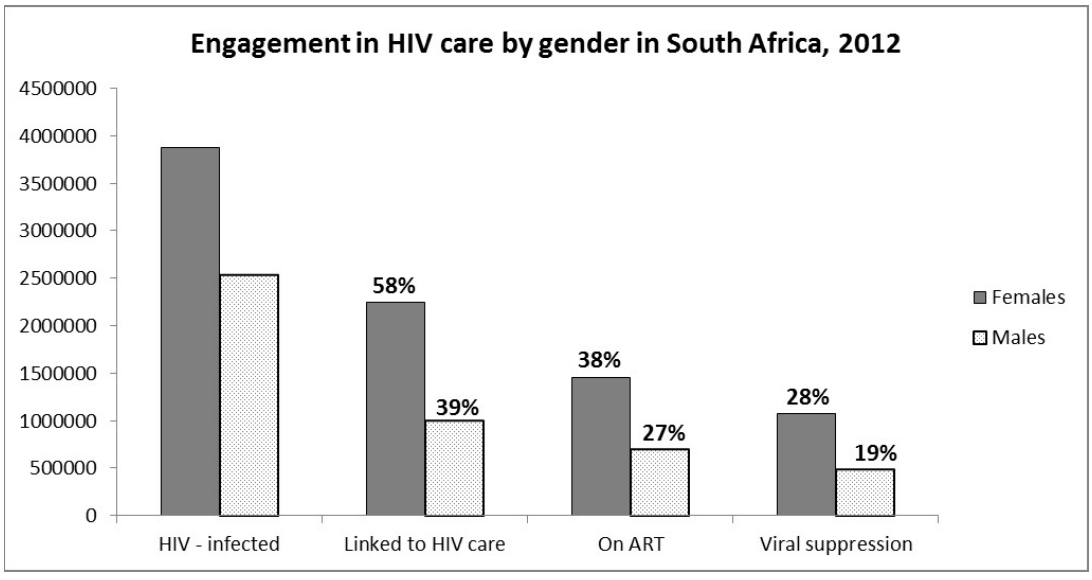


Figure 2.3 HIV treatment cascade in South Africa by gender, 2012 (Source: CROI 2015, Seattle)

Low perception of risk, concerns about confidentiality and fear of disclosure, stigma and discrimination have been suggested as explanations for the low testing rates in SSA. Gender inequity that leaves women economically dependent on men may undermine the ability of women to seek HIV testing (120-122).

2.3.3 Linkage to care

Further, studies have shown a huge drop between the number of people taking an HIV test and those linked to care. A systematic review and meta-analysis of eleven studies in SSA estimated that only 57% (95% CI, 48-66) of those diagnosed HIV positive are linked to care (123). Substantially higher numbers of individuals need to be linked to care for treatment assessment to realise the goal of HIV elimination. Within the TasP trial in rural KwaZulu-Natal, only 36% of HIV-positve individuals linked to care within six months of being referred (124). A review of 42 studies from 12 Countries in sub-Saharan Africa, 16 of which were from South Africa, examined the barriers to linkage to care. The most commonly identified factors were transport costs and distance to clinics. Others include concerns about disclosure and stigma, staff shortages, long clinic waiting times, male sex and younger age (125).

2.3.4 Retention in care

Retention in HIV care takes two forms: pre-ART retention refers to retention in care of individuals not yet eligible for ART while retention on ART refers to individuals who remain in care after initiating ART. Individuals need to be retained in care long enough to initiate ART, achieve virological suppression and should continue in care to maintain this. A review of four studies in South Africa and one in Malawi estimated that the median proportion of patients retained in pre-ART care was 45% when CD4 eligibility threshold was 200 cells/mm^3 (123). A review of 14 studies reporting

proportions of patients retained in care from ART eligibility to ART initiation estimates a median of 68% (range 14-84%) (126). For retention in care after initiating ART, a systematic review of 33 studies reporting on 39 patient cohorts estimated that 65% of patients were retained in ART care (range 58%-72%) after 36 months (127). Factors that impact retention in care include challenges that relate to housing, transportation to clinics, mental health and drug abuse which would need to be addressed in affected individuals. Provider-patient relationship and clinic opening hours are other issues that need to be addressed to improve retention in care (128).

2.3.5 Adherence

The WHO defines adherence as "the extent to which a person's behaviour - taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider" (129). In HIV, this refers to the initiation and maintenance of appropriate ART combination to achieve virological suppression and prevent disease progression. It was demonstrated in a study using protease inhibitors that up to 95% adherence is necessary to achieve virological suppression (130). Adherence to ART is vital for viral suppression (130), which is important for optimal treatment outcomes and for prevention of HIV transmission (20).

If patients perceive they are being treated primarily to prevent transmission to others, they may not maintain the same motivation for adherence as they would if they were being treated for their own health. Studies reporting on routine treatment programmes with differing ART initiation CD4 thresholds have shown that individuals starting treatment at higher CD4 counts are less likely to adhere consistently than individuals starting at lower CD4 counts (131, 132). However in the HPTN 052 on stable serodiscordant couples, adherence measured by pill count of at least 95% was seen in 79% of participants in early therapy group (CD4 350-550 cells/mm^3) compared to 74% in the delayed therapy group (CD4<250

cells/mm^3) (21). This may not be reflective of real life situations. Furthermore, some studies suggest that adherence wanes over time (133, 134).

In the pre-exposure prophylaxis studies in which participants were aware that they were using the prescribed medications to prevent HIV transmission, adherence measured by drug levels was poor (30, 31). A meta-analysis involving 37 qualitative and 47 quantitative studies on barriers and facilitators to adherence identified fear of disclosure, concomitant substance abuse, forgetfulness, suspicions of treatment, complex regimens, high pill burden, decreased quality of life, work and family responsibilities, falling asleep and access to medications as the main adherence barriers (135). The barriers to treatment adherence are summarised in the Table 2.4 (136)

Non-adherence is not always a premeditated decision not to take one's prescribed medicines. The barriers to adherence listed in Table 2.4 can be further classified as resulting in intentional or unintentional non-adherence (137). Unintentional non-adherence occurs when a patient is unable to adhere to their medications due to circumstances beyond their control, for example they are too old to remember the dosing instructions given by the doctor or unable to afford cost of transportation to go to the clinics for a refill. With intentional non-adherence, the patient makes a conscious decision not to take the prescribed medications perhaps influenced by their beliefs/perceptions about their own health or the prescribed medicines. However, these two forms of non-adherence are not mutually exclusive as they may exist in the same individual on different occasions. Drug stock-outs have also been identified as a cause of medication non-adherence in many sub-Saharan African Countries (138, 139). For an adherence intervention to be effective, it should address the patient's beliefs as well as other practical issues that facilitate or hinder their ability to adhere. Horne et al demonstrated that beliefs about medicine can be analysed using the necessity- concerns framework (140) in which patients try to balance the necessity of the medications prescribed to them for their health against other concerns they may have against them such as the fear of

Table 2.4 Barriers to medication adherence (136)

Patient factors	System factors	Community Factors	Medication Factors
Psychosocial • *Depression* • *Stigma* • *Substance/alcohol abuse*	Access • *Distance* • *Long wait times* • *Cost of co-pay medication*	Lack of knowledge/awareness	Pill burden
Socioeconomic • *Cost of transport* • *Food insecurity* • *Lower literacy*	Environment	stigma	Dose frequency
Demographics • *Younger age*	Provider relationship		Dietary restrictions/requirements
Clinical • *Prior and/or current medical comorbidities*	Support services		Side effects

side-effects. Quantifying these beliefs has been shown to predict adherence in a number of chronic disease conditions (141-143).

A meta-analysis of 94 studies comprising 25,072 individuals from developed countries which used the beliefs about medicines questionnaire to quantify the association of necessity and concerns with adherence showed that the odds of adherence increased by 1.7 per unit standard deviation increase in necessity scores. A unit decrease in concerns scores was associated with twice the odds of adherence (144).

2.3.5.1 Monitoring adherence to ART

There is no gold standard method for the accurate measurement of ART adherence but a number of approaches are in use in clinical settings despite their limitations. These various methods can result in discrepancies in rates of adherence as well as in predictors of adherence (145). Measuring adherence is important for identifying patients who require interventions. Common methods include indirect measures such as self-reports, pill counts, pharmacy refill records and electronic drug monitoring using Medication Event Monitoring System (MEMS) and direct measures which include the detection of drug or drug metabolites in plasma. The advantages and disadvantages of the various methods are summarised in Table 2.5.

2.3.5.1.1 Self-reports

Self-reports are the most commonly used adherence measures in the clinical setting. These can take the form of short questionnaires asking patients about missed doses over a recall period. A visual analogue scale with values ranging from 0 to 100% where 0 represents no pill taken and 100 represents that all pills were taken over a given recall period. This has been validated in resource-constrained settings (146).

Table 2.5 Characteristics of the different adherence measurement methods (136, 145, 147)

Methods	Advantages	Disadvantages	Correlation with Viral load
Self-report	Cheap	Overestimates adherence	Moderate correlation with VL
	Easy to implement	Relies on accurate recall and honesty	
	Low participant burden	Problem of social desirability bias	
	Questionnaires can suggest reasons for non-adherence	No standardised questions	
		Poor sensitivity	
		No information on timing of doses	
Pill count	Cheap	May overestimate adherence	Moderate correlation with VL
	Easy to implement	Pill dumping prior to appointments	
		No information on timing of doses	

Methods	Advantages	Disadvantages	Correlation with Viral load
		Assumes no alternative source of medication supply	
Pharmacy refill	Cheap	Overestimates adherence	
	Easy to implement	Assumes only one source of medication supply	
	Objective	Equates refills to adherence	
	Allows for population level analyses	No information on timing of doses	
	Not subject to social desirability bias and recall bias		
Medication Event Monitoring System	Allows analyses of adherence over prolonged periods	May underestimate adherence	Strongest correlation with VL
	Gives information about the timing of doses	Expensive and not feasible for most clinical settings or large scale use	

Methods	Advantages	Disadvantages	Correlation with Viral load
		May malfunction High participant burden Inaccuracies if multiples doses are removed at once (Pocket dosing) or if cap opened but no pills are removed (curiosity opening)	
Therapeutic drug monitoring	Direct method of measuring adherence Additional use for the monitoring and prevention of drug-related toxicity May inform drug dosing in special groups such as pregnant women, in situations of drug-drug interactions	Expensive and invasive Does not account for individual variability in pharmacokinetics Measures adherence over a limited period Limited use for assessing nucleoside reverse transcriptase inhibitor	Strong correlation with viral load

Methods	Advantages	Disadvantages	Correlation with Viral load
		plasma levels as activity is intracellular Non-adherent patients may take medications just prior to measurement of plasma levels	

2.3.5.1.2 Pill counts

This can be undertaken in health care facilities or can take the form of unannounced pill counts in patients' homes or telephonically. The number of remaining pills are counted and used to estimate adherence over a specific period of time based on the refill date and daily dosage.

2.3.5.1.3 Pharmacy refill records

Patients who collect their medications from the pharmacy on the due dates are assumed to be adherent to medication. Prolonged periods between refills are an indication that patients are either missing doses or not taking the medications at all. However, this is only valid if there is only one source of ART supply as patients may be obtaining ART from multiple sources (145). Adherence rates are estimated either by comparing actual to expected refill dates (148) or by identifying gaps in medication collection revealed by gaps in which the patient's medication is assumed to have been exhausted (149). This method of measuring adherence requires an effective record keeping system (136).

2.3.5.1.4 Medication event monitoring system (MEMS)

This is a device with a special bottle cap fitted with a microprocessor which records the time and date each time the cap is opened and closed. The data stored by the cap are downloaded on to a computer for analysis. MEMS are often treated as the gold standard method for measuring medication adherence because it correlates more closely with viral loads than other measures of adherence (150, 151).

2.3.5.1.5 Therapeutic drug monitoring

Adherence to ART can also be measured by estimating the levels of drug in blood or urine. This can either be done by adding a molecule to a drug known as a marker which is easy to detect in urine or by direct measurement of the levels of the drug in blood or urine. These assays can only measure recent doses of drug taken hence provide limited information on long term adherence. The absence of the drug in the blood or levels below certain defined threshold can be taken as an indication of non-adherence (152, 153). One of the short-comings of this method is

that patients who are aware that they will be having drug levels measured can take their medication just before the test, unless this is unannounced. Measuring drug levels in hair overcomes the limitation of “snapshot” adherence when adherence is measured in blood samples because ART levels in hair reflects adherence over periods of weeks to months, thereby providing an opportunity to intervene before virological failure occurs (154)

2.3.5.1.6 Composite adherence measures

The WHO recommends a multi-method approach which combines self-reporting and other objective measures as the current gold standard in the measurement of adherence behaviour (129). This is because combining information from multiple methods reduces the error associated with any single measure. For example, adherence measured by pill counts or pharmacy refills tend to be lower than self-reports but higher than adherence measured using MEMS (145) suggesting there is a measurement error inherent in each method; therefore true adherence would be better represented by a composite measure. However there are no data on how best to combine measures and which measures to combine (155-157).

2.3.6 Virological failure

In the industrialised world, virological failure is defined as sustained viral load above 50 copies/mL after about six months on ART and usually associated with progressive rise in viral load measured in plasma (158) Progressive rise in viral load has been shown to be associated with treatment failure, development of resistance and disease progression. Virological failure could also be indicated by a sustained increase in viral load >50 copies/mL after a previous viral load <50 copies/mL. This is different from viral load “blips” which typically refers to one elevated viral load greater than 50 copies/mL, and usually less than 1000 copies/mL, preceded and followed by “undetectable” viral load in plasma. Sometimes the reason for this is unexplained; however it can also be due to random variation in the viral load assays used (158-160). Most studies have shown

that blips are not associated with treatment failure or development of resistance but may be associated with decreased adherence (161-163). In one study, however, in which there was stratification according to the level of viral load blips, an increase in subsequent failure was seen in patients with a blip over 500 copies/mL (164).

The definition of treatment failure in resource-limited settings is broader and depends on the availability of viral load monitoring. In the absence of viral load monitoring, WHO defined a set of immunological and clinical criteria for defining treatment failure. The immunological definition for treatment failure refers to a 50% fall in CD4 cell count from on treatment peak value or persistent CD4 cell count below 100 cells/mm^3 after 24 weeks or more on treatment. The clinical definition of failure refers to a new or recurrent WHO stage 4 condition which is not immune reconstitution inflammatory syndrome. In settings where viral load could be monitored, virological failure was defined as viral load above 5000 copies/mL (165). Studies have shown that the 2010 WHO immunological and clinical criteria lack sensitivity and specificity for predicting virological failure, thus resulting in continuation of failing regimen in individuals with virological failure or in treatment switches in those who do not need them (166-169). The South African ART guidelines (170) define treatment failure as two consecutive viral load measurements exceeding 1000 copies/mL within a two month interval after 6 months on ART.

A systematic review that included 89 studies in sub-Saharan Africa prior to June 2009 with varying design showed that in on-treatment analysis in individuals eligible for treatment, 7413 (76%) of 9794 patients experienced virological suppression (VL<50 copies/mL) after 12 months of ART and 3840 (67%) of 5690 after 24 months (171). Studies from South Africa (172), Malawi (173), Uganda (174) and Botswana (175) showed that 15-25% of patients have HIV-RNA >400 copies/mL 6-36 months after starting first-line ART(176). This figure is consistent with findings from the Hlabisa HIV Treatment and Care programme (where my

research is based) in which an estimated 15% of patients have HIV RNA >400 copies/mL 12 months after starting ART (26).

A number of factors have been shown to be associated with treatment failure. These are higher baseline or pre-ART viral load or higher viral load at the time of regimen change (130) poor adherence (130, 177) or other causes of sub-optimal drug exposure such as poor absorption, drug-drug interactions (158), depression and younger age (178).

2.3.7 HIV Drug resistance

ART scale-up in sub-Saharan Africa has been rapid with an estimated 13.6 million individuals on ART by June 2014 (179). The increased expansion in the indication for ART use would result in earlier initiation of treatment and if adherence is sub-optimal, this could lead to virological failure and the likely development of drug resistance. The likelihood of transmission of resistant virus will depend on ART coverage, duration of ART roll-out and the proportion and absolute numbers of individuals with virological failure (28, 180, 181). Conditions that would promote transmission of resistant virus include proportion of failures with resistant virus, time spent on failing regimen, viral load of patients with resistant virus, fitness of the resistant virus and the transmission probability compared with ART-naïve individuals (28). Renewed interest in the use of ART to prevent HIV transmission has resulted in the expansion of the indication for ART initiation (105) and there are on-going population randomised trials of HIV test and treat (25, 94, 95) evaluating the impact of universal HIV testing and immediate ART initiation on HIV incidence. In a clinical trial setting with virological monitoring, individuals failing ART would potentially be identified early and switched to alternative suppressive ART. In real life setting, as many sub-Saharan Africa countries adopt the most recent WHO guidelines (105), as is the case in South Africa (15), it is plausible that high levels of transmitted resistance may occur. This could result from the limited use of virological monitoring in many countries and the increased reliance on WHO

immunological and clinical criteria for identifying treatment failure (166). This scenario could threaten the effectiveness of ART programmes, despite the increasing availability of the more expensive second-line regimen and make HIV elimination difficult.

Limited available data from North America and the United Kingdom do not support that earlier ART initiation will lead to increased resistance (182, 183). Analysis of resistance data in individuals with virological failure in the HPTN 052 trial which included participants from the African sites showed that frequency of resistance at ART failure was significantly higher in the delayed ART arm compared to the early ART arm (184). It could be speculated that individuals with higher CD4 count are likely to be in an earlier stage of HIV infection compared to those with lower CD4 counts, thus having less diverse viral quasispecies because error-prone reverse transcriptase replication has occurred for a shorter duration. Hence those with higher CD4 counts would be likely to have a lower population of low frequency spontaneously generated drug mutants. It could also be due to yet an unexplained interaction between a relatively preserved immune system in those with higher CD4 counts and the HIV virus which reduces the likelihood of resistance at virological failure.

2.3.7.1 Determinants of HIV drug resistance

2.3.7.1.1 Type of regimen

Standard first line ART recommended by WHO for adults comprises two nucleoside reverse transcriptase inhibitor (NRTI), usually zidovudine or tenofovir and one non nucleoside reverse transcriptase inhibitor (NNRTI), typically nevirapine or efavirenz. However NNRTIs have a lower genetic barrier (defined as the number of mutations required to render a drug ineffective) to resistance than boosted protease inhibitor. Individuals failing on NNRTI-based regimen tend to develop resistance more often than seen in failure on boosted protease inhibitors (185-187). Other regimen-related factors include drug-drug interaction which can

result in decreased exposure to ART from induction of increased metabolism or impaired absorption resulting in virological failure and the selection of resistance, for example, proton pump inhibitors reduce the absorption of atazanavir (188). While the use of a fixed dose combination can improve adherence (189), the use of regimen with high pill burden can reduce adherence and result in development of resistance.

2.3.7.1.2 Patient factors

The most important patient-related factor for the success of ART is adherence. Adherence is also vital for the success of treatment programmes. Poor adherence can result in virological failure as well as the selection of drug-resistant virus. The individual benefits of optimal ART adherence have already been highlighted as well as the factors/barriers of adherence.

2.3.7.1.3 Programmatic factors

Programmatic factors such as inadequate staffing, poor infrastructure and weak procurement and supply management systems can act as barriers to ART adherence and retention in care thereby contributing to the development of resistance. Heavy patient load coupled with inadequate staffing can increase patient waiting times and sometimes discourage clinic attendance. High patient load can also decrease the time spent with each patient, leaving inadequate time for adherence counselling. Weak supply management systems can result in drug stock-outs and treatment interruptions (138, 139). Other factors such as the cost of assessing care, for example, transportation to clinics and food insecurity may also be barriers to ART adherence (190, 191).

Programmes that routinely monitor viral load are more likely to detect virological failure and switch ART timely compared to those relying on immunological and clinical criteria to identify failure. Individuals left on failing regimens are more likely to accumulate resistance mutations (27).

2.3.7.1.4 Virus factors

The HIV-1 subtype genetic variation can influence the frequency and mutational patterns of drug resistance as well as their susceptibility to ART. Some data have suggested that the K65R mutation is readily selected in subtype C viruses, which is more common in South Africa (192) compared to subtype B. This has been demonstrated to be related to the template nucleotide sequence and the tendency of reverse transcription to pause at position 65 (193-195). Individuals who harbour viruses with PDR are more likely to experience virological failure to first-line ART and this is most critical in situations where multiple mutations are present which confer multi-class drug resistance and in turn makes accumulation of new resistance mutations more likely (196-198).

2.3.7.2 Acquired resistance

This refers to resistance mutations which occur as a result of drug-selective pressure in individuals receiving ART.

In a WHO report on drug resistance in low and middle-income countries (187) 29 of 40 surveys done in 12 countries from 2006 to 2010 contributed data on resistance at 12 months among people failing ART. Of the 4764 individuals from the 29 completed surveys, 3475 were alive and receiving first line ART at 12 months, 294 had transferred out and 362 had died. The others had either stopped ART, switched to second-line or were unclassified. 3219 of the 3475 individuals alive had viral load data. 87% of individuals received a thymidine analogue-based ART such as zidovudine or stavudine and 12% were on tenofovir-based ART. About 5.1% of those initiating ART, excluding deaths and those transferred out, had evidence of resistance at 12 months. The prevalence of drug resistance was 72.1% among those failing first-line ART (69.5% to NNRTI, 62.5% to NRTI and 59.9% to both NNRTI and NRTI).

The frequency of the different NRTI and NNRTI mutations is summarised in Figure 2.4. 15.6% of individuals with treatment failure had one or more thymidine analogue associated mutations (TAM) which are M41L, D67GN, K70ER, L210W, T215IFNSY, K219ENQ.

The remaining 27.9% failed without resistance mutations. Possible explanations for this could have been from treatment interruption or very poor adherence resulting in no drug selective pressure on the virus

These results are similar to another systematic review of virological outcomes of 89 studies carried out before June 2009 in sub-Saharan Africa, in which 7413 (76%) of 9794 patients had VL<400 copies/mL after 12 months (171). 29 studies had information on genotypic resistance test in 734 patients. The majority of the patients (82%) were on NNRTI-based first-line therapy. The most prevalent resistant mutation was the M184V which confers resistance to lamivudine and emtricitabine, present in 478 patients (65%). D67N was the most prevalent TAM in 118 (16%) of patients. Of the 600 patients exposed to NNRTI who had genotypic resistance tests, the K103N mutation was the most prevalent and seen in 310 (52%) patients. The K65R which is associated with tenofovir use, a backbone component of the first-line ART recommended in South Africa, and TAMS associated with the use of zidovudine and stavudine were less common. 116 patients exposed to protease inhibitors (PI) had a resistance test. The L90M and the V82A/F/T/S mutations were the most common, both present in 18 (16%) patients.

Another systematic review comprising 8376 patients from eight cohorts and two prospective studies (6500 patients from seven low and middle income countries and 1876 from UK, Canada and Switzerland) reporting resistance in patients infected with HIV who received treatment consisting of 2 NRTI and an NNRTI at a CD4 < 200 cells/mm^3 covering the periods 1994 to 2009 compared the effect of monitoring frequency on the emergence of drug resistance (199).

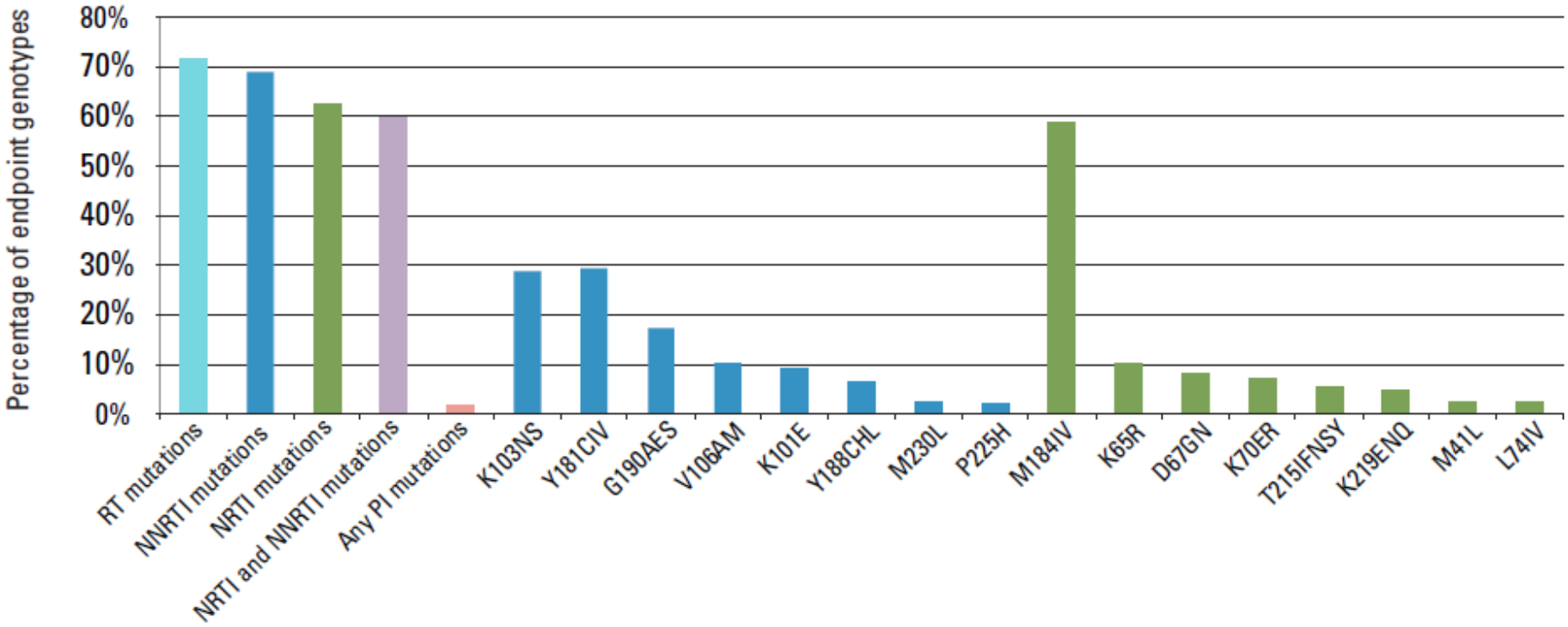


Figure 2.4 Prevalence of HIV-associated mutations amongst people experiencing treatment failure at 12 months

(Source: WHO HIV Drug resistance report 2012)

Resistance at treatment failure to NNRTI at 12 months was 88.3% and 61% in infrequently and frequently monitored patients, respectively. Lamivudine resistance was 80.5% and 40.3%; and the prevalence of at least one TAM was 27.8% and 12.1% respectively. The most likely explanation for the difference in prevalence of resistance observed between frequently and infrequently monitored patients is the time left on failing ART regimen. This situation could possibly be mitigated by the availability of cheap point-of-care viral load tests making viral load tests accessible with immediate availability of results.

A recent systematic review and meta-analysis of studies published between January 2006 and May 2013 (69% of the cohorts were from sub-Saharan Africa) on virological outcomes in low- and middle-income countries estimated virological suppression for all time-points (6, 12, 24, 36, 48, 60 months) up to 5 years to be >80% in the on treatment analysis after excluding the number of people who died, lost to follow up and who discontinued ART from the denominator of the estimate. The estimate was much lower when these indices were included in the denominator in an intention-to-treat analysis. This ranged from 61.8% (95% CI: 44.0-79.7) at 48 months to 74.7% (95% CI: 72.2-77.2) at 6 months (200). A study from the same sub-district as that in which the TasP trial was implemented reported virological suppression rates of 86% at 12 months in individuals who started ART between 1 August 2004 and 31 October 2009 (26).

A study from the Hlabisa sub-district, where my research was conducted, showed that 191 (86%) of 222 individuals failing first-line ART with detectable viral load had at least one drug-resistant mutation (27). (NNRTI 181 (82%); NRTI 179 (81%)). M184V was the most common NRTI mutation and present in 173 (78%) patients followed by the NNRTI mutation, K103N seen in 101 (45%) patients. TAMs were detected in 88 (40%) patients with the D67N being the most prevalent seen in 22% of patients. The K65R mutation was seen in 13 (6%) patients. This was likely due to the earlier exposure of some of the patients to stavudine. Some patients failing on stavudine develop the K65R mutation (201). The median time spent on a failing regimen was 27 months (IQR 17-41). The long duration spent on failing regimen

could be the reason for the higher frequency of TAMs seen in this study. Thirty four patients (15%) had a calculated genotypic susceptibility score (the number of active drugs in a regimen) of < 2 for the second-line regimen recommended by local guidelines; response to second-line ART may thus be sub-optimal.

2.3.7.3 Transmitted resistance

This refers to infection of HIV-negative individuals with drug-resistant virus, usually from individuals failing ART. Some studies would use the term “transmitted resistance” to refer to resistance seen in only recently infected individuals, while other studies would include individuals with longer duration of infection, classed as chronically infected. This distinction arises because mutations tend to revert to wild type with increasing duration of infection at variable rates, or may become archived and not be detected in majority virus during standard genotype testing (202-204).

WHO reports (187) 72 surveys of transmitted resistance conducted in 26 low- and middle-income countries from 2004 to 2010; 43/72 (60%) were from the African region. 52/72 (72%) surveys showed low prevalence (<5%) to all drug classes and 28% had a moderate prevalence (5-15%) classification. There was no survey with high prevalence classification. The proportion of surveys reporting a moderate prevalence of transmitted resistance to any drug class increased from 18% in the period 2004-2006 to 32% in the period 2007-2010 and this was driven by a rise in the prevalence of NNRTI resistance. The rise in moderate prevalence of transmitted resistance is mostly represented by increases from the African region from 17.6% to 40.7% in the respective periods.

Figure 2.5 shows that higher levels of ART coverage (proportion of all HIV positive individuals on ART) are associated with increased prevalence of transmitted drug resistance to NNRTI after adjusting for regional variability (OR 1.49, 95% CI 1.07-2.08; p-value adjusted for region 0.039). Figure 2.6 shows the prevalence of transmitted drug resistance mutations in individuals in the surveys, 2004 – 2010.

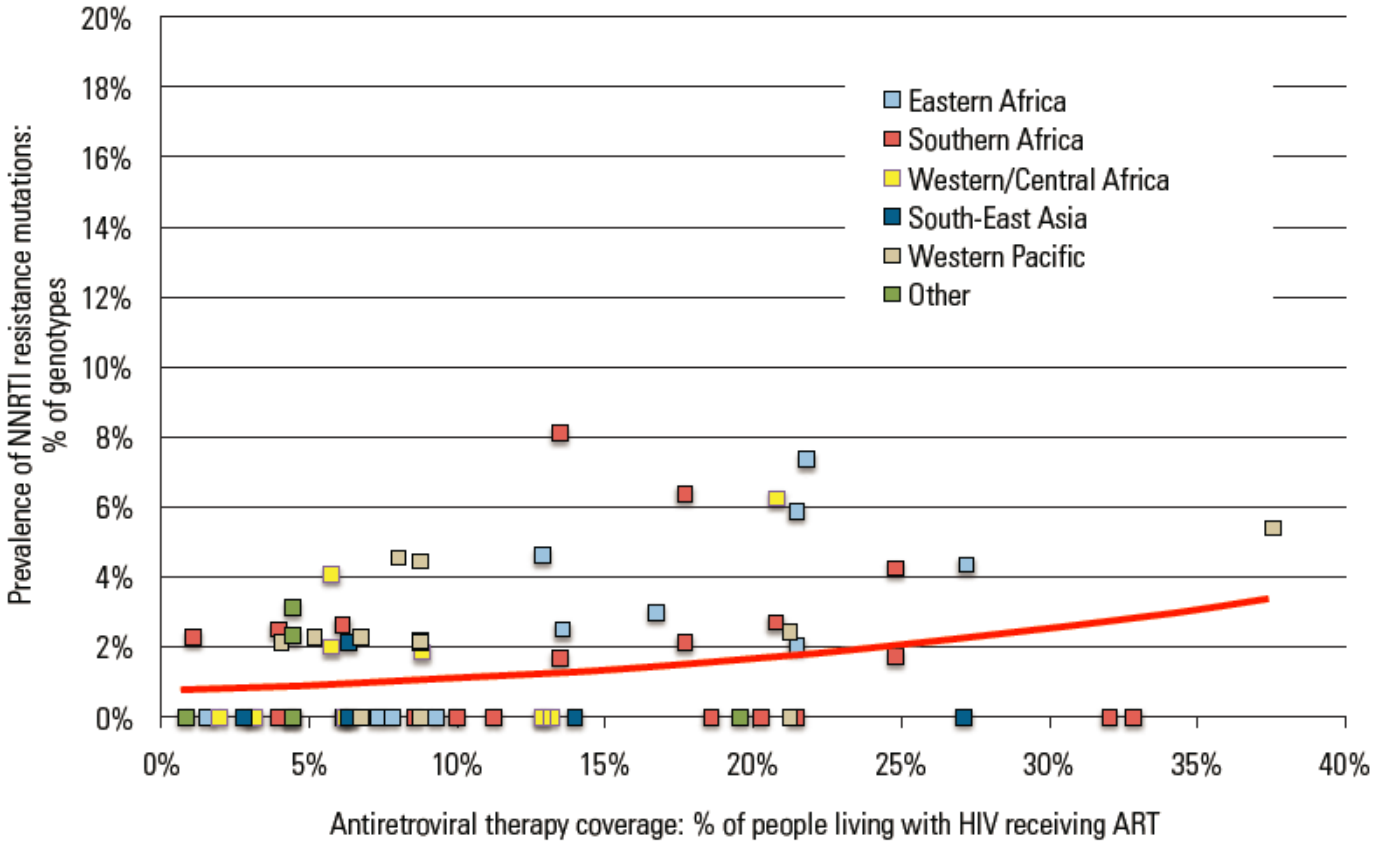


Figure 2.5 Relationship between ART coverage and prevalence of transmitted NNRTI resistance mutations (Source: WHO HIV Drug resistance report 2012

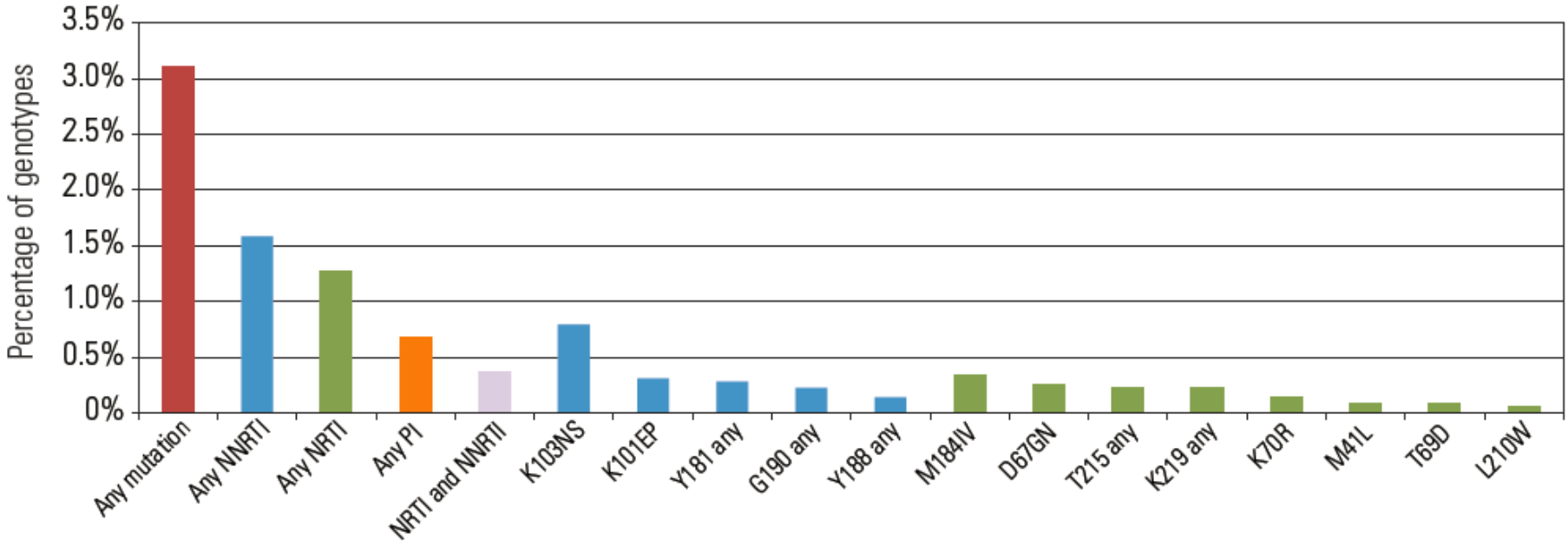


Figure 2.6 Prevalence of drug resistance mutations in individuals included in WHO transmitted HIV drug resistance. (Source: WHO HIV Drug resistance report 2012)

A meta-analysis of studies from Jan 2001 to July 2011 comprising 26,102 ART naïve individuals from sub-Saharan Africa, Asia and Latin America estimated the prevalence of transmitted drug resistance since ART roll-out (205). The prevalence of any drug resistance in East Africa increased from 1.0% (95% CI 0.6 -1.9) at roll-out to 7.4% (4.3 -12.7) 8 years after roll out; an increase of 29% per year (15 - 45). The prevalence of any drug resistance in Southern Africa and West/Central Africa at roll out was 1.4% (0.8 - 2.3) and 3.1% (1.7 - 5.6) respectively with estimated yearly increases of 14% (0 - 29; p = 0.054) and 3% (-0.9 – 16; p=0.618) respectively.

ART coverage (p=0.0013) and time since roll-out (p=0.0006) were associated with an increase in the prevalence of drug resistance in East Africa, but only time since roll-out (p=0.0006) and not coverage (p=0.88) were associated with prevalence of drug resistance in Southern Africa. Neither time since roll-out (p=0.43) nor ART coverage (p=0.55) were associated with prevalence of drug resistance in West/Central Africa

However, despite these high relative increases with time since roll-out, the overall prevalence of resistance to any drug class remains low: 5.1% for NNRTI resistance in East Africa eight years after roll out (increase of 36% per year) and.an increase of 23% per year in Southern Africa. The prevalence of TAM increased by 31% per year since roll-out in East Africa with no significant changes documented in other regions.

Another evaluation of transmitted resistance from 2007 to 2009 in 11 regions in six sub-Saharan African countries, including South Africa, showed that the prevalence of transmitted drug resistance in South Africa overall was low at 1.1%, but as high as 12.3% in Kampala, Uganda (206). The prevalence of drug resistance was 3.3% for NNRTI; 2.5% for NRTI, 1.3% for protease inhibitors and 1.2% for both NRTI/NNRTI.

A study of transmitted resistance on 701 ART-naïve individuals in the Africa Centre demographic surveillance area in the Hlabisa sub-district (adjacent to the TasP trial

communities) comprising 67 samples from 2010 although sample size was small, 381 (2011) and 253 (2012) showed an overall prevalence of transmitted resistance of 5%.There were no resistance mutations observed in 2010 although sample size was low; prevalence was 5% in 2011 and 8% in 2012. The most common NNRTI mutation was the K103N mutation present in 5% of samples. NRTI mutations were detected in 1.6% of the participants (207).

The collective studies presented here suggest that there is a moderate increase in transmitted resistance with increasing duration since roll-out of ART as well as with increase in ART coverage.

2.3.7.3.1 Persistence and transmission of drug-resistant virus

HIV drug-resistant virus may impair replicative capacity of the mutant; hence there is a fitness cost to the virus to remain in the mutant form; replacement with wild-type virus or the development of compensatory mutations may restore replicative capacity and confer a survival advantage to the virus. Since the viral population in transmitted resistance comprises mainly of resistant viruses with no co-existing wild-type virus, whether the virus reverts to wild-type would depend, in addition, to the number of back mutations required to do so, the fitness of back mutated viruses and the rate of viral turnover (202-204, 208, 209). It is plausible to assume more persistent mutations and fit viruses would be more likely to be transmitted compared to those mutations which readily revert to wild-type. In a study of the persistence of individual transmitted resistance mutation involving 313 individuals (203) the rate of loss of mutations was estimated. The M184V was the quickest to disappear at a rate of 71 (34 – 149) per 100 pyrs. Also, rapid in disappearing is the K70R at a rate of 38 (17-83) per 100 pyrs and the T215Y/T215F to one of the T215 revertants that are very stable with loss rates of 5 (3-11) per 100 pyrs. The TAMs (M41L D67N L210W and K219Q/N) are highly persistent with loss rates ranging from 4 (1-19) per 100 pyrs for K219Q to 15 (3-72) per 100 pyrs for K219N. The K103N mutation was the most common NNRTI mutation present with a loss rate of 18 (10-34) per 100 pyrs and this was not significantly different from the rate of loss of other NNRTI mutations. Hence they are not as persistent as the TAMs except

for the K70R and T215Y/F which disappear more rapidly. PI mutations have a rate of loss that is fairly uniform across the different mutations and similar to that of the NNRTI. The L90M mutation was the most common with a loss rate of 12 (5-31) per 100 pyrs.

Another study of persistence of transmitted drug resistance mutations evaluated 75 individuals with 195 mutations (202). In this study, the different drug classes were examined as a group apart from the M184V/I which was found to be lost quicker than the NNRTI. The NNRTI and PI were more persistent with loss rates that were similar to each other. The Castro study described above, involving 313 individuals, showed that even within drug classes the rate of loss of mutations could be variable. This has implications for the pattern of mutations that are likely to be transmitted.

Neither of the two studies above discussed the K65R mutation which is particularly relevant in the South African setting with the current widespread use of tenofovir-containing regimen and the observation in certain studies that this mutation is more readily selected in subtype C viruses (210, 211). However, a study that examined 19,823 sequences from ART-naïve individuals with diverse HIV-1 subtypes (212) showed that only 20 (0.4%) sequences had the K65R mutation. No significant difference was found in the prevalence of K65R in subtype C (3/3198, 0.09%) compared to non-subtype C sequences (17/16,608, 0.10% p=1). One possible explanation for the very low prevalence of K65R mutation in the analysed sequences could be due to the potential reduction of viral replication and fitness as a result of this mutation (213, 214). This suggests that despite widespread use of tenofovir in South Africa, transmission of the K65R mutation may be rare. However this assumption is being challenged by other studies that have not found in a reduction in the fitness of viruses harbouring the K65R mutation (215). I will be addressing this question in my research.

2.3.7.4 Pre-treatment drug resistance

With the maturing of the ART programme in many sub-Saharan African countries, it becomes increasingly difficult to be certain that individuals presenting for ART initiation have not had prior ART exposure. Pre-treatment drug resistance refers to resistance present in individuals starting ART and can be either transmitted or acquired due to previous ART, especially in those who defaulted from care but re-engaged or as part of prevention of mother to child transmission (216). The WHO now recommends monitoring pre-treatment drug resistance to provide information about the appropriateness of first-line ART being prescribed in an ART programme (216)

2.4 Discussion

Many tools are available for HIV prevention. Although the focus of this chapter is biomedical HIV prevention, all prevention methods require behaviour change (217) as individuals need to have the agency to decide which of the prevention methods best meets their needs at any particular point in time. As none of the prevention methods is 100% efficacious, it has been suggested that the optimal way to tackle the epidemic is through a combination of these methods focussed to areas of high transmission and people at most risk of infection, including key populations (13). There is increased interest in the use of ART, not just for individual health but also for the anticipated population benefit of decreased HIV transmission. ART guidelines in many countries now recommend ART regardless of CD4 cell count (15) but concerns have been expressed about ART adherence in individuals with high CD4 count who are mainly asymptomatic. If adherence is sub-optimal, this could lead to virological failure and the emergence and transmission of drug-resistant HIV. The relationship between CD4 count at initiation, adherence, virological response and drug resistance is conceptualized in Figure 2.7. Even under more restrictive ART guidelines, WHO surveys and many studies (187, 205) suggest there is a moderate increase in risk of transmitted resistance with

increasing duration since roll-out of ART as well as with increase in ART coverage. The question that arises as a result of this is whether the prevalence and pattern of drug-resistant HIV will attenuate the beneficial impact of ART at the individual- and population-level and hinder HIV elimination. Individuals harbouring viruses with transmitted resistance would require more complex and expensive ART regimens and care. Increased pill burden will make adherence to ART more difficult resulting in virological failure, thus creating a vicious cycle. The WHO recognizes HIV drug resistance as a threat to the elimination of HIV (216). To optimize individual- and population-level ART outcomes, WHO recommends HIV drug-resistance surveillance through the monitoring of clinic level early warning indicators (EWI). These EWI give insight about the quality of an ART programme and identify gaps that could result in the development of drug resistance (32) and therefore require interventions to mitigate them. These EWI and their targets are summarized in Table 2.6. WHO also recommend surveys of pre-treatment drug resistance in individuals initiating ART to inform choice of first-line ART, and surveys of acquired drug resistance in individuals receiving ART to inform choice of second-line ART as well as third-line ART. My research will be addressing two of these early warning indicators; on-time pill pick up, which is a proxy measure of adherence, and virological suppression. I will also estimate the prevalence of pre-treatment and acquired drug resistance.

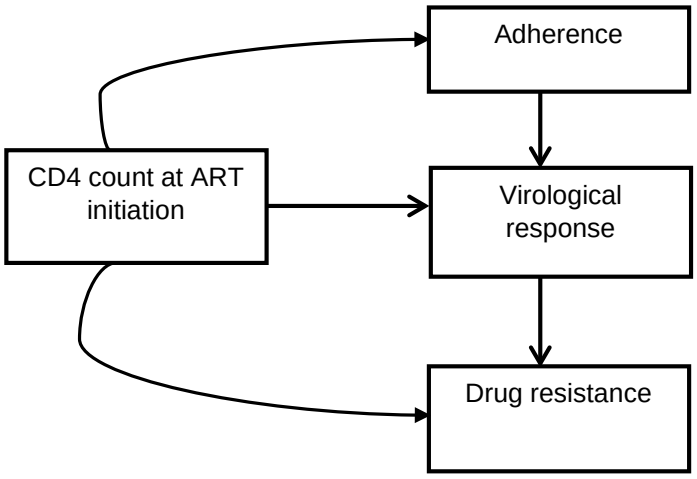


Figure 2.7 Conceptualised framework of CD4 count at initiation, adherence, virological response and drug resistance

Table 2.6 WHO HIV drug resistance early warning indicators and targets (32)

Early warning indicators	Excellent performance Fair performance Poor performance
Prescribing practices % of ART prescriptions congruent with national/international guidelines	100% <100%
LTFU at 12 months % of patients LTFU 12 months after ART initiation	<15% 15-25% >25%
Retention at 12 months % of patients retained in care 12 months after ART initiation	>85% 75-85% <75%
On-time pill pick-up % of patients with 100% on-time drug pick-up during the first 12 months of ART or during a specified time period	>90% 80-90% <80%
On-time appointment keeping % of patients attending all clinic appointments on time during the first 12 months of ART or during a specified time period	>80% 70-80% <70%
Drug stock out % of months with any day(s) of stock out of any routinely dispensed ARV drug	0% <0%
Viral load suppression % of patients with viral load <1000 copies/mL 12 months after ART initiation	>90% 80-90% <80%
Viral load completion % of patients with a 12-month viral load test result available	≥70% <70%

Chapter 3 Methods

This chapter describes firstly the procedures of the HIV Treatment as Prevention cluster randomised trial and secondly, the procedures of the MD research nested within it.

The hypothesis of the TasP trial was that HIV testing of all adult members of a community followed by immediate offer of ART to those HIV-positive regardless of immunological or clinical staging will prevent onward transmission and reduce population HIV incidence.

3.1 The HIV Treatment as Prevention trial

3.1.1 Study setting

The TasP trial was hosted by the Africa Health Research Institute (AHRI), previously known as the Africa Centre. The AHRI receives core funding from the Wellcome Trust and the Howard Hughes Institute and has two research sites; the Durban site located at the Medical School, University of KwaZulu-Natal and the Somkhle site situated in Hlabisa sub-district, uMkhanyakude district, northern KwaZulu-Natal, South Africa (Figure 3.1). The TasP trial was implemented in the Hlabisa sub-district which covers an area of 1430 km^2 and has a population of 220,000 Zulu speaking people.

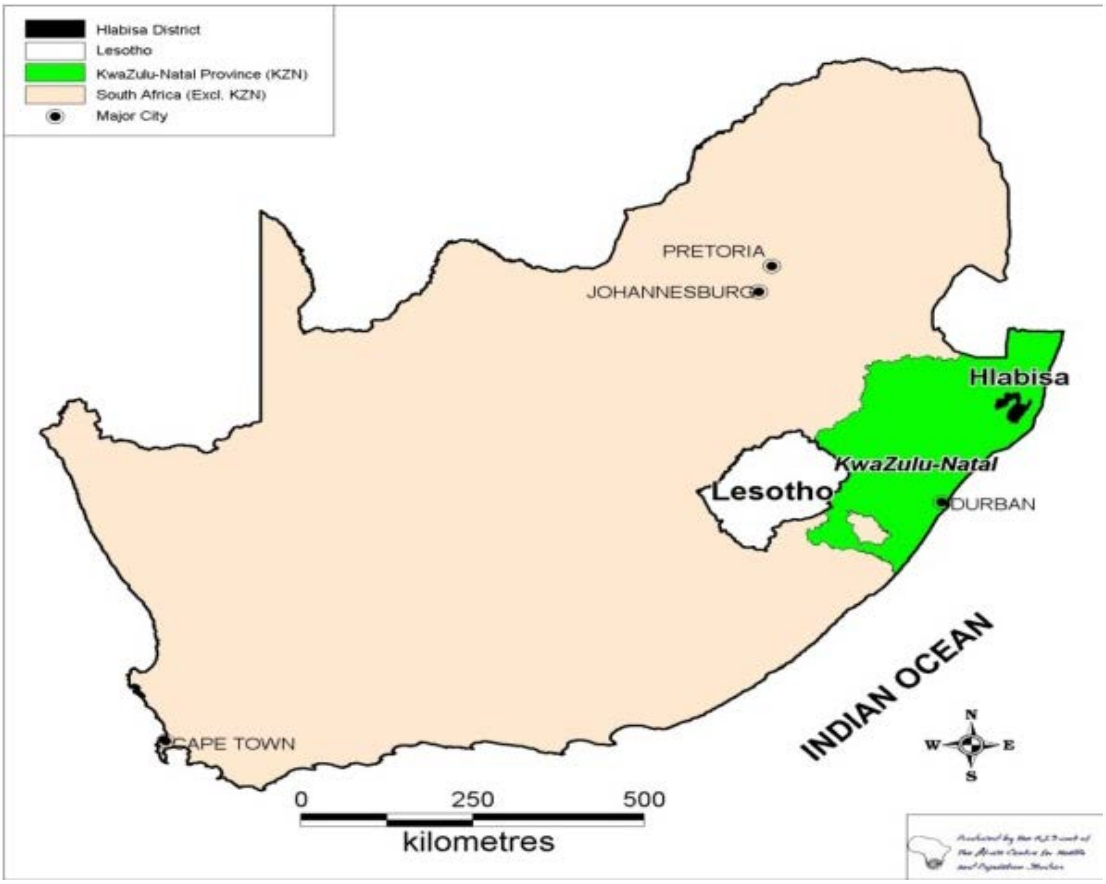


Figure 3.1 Map of South Africa showing locations of AHRI sites in Durban and Hlabisa sub-district

The AHRI carries out population-based research in an area of the sub-district known as the Population Intervention Platform Study Area (PIPSA) which measures 825 km^2 with a mid-year population of 151,441 in 2015 (Figure 3.2). The PIPSA was implemented in January 2017 as a newly expanded research platform combining the former Africa Centre HIV surveillance area and the TasP trial area (Figure 3.2).

The crude HIV incidence in the demographic/HIV surveillance area in the period 2004-2011 was estimated at 2.63 new infections per 100 person-years (95% CI 2.50-2.77) in people 15 years and older (14). Estimated HIV prevalence was 29% in 2011 for those aged 15-49 years (218). At the time of initiation of my research, the ART coverage within the demographic/HIV surveillance area was 37% of all HIV-infected individuals (14).

The TasP trial was conducted in communities outside of the former Africa Centre demographic/HIV surveillance area on the northern side, as illustrated in Figure 3.2. The Hlabisa public ART programme primary health care clinics are indicated by the crosses in Figure 3.2. Three of these 17 clinics are situated within the TasP trial communities. In addition to these three clinics, there is a TasP trial clinic located in each cluster as illustrated by the vehicle icon in Figure 3.3, a map showing only the trial communities.

3.1.2 Study design

The TasP trial is a two-arm cluster randomised trial implemented in 22 clusters (2 x11) to investigate the impact of population ART on HIV incidence. The main trial has two components: the first is a population-based home survey comprising the offer of six-monthly home HIV testing using rapid test technology and the referral of those identified HIV-positive to the trial clinic in their cluster while the second relates to the clinical care of HIV-positive individuals who linked to the trial clinics following referral.

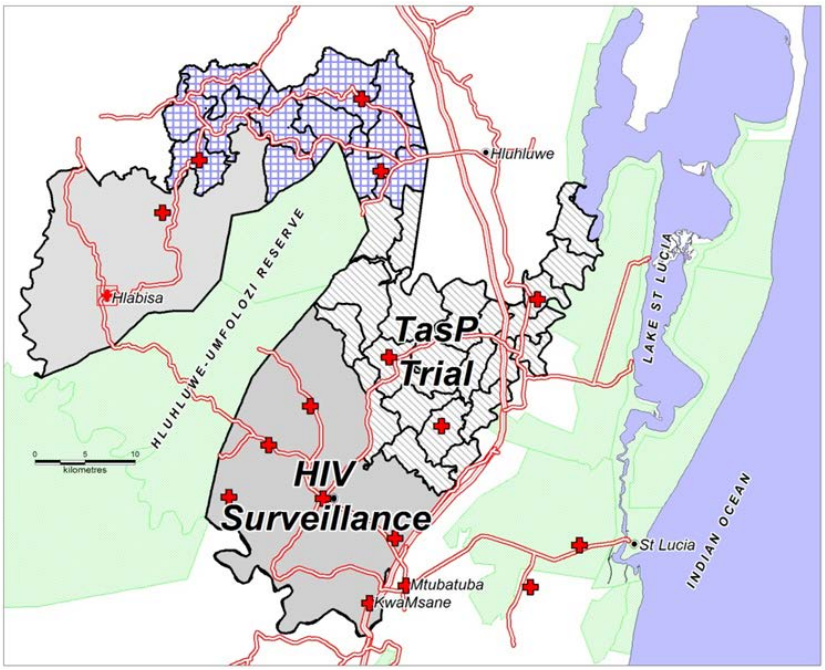


Figure 3.2 Map of the Hlabisa subdistrict showing the TasP trial clusters and previous Africa Centre HIV surveillance area (Combined as PIPSA)

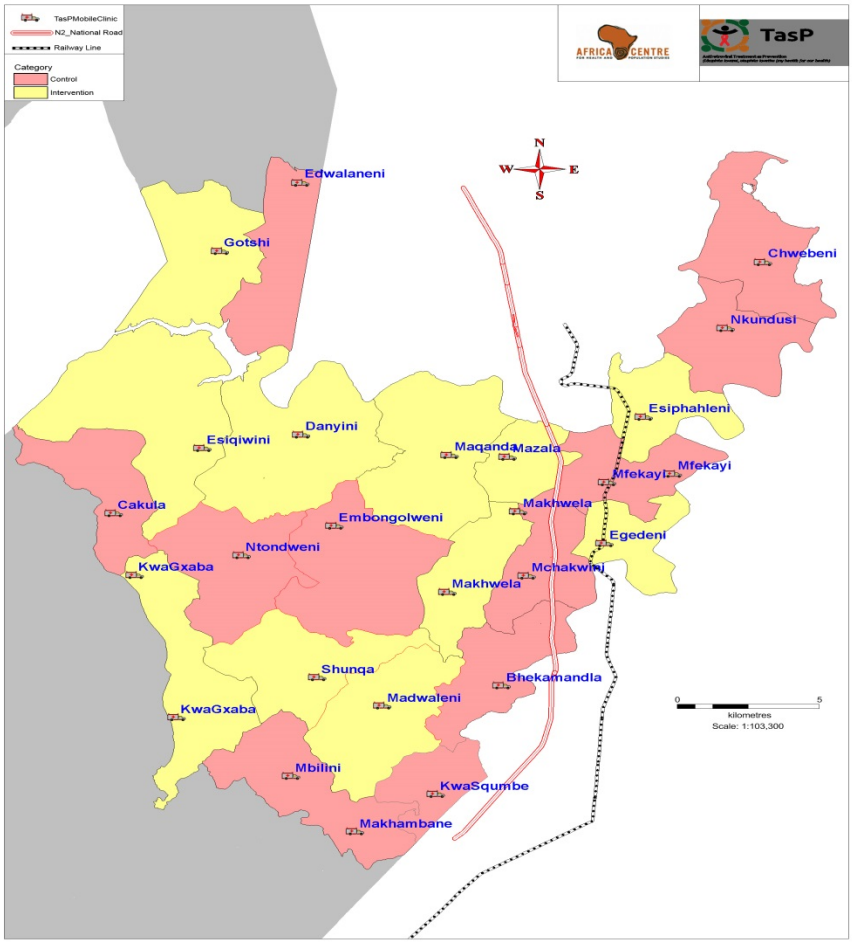


Figure 3.3 Map of the TasP trial clusters showing the location of the clinics

At the time of referral, HIV-positive individuals were either newly diagnosed or already knew (and reported) their status to be HIV-positive. Some of the latter individuals were receiving ART from the Hlabisa public ART programme.

ART-naïve HIV-positive adults in the intervention arm were offered immediate ART initiation upon enrolment in trial clinics. The HIV-positive adults in the control arm were offered ART according to the prevailing national guidelines (CD4 ≤350 cells/mm^3, WHO stage 3 or 4 disease or MDR/XDR TB (219). In January 2015, following revised WHO guidelines, the new South African national department guidelines recommending initiation of ART at a CD4 ≤500 cells/mm^3 was adopted in the control arm of the trial (170).

3.1.3 Eligibility criteria

Individuals 16 years and above, who are resident members of the trial communities and could give informed consent were eligible for inclusion in the trial.

For individuals between 16 and 18 years of age consent was obtained from a parent or a responsible guardian for participation in the main trial during the home survey as the age of consent for research in South Africa is 18 years. The individual could then give assent to participate in the trial. The parents were neither informed if participants gave assent for an HIV test nor were HIV test results discussed with them. No further parental consent was sought for this group if they enrolled in trial clinics following an HIV-positive result.

All HIV-positive individuals aged 16 years and under, non-resident members in the trial clusters and those unable to provide written informed consent were excluded from the research.

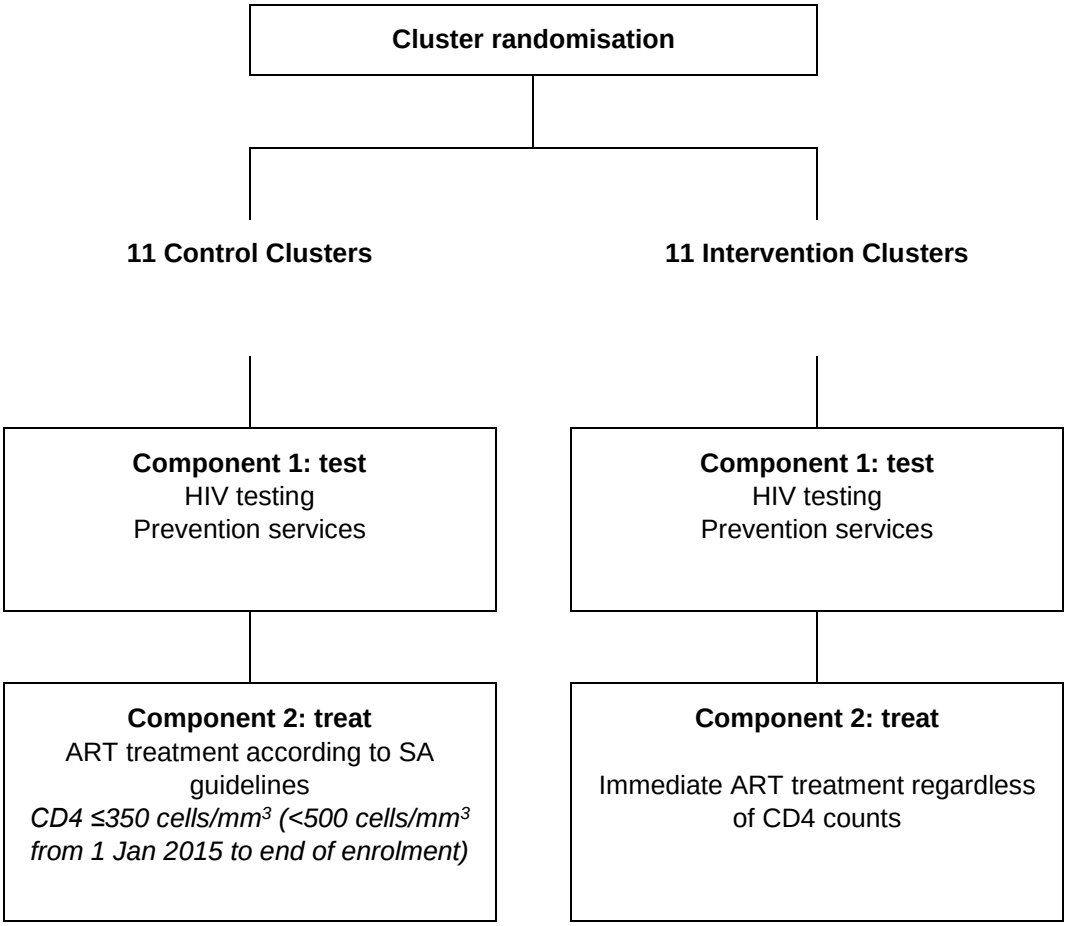


Figure 3.4 Design of the TasP trial

3.1.4 Study procedures

3.1.4.1 *Household entry and procedures*

All households within the Hlabisa sub-district were mapped in 1999 as part of the establishment of the former Afica Centre and each homestead was assigned a bounded structure identification number (BSID). All homesteads newly constructed since then were mapped by the Africa Centre geographic information system team before the implementation of the TasP trial.

Household procedures were carried out by a team of fieldworkers all of whom were trained in HIV counselling and testing. I trained them in the study protocol as well as in other procedures relevant to the trial and conducted a refresher training in HIV counselling and testing (HCT).

These fieldworkers approached each household and sought permission from the household head (or most senior resident household member present if head was absent) to enter the household. The fieldworkers explained the trial and the procedures to the household head and sought their permission to offer trial participation and HCT to adult members of the household. Specific information sheets for each component were provided to all individuals. Once permission to proceed was granted, all members of the household 16 years and above were enumerated and registered. Following this, a private space was identified and written permission was sought from all eligible resident household members to complete the *TasP home-based individual questionnaire* (IQ) with or without collection of dried blood spot (DBS) (Appendix A), and to undergo confidential HIV counselling and testing using rapid HIV test technology. The home-based questionnaire enquired about socio-demographic information, sexual behaviour and attitudes about HIV testing. Individual household members could consent to either component alone (IQ with or without DBS) or both components (IQ+/-DBS and HIV testing). Two consent forms; one for *Home-Based Individual Questionnaire/DBS and the second for Home-Based HIV Testing* were also completed during the process (Appendices B & C). People who did not want to be

tested for HIV in the household could attend any department of health or trial clinic for testing and were informed of this possibility.

The majority of individuals enrolled into the main trial agreed to provide dried blood spots, but some refused rapid HIV testing. The dried blood spots collected every six months were used for HIV ELISA antibody testing in the AHRI laboratory for establishing HIV seroconversion, these results were not fed back to participants. Only participants who consented to rapid HIV testing were informed of their results and referred to trial clinics if HIV-positive.

In each round of testing, the team was able to return on up to two more occasions to offer trial participation and/or HIV testing to members of the household who were not present during the initial visit, after which the untested members of the household would receive a written invitation to attend the trial clinics where HIV counselling and testing was also available.

3.1.4.2 *HIV counselling and testing*

Participants underwent counselling at home prior to HIV test. Participants who were reactive to an initial screening test underwent a confirmatory HIV rapid test immediately, using a different rapid test kit. If this was reactive, the participant was informed they were HIV-positive. They were given a trial card which contained a unique number and referred to the trial clinic where they presented the card. This unique number became their study identity number for the duration of the trial. Participants who tested HIV negative were informed they would be tested again in a subsequent HIV testing round. The HIV status of the participants was documented in the fieldworkers netbooks and uploaded to the Africa Centre server at the end of each working day.

3.1.4.3 *Care of HIV-positive participants in the TasP clinics*

All participants identified as HIV-positive during any of the home-based HCT rounds were referred to their trial clinic for immediate further assessment.

Trial clinics (one per cluster) were situated in close proximity to residences. Each trial clinic was staffed by a counsellor and a nurse. I visited a trial clinic at least once a week to consult complex patients referred to me by the nurses. At the trial clinics, all HIV-positive participants received information regarding the treatment procedures from the nurse, and were also screened for eligibility for treatment initiation and were asked to provide informed consent to receive care and/or treatment within the trial. Point-of-care CD4 machines were available in each of the trial clinics, to inform treatment eligibility. Participants already established on ART from the Hlabisa HIV Treatment and Care Programme (Public ART programme) who were residing in the trial clusters were encouraged to enroll in the trial and to transfer their care to the trial clinics.

3.2 MD research

3.2.1 Aims and objectives

The overarching aim of my research is to test the hypothesis that initiation of ART at high CD4 would result in the emergence of drug resistance of the form and prevalence that could hinder the elimination of HIV.

The specific objectives are:

- To examine the association between CD4 count at ART initiation and adherence levels.
- To examine virological response by CD4 count at ART initiation and extent of acquired resistance following virological failure.
- To estimate the level of pre-treatment drug resistance in ART naïve individuals and examine its association with virological response to first-line ART.

3.2.2 Study setting

My MD research was nested within the cluster-randomised trial of HIV treatment as prevention described earlier.

3.2.3 Study design

My MD focusses on a cohort analysis of data from all HIV-positive participants enrolled in TasP trial clinics in all 22 clusters from March 2012 until June 2016. I compared data from all individuals who initiated ART at a CD4 ≤350cells/mm^3 with data from those who started at CD4>350 cells/mm^3 irrespective of whether in the control or intervention clusters (Figure 3.5). The CD4 count of 350 cells/mm^3 was chosen as the cut-off point as the majority of the participants enrolled in the trial were offered ART based on previous South African ART guidelines threshold of 350cells/mm^3. From March 2012 to December 2014 (33 months), CD4 ≤350 cells/mm^3 was the threshold for ART initiation and from January 2015 to June 2016 (18 months), this threshold increased to 500 cells/mm^3.

3.2.1 Eligibility criteria

Individuals identified as HIV-positive who were ≥16 years, gave written informed consent, recruited from a household within the TasP trial clusters and received their care from one of the TasP trial clinics were eligible for inclusion in my research.

3.2.1 MD study population

Eligible community members who self-reported to be HIV-positive or were newly identified as HIV-positive through rapid HIV test technology and received their HIV care from the TasP trial clinics formed the basis of my analysis. The HIV positive individuals comprised two categories: The first are those ART-naïve at entry into the TasP trial clinics and the second were ART-experienced at their first trial clinic visit.

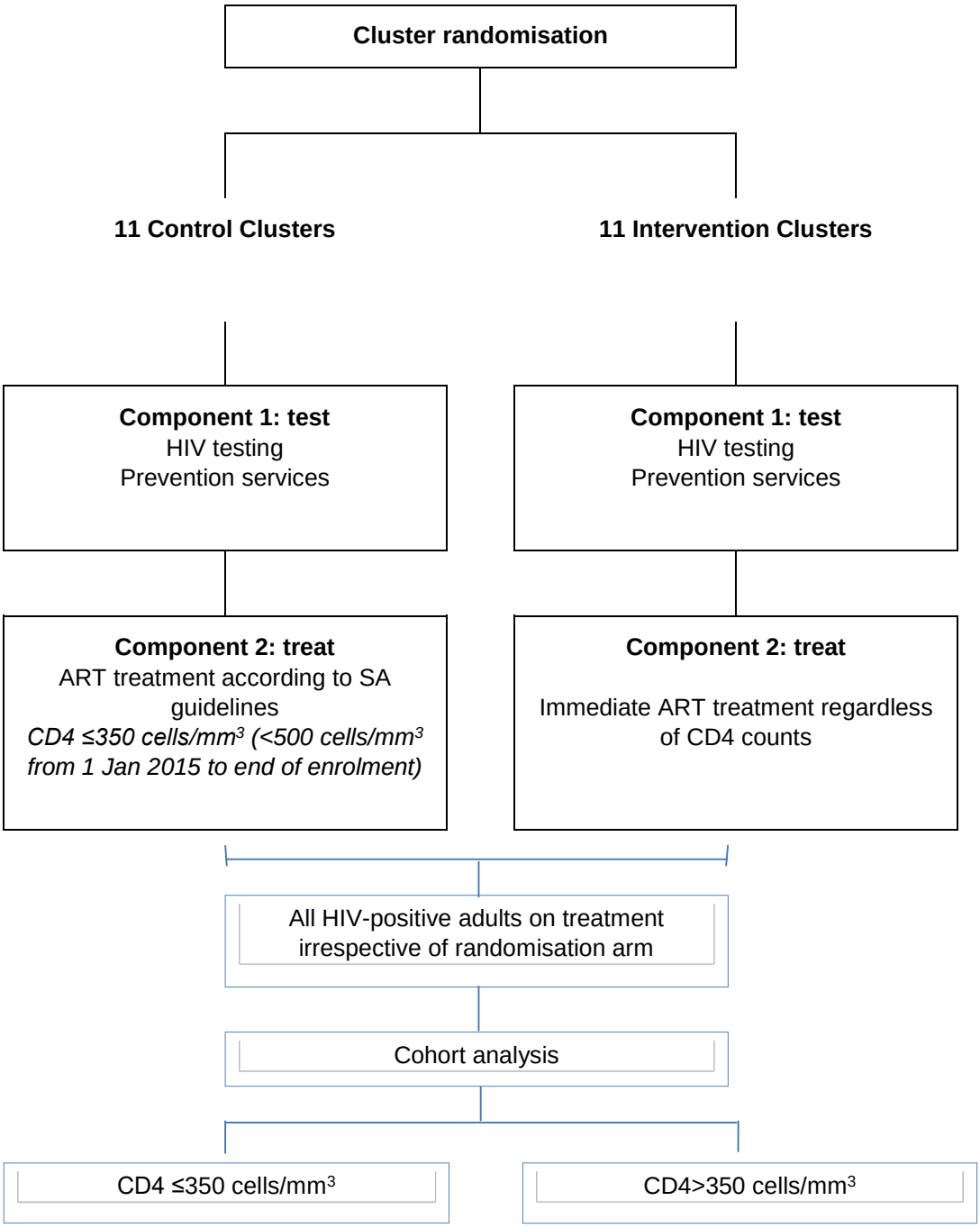


Figure 3.5 Study design of MD research

A database containing information on those ART-experienced at entry had been maintained by the Africa Centre since 2004 when the public ART programme was rolled out in the Hlabisa sub-district. I obtained ethics approval to link the TasP trial database with the Hlabisa ART programme database.

The analysis on pre-treatment drug resistance was not restricted to the ART-naïve group who linked to trial clinics. Participants who gave blood samples on filter paper as dried blood spots in the home surveys and were confirmed HIV-negative through a laboratory HIV ELISA antibody test on the very first sample taken from them were eligible for inclusion if subsequent samples confirmed HIV seroconversion during the course of the trial (and were therefore known to be recent infections). These individuals were not necessarily aware of their HIV-positive status, as they could only be informed about their HIV status if they consented to a point of care HIV test as well. It was necessary to use the dried blood spots for assessing pre-treatment drug resistance because the majority of seroconverters did not link to the trial clinics (either because they were not aware of their HIV-positive status or for other reasons if knew their status), hence no plasma samples were available on them, but on the small subset that linked to care, and provided plasma samples, this was used in place of the dried blood spots.

3.2.2 Study procedures

I set up a trial clinic in each of the 22 trial clusters for the management of all HIV-positive individuals referred to the trial clinics as part of the main trial and within the clinics set up procedures that allow the collection of data for my research.

3.2.2.1 Clinic baseline visit

I developed the questionnaires administered to participants in the trial clinics (Appendices D, E & F), drafted the standard operating procedures for their use and trained the clinic staff on how to complete the forms.

The counsellor administered a baseline questionnaire documenting anthropometric details and previous ART history. A detailed clinical history and examination questionnaire was completed by the trial nurse. A total of 30mL of blood was drawn by the nurse; 10 mL of blood was sent to the National Health Laboratory Service (NHLS) for routine haematology (4mL EDTA) biochemistry and HBV testing (6mL lithium heparin) (these samples were not stored), 20 mL of blood (2×10 mL EDTA tubes) was sent to the AHRI laboratory in Durban for plasma viral load testing and storage at -80°C. Some of the blood sent to the laboratory was also used for performing genotypic resistance tests in participants whom I identified to be experiencing virological failure (see Chapter 6).

3.2.2.2 Scheduled follow up visits

Individuals initiated on ART within the trial clinics were seen after two weeks for adherence check and assessed for complaints of side-effects. They were again seen at 4 weeks post-ART initiation and then monthly thereafter. For example, an individual on ART for 6 months, should have seven adherence measurements post-ART initiation (2 weeks, 4 weeks, 8 weeks, 12 weeks, 16 weeks, 20 weeks, 24 weeks).

Individuals already established on ART prior to their first trial clinic visit, were seen monthly. Those not yet eligible for ART were followed up every 4-6 months.

During the follow up visits, the counsellor administered a follow-up questionnaire enquiring about ART adherence in those on ART. The nurse completed a clinical history and examination form each time a participant visited the clinic.

Blood tests were done according to the schedule in Table 3.1; beyond 12 months, the blood tests were done six monthly.

Interval visits were permitted for participants who required clinical attention for whatever reason between scheduled appointment dates.

Table 3.1 Blood tests schedule

Laboratory	Baseline	3 months	6 months	12 months
HIV Viral load	✓	✓	✓	✓
CD4 point-of-care	✓		✓	✓
Genotyping			If meets definition for virological failure	

3.2.2.3 Antiretroviral therapy

Atripla, a fixed dose combination of tenofovir, emtricitabine and efavirenz, was prescribed as first-line ART to all those eligible for treatment. Other first-line drugs such as zidovudine, abacavir and lamivudine were also available depending on clinical indication. Participants failing first-line ART were prescribed second-line regimen which is based on a boosted protease inhibitor. Switch to second-line ART was informed by the results of genotypic resistance test which I requested, interpreted the results and made recommendation for second-line ART. The genotype tests were performed by the laboratory staff at the AHRI laboratory in Durban. I chaired a clinic multidisciplinary meeting every Friday afternoon involving doctors, nurses and pharmacists to discuss all patients initiating or switching ART within the trial. I also supervised a computerised ‘hot-list” meeting from 8-9 am daily attended by trial doctors and the three nurse team leaders. This was to discuss the management plan for trial patients who were acutely unwell/admitted in hospital or had complex care needs.

3.2.2.4 Virtual clinics

I was responsible for two different types of virtual clinics during the duration of my research. Once a week, I chaired a multidisciplinary virtual clinic comprising, nurses, clinicians and pharmacist to discuss all participants recruited to the clinics the previous week. I reviewed baseline blood results and made ART treatment recommendations.Participants already on ART with adherence issues or

experiencing virological failure are also discussed in this meeting following which I recommend a plan of management.

Furthermore, I set up a “hot-list” meeting which took place daily before the start of clinic. I developed a set of clinical and laboratory criteria for enlisting participants into this hot-list. The criteria were set at a threshold to flag up participants before their conditions became serious. These included but not restricted to the following:

- All sick patients, including those admitted to hospital
- Any patient the nurse is concerned about
- Participants diagnosed with TB
- Participants with abnormal creatinine
- CD4 < 100 cells/µL
- Hb < 7g/dL
- ALT >= 2 X ULN
- ALP>= 2 X ULN
- Patients with abnormal cervical smears

I supervised the preparation of a standard operating procedure to guide the operation of the hotlist and subsequently approved it.

3.2.2.5 Laboratory procedures

3.2.2.5.1 CD4 count measurement and quality control

For all patients, the CD4 count was measured by the nurses using the point-of-care PIMA machine (Alere Inc. Waltham, Maryland, USA). About 5 µL of capillary blood was collected from a finger-prick into a PIMA cartridge taking care to avoid air bubbles. The cartridge was inserted into the PIMA machine and an absolute CD4 count result displayed on the monitor after 20 minutes. No CD4 percentage was provided. Prior to enrolment in the main trial, participants ART-experienced at trial entry had their CD4 counts measured in the public health laboratory and would return at a future date (usually two weeks later) for the results.

There was both internal and external/continuous quality assessment of the point-of-care CD4 testing throughout the duration of my research. The quality assessment incorporated three different aspects:

- Training of nurses, observation of performance of POC CD4 tests and adherence to SOP. Refresher training organised by the supplier of the PIMA machines (Alere)
- Internal instrument quality control (QC): the PIMA machine has two PIMA Bead Standard for daily internal QC. This comprises two ready to use test cartridges (PIMA Beads 'Normal' and PIMA Beads 'Low') with set amounts of immobilised fluorescent beads. A QC test was done daily before any sample measurements and a reading within the range indicated on the beads suggests that the machine is working well. Daily records of the QC results were kept and reviewed weekly either by myself or the research nurse manager.
- Continuous quality assessment – 10% of participants having a PIMA CD4 test had paired venous samples sent to the Hlabisa NHLS laboratory for testing on the Beckman Coulter EPICS ® XL flow cytometer. The 10% of participants were identified through systematic random sampling, that is every 10th participant registering in each clinic was recruited to this QC sample. The aim will be for >80% of PIMA™ CD4 results to fall within the range ±20% of the result from the Beckman Coulter EPICS ® XL flow cytometer.

3.2.2.5.2 Viral load measurement

The Abbott M2000 platform (Abbott Laboratories©. Abbott Park, Illinois, U.S.A) was used in the AHRI laboratory for HIV-1 viral load determination from human plasma of HIV-positive individuals in the range of 40- 10 000 000 copies/mL. RNA extractions were performed by laboratory staff using the Abbott m2000sp automated extraction instrument and the Abbott ASPS sample preparation kit (Cat#). The setup of the Abbott RealTime HIV-1 assay [an in vitro reverse

transcription-polymerase chain reaction (RT-PCR) assay for the quantification of Human Immunodeficiency Virus type 1 (HIV-1)] was done using the m2000sp instrument. The target region of the assay is the integrase region of the polymerase gene and is suitable for detection of Group M subtypes A—H, Group O and Group N. The Abbott m2000RT is the RealTime detection instrument used in this closed workflow.

For the Internal QC, each plasma sample is spiked with an internal control which is co-extracted with the viral RNA. The internal control is also detected during the viral load assay and if it is not detected the test is deemed invalid. A positive and negative control is also tested in each run.

The AHRI laboratory subscribes to the QCMD Human Immunodeficiency Virus RNA EQA programme (for RNA quantification performed using the Abbott m2000sp and Abbott realtime HIV-1 kit)

3.2.2.5.3 HIV Drug resistance Protocol

The SATuRN genotyping system (220) developed by the SATuRN community is used in the AHRI laboratory. The genotypic protocol is based on the Life Technologies Sanger sequencing system. Sequencing of the extracted and amplified virus is done on the ABI 3130XL Genetic Analyzer instrument (Applied Biosystems, Foster City, CA 94404 USA). The pol sequences generated covers all the 99 Protease codons and the first 300 Reverse transcriptase codons. The informatics protocol uses a relational database to manage patient treatment and monitoring history as well as viral isolates for drug resistance. The database also generates drug resistance reports that combine treatment history (changes in regimens) and surrogate markers (CD4s and viral loads) with genotypic resistance data to give a more comprehensive picture of the participant's status.

For internal QC, each reverse transcription and PCR amplification run includes a positive and negative control. Should either amplify or not as expected the run is deemed invalid. The sequencing reaction does not include a control since the quality of the reaction is noted at the analysis step. Raw sequence data is imported

into Geneius software where the quality of the electropherogram at each base is provided as a percentage. Only reads > 70% in quality are analysed. Poor quality reads are trimmed before the remaining reads are mapped to a reference. The translation of the reads is assessed and only accepted if the Pol and RT signature amino acid sequence is noted. Mixed bases are noted and ambiguities are resolved based on the intensity/ height of the eletropherogram peaks. The resultant consensus is exported and submitted to Stanford where quality checks of the sequence is also undertaken. This includes a check of stop codons, ambiguities and the length of the sequence covering Pol and RT. If these checks fail the sequence is not analysed or partially analysed with an error message.

For external QC, the laboratory also subscribes to the QCMD ENVA HIV Drug Resistance Typing EQA programme (for determination of HIV drug resistance mutations in the HIV-1 protease and reverse transcriptase genes).

3.3 Exposures and outcomes

Exposures and outcomes for all three objectives are briefly described below. Exposures are categorized taking into consideration biological grouping (e.g sex), validated scores (PHQ4), number of observations within groups or according to clinical relevance. A number of approaches were used for quantitative exposure variables, CD4 count and age were included in the model as continuous covariates, Distance to TasP clinic, self-reported health status and visit frequency were transformed to binary variables above and below their median values, except where otherwise indicated.

PHQ-4 was presented in its pre-specified validated categories for the measurement of depression.

Objective 1

- To examine the association between CD4 count at ART initiation and adherence

Outcome: Adherence at each visit during the first12 months after ART initiation categorized into optimal or non-optimal adherence using a cut-off of 95% at each visit. This is the conventional cut-off in ART adherence literature (130).

Exposures: CD4 cell count at ART initiation (3 categories for descriptive analysis, ≤350, 350-500, >500, to reflect change in ART guidelines but modelled as a continuous covariate), Sex (male/female) , Age at ART initiation (4 categories for descriptive analysis, but modelled as continuous), Visit frequency defined as number of visits in the first 12 months after initiating ART (binary by median visits), Disclosure of HIV status to anyone (yes/no), Disclosure of HIV status to current partner (yes/no), Employment status (employed, student, unemployed), Marital status (never married, married, divorced/separated), Educational attainment (primary or less, some secondary, secondary or higher), Regimen type (whether fixed dosed combination or not), Distance to the nearest TasP clinic, obtained by measuring the distance as the crow flies from the participant's home (GPS coordinates) to the trial clinic (GPS coordinates) in their cluster (binary covariate around median), Depression (assessed using the validated PHQ-4 scale rated as normal (0-2), mild (3-5), moderate (6-8) and severe (9-12), (221), Self-reported health status measured using a scale ranging from 0 to 100% in which 0 represents poor health and 100% represents excellent health (binary around median value), Food insecurity (measured by whether skipped meals in last 12 months or not), ART treatment perception (through three questions concerning the participant's attitudes about ART as displayed on results tables). Missing observations were indicated within each covariate for descriptive purposes but were dropped during model building.

Objective 2

- To examine virological response by CD4 at ART initiation and extent of acquired resistance following virological failure

Outcome 1: virological suppression at 6 months in individuals who initiated ART within the trial

Primary exposure: CD4 count at ART initiation (as described above)

Secondary exposure: Adherence measured by both VAS and PC

Other exposures of interest: Baseline viral load categorised so the number of observations in each category are similar (<10,000, 10,000-100,000, >100,000). This is in addition to the exposures described in objective 1.

Outcome 2: virological suppression at first trial clinic visit in ART-experienced individuals

Primary exposure: CD4 count at ART initiation

As this was amongst individuals who initiated ART according to SA guidelines, the three CD4 thresholds were ≤200, 200-350, >350 to capture the changes in guidelines. This was used for descriptive analysis but CD4 included in models as continuous covariate.

No adherence data were available for those who were receiving ART from the public ART programme at the time of transfer to trial clinics. Furthermore, baseline viral load was not routinely measured prior to ART initiation. This was only used to monitor response to ART.

Other exposures of interest: In addition to exposures described in objective 1**,** other exposures were**,** distance to nearest public ART clinic (categorized into quartiles) was used in place of distance to trial clinic for this analysis as it was assumed that participants received care from the public ART programme closest to them prior to first trial clinic visit, duration on ART (categorized into quartiles)

Outcome 3: Proportion with acquired resistance among individuals with virological failure (VL >1000 copies/mL ≥6 months post-ART initiation) stratified according to whether individuals initiated ART within the trial or ART-experienced at entry in trial

Due to small sample size of genotypes, only descriptive analysis was undertaken summarized for both groups according to the following: CD4 count at initiation (two categories, ≤350, >350 due to small sample size), median viral load at time of

genotype, median ART duration on first-line ART, median time on failing regimen, regimen type at time of genotype, previous ART substitution, median age and sex

Objective 3

- To assess the level of pre-treatment drug resistance (PDR) in individuals seroconverting during the study period and the response to first-line ART.

Outcome 1: Descriptive analysis of proportions with pretreatment drug resistance amongst recently and chronically infected ART naïve individuals.

Outcome 2: risk factors for the presence of pre-treatment drug resistance in ART naive

Exposures: Type of infection (recent vs. chronic), CD4 count at presentation (≤350, 350-500, >500, included in model as continuous covariate), age at enrolment, sex, viral load at presentation, education, marital status and employment were categorised as previously described

Outcome 3: time to virological suppression

Primary exposure: presence of any pre-treatment drug resistance with three categories -no mutations present, minority mutations only, majority mutations

Other exposures of interest: Age at ART initiation, Sex, CD4 count at ART initiation, viral load, adherence measured by VAS.

3.4 Data sources

The data sources for my research were based on the questionnaires administered in the home-survey by the fieldworkers as well as those administered in the clinic by the nurses and counsellors. A social science sub-study which was not part of my study was also embedded within the clinic and participants were consented separately for this. This study was overseen by a team referred to as independent interviewers who were not part of the staff providing clinical care to the participants. They enquired about symptoms of depression, HIV status disclosure, use of

alternative source of health care etc. The Patient Health Questionnaire for depression and anxiety (PHQ4), HIV status disclosure and food security variables were extracted from the questionnaire used in this sub-study. The relevant variables I used for my analyses and their sources are summarized in Table 3.2. I developed the questionnaires marked in asterisk on the table. I obtained ethics approval to link data from the public ART programme with the TasP trial database

3.5 Data management

Electronic and paper-based case report forms were used for data collection. All questionnaires were barcoded and scanned at every stage of their life cycle from the time they left the AHRI until returned thereby maintaining a chain of custody. All completed forms including signed copies of consent forms underwent quality control after which data were entered into Microsoft access database by a team of data capturers. All data were stored in a MS-SQL Server database located on one of the AHRI database servers, managed by professional database administrators.

Laboratory results (viral loads, genotypes) were transmitted, using already-established procedures, directly into the database, via a secure (https) connection from the AHRI Laboratory's Information System. Point of care CD4 count was documented directly on study case report form.

All datasets used for analysis were anonymised and covered by formal, signed Data Use Agreements, which cover acceptable use, security, destruction after use.

All analytical datasets were documented and placed on the AHRI Data Repository with access restriction.

Table 3.2 Data sources used for analysis in this MD research

TasP trial database: ART naïve and ART-experienced participants at trial entry	
TasP home-based individual questionnaire administered in home survey	ID number
	Age
	Sex
	Marital status
	employment
	educational level
	date of HIV diagnosis if new
	distance to TasP clinic in own cluster health beliefs about ART
	self-reported health status
**Clinical history and examination form*	Point-of-care CD4
	ART history
	ART initiation date
	comorbidity such as previous and current tuberculosis
	WHO staging[#]
**Clinic baseline form*	Anthropometric details: Weight, height
	date of HIV diagnosis if known positive ART history
	date of ART initiation
**Clinic follow up form*	Adherence data: • visual analogue scale • pill count
Social science sub-study form	PHQ4 scale to measure depression
	HIV status disclosure
	food security
Laboratory data	Viral load
	genotypic resistance mutations

Hlabisa HIV treatment and care database **(Public ART programme): ART-experienced participants only**	
Basic details	Age, sex, ID number, treatment clinic
ART record	ART regimen at initiation, changes in ART regimen during treatment
Laboratory data	Baseline and 6-monthly CD4 and HIV viral load

*Questionnaires that I develop, # represents the highest WHO staging ever attained

Access to read, enter, modify or delete data was granted via the standard authentication and access-control features of MS-SQL Server and MS-Windows. All staff were trained in good clinical practice and signed a confidentiality clause based on AHRI policy when employed by the trial. The completed questionnaires, case report forms and supporting documentation were kept securely in locked cabinets at the Somkhele site of AHRI. These source documents were digitally archived at the end of each round of fieldwork by following a standard operating procedure. After a quality assurance process comparing digitalised forms with physical forms to ensure all forms had been digitalised, the physical forms were then destroyed.

3.6 Conceptual framework and analysis plan

The analysis plan is described in detail in the respective chapters. I hypothesized that individuals with a high CD4 count at ART initiation would have a lower ART adherence as a result of not perceiving ART to be beneficial to their health resulting in poorer virological outcome and the emergence and transmission of drug resistant HIV. To test this hypothesis, I therefore examined the relationship between CD4 count at ART initiation and adherence, and between CD4 count at initiation and virological suppression. To capture the full effect of CD4 count, I fit an initial model that excluded adherence (Fig 3.6, red arrow). A subsequent model that included adherence, would allow an estimate of the effect of CD4 count on virological suppression to be made allowing for that effect to be mediated through adherence (Fig 3.6, blue arrow).

I also adjusted for potential confounders (associated with the exposure and outcome but not on the causal pathway) of the association between CD4 count at ART initiation and virological suppression (Brown arrow). Many of the potential confounders considered for inclusion in the model are proximally related to adherence but distally related to virological suppression (broken arrows). For example, depression (PHQ4) can impact adherence negatively which can result in

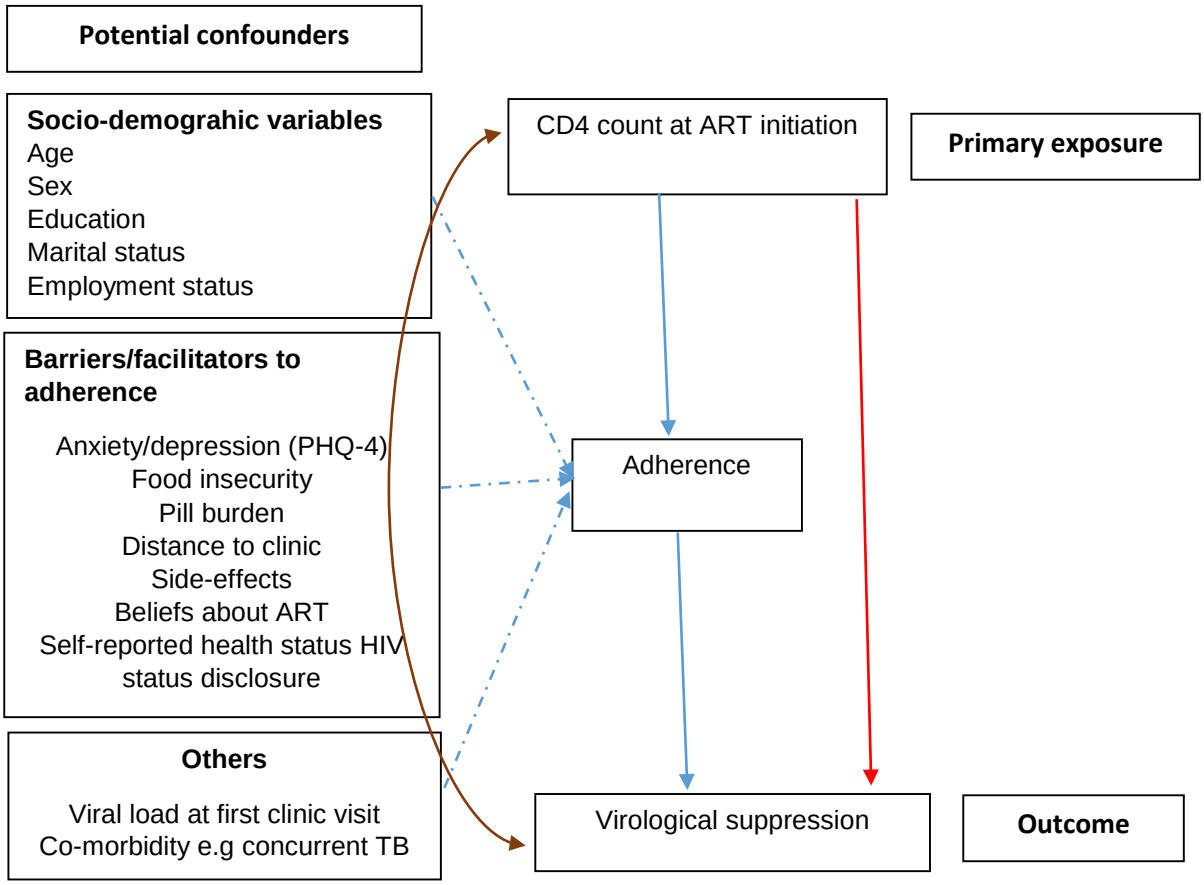


Figure 3.6 Conceptual framework of the relationship between CD4 at initiation, adherence and virological suppression

poor virological suppression. It is also possible that some of the confounders could be associated with virological suppression directly. For example, a high pill burden from treating multiple co-morbidities, apart from impacting adherence, could result in drug-drug interactions which could result in sub-optimal levels of antiretroviral drugs in the plasma and the potential to compromise virological response. Many of these factors are cited in the literature as being associated with virological suppression (136, 177), hence I adjusted for them in the model.

3.6.1 Statistical analysis

I used median and interquartile ranges to summarise continuous exposure variables with skewed distributions and mean to summarise continuous exposure variables with normal distributions. I reported frequencies and percentages for categorical exposure variables and used the Chi-squared tests to examine associations between categorical variables and Mann-Whitney test to compare medians for continuous variables.

The primary exposure of interest was CD4 count at ART initiation for all analyses undertaken, except when examining the association between pre-treatment drug resistance and virological suppression. In one analysis, I examined the association between CD4 count at initiation and adherence (objective 1), whilst in objective 2, I examined the association between CD4 count at ART initiation (primary exposure), adherence (secondary exposure) and virological suppression. More detail concerning the statistical analyses strategy employed is described within the respective chapters. I carried out all statistical analyses with Stata 14.2 (StataCorp LLC, College Station, Texas 77845, USA).

3.6.2 Handling of missing data

Descriptive summary of all covariates/exposures showed the proportion of missing data within each. The degree and pattern of "missingness" was variable and was high particularly for variables about ART perception, disclosure and PHQ-4. There was no imputation of missing data, hence I undertook a complete case analyses for all analyses, with the implicit assumption that data were missing at random. For covariates with large proportions of missing observations, this was around the 3% mark, hence covariates with missing observations ≥3% were not considered for inclusion in the multivariable model. Including them would have meant large amount of data being dropped from the multivariable model to allow a complete case analysis. This would have made the risk-set in the multivariable model very different from the univariable analyses. However the proportion of missing data within each covariate differed according to the study population being used to address that objective, and if there was <3% missing, that covariate was considered for inclusion in the multivariable model for that particular objective.

3.7 Ethical safeguards

I applied for and obtained ethics approval from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal for the control clusters on 2 February 2012 (Appendix G) and on 6 July 2012 for all clusters in the main trial (Ref: BFC104/11)- Appendix H. This is recertified yearly for the duration of the trial. Last recertification is appended (Appendix I) I also secured approval from the Medicines Control Council of South Africa on 28 June 2012 (Ref: N2/19/8/2) to comply with regulatory approval (Appendix J). My research received provisional approval on 25 February 2013 and full approval in a letter dated 9 September 2013

from Brighton and Sussex Medical School Research Governance and Ethics Committee (Ref: 13/033/NEW)-(Appendix K). Full approval was received from UCL Research Ethics Committee on 18 June 2015 (Project ID: 6604/001) (Appendix L). All participants recruited to my research gave written informed consent.

3.8 Funding

The tuition fees for this thesis was funded through discretionary funding provided to the Director of the former Africa Centre and continued by Africa Health Reseach Institute through the Welcome Trust. The salary of Collins Iwuji was paid by the Agence Nationale de Recherches sur le Sida et les Hépatites Virales (ANRS) as project coordinator of the ANRS 12249 Treatment as Prevention Trial. Collins also received additional funding from the People Programme (Marie Curie Actions) of the European Union's seventh Framework Programme FP7/2007-2013 under REA grant agreement n° 612216.

Chapter 4 Cohort profile

4.1 Background

This short descriptive chapter presents a flow chart of participant recruitment in the main trial and the number of individuals identified as HIV-positive during the home survey. A proportion of those identified HIV-positive linked to the trial clinics and formed the cohort for addressing the first two objectives of my thesis. Individuals identified as recently infected, as defined in Chapter 3, who contributed to the analysis of pre-treatment resistance (third objective) are also presented.

4.2 Statistical analysis

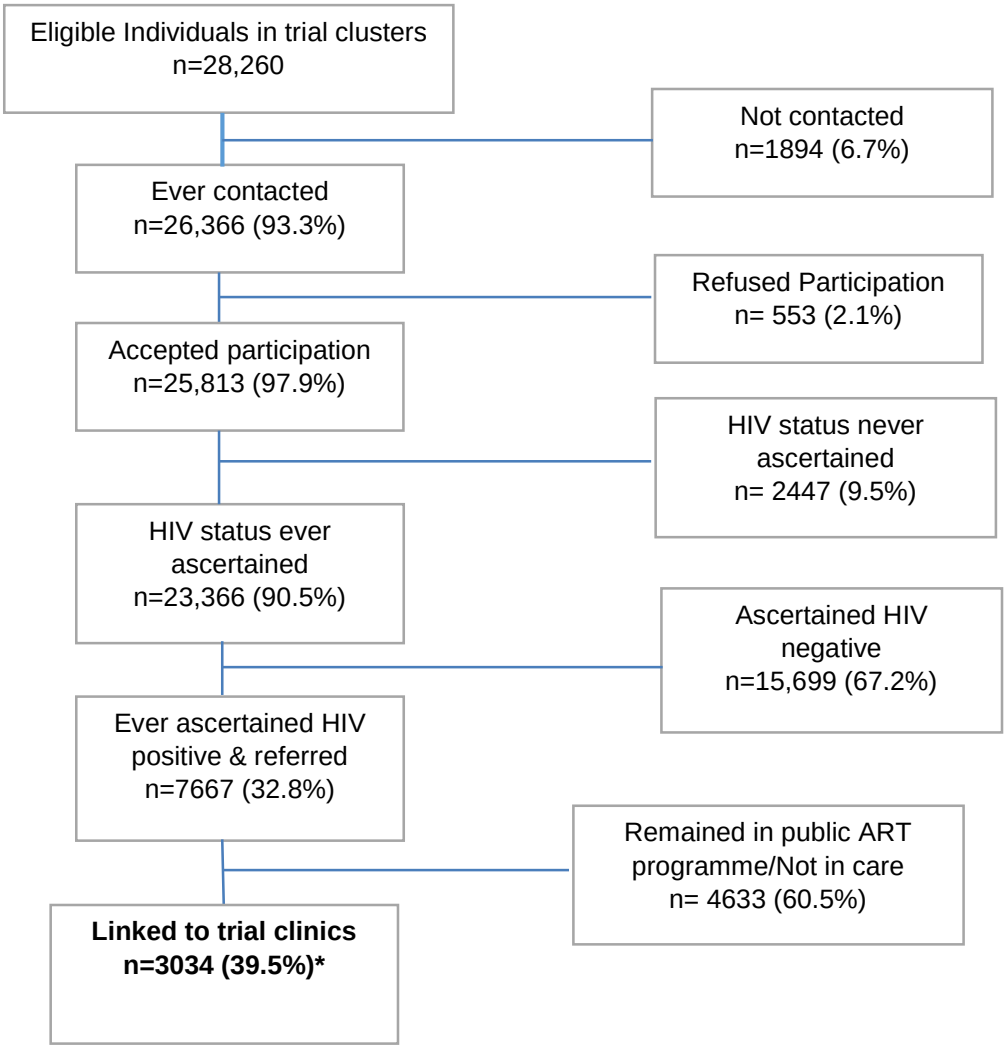
The baseline characteristics of the trial clinic cohort are presented according to whether the individual was ART-naïve or experienced at the time of first visit to the clinic. Characteristics of recently infected participants identified through dried blood spots are also summarised.

Continuous measures are summarised using median and interquartile range. Frequencies and percentages are reported for categorical variables.

4.3 Results

4.3.1 Cohort profile

Figure 4.1 shows that, of 28,260 individuals eligible for inclusion in the main trial, 26,366 (93.0%) were contacted at home at least once; of these 25,813 (97.9%) agreed to participate in the trial. HIV status was ascertained for 23,366 (90.5%) through rapid HIV testing. Of these, 7667 (32.8%) were HIV positive of whom 3034 (39.5%) linked to the trial clinics and formed the basis of my research analyses (Main objectives 1 and 2). HIV-positive individuals who enrolled in TasP clinics were more likely to be older (median age 37.1 (IQR 28.4, 48.6) vs. 31.9



*MD research cohort

Figure 4.1 Cohort Profile - March 2012 to June 2016

(IQR 25.2, 41.9) years), of male sex, have a lower educational attainment, more likely to be divorced/separated as well as unemployed. Those who enrolled in TasP clinics were also more likely to already be in care in the government ART programme or were returning to care having previously been lost to follow up (Table 4.1).

Further, of the 25,813 who agreed to participate, 17,845 (69.1%) tested HIV negative in the very first dried blood spots sample obtained from them at enrollment, of whom 503 (2.8%) seroconverted (recent infections) in a subsequent sample. 277 (55.1%) of these 503 participants were successfully sequenced and contributed to the analysis of pre-treatment drug resistance (Main objective 3).

4.3.2 Characteristics of my research cohort

Of the 3,034 individuals who visited a trial clinic at least once (Table 4.2), 1577 (51.3%) were ART-naïve at presentation to the trial clinic. The median age of this cohort was 37.1 years (IQR 28.4-48.6). The majority were female (73.0%), with completed primary education or less (48.4%) and unmarried (83.6%). The median CD4 count at the first clinic visit was 451 cells/mm^3 (IQR 297, 628). 44.8% of individuals had a viral load <400 copies/mL. In ART-experienced individuals, the median duration spent on ART at the time of the first clinic visit was 3.85 years (IQR 1.92-5.89).

The median age those recently infected was 22.4 years (IQR 19.0-27.7). The majority were female (86.3%), never been married (93.05) and unemployed (62.6%)-Table 4.3

Table 4.1 Characteristics of HIV-positive participants by whether or not they enrolled in TasP clinics.

Socio-demographic characteristics	Enrolled in TasP clinic n=3,034 (%)	Not enrolled in TasP n=4,633 (%)	P value
Age			<0.001
Median (IQR) years	37.1 (28.4, 48.6)	31.9 (25.2, 41.9)	
16-29	907 (29.9)	1,792 (38.7)	
30-39	827 (27.3)	1,177 (25.4)	
40-49	614 (20.2)	684 (14.8)	
>50	671 (22.1)	515 (11.1)	
Missing	15 (0.5)	465 (10.0)	
Sex			0,003
Female	2216 (73.0)	3523 (76.0)	
Male	818 (27.0)	1,110 (24.0)	
Educational attainment			<0.001
Primary or less	1469 (48.4)	1,444 (31.2)	
Some Secondary	981 (32.3)	1,819 (39.3)	
At least completed secondary	565 (18.6)	1,301 (28.1)	
Missing	19 (0.6)	69 (1.5)	
Marital status			
Never married	2,536 (83.6)	3,905 (84.3)	<0.001
Married	328 (10.8)	520 (11.2)	
Divorced/Separated	150 (4.9)	139 (3.0)	
Missing	20 (0.7)	20 (0.7)	
Employment status			<0.001
Employed	386 (12.7)	623 (13.5)	
Student	113 (3.7)	414 (8.9)	
Unemployed/Inactive	2,510 (82.7)	3,508 (75.2)	
Missing	25 (0.8)	88 (1.9)	
Care status at referral			<0.001
In care in DoH clinics	1,326 (43.7)	1,559 (33.7)	
Lost to follow up from DoH	616 (20.3)	622 (13.4)	
Never been in care	1,070 (35.3)	2,452 (52.9)	
Missing	22 (0.7)	0 (0.0)	

Table 4.2 Characteristics of participants enrolled in trial clinics at presentation

Socio-demographic characteristics	ART-naïve n=1557	ART-experienced n=1477	Total N=3034
Age			
Median (IQR) years	33.0 (25.8, 45.3)	40.5 (31.9, 50.6)	37.1 (28.4, 48.6)
16-29	624 (40.1)	283 (19.2)	907 (29.9)
30-39	395 (25.4)	432 (29.3)	827 (27.3)
40-49	246 (15.8)	368 (24.9)	614 (20.2)
>50	281 (18.1)	390 (26.4)	671 (22.1)
Missing	11 (0.7)	4 (0.3)	15 (0.5)
Sex			
Female	1123 (72.1)	1093 (74.0)	2216 (73.0)
Male	434 (27.9)	384 (26.0)	818 (27.0)
Educational attainment			
Primary or less	655 (42.1)	814 (55.1)	1469 (48.4)
Some Secondary	570 (36.6)	411 (27.8)	981 (32.3)
At least completed secondary	317 (20.4)	248 (16.8)	565 (18.6)
Missing	15 (1.0)	4 (0.3)	19 (0.6)
Marital status			
Never been married	1357 (87.2)	1179 (79.8)	2,536 (83.6)
Married	123 (7.9)	205 (13.9)	328 (10.8)
Divorced/Separated	62 (4.0)	88 (6.0)	150 (4.9)
Missing	15 (1.0)	5 (0.3)	20 (0.7)
Employment status			
Employed	215 (13.8)	171 (11.6)	386 (12.7)
Student	810(5.1)	33 (2.2)	113 (3.7)
Unemployed/Inactive	1244 (79.9)	1266 (85.7)	2,510 (82.7)
Missing	18 (1.2)	7 (0.5)	25 (0.8)
Clinical characteristics			
CD4 at first clinic visit			
Median (IQR) cells/mm^3	429 (279, 591)	473 (312, 653)	451 (297, 628)
≤350	551 (35.4)	444 (30.1)	995 (32.8)

Socio-demographic characteristics	ART-naïve n=1557	ART-experienced n=1477	Total N=3034
350-500	392 (25.2)	339 (23.0)	731 (24.1)
>500	569 (36.5)	672 (45.5)	1241(40.9)
Missing	45 (2.9)	22 (1.5)	67 (2.2)
Viral load at first clinic visit			
<400 copies/mL	187 (12.0)	1173 (79.4)	1360 (44.8)
≥400	1324 (85.0)	298 (20.1)	1622 (53.5)
Missing	46 (3.0)	6 (0.4)	52 (1.7)
ART duration at first visit			
Median (IQR) years	-	3.85 (1.92-5.89)	
WHO Stage (%)			
1	1016 (65.3)	764 (51.7)	1780 (58.7)
2	302 (19.4)	217 (14.7)	519 (17.1)
3	148 (9.5)	372 (25.2)	520 (17.1)
4	17 (1.1)	60 (4.1)	77 (2.5)
Missing	74 (4.8)	64 (4.3)	138 (4.6)
Past history of TB			
Yes	173 (11.1)	535 (36.2)	708 (23.3)
No	1143 (73.4)	797 (54.0)	1940 (63.9)
Missing	241 (15.5)	145 (9.8)	386 (12.7)
Current TB			
Yes	15 (1.0)	39 (2.6)	54 (1.8)
No	1487 (95.5)	1,383 (93.6)	2,870 (94.6)
Missing	55 (3.5)	55 (3.7)	110 (3.6)

Table 4.3 Characteristics of recently Infected HIV-positive indivduals

Socio-demographic characteristics	N=503 (%)
Age	
Median (IQR) years	22.4 (19.0-27.7)
16-29	383 (76.1)
30-39	42 (8.4)
40-49	19 (3.8)
>50	35 (7.0)
Missing	24 (4.8)
Sex	
Female	434 (86.3)
Male	69 (13.7)
Educational attainment	
Primary or less	85 (16.9)
Some Secondary	228 (45.3)
At least completed secondary	189 (37.6)
Missing	1 (0.2)
Marital status	
Never been married	468 (93.0)
Married	30 (6.0)
Divorced/Separated	5 (1.0)
Employment status	
Employed	34 (6.8)
Student	153 (30.4)
Unemployed/Inactive	315 (62.6)
Missing	1 (0.2)

4.4 Discussion

In this chapter, I described the population of HIV positive individuals identified during the trial, the proportion of those who linked to care and formed the basis of my research, and how they compare to the total population of HIV-positive individuals identified in the community.Nearly 40% enrolled in the TasP clinics, and of those who did not enrol, about one-third had no prior or current engagement in TasP or DoH clinics.

Just over half of those who enrolled in TasP clinics were ART-naïve at presentation with median CD4 count at first clinic visit of 429 cells/mm^3. The majority of the cohort were in an early stage of HIV infection as reflected by their WHO staging.

I observed a higher CD4 count at presentation amongst ART-naïve individuals than reported in sub-Saharan Africa. A meta-analysis of 56 studies covering the period January 2002 –December 2013 comprising 295,455 HIV-positive individuals in sub-Saharan Africa reported the mean CD4 count at presentation to care to be 250 cells/mm^3 (95% CI 147–354 cells/mm^3) in 2002 and 309 cells/mm^3 (95% CI 237–381 cells/mm^3) in 2012 (222). The yearly increase in CD4 count at presentation was not significant (β = 5.8 cells per year; 95% CI −10.7 to 22.4 cells/year; P = .48). A subgroup analysis restricted to only studies done in South Africa showed that CD4 count at presentation increased significantly by 39.9 cells/year (95% CI, 9.2–70.2 cells/year; P = .02). This metanalysis must have included studies of individuals diagnosed in facilities with symptoms of more advanced disease. My study has not accounted for the CD4 count at presentation of those not in care who could be in a more advanced stage of HIV disease.

In another review that contained results from searches up to 4 March 2013 that compared CD4 count of individuals diagnosed HIV-positive using community testing approaches with that of those diagnosed in health care facilities, more participants in community-based testing approaches had CD4 counts >350

cells/mL than in facility-based approaches (RR 1.42, 95% CI 1.16–1.74). One of the strengths of community-based approaches, such as the home-based testing offered in the TasP trial, is earlier HIV diagnosis compared to facility-based approaches.

The characteristics of the 60% of individuals who were not receiving their care from the TasP clinics (53% no prior history of care, 47% with current or previous care) differed from those who engaged with the TasP clinics. Whether these differences would influence the inference I make on the overall population of people living with HIV in the community would depend on whether these covariates are associated with adherence and virological suppression in the sample that enrolled in the TasP clinics.

In subsequent chapters, I will examine the association between CD4 count at ART initiation and adherence using data from the ART-naïve participants. I will also examine the association between CD4 count at ART initiation and virological suppression as well as the development of drug resistance. These analyses will use data from both ART-naïve and ART-experienced participants. For estimating the proportion with pre-treatment drug resistance, I will use data from ART-naïve participants, including those recently infected with HIV who did not link to trial clinics. And, finally, in order to examine the association between CD4 count at ART initiation and the presence of pre-treatment drug resistance mutations on virological response, I will use data from ART-naïve participants.

Chapter 5 Does high CD4 count at ART initiation impact adherence?

In this chapter, I describe the levels of adherence in ART-naïve participants and examine the association between CD4 count at ART initiation and adherence. I also examine the risk factors associated with non-optimal adherence and assess the predictive validity of the tools used to measure adherence.

5.1 Background

Concern has been expressed that individuals offered ART at CD4 counts higher than the ART eligibility CD4 threshold may not be motivated to adhere to ART long-term. The reasoning being that individuals initiating at higher CD4 counts would be asymptomatic and healthy and may not perceive ART to be beneficial for their own health and may have other competing priorities.

As a first step, I conducted a scoping review of the published literature on adherence in individuals initiated on ART in Africa at CD4 count >350 cells/mm^3. Secondly, I examined the relationship between CD4 count at ART initiation and adherence in individuals initiating ART within the trial and finally, I assessed the validity of the adherence measurement tools I used to estimate adherence.

5.2 Literature review

The aim of the scoping review was to identify published studies of individuals who initiated ART in Africa with CD4 >350 cells/mm^3 in which adherence was estimated, and summarise the findings.

I searched Pubmed for studies published before 4 February 2017 using the following search terms resulting in the references described:

- Search ((High) OR Earl*) AND CD4 (40,202)
- Search (Adherence) OR Compliance (230,657)

- Search (ART) OR agents, antiretroviral[MeSH Terms] (147,514)
- Search Africa (285,075)
- Combining all search criteria above ((((((High) OR Earl*) AND CD4)) AND ((Adherence) OR Compliance)) AND ((ART) OR agents, antiretroviral[MeSH Terms])) AND Africa (206)

I read the titles of all 206 articles and excluded 182 studies that were not related to my topic of interest. Since the search was executed in all fields (title and body of article), some of the articles returned included one or more criteria specified in the search but not all. Excluded articles were studies that used qualitative methods, focussed on children, early HIV infection, cost-effectiveness and outcomes of ART in general. I reviewed the abstracts of the remaining 24 studies and excluded 20 papers; 2 were on systematic reviews , 2 on opinion pieces, 6 on treatment outcomes, 2 on protocols, 3 on lower CD4 count at initiation, with the remaining being either on retention or mathematical models. I reviewed the full manuscript of the remainingfour papers that reported the relationship between the CD4 threshold of interest (>350 cells/mm^3) and adherence; one examined high current CD4 rather than CD4 count at initiation, the other turned out not to have examined CD4 count at initiation; both these papers were excluded (223, 224). Of the remaining two papers, one reported on adherence at high CD4 count >350 cells/mm^3 (225) and the other was a systematic review of impact of CD4 count at initiation on ART adherence between the periods 1 January, 2004 and 30 September 2015 (226). There was no country/region restriction placed on the articles included in that systematic review. I identified two further studies (227, 228) from Africa in the references of the systematic review. In total, my literature review yielded three individual studies from Africa (Table 5.1) that examined the association of high CD4 count at initiation and adherence.

5.3 Discussion of review

The small study by Jain et al (225) estimated adherence at 4, 8, 12, 24 and 48 weeks following ART initiation and averaged this over the period. Adherence was 98% during the first 48 weeks of ART in individuals who initiated ART at CD4>350 cells/mm^3. These were patients enrolled in a prospective trial of streamlined model of care to reduce patient clinic waiting time.

Each patient in the study was given a phone number of a clinician to facilitate access to care in case of any health related matters. An important weakness of this study is the absence of a comparator arm. The other two studies by Charurat et al (227) and Memiah et al (228) compared adherence in individuals with CD4 >350 cells/mm^3 with those of individuals with more advanced HIV disease at time of ART initiation. The average CD4 count in both studies were low. No African studies comparing adherence in individuals with CD4 >350 cells/mm^3 versus CD4 ≤350 cells/mm^3 were identified. The systematic review identified during the literature search reported a sub-analysis to examine adherence in individuals with CD4 >350 cells/mm^3 compared to lower CD4 counts. Three studies were identified, two of which were the African studies (227, 228) described above and the third study was done in the USA (229) and the reference group had CD4 <200 cells/mm^3, (pooled OR 0.85; 95% CI: 0.73 to 0.97 for all three studies). This indicates slightly lower probability of being adherent in those with CD4 > 350 cells/mm^3. Another sub-analysis comprising two studies from high income countries (183, 230) compared adherence in individuals who initiatied ART with CD4 >500 cells/mm^3 versus CD4 <500 cells/mm^3. There was no evidence of a difference in adherence between the two groups (pooled OR, 1.01 (95% CI 0.97-1.05).

Table 5.1 Studies of ART adherence in Africa with CD4 at ART initiation >350 cells/mm^3

Author	Study setting	Study population/sample size	Study design	CD4 groups and median/mean CD4 at ART initiation	Adherence outcome	Effect estimate
Jain V (225)	Uganda	>18 years, 197 patients	Prospective	CD4 ≥ 350 cells/mm^3, no comparison group; median CD4 569 (IQR 451-716)	Self-reported adherence, 3-day recall test of no missed pills within previous three days for 48 weeks	98% averaged over 48 weeks
Charurat, M (227)	Nigeria	Median age 35 years (IQR 29-41) 4529 patients	Retrospective analysis	>350 cells/mm^3 vs. 100-200 cells/mm^3 Median CD4 121 (IQR not given)	Pharmacy refill adherence rate <95% during first 12 months	aOR 1.25 (95% CI 1.05-1.49)
Memiah P (228)	Nigeria, Uganda, Zambia and Tanzania	Mean age 38 years, 2344 patients	Cross sectional analysis	>350 cells/mm^3 vs. <50 cells/mm^3 Mean CD4 227 cells/mm^3	Composite of dose and schedule adherence (timing of medication) ≥95% of doses correctly taken in previous 7 days	aOR 1.07 (95%CI 0.73-1.58)

5.4 Aims and objectives

The primary aim of the analyses presented in this chapter was to examine the association between CD4 count at ART initiation and adherence and to assess the validity of the tools used to measure adherence.

The objectives were to:

- Examine CD4 count at ART initiation and other potential risk factors for non-optimal adherence during the first 12 months of ART
- Assess whether measures of adherence adequately reflect virological suppression at 12 months

5.5 Methods

5.5.1 Study population

Data from participants who were ART-naïve at entry into the trial were used for this analysis. Individuals were eligible for inclusion in this analysis if they initiated ART within the trial and had been on ART for 12 months by the time of database closure on 30 June 2016.

5.5.2 Definition of outcome and exposure variables

5.5.2.1 Objective 1

Outcome 1: Adherence at each visit during the first12 months after ART initiation categorized into optimal or non-optimal adherence using a cut-off of 95% at each visit.

Adherence was measured using a visual analogue scale (VAS) and pill counts (PC).

The VAS is a scale expressed as a percentage, the participant was asked to put a mark on the scale which best reflects their adherence in the previous four days.

Pill count adherence (%) = ((Amount of pills dispensed - Amount returned)/Amount expected to be taken) x 100

If the estimated PC score was <95%, adherence was considered non-optimal, if between 95-105%, adherence was considered to be optimal and if >105%, this was considered as non-optimal adherence. Hence two categories: optimal adherence (95-105%) and non-optimal adherence (<95% or >105%).

Primary exposure: CD4 cell count at ART initiation

Other exposures of interest

Sex (male/female)

Age at ART initiation

Visit frequency (number of visits in the first 12 months after initiating ART)

Disclosure of HIV status to anyone (yes/no)

Disclosure of HIV status to current partner (yes/no)

Employment status (employed, student, unemployed)

Marital status (never married, married, divorced/separated)

Educational attainment (primary or less, some secondary, secondary or higher)

Regimen type (whether fixed dosed combination or not)

Distance to the nearest TasP clinic: obtained by measuring the distance as the crow flies from the participant's home (GPS coordinates) to the trial clinic (GPS coordinates) in their cluster

Depression (assessed using the PHQ-4 scale rated as normal (0-2), mild (3-5), moderate (6-8) and severe (9-12), (221))

Self-reported health status (as measured using a scale ranging from 0 to 100% in which 0 represents poor health and 100% represents excellent health)

Food insecurity (as measured by whether skipped meals in last 12 months or not)

ART treatment perception (through three questions concerning the participant's attitudes about ART as displayed on results tables)

5.5.2.2 **Objective 2**

To assess whether measures of adherence adequately reflect virological suppression (viral load < 400 copies/mL) at 12 months.

5.5.3 Statistical analysis

I used median and interquartile ranges to summarise continuous variables and reported percentages for categorical variables.

For the main analysis of my first objective, I examined the association between CD4 count at ART initiation and other potential risk factors and non-optimal adherence (measured by VAS) at 12 months. I first compared the characteristics of individuals who were eligible for inclusion in this analysis but were excluded, because no adherence data were available, with the individuals who were included by using a chi-squared test to derive p values. Median values were compared using the Mann-Whitney U test.

I then used a random effects logistic regression model to examine the association between the exposures of interest and non-optimal adherence at each visit accounting for the correlation of repeated measurements within each participant. I opted for logistic regression models and the first 12 months in order to address the issue of adherence as an early warning indicator for development of drug resistance using the WHO proxy of percentage of patients with 100% on-time drug pick-up during the first 12 months of ART.

I undertook a complete case analysis, so visits with missing VAS adherence were dropped. At 12 months, 13 visits were expected to have adherence documented (2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48 weeks post ART initiation). I examined the association of visit frequency with adherence for inclusion in the modelling because individuals who attended more frequently would contribute more data to analysis and therefore potentially bias estimates of the effects of other variables.

I then used the likelihood ratio tests to find the model which best fitted the data. I examined CD4 count at ART initiation as both a categorical and continuous variable. I also did the same for age at initiation. As there was no evidence of a departure from linearity for these two variables, they were subsequently included in the model as linear variables. I transformed other continuous variables (distance to clinic, self-reported health status, visit frequency) into binary variables above and below their median values. I used the validated scores for the PHQ4 scale for screening of depression published in the literature (221).

Age at ART initiation and sex were considered a priori as confounders and were forced into the the multivariable model irrespective of whether their association with non-optimal adherence in the univariable model was significant or not. For the model including age and sex, I forward fitted other potential risk factors that were significant at $p<0.15$ in the univariable model one at a time to the multivariable model. I used likelihood ratio tests to derive p values for each association. Variables with missing observations ≥3% [psychological distress (PHQ4), and agreement with the statement that ART will reduce infectiousness] were excluded from the multivariable model. This allows the risk-set in the multivariable model to be similar to the univariable model as I had undertaken a complete case analysis

For the sensitivity analysis, I repeated the analysis by assessing the association between CD4 count at ART initiation and VAS adherence at six months. I undertook further sensitivity analysis by repeating the analysis examining the

association between CD4 count at ART initiation and adherence measured by PC during the first 12 months.

To address my second objective, assessing whether VAS scores and Pill count scores adequately reflect virological suppression at 12 months, firstly I sought to establish that they were both measuring the same construct by checking the correlation between them. I used a two sample t-test for means to test the hypothesis that there is no association between VAS scores and pill count scores at each visit. I used bootstrap techniques to derive the 95% confidence interval for the correlation coefficient. I plotted a scatter graph between PC and VAS scores and predicted the values of Pill score based on VAS scores using linear regression and plotted the line that gives the best fit to the data.

I also assessed whether the mean VAS score in each individual during the first 12 months of ART was predictive of virological suppression at 12 months. The mean VAS scores was estimated by taking the mean of the adherence scores in visits in which adherence was measured. Missing adherence scores were omitted.

5.6 Results

5.6.1 Comparison of included and excluded participants

From 10 March 2012 to 30 June 2016, 1557 ART-naïve individuals were enrolled in the TasP trial; of whom 1198 initiated ART. Of the 926 on ART for 12 months at database closure, 198 (21.4%) were excluded for reasons indicated in Figure 5.1 leaving 728 individuals for inclusion in the analyses.

Individuals included in the 12 months analysis were older than those not included, (median 36.3 years (IQR 28.5-48.4) versus 30.7 years (IQR 25.7-41.8) respectively. Included individuals were also more likely to be female (Table 5.2). There was no evidence of a difference in median CD4 count at ART initiation, the primary exposure of interest, between included and excluded individuals.

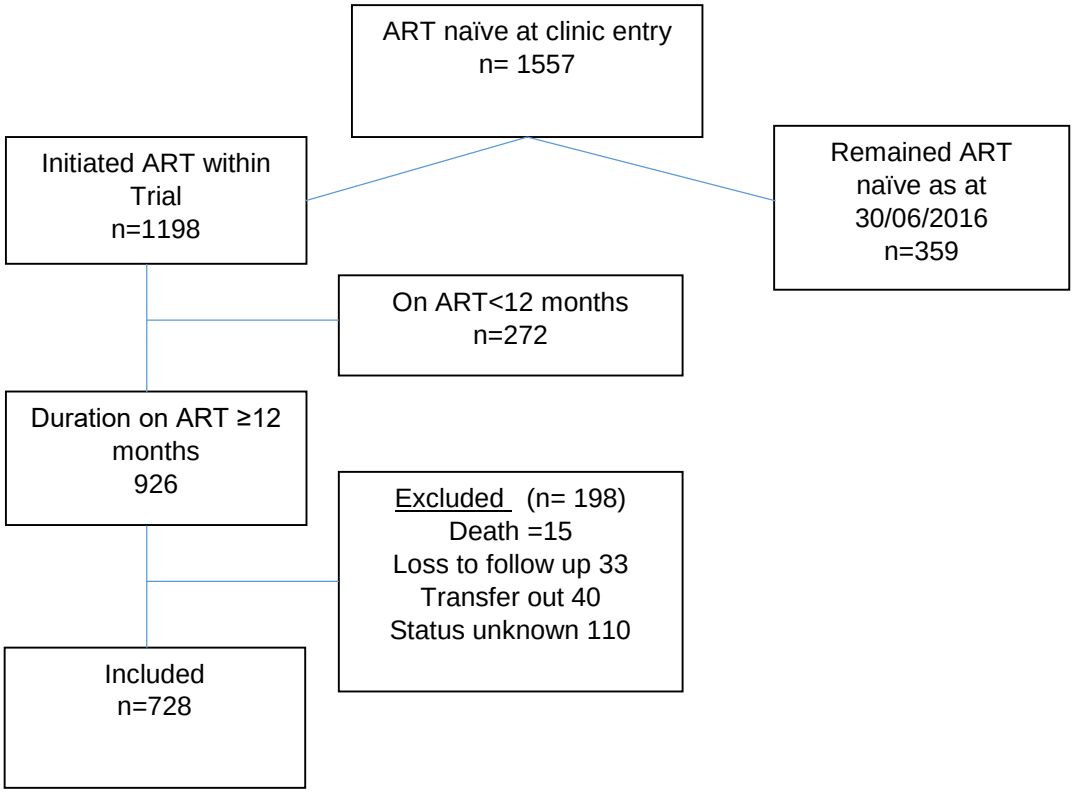


Figure 5.1 Included and excluded participants for the 12 months VAS analysis

Table 5.2 Characteristics of individuals included and excluded from the 12 months adherence analysis using VAS

Socio-demographic	12 months (VAS)		
	Excluded n=198	**Included n=728**	**P value**
Age at initiation (Years)			0.01
Median age (IQR)	30.7 (25.7-41.8)	36.3 (28.5-48.4)	
16-29	93 (47.0)	222 (30.5)	
30-39	49 (24.8)	213 (29.3)	
40-49	26 (13.1)	131 (18.0)	
>50	30 (15.2)	161 (22.1)	
Missing	0 (0.0)	1 (0.1)	
Sex			0.01
Female	127 (64.1)	534 (73.4)	
Male	71 (35.9)	194 (26.7)	
Educational attainment			0.260
Primary or less	74 (37.4)	327 (44.9)	
Some Secondary	116 (58.6)	369 (50.7)	
Secondary or higher	7 (3.5)	29 (4.0)	
Missing	1 (0.5)	3 (0.4)	
Marital status			0.330
Never married	178 (89.9)	624 (85.7)	
Married	11 (5.6)	69 (9.5)	
Divorced/Separated	8 (4.0)	33 (4.5)	
Missing	1 (0.5)	2 90.3)	
Employment status			0.09
Employed	22 (11.1)	116 (15.9)	
Student	12 (6.1)	24 (3.3)	
Unemployed	163 (82.3)	587 (80.6)	
Missing	1 (0.5)	1 (0.1)	
Clinical characteristics			

Socio-demographic	12 months (VAS)		
	Excluded n=198	**Included n=728**	**P value**
CD4 at Initiation (cells/mm^3)			0.932
Median (IQR)	352 (226, 514)	351 (236, 502)	
≤350	97 (49.0)	364 (50.0)	
350-500	46 (23.2)	181 (24.9)	
>500	52 (26.3)	183 (25.1)	
Missing	3 (1.5)	0 (0.0)	
Viral Load at first clinic visit (Log_{10} copies/mL)			0.374
Median (IQR)	4.6 (3.7, 5.2)	4.5 (3.8, 5.2)	

5.6.2 Association between CD4 count at initiation, other factors and VAS adherence <95% during the first 12 months

Of the 728 individuals who had been on ART for 12 months, 723 had visits with at least one adherence measurement documented. Of the expected 9399 visits, 7782 (82.8%) visits had adherence measurements. Adherence was optimal in 6675 (85.8%) of these 7782 visits. The median number of visits per individual was 11 visits (IQR 10-12).

In the unadjusted analysis (Table 5.3), there was no evidence of a significant association between CD4 count at ART initiation and adherence <95% during the first 12 months of ART.

Factors associated with increased probability of adherence <95% were male sex, (male vs. female, OR 2.23, 95% CI 1.71-2.90), not knowing whether ART could reduce infectiousness (OR 1.61, 95% CI 1.15-2.26 comparing those who did not know with those who agreed with the statement). Factors that were inversely associated with adherence <95% were being on a fixed dose ART combination (OR 0.80, 95% CI 0.66-0.96 compared with those not on a fixed dose combination) and food security (OR 0.68, 95% CI 0.52-0.88). Increased visit frequency was also strongly associated with a decreased probability of adherence <95% (OR 0.48, 95% CI 0.38-0.62 for >11 vs. ≤ 11 visits). There was weak evidence of an association between travel distance to the trial clinic and odds of adherence <95%; (OR 0.79, 95% CI 0.62-1.01 for >1.3km vs. ≤ 1.3km).

In the adjusted analysis, there was no significant association between CD4 count at ART initiation and VAS <95% during the first 12 months of ART.

Male sex was independently associated with increased odds of VAS adherence <95% (male vs. female, aOR 2.24, 95% CI 1.73-2.91).

Factors that were independently inversely associated with VAS adherence <95% were being on a fixed dose ART combination (aOR 0.45, 95% CI 0.26-0.79

compared with those not on a fixed dose combination) and food security (aOR 0.63, 95% CI 0.49-0.81). Increased visit frequency was also strongly associated with a decreased probability of adherence <95% (aOR 0.50, 95% CI 0.39-0.64 for >11 vs. ≤ 11 visits). Living farther away from the trial clinic was weakly associated with decreased odds of adherence <95%, aOR 0.81 (95% CI 0.64-1.02, p=0.07). Exploring this further, 52% of those living >1.3km away from the clinic had >11 visits to the clinic compared to 43% in those living ≤1.3km and increased visit frequency is associated with decreased probability of adherence <95%.

5.6.3 Sensitivity analyses

5.6.3.1 Association between CD4 count at initiation, other factors and VAS adherence <95% during the first 6 months

Of the 983 individuals who had been on ART for 6 months, 976 had visits with at least one adherence measurement documented. Of the expected 6832 visits, 5597 (81.9%) visits had adherence measurements. Adherence was optimal in 4833 (86.4%) of 5597 visits. The median number of visits per individual was 6 visits (IQR 5-7).

In the unadjusted analysis, there was no evidence of an association between CD4 count and adherence measured by visual analogue scale during the first 6 months of ART (OR = 0.98; 95% CI 0.93-1.03 for every 100 cells/mm^3 increase).

In a multivariable logistic regression model that adjusted for age, sex, marital status, fixed dose combination ART, food insecurity and visit frequency, there was no evidence that CD4 count at ART initiation was associated with adherence <95% (OR = 0.99, 95% CI 0.94-1.05 for every 100 cells/mm^3 increase).

Table 5.3 Association between CD4 count at initiation and other risk factors with <95% VAS adherence during the first 12 months of ART

Characteristics	**Adherence <95%** **N visits/Total visits**	**Crude odds ratio (95% CI)**	**P value**	**[&]Adjusted odds ratio (95% CI)**	**P value**
Age at initiation n= 7772					
16-29	378/2357 (16.0)				
30-39	313/2224 (14.1)	0.97 (0.93-1.02)[#]	0.211	0.98 (0.93-1.03)[#]	0.414
40-49	196/1518 (12.9)				
>50	220/1673 (13.2)				
Sex n=7782			<0.0001		<0.0001
Female	698/5743 (12.2)	1		1	
Male	409/2039 (20.1)	2.23 (1.71-2.90)		2.24 (1.73-2.91)	
CD4 at Initiation (cells/mm^3) n =7782					
≤350	573/3951 (14.5)				
350-500	284/1953 (14.5)	0.98 (0.94-1.03)*	0.494	0.99 (0.95-1.04)*	0.731
>500	250/1878 (13.3)				
				-	

Characteristics	Adherence <95% N visits/Total visits	Crude odds ratio (95% CI)	P value	&Adjusted odds ratio (95% CI)	P value
Educational attainment n=7749			0.439		
Primary or less	480/3612 (13.3)	1			
Some Secondary	580/3835 (15.1)	1.18 (0.92-1.52)			
At least completed secondary	42/302 (13.9)	1.05 (0.55-2.02)			
Marital status n= 7760			0.103		0.168
Never been married	975/6639 (14.7)	1		1	
Married	87/745 (11.7)	0.68 (0.44 -1.06)		0.66 (0.43-1.03)	
Divorced/Separated	40/376 (10.6)	0.65 (0.35-1.20)		0.78 (0.42-1.44)	
Employment status n= 7771			0.596		
Employed	183/1241 (14.8)	1		-	
Student	46/253 (18.2)	1.24 (0.59-2.57)			
Unemployed	875/6277 (13.9)	0.91 (0.65-1.27)			
First line Regimen n= 7753			0.02		0.006
Non Fixed dose combination	72/346 (20.8)	1		1	
Fixed dose combination	1021/7407 (13.8)	0.80 (0.66-0.96)		0.45 (0.26-0.79)	

Characteristics	Adherence <95% N visits/Total visits	Crude odds ratio (95% CI)	P value	&Adjusted odds ratio (95% CI)	P value
ART treatment perception					
Agree that ART will improve health n=7668			0.746	-	
Yes	1041/7394 (14.1)	1			
No	14/78 (18.0)	1.53 (0.47-5.01)			
Don't know	33/196 (14.2)	1.14 (0.51-2.52)			
Worried about side effects of ART n=7618			0.707	-	
Yes	907/6534 (13.9)	1			
No	51/330 (15.5)	1.12 (0.60-2.09)			
Don't know	119/754 (15.8)	1.18 (0.78-1.79)			
Agree that ART will reduce infectiousness n=7541			0.012	-	
Yes	755/5853 (12.9)	1			
No	91/541 (16.8)	1.45 (0.90-2.34)			
Don't know	210/1147 (18.3)	1.61 (1.15-2.26)			
				-	

Characteristics	Adherence <95% N visits/Total visits	Crude odds ratio (95% CI)	P value	&Adjusted odds ratio (95% CI)	P value
HIV status disclosure to anyone n= 7661			0.346		
Yes	924/6557 (14.1)	1			
No	173/1104 (15.7)	1.18 (0.83-1.68)			
HIV status disclosure to current partner n=7489			0.595	-	
Yes	613/4217 (14.5)	1			
No	286/2088 (13.7)	0.88 (0.65-1.18)			
Not applicable (No partner)	178/1184 (15.0)	1.05 (0.74-1.51)			
Food insecurity n= 7691			0.015		0.001
Yes	779/4966 (15.7)	1		1	
No	310/2649 (11.7)	0.68 (0.52-0.88)		0.63 (0.49-0.81)	
Don’t know	9/76 (11.8)	0.69 (0.19-2.52)		0.53 (0.15-1.81)	

Characteristics	Adherence <95% N visits/Total visits	Crude odds ratio (95% CI)	P value	&Adjusted odds ratio (95% CI)	P value
Psychological distress (PHQ4) n=7531			0.105		
None	845/6154 (13.7)	1		-	
Mild	219/1224 (17.9)	1.51 (1.09-2.09)			
Moderate	10/81 (12.4)	0.94 (0.29-3.01)			
Severe	9/72 (12.5)	1.02 (0.27-3.83)			
Self-reported health status n= 7782			0.888	-	
≤80	676/4860 (13.9)	1			
>80	431/2922 (14.8)	1.02 (0.79-1.32)			
Distance from home to trial clinic (Km) n= 7782			0.06		0.074
≤1.3	589/3883 (15.2)	1		1	
>1.3	518/3899 (13.3)	0.79 (0.62-1.01)		0.81 (0.64-1.02)	
Visit frequency n=7782			<0.0001		<0.0001
≤11	713/4088 (17.4)	1		1	
>11	394/3694 (10.7)	0.48 (0.38-0.62)		0.50 (0.39-0.64)	

#Odds ratio for a 5 unit increase in age modelled as linear association with a continuous covariate; *odds ratio for a 100 unit increase in CD4 count at initiation, modelled as linear association with a continuous covariate; & Adjusted for age, CD4 count at initiation, sex, marital status, whether on fixed dose combination of ART, food insecurity, distance to clinic and visit frequency.

5.6.3.2 Association between CD4 count at initiation, other factors and PC adherence <95% during the first 12 months

Of the 728 individuals who had been on ART for 12 months, 721 had visits with at least one adherence measurement documented. Of the expected 9373 visits, 7011 (74.8%) visits had adherence measurements. Adherence was optimal in 5589 (79.7%) of 7011 visits. The median number of visits per individual was 11 visits (IQR 9-12).

In the unadjusted analysis, there was no evidence of an association between CD4 count and adherence measured by pill count during the first 12 months of ART (OR = 1.01; 95% CI 0.97-1.05 for every 100 cells/mm^3 increase).

After adjusting for age and sex, educational attainment, distance to clinic and visit frequency, there was no evidence that CD4 count at ART initiation was associated with adherence <95% (OR = 1.02, 95% CI 0.98-1.06 for every 100 cells/mm^3 increase).

5.6.4 Pearson correlation coefficients between PC, VAS and viral loads at 12 months

Figure 5.2 shows a scatter plot of the relationship between adherence measured by VAS and pill count.

The median Pill count was 100% (IQR 98.5-100) with 6% of visits recording pill count adherence >105% which was the cut-off for optimal adherence. Extreme values of ≥200% were documented in 5 visits.

Adherence measurements with VAS were within 0-100% limits of the scale. The median VAS adherence was 100% (IQR 100, 100). VAS adherence was <100% in 25% of visits. The predicted values of pill count adherence given VAS adherence showed a linear relationship (Figure 5.2). There was a significant positive correlation between adherence measured by pill count and VAS at 12 months (r =0.19, 95% CI 0.13-0.25; P=0.0003) and a significant negative correlation between mean VAS adherence and viral load at 12 months (Table 5.4).

99.0% of individuals with adherence ≥95% achieved virological suppression at 12 months compared to 90% in those with <95% adherence (p<0.001 for comparison of the three VAS adherence strata in Figure 5.3)

5.6.4.1 Sensitivity analyses

I undertook a series of sensitivity analyses comparing adherence scores from VAS and PC during the first 6 months as well the correlation between mean VAS and viral load at 6 months as well as mean PC and viral load at 6 and 12 months and found similar results (Table 5.4). Similarly, the proportion of individuals with virological suppression at 6 months increased with adherence as measured by VAS

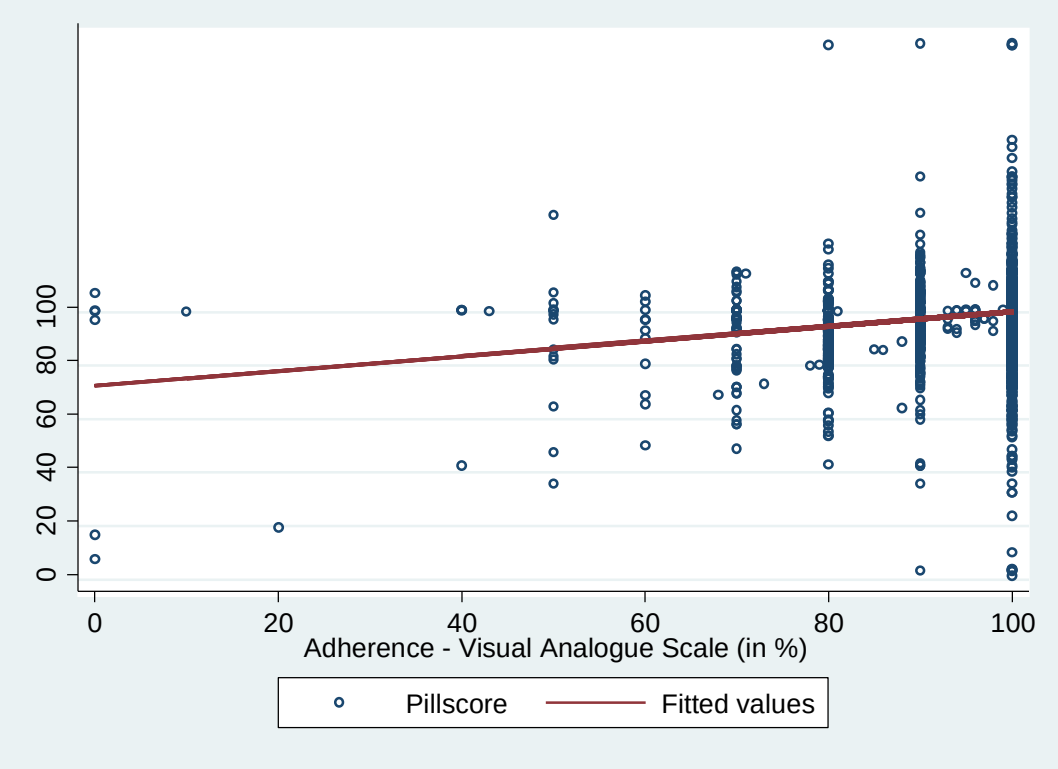


Figure 5.2 Scatter plot showing the relationship between adherence measured by pill count and VAS during each visit at 12 months

Table 5.4 Pearson correlation coefficinets between VAS, PC and virological suppression at 6 and 12 months

	Time period (months)	Sample size	Correlation coefficient (95% CI)	P value
Main analyses				
VAS and PC	12	7802 visits	0.19 (0.13-0.25)	0.0003
VAS and viral load	12	723 participants	-0.07 (-0.22 to 0.07)	0.07
Sensitivity analyses				
VAS and PC	6	5603 visits	0.22 (0.13-0.28)	<0.0001
VAS and viral load	6	976 participants	-0.09 (-0.22 to 0.05)	0.009
PC and viral load	6	968 participants	-0.01 (-0.06 to 0.05)	0.834
	12	721 participants	-0.09 (-0.21 to 0.03)	0.02

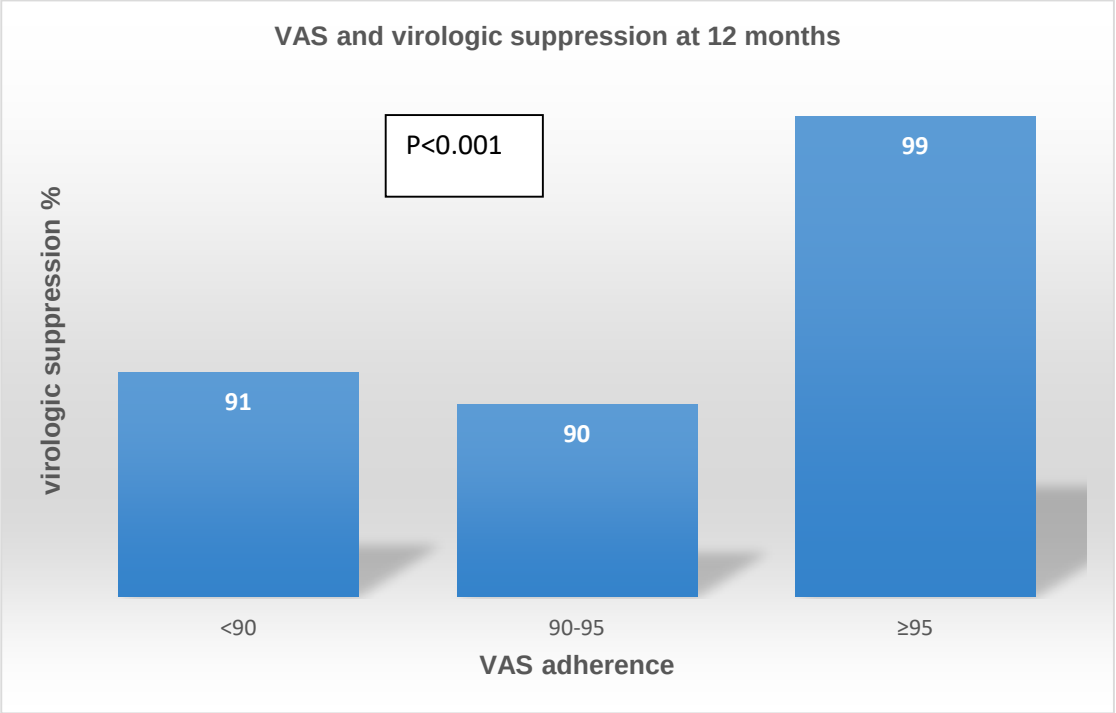


Figure 5.3 Relationship between adherence levels measured by VAS and virological suppression at 12 months

5.7 Discussion

This is a cohort analysis of participants enrolled in a cluster randomised trial investigating whether there is an association between CD4 count at ART initiation and adherence. Adherence measured by VAS was optimal (≥95%) in 86% of visits during the first 12 months of ART. I found no evidence of a significant association between CD4 count at ART initiation and adherence measured by VAS during the first 12 months of ART. Furthermore, male sex was a risk factor for non-optimal adherence. Being on a fixed dose combination ART, food security, high clinic visit frequency and living farther away from the clinic were associated with decreased probability of non-optimal adherence. VAS as a tool to measure adherence was predictive of virological suppression.

The literature review identified only two studies in Africa (227, 228) comparing individuals with CD4 count >350 cells/mm^3 with those who had lower CD4 counts. The findings from these two studies were contradictory. They used different adherence measurement tools, different reference groups and comprised individuals with advanced HIV disease based on the median CD4 count at ART initiation. My cohort comprised individuals with a higher median CD4 count at ART initiation and findings corroborate that seen in high income countries reported in the systematic review by Bock et al (226). WHO recommends universal test and treat for HIV(105); South Africa has already adopted this policy (15) but there are no data on adherence in people initiating ART at high CD4 counts. With this new policy, the median CD4 count at which individuals initiate ART is likely to rise to levels observed in this research. However, a meta-analysis covering the period from January 2002 to Dec 2013 showed that the CD4 count at presentation has increased in South Africa but the CD4 count at ART initiation has remained unchanged at a mean of 123 cells/mm^3 (226).

One of the WHO early warning indicators for development of HIV drug resistance is the monitoring of proportion of patients with 100% of pills picked up on time during the first 12 months of ART and serves as a proxy for adherence. The overall adherence of 86% observed in this research for visits during the first 12 months falls just under the >90% WHO recommendation (32).

Men have more than double the odds of low adherence compared to women with similar findings reported in two studies in Tanzania (223) and South Africa (132). The majority of the studies have reported no gender difference with respect to adherence (130, 131, 138, 183, 231, 232) with one meta-analysis reporting a marginal association of male gender with higher adherence (233).

I found that being food-insecure was associated with lower adherence. A study conducted in Namibia observed a high proportion of individuals attending a public ART programme to be food-insecure and this was associated with poor adherence as measured by medication possession ratio (190). The relationship between food-insecurity and poor adherence has also been reported in high-income countries (234, 235). Patients who have missed doses have often cited not having food at home as a reason for missing doses as they did not want to take their drugs on an empty stomach. This anecdotal observation has been confirmed in formal qualitative studies (236, 237).

There was weak evidence that poor adherence was more likely in those living closer to trial clinics. A systematic review of studies in sub-Saharan Africa settings examined the impact of geographic and transportation-related barriers (travel distance, travel time, transportation cost, urban vs. rural) on a number of HIV outcomes which included adherence (238). Seven of the studies included in the review specifically examined the association between travel distance and ART adherence; two studies found a significant association between increased distance from clinic and poor adherence whilst five of the studies found no evidence of an association. Travel distance was measured by self-reported estimates in six of the studies and by the straight line distance in one of the studies. This research is the only study that has reported an association of increased travel distance to the clinic and better adherence, although the evidence for this was weak. Visit frequency was greater in those who lived farther away from the clinic and the data showed that individuals with higher visit frequency had better adherence. Previous publication from the trial showed that those who lived farther away from the clinic were less likely to link to care (124). It could be that individuals who lived farther away from the clinic and linked to care were more motivated to adhere than those who lived closer to the clinics. In addition, they may have been less worried about

being recognised by their neighbours than those who lived very close to the clinics (239-241).

There is no gold standard measure of adherence. In this research, I demonstrated a correlation between adherence measured by VAS and pill count and both were predictive of virological suppression. This is suggested by a significant correlation between VAS adherence during the first 12 months on ART and virological suppression. There was also increased virological suppression with increasing adherence as measured by VAS. These findings are robust to a number of sensitivity analyses using another time-point such as average VAS adherence in the first 6 months and virological suppression at 6 months, as well as the significant correlation between average pill count in the first 12 months and virological suppression at 12 months.

This research study has a few limitations. About 20% of individuals eligible for my main analysis were excluded either because they were dead, lost to follow up, transferred their care or their status was unknown. Included individuals were more likely to be female and older. These characteristics have been found to be associated with good adherence in a number of studies (130, 223, 228, 233). Although there was no difference in the median CD4 count at initiation between included and excluded individuals, the more favourable adherence characteristics of included individuals may have biased my estimate of the association between CD4 count at ART initiation and adherence. Amongst individuals included in the analysis, adherence measures were missing in nearly 20% of visits, either because the visit did not happen or the measurement was not taken. I assumed that missing adherence measures were missing completely at random in my analysis and so undertook a complete case analysis. This might not be the case as my data showed that individuals with fewer than expected number of visits to the clinics were more likely to have non-optimal adherence, hence excluding missed visits could have resulted in a biased estimate of adherence. Furthermore, in the sensitivity analyses I conducted using pill count adherence, I had assumed that individuals with pill count adherence >105% had non-optimal adherence. This could have resulted in misclassification of the adherence in some individuals, although only just over 5% of visits were affected by this ‘overestimates’ of adherence.

The main strength of this analysis is that it is was nested within a randomised trial, so that individuals who started ART at different CD4 counts did so at random. This mitigates against any bias that might be introduced if those with higher CD4 counts were more motivated and hence more likely to adhere. This analysis is also novel in the African setting.

To summarise, I found no evidence of a significant relationship between CD4 count at ART initiation and adherence during the first 12 months of ART. With two large trials showing individual health benefits of initiating ART early (16, 17) and the WHO 2015 ART guidelines recommending HIV treatment regardless of CD4 count (105), a policy already adopted by South Africa (15), this result should allay any anxieties about adherence in individuals initiating ART at higher CD4 counts.

Chapter 6 Virological suppression and acquired resistance

This chapter examines virological suppression in individuals ART-naïve at entry into the trial who subsequently initiated ART and in those who were ART-experienced at their first trial clinic visit. I also examine the association between CD4 count at ART initiation and virological suppression and assessed acquired resistance in individuals with virological failure. Amongst individuals with drug resistant mutations at virological failure, mutations observed are compared with mutations present pre-treatment in cases with data available.

6.1 Background

Good adherence to ART is required to halt viral replication and avoid the development of resistance mutations (130, 242). However, maintaining sustained adherence could sometimes be challenging due to either individual or system-related factors resulting in virological failure and drug resistance (158, 243). The close relationship between adherence and virological suppression creates a similar concern that motivation for adherence may not be as high in individuals started on ART at high CD4 counts who may not be experiencing symptoms of ill-health. More so, if informed that the primary reason for the offer of ART was to prevent transmission to sexual partners. If this was the case, then this could result in virological failure and the development of drug resistance.

With increasing number of treatment guidelines recommending ART initiation at higher CD4 counts including South Africa since 1 September 2016 (15), high numbers of asymptomatic individuals would be starting ART. With the error-prone character of reverse transcriptase, and the high mutation rate of HIV, drug resistance is likely to occur both in poorly adherent and some adherent individuals due to drug selective pressure from ART. These drug resistant mutations could be transmitted resulting in a greater proportion of individuals seroconverting to HIV being infected with drug resistant virus (29).

The K65R and the K103N mutations warrant surveillance as the currently recommended first-line ART in South Africa is based on a fixed dose combination of tenofovir, lamivudine or

emtricitabine and efavirenz. Individuals who develop resistance with these mutations can potentially transmit them thereby compromising the public health ART programme. However there are limited data on virological suppression and resistance in the African setting on individuals starting ART at CD4 > 350 cells/mm^3. Therefore, I conducted two scoping reviews of the published literature on virological suppression on the one hand and then again on acquired resistance in individuals initiating ART at CD4 >350 cells/mm^3. Secondly, I examined the relationship between CD4 count at initiation and virological suppression in both ART-naïve individuals who initiated ART within the trial and those ART-experienced at entry. Thirdly, I examined acquired resistance in individuals with virological failure.

6.2 Literature review

The aim of the scoping review was to summarise current knowledge in the African setting on the association between high CD4 count (>350 cells/mm^3) at ART initiation and virological outcomes (suppression/failure) as well as acquired resistance.

6.2.1 Virological outcomes

I searched Pubmed for studies that reported on the association between high CD4 count (>350 cells/mm^3) and virological outcomes on ART published before 9 February 2017 using the search criteria below:

Search (High) OR Earl* initiation (61,234)

Search CD4 (145,475)

Search (((ART) OR Antiretroviral) OR HIV Therapy) OR agents, antiretroviral[MeSH Terms] (213,381)

Search (Viral) OR Virological* (706,851)

Search ((Suppression) OR Failure) OR Outcomes (1,512, 955)

Search Africa (285,573)

Combining all search criteria above: Search (((((((High) OR Earl* initiation)) AND CD4) AND ((((ART) OR Antiretroviral) OR HIV Therapy) OR agents, antiretroviral[MeSH Terms])) AND ((Viral) OR Virological*)) AND (((Suppression) OR Failure) OR Outcomes)) AND Africa (126)

I read the titles of all 126 articles and excluded 111 articles that were clearly unrelated to the question of the scoping review; addressing broad ranging issues on HIV subtypes and ART response, mortality outcomes, retention, linkage to care, modelling, other viruses such as hepatitis B and paediatric HIV, amongst other reasons. I read the abstract of the remaining 15 and selected nine studies for full review that appeared to have examined the association of CD4 count with virological outcomes.

Of the nine studies remaining eight were excluded after reading the full article. Excluded studies include the following: one related to the initial results of the main trial (TasP) (96) two were on community HIV testing and linkage with ART initiated at CD4 <350 cells/mm^3 (244, 245), one was on drug resistance outcome in individuals initiating ART at high CD4 counts (HPTN 052), included participants from Africa, Asia and America (184), one examined immunological failure (246), one reported on mortality (247), one had no data on virological suppression (248) and one compared CD4 >150 cells/mm^3 with those of lower CD4 counts (249).

The only study meeting the criteria of the scoping review was the TEMPRANO study of early antiretroviral therapy in Africa (17).

6.2.2 Acquired resistance

I searched pubmed for studies that reported on the association between high CD4 count (>350 cells/mm^3) and acquired resistance for individuals with virological failure (as defined by the study) published before 10 February 2017 using the search criteria below:

Search (High) OR Earl* initiation (61,234)

Search (((ART) OR Antiretroviral) OR HIV Therapy) OR agents, antiretroviral[MeSH Terms] (213,381)

Search (Viral) OR Virological* (706,851)

Search ((Suppression) OR Failure) OR Outcomes (1,512,955)

Search Africa (285,573)

Search ((drug resistance) OR Acquired resistance) OR antiviral drug resistance[MeSH Terms] (440,539)

Combining all search criteria above: Search (((((((High) OR Earl* initiation)) AND ((((ART) OR Antiretroviral) OR HIV Therapy) OR agents, antiretroviral[MeSH Terms])) AND ((Viral) OR Virological*)) AND (((Suppression) OR Failure) OR Outcomes)) AND Africa) AND (((drug resistance) OR Acquired resistance) OR antiviral drug resistance[MeSH Terms]) (37)

I read the titles of all 37 articles identified and excluded 27 articles that were clearly unrelated to the topic of interest. The search was executed on all fields, hence articles that did not meet all search criteria were occasionally returned if the search terms were mentioned in the body of the article. The excluded articles were studies on children (12), pre-treatment drug resistance (2 studies), mathematical models (2 studies) and a range of individual studies that did not meet all the search criteria above (11).

I read the abstract of the remaining 10 andexcluded a further five studies. These 5 studies described drug resistance in individuals failing ART but did not examine the association between CD4 count at initiation and emergence of acquired resistance. These were also in individuals initiating ART at a CD4 count <350 cells/mm^3. Of the remaining 5 studies of which I read the full manuscript, three of them satisfied the criteria of the scoping review and are summarised in Table 6.1 ((184, 250, 251). Of the two that did not fulfil the criteria, one compared CD4 <100 vs. ≥100 (252) while the effect of CD4 count was not examined in the second (253).

6.3 Discussion of literature review

My first literature review identified one published study reporting on virological suppression in individuals who initiated ART at high CD4 counts (17). The TEMPRANO study conducted in Ivory Coast between March 2008 and January 2015 randomised 2056 individuals with CD4 ≤ 800 cells/mm^3 to either immediate ART or deferred until WHO criteria for starting ART was met (initially CD4 < 200 cells/mm^3 until 2013, then 500 cells/mm^3 afterwards). 84% of 911 individuals had undetectable viral load 12 months post-ART initiation in the immediate arm

and 80% of 331 individuals had undetectable viral load 12 months post-ART initiation in the deferred therapy arm. This study did not examine the independent effect of CD4 count at initiation on virological suppression as it was not one of the study outcomes.

Through the second review, I identified three studies on acquired resistance that included individuals initiating ART at high CD4 counts (CD4 > 350 cells/mm^3). The study by Fogel et al (184) included participants with virological failure from the HPTN 052 study (21). Of the 85 participants with virological failure from the immediate arm, 42 were from Africa; 15 (36%) of whom developed resistance at virological failure. 8 participants developed virological failure in the deferred arm, seven of whom had drug resistance mutation.The frequency of drug resistance mutation was lower in the immediate arm in which individuals initiated ART at higher CD4 count. Because only few people had virological failure in the delayed arm, factors associated with resistance mutation was only examined in the 85 individuals with virological failure in the immediate arm.

The cohort study by Hanson et al (250) used a survival time ratio approach to examine the factors associated with the emergence of drug resistance. In this approach, an STR <1 meant an increased risk of resistance. There was no difference in risk between those initiating at ≥350 vs. 200-349 but there was increased risk of drug resistance emergence in those initiating in the lower CD4 counts strata compared to 350. Nearly half of the cohort were on PI-based first-line ART, hence differed from my research cohort in this respect.

The study by Hong et al (251) found no difference in the emergence of drug resistance according to CD4 count at initiation. The estimated odds ratio were not adjusted possibly because only 12 (5%) of the 245 individuals with viral load data available12 months post-ART initiation had drug resistance mutation. There is a paucity of high quality studies in the African setting to address the question of whether high CD4 count at ART initiation is associated with the emergence of drug resistance mutation at virological failure.

Table 6.1Studies of acquired resistance in Africa comprising individuals initiating ART at CD4>350 cells/mm^3

Author	Study setting	Study population/sample size	Study design & ART regimen	Outcome	CD4 groups at ART initiation (Cells/mm^3)	Effect estimate
Fogel J (184)	Asia/Africa/Americas	≥18 years / 85 individuals; 42 from Africa	Randomised trial	Proportion with drug resistance at virological failure	350-550 vs. ≤ 250	early ART arm vs. delayed ART arm 30/85 [35.2%] vs. 7/8 [87.5%]
						p=0.006
Hanson D (250)	Ivory Coast	≥16 years, 645 individuals	Cohort study;	Factors associated with emergence of drug resistance using survival time ratio		STR* (95% CI)
			Dual or triple NRTI (19%), PI-based (48%), NNRTI-based (33%)		≥350 reference	1
					200-349	0.80 (0.42, 1.55)
					50-199	0.52 (0.28, 0.96)
					0-49	0.46 (0.24, 0.86)
Hong S (251)	Namibia	≥18 years, 394 individuals; 245 had VL data at 12 months	Prospective cohort;	Factors associated with HIVDR or possible HIVDR at 12 months		Odds ratio (95% CI)
			NNRTI-based ART with either ZDV, D4T or TDF		<200	1
					200-350	0.9 (0.5, 1.6)
					>351	1.1 (0.3-3.8)

*Interpretation of survival time ratio (STR): estimates >1 relate to longer survival time before developing drug resistance whereas, estimates <1 mean shorter survival time (greater risk for drug resistance).

Outside of this literature review, a few studies in high-income countries have reported a lower frequency of emergent drug resistance at virological failure in individuals who initiated ART at high CD4 counts (182, 183, 254) while one study reported no difference (255).

6.4 Aim and objectives

The aim of this chapter was to examine the association between CD4 count at ART initiation and virological suppression and to estimate acquired resistance rates in individuals with virological failure.

The primary objectives of this chapter were:

- To estimate virological suppression at 6 and 12 months in ART naïve individuals and to examine if CD4 count at ART initiation is associated with virological suppression
- To examine virological suppression in individuals already established on ART on entry into the trial, who initiated ART in the public health ART programme according to South Africa guidelines prior to 30 June 2016 when follow up ended (9)
- To describe acquired resistance in individuals with virological failure (VL>1000 copies/mL after at least 6 months on ART) and
    - to assess the potential transmissibility of the K65R mutation associated with tenofovir and historical use of stavudine in this population.
    - to assess the appropriateness of second-line ART that would have been prescribed using the public health approach in individuals with virological failure and acquired resistance to the recommended first-line (zidovudine and tenofovir) and NNRTI
    - to compare baseline resistance (pre-treatment drug resistance) with acquired resistance in individuals who had both results available

6.5 Methods

6.5.1 Study population

The study population comprises individuals who were ART-naïve at entry into the trial but initiated ART within the trial and individuals who were already ART-experienced at entry into the trial. Individuals who were ART-experienced at entry into the trial were more typical of real-life patients as they transferred their care to the trial from the public sector ART programme. Both groups were combined for the analysis on acquired resistance.

6.5.2 Study procedures

Briefly, HIV-positive participants who visited the trial clinics were registered at the first clinic visit as clinic participants. Clinic questionnaires were administered by the study nurses enquiring about current and past medical history. A physical examination was also undertaken. Participants who were ART naïve at study entry were assessed for ART eligibility according to study protocol. A point of care CD4 count was obtained for all participants. Participants in the control arm were offered ART based on South African guidelines whilst those in the intervention arm were eligible for ART regardless of CD4 count. A venous blood sample was obtained from all participants at the baseline visit and subsequently according to schedule described in Chapter 3. Blood samples were used for storage and HIV viral load testing and genotype tests at the AHRI laboratory as indicated. Further blood samples were sent to the National Health Laboratory Service for monitoring of toxicity in individuals on ART. Partipants who initiated ART within the trial and those who were already established on ART prior to trial entry were seen monthly in the clinic for review, adherence support and ART prescription. Participants from both groups who were unwell, or with virological failure (VL > 1000 copies/mL after 6 months on ART measured three months apart) were reviewed by me. The first viral load above 1000 copies/mL was sent for genotype tests in those satisfying the criteria for virological failure. In ART-experienced patients, this could be the viral load taken at the first clinic visit or subsequently

if virological failure happened at a later date. No participants, whether ART naïve or experienced, had stored blood samples prior to enrolment in the trial. The genotype tests were done by staff at the AHRI laboratory in Durban as described in Chapter 3.

6.5.3 Definition of variables and outcomes

6.5.3.1 Objective 1

Outcome 1: Virological suppression (VL <400 copies/mL) at 6 and 12 months in individuals who initiated ART within the trial.

Primary exposure: CD4 cell count at ART initiation

Secondary exposure: Adherence measured by both VAS and PC

6.5.3.2 Objective 2

Outcome 2: Virological suppression (VL <400 copies/mL) at entry into the trial in individuals on ART ≥ 6 months. Six months was used as the cut-off to allow time to achieve suppression. The viral load measurement at the first trial clinic visit was used for this analysis.

Primary exposure: CD4 cell count at ART initiation

6.5.3.3 Objective 3

Outcome 3: Proportion with acquired resistance among individuals with virological failure (VL >1000 copies/mL ≥6 months post-ART initiation)

Primary exposure: CD4 cell count at ART initiation

Other exposures of interest for the first two outcomes

Sex (male/female)

Age at ART initiation

Baseline viral load (<10,000, 10,000-100,000, >100,000)

Disclosure of HIV status to anyone (yes/no)

Disclosure of HIV status to current partner (yes/no)

Employment status (employed, student, unemployed)

Marital status (never married, married, divorced/separated)

Educational attainment (primary or less, some secondary, secondary or higher)

Regimen type (whether fixed dosed combination or not)

Distance to the nearest TasP clinic was measured for those ART-naïve at entry (divided into quantiles)

Distance to nearest DoH clinic was measured for ART-experienced at entry*;* obtained by measuring the distance as the crow flies from the participant's home to the public ART clinic closest to their home. This distance was used for these participants as it represented their travel distance to receive care prior to first clinic visit in the trial (divided into quartiles)

Duration on ART prior to first trial clinic visit in those ART-experienced (divided into quartiles)

Depression was assessed using the PHQ-4 scale rated as normal (0-2), mild (3-5), moderate (6-8) and severe (9-12), (221)

Self-reported health status using a scale ranging fom 0 to 100% in which 0 represents poor health and 100% represents excellent health.

Food insecurity measured by whether skipped meals in last 12 months or not (yes/no)

ART treatment perception (through three questions concerning the participant's attitudes about ART as displayed on results tables)

6.5.4 Statistical analysis

Median and interquartile ranges were used to summarise continuous exposure variables with skewed distributions and mean was used to summarise continuous exposure variables with normal distributions. Frequencies and percentages were reported for categorical exposure

variables. I used the Chi-squared tests to examine association between categorical variables whilst Mann-Whitney test was used to compare medians for continuous variables.

In the analysis involving individuals who initiated ART within the trial; I examined the associtation between CD4 count at ART initiation, adherence and virological suppression at 6 months. Sex and age distribution of individuals included in the analysis were compared to those excluded from the analysis to assess for baseline differences. For the outcome (virological suppression), I allowed a three-month window on either side of the date the viral load was measured to capture viral loads not measured on the exact dates they were due. Thus, to estimate virological suppression at 6 months, I considered viral loads measured between 3 and 9 months, and the closest viral load to 6 months was used. Individuals without viral load data at 6 months were dropped (complete case analysis) with no imputation of missing data.

Multivariable logistic regression was used to assess the associations between CD4 count at initiation, adherence (measured by both VAS and PC) and other factors with virological suppression at 6 months. Odds ratios were reported with 95% confidence intervals and p values for association between the factors included in the model and virological suppression was derived using the likelihood ratio tests. I used logistic regression models for my analysis to allow comparison with the EWI targets set by the WHO for assessing the performance of an ART programme (32) as this better reflects the overarching question of my research. Adherence was measured using VAS and PC and mean adherence was computed from monthly adherence measured during the first 6 months of ART. Any missing adherence measures were omitted for calculation of mean adherence.

Analysis using VAS was presented as the main analysis, whilst the analysis with PC was presented as a sensitivity analysis. I used the likelihood ratio tests to find the model which best fitted the data. I examined CD4 count at ART initiation as both a categorical and continuous variable. I also did the same for age. There was no evidence of a departure from a linear trend for these two exposure variables in the multivariable model. I transformed other continuous exposure variables (distance to clinic, self-reported health status) into binary variables above and below their median values. I added variables that were significant

at p<0.15 in the univariable model one at a time to the multivariable model. To capture the full effect of CD4 count, I fit an initial model that excluded adherence. A subsequent model that included adherence, would allow an estimate of the effect of CD4 count on virological suppression to be made allowing for that effect to be mediated through adherence

I used likelihood ratio tests as explained above to select the model with the best fit and to derive p values. I included age and sex a priori in the model because of their known association with virological suppression in the published literature (256, 257).

I restricted the risk factor analysis to 6 months post-ART initiation as nearly all (97.2%; 630/648) individuals with available viral load were suppressed at 12 months post-ART initiation.

I undertook a sensitivity analysis by replacing any viral loads missing from the three month window (window of 3 months to 9 months for the 6 months viral load) with the viral load closest to the lower bounds of the window and repeated the analysis.

In the analysis involving individuals who were ART-experienced at entry into the trial, I used multivariable logistic regression to assess the associations between CD4 count at initiation and virological suppression at entry into the trial. Individuals were eligible for inclusion if they had been on ART for at least six months to allow time to achieve virological suppression. The viral load measured at the first clinic visit was used for this outcome. Prior to the trial clinic visit, these individuals received their care in the public ART programme where measurement of viral loads was erratic, precluding assessment of virological suppression at specific time points. For example, I found that only 379 (30.1%) of the 1259 individuals who had been on ART for 12 months or longer had viral load measurements at 12 months (window 9-15 months). Hence, I decided to undertake a cross-sectional analysis assessing virological suppression at the time of the first clinic visit in the trial. There were no adherence data prior to trial entry. Similarly, a large proportion of individuals have no CD4 count at initiation documented in the public ART programme database in the trial area, possibly because they transferred their care from a clinic located outside the sub-district to the trial area whilst already on ART. Because CD4 count at initiation was my main exposure variable

and to ensure the univariable and multivariable models were comparable, I excluded all individuals with missing CD4 count.

I followed the modelling approach described for those who initiated ART within the trial. Odds ratios were reported with their 95% confidence intervals and p values for association between the factors included in the model and virological suppression were derived using the likelihood ratio tests. Duration on ART and Distance to the public ART clinic exposure variables were categorised into quartiles (approximately equal number of individuals in each group).

Both groups were combined for the analysis on acquired resistance and descriptive statistics presented as described above. I could not examine the association between CD4 count at initiation and development of acquired resistance due to the small numbers of people with acquired resistance who initiated ART at CD4 >350 cells/mm^3.

In individuals who have a combination of resistance mutations that can compromise both tenofovir and zidovudine in combination with mutations that affect the efficacy of NNRTI, I assessed the efficacy of second-line ART that would have been prescribed by the public health approach (that is not informed by results of resistance test).

Finally, drug resistance mutations detected in individuals who initiated ART within the trial were compared to drug resistance mutations present pre-treatment as the majority of these participants were assessed for pre-treatment drug resistance using stored samples collected at their first clinic visit. This was to assess if individuals failed as a result of pre-treatment drug resistance and to check for development of new mutations. The genotype results from the pre-treatment samples were not used to inform regimen choice during initiation of first-line ART as this was prescribed using the public health approach. Furthermore results of pre-treatment drug resistance were not available in real time.

6.6 Results

6.6.1 Objective 1

6.6.1.1 Virological suppression

Table 6.2 shows the baseline characteristics of the exposure variables and the frequency of missing data.

Of the 983 individuals on ART for at least 6 months, 75 had missing viral loads. Of the remaining 908 individuals, 851 (93.7%) achieved virological suppression 6 months post-ART initiation . There was no difference in the sex distribution of those with missing VL vs. those with complete data (32.4% vs. 26.9% for males; p=0.302) and the median age was also similar [missing vs. complete; 35.8 years (IQR 27.1-46.0) vs. 34.9 years (IQR 27.7-46.6); p=0.465].

6.6.1.2 Relationship between CD4 count at initiation, adherence and virological suppression at 6 months

In the univariable regression model including data from the 908 individuals with viral loads at 6 months (Table 6.3), the odds of virological suppression (VL<400 copies/mL) increased with every 100 unit increase in CD4 count at initiation, [OR 1.49 (95% CI 1.25-1.77), p<0.0001]. Individuals with a VAS adherence of ≥95 were >3 times more likely to achieve virological suppression than those with adherence of <95%, [≥95 vs. 95%; OR 3.21, (95% CI 1.75-5.89)]. Other factors significantly associated with an increased probability of virological suppression were being prescribed atripla (fixed dose combination of tenofovir, emtricitabine and efavirenz) compared to separate tablet regimen and having a high self-reported health status.

Factors associated with a decreased probability of virological suppression were having a high baseline viral load (OR 0.10, 95% CI: 0.04-0.27 and OR 0.29, 95% CI: 0.11-0.81 for VL >100,000 and 10,000-100,000 respectively vs. <10,000), being male OR 0.52, 95% CI 0.30-0.90) and being a student compared to being employed (student vs. employed OR 0.28, 95% CI 0.10-0.83).

Table 6.2 Characteristics of individuals included in the analysis

Socio-demographic characteristics	N=908 (%)
Clinical characteristics	
CD4 at Initiation (cells/mm^3)	
Median (IQR)	359 (244-498)
≤350	440 (48.5)
350-500	242 (26.7)
>500	224 (24.7)
Missing	2 (0.2)
Adherence (VAS) %	
<95	121 (13.3)
≥95	784 (86.3)
Missing	3 (0.3)
Viral load at presentation (copies/mL)	
Median	26,734 (4,513-141,575)
<10,000	320 (35.2)
10,000-100,000	313 (34.5)
>100,000	275 (30.3)
First line Regimen	
Non Fixed dose combination	34 (3.7)
Fixed dose combination (Atripla)	869 (95.7)
Missing	5 (0.6)
Age at initiation (Years)	
16-29	301 (33.2)
30-39	266 (29.3)
40-49	153 (16.9)
>50	187 (20.6)
Missing	1 (0.1)
Sex	
Female	664 (73.1)
Male	244 (26.9)

Socio-demographic characteristics	N=908 (%)
Educational attainment	
Primary or less	396 (43.6)
Some Secondary	465 (51.2)
At least completed secondary	44 (4.9)
Missing	3 (0.3)
Marital status	
Never been married/Engaged	792 (87.2)
Married	76 (8.4)
Divorced/Separated	37 (4.1)
Missing	3 (0.3)
Employment status	
Employed	140 (15.4)
Student	36 (4.0)
Unemployed	729 (80.3)
Missing	3 (0.3)
Distance from home to trial clinic (Km)	
Distance to trial clinic/unit increase	
Median (IQR)	1.3 (0.74-1.87)
≤1.3	453 (49.9)
>1.3	455 (50.1)
Other characteristics	
Self-reported health status	
Median (IQR)	80 (70-90)
≤80	534 (58.8)
>80	373 (41.1)
Missing	1 (0.1)
HIV status disclosure to anyone	
Yes	769 (84.7)
No	125 (13.8)
Missing	14 (1.5)

Socio-demographic characteristics	N=908 (%)
HIV status disclosure to current partner	
Yes	482 (53.1)
No	260 (28.6)
Not applicable (No partner)	133 (14.7)
Missing	33 (3.6)
Food insecurity	
Yes	571 (62.9)
No	318 (35.0)
Don’t know	7 (0.8)
Missing	12 (1.3)
ART will improve health	
Yes	863 (95.0)
No	12 (1.3)
Don’t know	18 (2.0)
Missing	15 (1.7)
Worried about side effects of ART	
Yes	762 (83.9)
No	39 (4.3)
Don’t know	86 (9.5)
Missing	21 (2.3)
Agree that ART will reduce transmission	
Yes	680 (74.9)
No	71 (7.8)
Don’t know	129 (14.2)
Missing	28 (3.1)
Psychological distress (PHQ4)	
None	713 (78.5)
Mild	145 (16.0)
Moderate	12 (1.3)
Severe	9 (1.0)
Missing	29 (3.2)

In multivariable regression, I adjusted for the variables shown in the table 6.3, initially estimating the full effect of CD4 at initiation in the association with virological suppression; CD4 count was associated with increased virological suppression (aOR 1.29, 95% CI 1.07-1.56 for every 100 cells/mm^3 increase)-not shown in table. In the full model that included adherence (Table 6.3) factors independently associated with an increased probability of virological suppression were CD4 cell count at initiation, aOR 1.26 (95% 1.05-1.53)), good adherence as measured by VAS (≥95 vs. 95%; aOR 2.41 (95%CI 1.20-4.84)), being on atripla (aOR 9.53 (95% CI 3.54-25.68) and having a high self-reported health status. Older age was also independently associated with increased probability of virological suppression (aOR =1.14, 95% CI 1.00-1.31 for every 5 years increase in age).

Factors associated with a decreased probability of virological suppression were high baseline viral load which showed a dose-response relationship with decreased virological suppression (aOR 0.16, 95% CI 0.06-0.45 and aOR 0.33, 95% CI 0.11-0.98 for VL >100,000 and 10,000-100,000 respectively vs. <10,000 p-trend <0.0001) and being a student. The association of male sex with decreased virological suppression was no longer significant after adjustment.

Table 6.3 The association between CD4 count at initiation, adherence measured by VAS and other factors with virological suppression at 6 months

	Virological suppression n (%)	Crude odds ratio (95% CI)	P value	*Adjusted odds ratio (95% CI)	P value
Clinical characteristics					
CD4 at Initiation (cells/mm^3) n=906					
CD4 at initiation/100 units					
≤350	396/440 (90.0)				
350-500	236/242 (97.5)	1.49 (1.25-1.77)	<0.0001	1.26 (1.05-1.53)	0.009
>500	217/224 (96.9)				
Adherence (VAS) % N=905			0.0005		0.018
<95	104/121 (86.0)	1		1	
≥95	746/784 (95.2)	3.21 (1.75-5.89)		2.41 (1.20-4.84)	
Viral load at presentation (copies/mL) **n=908**			<0.0001		<0.001
<10,000	315/320 (98.4)	1		1	
10,000-100,000	297/313 (94.9)	0.29 (0.11-0.81)		0.33 (0.11-0.98)	
>100,000	239/275 (86.9)	0.10 (0.04-0.27)		0.16 (0.06-0.45)	

	Virological suppression n (%)	Crude odds ratio (95% CI)	P value	*Adjusted odds ratio (95% CI)	P value
First line Regimen n=903			<0.0001		<0.0001
Non Fixed dose combination	24/34 (70.6)	1		1	
Fixed dose combination (Atripla)	822/869 (94.5)	7.29 (3.29-16.12)		9.53 (3.54-25.68)	
Age at initiation/5 years (Years) **n=907**					
16-29	283/301 (94.0)				
30-39	249/266 (93.6)	1.00 (0.90-1.11)	0.996	1.14 (1.00-1.31)	0.047
40-49	139/153 (90.9)				
>50	179/187 (95.7)				
Sex n=908			0.023		0.430
Female	630/664 (94.9)	1		1	
Male	221/244 (90.6)	0.52 (0.30-0.90)		0.76 (0.39-1.50)	
Educational attainment n=905			0.873		
Primary or less	371/396 (93.7)	1			
Some Secondary	435/465 (93.6)	0.98 (0.56-1.69)		-	
At least completed secondary	42/44 (95.5)	1.42 (0.32-6.19)			

	Virological suppression n (%)	Crude odds ratio (95% CI)	P value	*Adjusted odds ratio (95% CI)	P value
Marital status n= 905			0.361		
Never been married/Engaged	739/792 (93.3)	1			
Married	73/76 (96.1)	1.74 (0.53-5.72)		-	
Divorced/Separated	36/37 (97.3)	2.58 (0.35-19.20)			
Employment status n=905			0.022		0.036
Employed	131/140 (93.6)	1		1	
Student	29/36 (80.6)	0.28 (0.10-0.83)		0.18 (0.05-0.69)	
Unemployed	688/729 (94.4)	1.15 (0.55-2.43)		0.82 (0.34-1.96)	
Distance from home to trial clinic (Km) n= 908					
Distance to trial clinic/unit increase			0.878		
≤1.3	424/453 (93.6)	1		-	
>1.3	427/455 (93.9)	1.04 (0.61-1.68)			
Other characteristics					

	Virological suppression n (%)	Crude odds ratio (95% CI)	P value	*Adjusted odds ratio (95% CI)	P value
Self-reported health status n=907			0.003		0.020
≤80	490/534 (91.8)	1		1	
>80	360/373 (96.5)	2.49 (1.32-4.69)		2.20 (1.10-4.42)	
HIV status disclosure to anyone n=894			0.946		
Yes	721/769 (93.8)	1			
No	117/125 (93.6)	0.97 (0.45-2.11)			
HIV status disclosure to current partner n=875			0.912		
Yes	454/482 (94.2)	1		-	
No	245/260 (94.2)	1.01 (0.53-1.92)			
Not applicable (No partner)	124/133 (93.2)	0.85 (0.39-1.85)			
Food insecurity n= 896			0.511		
Yes	532/571 (93.2)	1			
No	301/318 (94.7)	1.30 (0.72-2.33)		-	
Don’t know	6/7 (85.7)	0.44 (0.05-3.74)			

	Virological suppression n (%)	Crude odds ratio (95% CI)	P value	*Adjusted odds ratio (95% CI)	P value
ART will improve health n= 893			0.298		
Yes	810/863 (93.9)	1			
No	11/12 (91.7)	0.72 (0.09-5.68)		-	
Don't know	15/18 (83.3)	0.33 (0.09-1.17)			
Worried about side effects of ART n=887			0.926		
Yes	713/762 (93.6)	1			
No	37/39 (94.8)	1.27 (0.30-5.43)		-	
Don't know	81/86 (94.2)	1.11 (0.43-2.87)			
Agree that ART will reduce transmission n= 880			0.858		
Yes	635/680 (93.4)	1			
No	66/71 (93.0)	0.94 (0.36-2.44)		-	
Don't know	122/129 (94.6)	1.24 (0.54-2.80)			

	Virological suppression n (%)	Crude odds ratio (95% CI)	P value	*Adjusted odds ratio (95% CI)	P value
Psychological distress (PHQ4) n=879			0.272		
None	670/713 (94.0)	1			
Mild	135/145 (93.1)	0.87 (0.42-1.77)		-	
Moderate	12/12 (100.0)	-			
Severe	7/9 (77.8)	0.22 (0.05-1.11)			

*Multivariable model adjusted for age, sex, CD4 count at initiation, fixed dose combination ART, VAS adherence, baseline viral load, employment status and self-reported health status

6.6.1.3 Sensitivity analysis

I recalculated the proportion of individuals with virological suppression at six months in a sensitivity analysis that allowed those with missing viral loads at the six month time point to be replaced by carrying forward the last measured viral load closest to the lower bounds of the allowed window (three to nine months). 92% (903) of 982 individuals achieved virological suppression with this approach. I explored the merits of these two approaches by checking the proportion of viral loads that were actually close to six months by subtracting the date of ART initiation from the date of the viral load within the window representing the 6 month point. Table 6.4 shows that in the approach in which missing viral loads were replaced, 25% of individuals amongst those not suppressed did not have a viral load after starting ART and the mean number of days was further from the six months. Hence the complete case approach was presented as the main results.

Table 6.4 Sensitivity analysis of two approaches considered for estimating virological suppression

Complete case analysis N=908 75 missing viral loads dropped	**Missing viral loads replaced N=982***
Suppressed n=851	**Suppressed n=903**
Median 176 days (IQR 168-196)	Median 175 days (IQR 167-195)
Mean 181 days; SD 29.0	Mean 175 days; SD 38.5
Not suppressed n=57	**Not suppressed n=79**
Median 177 days (IQR 169-202)	Median 172 days (IQR 0-195)
Mean 186 days; SD 27.2	Mean 118 days; SD 124.5)

*One person did not have any viral load documented

The association between CD4 count, and adherence and virological suppression was repeated in a regression model that used pill count as the adherence measure (Appendix M). The findings were qualitatively similar to analysis using VAS adherence.

6.6.2 Objective 2

6.6.2.1 Cohort profile and characteristics of ART-experienced individuals at entry

Of the 1477 individuals who were on ART when transferring their care to the trial clinics, 1397 (94.6%) had been on ART for at least 6 months and were eligible for inclusion in the analysis (Figure 6.1). Of these, 496 (35.5%) individuals were excluded (four had missing viral load data at entry, 17 were on second-line ART and 475 had no CD4 documented at the time of ART initiation in the public health ART programme). Individuals with missing CD4 count at ART initiation were excluded because it was my main exposure of interest. Hence, 901 individuals contributed data to the analysis.

The median duration on ART was significantly higher in individuals with missing CD4 count at initiation than in those without missing CD4 count (missing vs. non-missing, 5.2 years (IQR 2.3-7.3) vs. 3.7 years (IQR 2.2-5.2), p<0.001) with 395/475 (83.2%) and 711/901 (78.9%) achieving virological suppression respectively (p=0.059). Missing CD4 count was more frequent in males than females (40.6% vs 32.4%, p =0.005). Individuals with missing CD4 count at initiation tended to be younger than those without missing CD4 count [median 35.6 years (IQR 28.6-44.4) vs. 37.2 years (IQR 28.9-47.4) p=0.06].

The characteristics of included participants are summarised in Table 6.5.The median age of the cohort was 37.3 years; (IQR 28.9, 47.4), three-quarters were female, 17.4% completed secondary school or higher education.The majority had never been married (78.5%) and 10.8% were employed. The median CD4 count at initiation was 176 cells/mm^3; (IQR 102, 260). The median duration on ART was 3.7 years; (IQR 2.2, 5.2). The median distance to a TasP clinic was 1.2 km compared to 5.3 km to a public ART clinic.

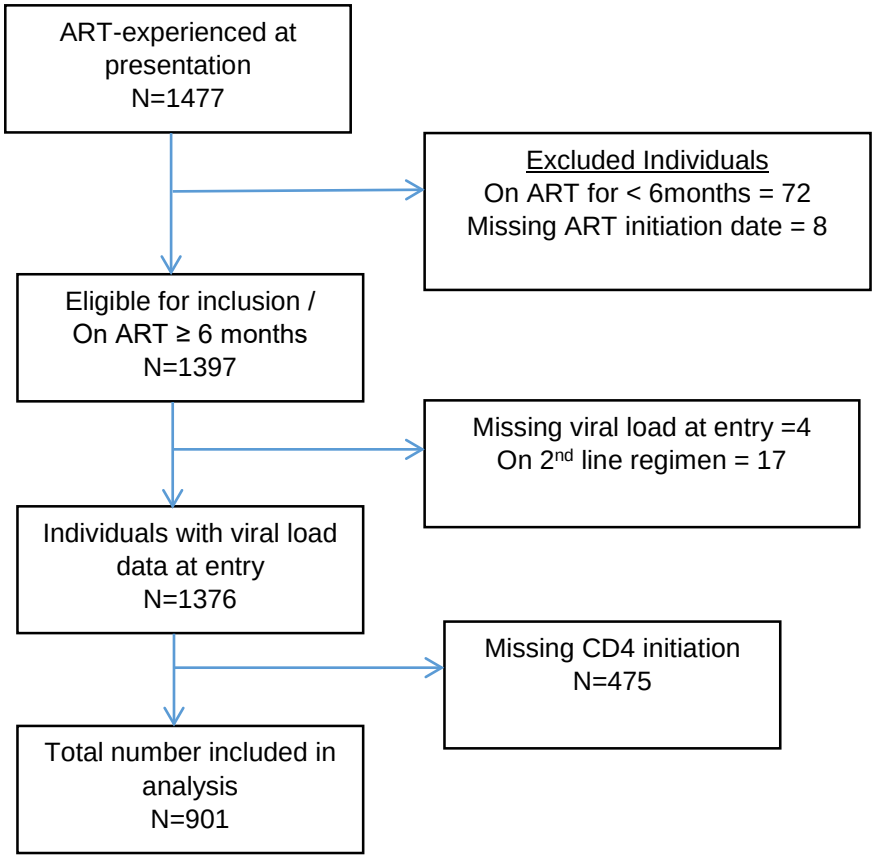


Figure 6.1Cohort profile of ART-experienced individuals included in the analysis of virological suppression at trial entry

Table 6.5 Characteristics of ART-experienced individuals included in the analysis of virological suppression at entry into the trial

Characteristics	N=901 (%)
CD4 at Initiation (cells/mm^3)	
Median CD4 at initiation (IQR)	176 (102-260)
<200	544 (60.4)
200-350	258 (28.6)
>350	99 (11.0)
First ever Regimen	
Non Fixed dose combination	847 (94.0)
Fixed dose combination (TDF + FTC + EFV)	54 (6.0)
Regimen at entry into TasP	
Non fixed dose combination	769 (85.4)
• ABC + 3TC +EFV	*3 (0.3)*
• AZT + 3TC + EFV	*29 (3.2)*
• AZT + 3TC + NVP	*5 (0.6)*
• D4T + 3TC + EFV	*172 (19.1)*
• D4T + 3TC + NVP	*47 (5.2)*
• TDF + 3TC + AZT	*1 (0.1)*
• TDF + 3TC + EFV	*490 (54.4)*
• TDF + 3TC + NVP	*22 (2.4)*
Fixed dose combination (TDF + FTC + EFV)	132 (14.7)
Duration on ART (years)	
Median duration on ART (IQR)	3.7 (2.2-5.2)
0.5-2.2	225 (25.0)
2.2 to 3.7	225 (25.0)
3.7 to 5.2	224 (24.9)
>5.2	227 (25.2)
First measured VL in Previous clinic, copies/mL	
<1000	602 (66.2)
≥1000	308 (33.9)

Characteristics	N=901 (%)
Age at initiation (Years)	
Median age (IQR)	37.3 (28.9-47.4)
16-29	262 (29.1)
30-39	257 (28.5)
40-49	212 (23.5)
>50	169 (18.8)
Missing	1 (0.1)
Sex	
Female	692 (76.8)
Male	209 (23.2)
Educational attainment	
Primary or less	507 (56.3)
Some Secondary	236 (26.2)
At least completed secondary	157 (17.4)
Missing	1 (0.1)
Marital status	
Never been married	707 (78.5)
Married	135 (15.0)
Divorced/Separated	57 (6.3)
Missing	2 (0.2)
Employment status	
Employed	97 (10.8)
Student	20 (2.2)
Unemployed	782 (86.8)
Missing	2 (0.2)
Self-reported health status	
Median (IQR)	75 (60-90)
≤75	485 (53.8)
>75	415 (46.1)
Missing	1 (0.1)

Characteristics	N=901 (%)
HIV status disclosure to current partner	
Yes	566 (62.8)
No	270 (30.0)
Not applicable (No partner)	23 (2.6)
Missing	42 (4.7)
HIV status disclosure to anyone	
Yes	871 (96.7)
No	15 (1.7)
Missing	15 (1.7)
Food insecurity	
Yes	630 (69.9)
No	252 (28.0)
Don’t know	7 (0.8)
Missing	12 (1.3)
ART will improve health	
Yes	875 (97.1)
No	3 (0.3)
Don’t know	7 (0.8)
Missing	16 (1.8)
Worried about side effects of ART	
Yes	837 (92.9)
No	20 (2.2)
Don’t know	23 (2.6)
Missing	21 (2.3)
Agree that ART will reduce infectiousness	
Yes	765 (84.9)
No	61 (6.8)
Don’t know	54 (6.0)
Missing	21 (2.3)

Characteristics	N=901 (%)
Psychological distress (PHQ4)	
None	691 (76.7)
Mild	164 (18.2)
Moderate	14 (1.6)
Severe	6 (0.7)
Missing	26 (2.9)
Distance from home to trial clinic (Km)/unit	
Median distance (IQR)	1.2 (0.7-1.9)
≤1.2	442 (49.1)
>1.2	459 (50.9)
Distance from home to nearest public clinic (km)	
Median distance (IQR)	5.3 (4.2-6.6)
0.2 to 4.2	224 (24.9)
4.2 to 5.3	224 (24.9)
5.3 to 6.6	227 (25.2)
>6.6	226 (25.1)

6.6.2.2 CD4 count at initiation and virological suppression in individuals ART-experienced at trial entry

In the univariable regression including the 901 ART-experienced individuals with viral load data at trial entry (Table 6.6), high CD4 count at initiation was associated with increased probability of virological suppression (VL<400 copies/mL) [OR 1.38, 95% CI 1.19-1.58 for every 100 cells/mm3 increase, p<0.0001]. Older age was associated with increased virological suppression (OR 1.26 (95% CI 1.17-1.36 for every 5 years increase). Being divorced or married compared to being single was also associated with an increased probability of virological suppression, (OR 4.26, 95% CI 1.52-11.94 and OR 3.02, 95% CI 1.66-5.48 for being divorced and married respectively, vs. being single).

Factors associated with a decreased probability of virological suppression at trial entry were being male, OR 0.49 (95% CI 0.34-0.69), completed secondary or higher education vs. primary or less, OR 0.42 (95% CI 0.28-0.64). Other factors significantly associated with a decreased probability of virological suppression were not disclosing HIV status to anyone, not being worried about side-effects. There is a weak evidence that psychological distress is associated with decreased probability of virological suppression.

In multivariable regression, I adjusted for CD4 at initiation, age, sex, educational attainment, marital status, HIV status disclosure to anyone, being worried about side effects and PHQ-4. Factors independently associated with an increased probability of virological suppression at trial entry were CD4 cell count at initiation (aOR 1.33, 95% CI 1.14-1.55) and age (aOR 1.33, 95% CI 1.19-1.48). Male sex was independently associated with a decreased probability of virological suppression (aOR 0.31, 95%CI 0.21-0.48), so was not disclosing HIV status to anyone (aOR 0.20, 95% CI 0.06-0.73) and not being worried about side-effects (aOR 0.19, 95% CI 0.07-0.52). The association between marital status and virological suppression was no longer significant after adjustment.

Table 6.6 CD4 count at initiation and other factors associated with virological suppression in ART-experienced patients at trial entry

	Virological suppression n (%)	Crude odds ratio (95% CI)	P value	*Adjusted odds ratio (95% CI)	P value
Clinical characteristics					
CD4 at Initiation (cells/mm^3) n= 901					
CD4 at initiation/100 units					
≤200	407/544 (74.8)				
200-350	214/258 (83.0)	1.38 (1.19-1.58)	<0.0001	1.33 (1.14-1.55)	0.0001
>350	90/99 (90.9)				
Regimen at first trial clinic visit n = 901			0.670		
Non Fixed dose combination	605/769 (78.7)	1		-	
Fixed dose combination (Atripla)	106/132 (80.3)	1.11 (0.70-1.75)			
Duration on ART (years) n=901			0.199		
0.5-2.2	182/225 (80.9)	1			
2.2 to 3.7	176/225 (78.2)	0.85 (0.54-1.34)			
3.7 to 5.2	184/224 (82.1)	1.09 (0.67-1.75)		-	
>5.2	169/227 (74.5)	0.69 (0.44-1.08)			

	Virological suppression n (%)	Crude odds ratio (95% CI)	P value	*Adjusted odds ratio (95% CI)	P value
Age at initiation (Years) **n=900**					
Age/5 units					
16-29	180/262 (68.7)				
30-39	192/257 (74.7)	1.26 (1.17-1.36)	<0.0001	1.33 (1.19-1.48)	<0.0001
40-49	186/212 (87.7)				
>50	152/169 (89.9)				
Sex n= 901			0.0001		<0.0001
Female	567/692 (81.9)	1		1	
Male	144/209 (68.9)	0.49 (0.34-0.69)		0.31 (0.21-0.48)	
Educational attainment n = 900			0.0002		0.09
Primary or less	421/507 (83.0)	1		1	
Some Secondary	183/236 (77.5)	0.71 (0.48-1.04)		1.25 (0.78-2.00)	
At least completed secondary	106/157 (67.5)	0.42 (0.28-0.64)		0.71 (0.43-1.19)	

	Virological suppression n (%)	Crude odds ratio (95% CI)	P value	*Adjusted odds ratio (95% CI)	P value
Marital status n= 899			<0.0001		0.06
Never married	535/707 (75.7)	1		1	
Married	122/135 (90.4)	3.02 (1.66-5.48)		1.86 (0.92-3.74)	
Divorced/Separated	53/57 (93.0)	4.26 (1.52-11.94)		3.17 (0.73-13.78)	
Employment status n = 899			0.243		
Employed	80/97 (82.5)	1			
Student	13/20 (65.0)	0.39 (0.14-1.14)		-	
Unemployed	616/782 (78.8)	0.79 (0.45-1.37)			
Distance, (km) n=901					
Home to nearest public clinic/km increase			0.190		
0.2 to 4.2	169/224 (75.5)	1			
4.2 to 5.3	182/224 (81.3)	1.41 (0.90-2.22)		-	
5.3 to 6.6	187/227 (82.4)	1.52 (0.96-2.40)			
>6.6	173/226 (76.6)	1.06 (0.69-1.64)			
Other characteristics					

	Virological suppression n (%)	Crude odds ratio (95% CI)	P value	*Adjusted odds ratio (95% CI)	P value
Self-reported health status n= 900			0.920		
≤75	382/485 (78.8)	1		-	
>75	328/415 (79.0)	1.02 (0.74-1.40)			
HIV status disclosure to anyone n=886			0.025		0.017
Yes	693/871 (79.6)	1		1	
No	8/15 (53.3)	0.29 (0.11-0.82)		0.20 (0.06-0.73)	
Food insecurity n = 889			0.875		
Yes	499/630 (79.2)	1			
No	198/252 (78.6)	0.96 (0.67-1.38)		-	
Don’t know	5/7 (71.4)	0.66 (0.13-3.42)			
ART will improve health n= 885			0.191		
Yes	693/875 (79.2)	1			
No	3/3 (100.0)	-		-	
Don’t know	5/7 (57.1)	0.35 (0.08-1.58)			

	Virological suppression n (%)	Crude odds ratio (95% CI)	P value	*Adjusted odds ratio (95% CI)	P value
#Worried about side effects of ART n = 880			0.023		0.007
Yes	671/837 (80.2)	1		1	
No	11/20 (55.0)	0.30 (0.12-0.74)		0.19 (0.07-0.52)	
Don't know	16/23 (69.6)	0.57 (0.23-1.40)		0.75 (0.23-2.45)	
Agree that ART will reduce transmission n=880			0.342		
Yes	612/765 (80.0)	1			
No	45/61 (73.8)	0.70 (0.39-1.28)		-	
Don't know	40/54 (74.1)	0.71 (0.38-1.35)		-	
#Psychological distress (PHQ4) n = 875			0.108		0.288
None	558/691 (80.8)	1		1	
Mild	119/164 (72.6)	0.63 (0.43-0.93)		0.70 (0.45-1.08)	
Moderate	10/14 (71.4)	0.60 (0.18-1.93)		0.43 (0.11-4.48)	
Severe	4/6 (66.7)	0.48 (0.09-2.63)		0.68 (0.10-4.48)	

*Multivariable model adjusted for CD4 at initiation, age, sex, educational attainment, marital status and HIV status disclosure to anyone, PHQ-4 and worried about side-effects .

6.6.3 Objective 3 Acquired resistance in both populations

6.6.3.1 Cohort profile and characteristics of individuals with acquired resistance

Of the 2,675 individuals initiated on ART, 2361 (88.3%) had been on first-line ART for ≥6 months and had viral load data available (Figure 6.2). Of these 217 (9.2%) met the criteria for virological failure (two consecutive VL ≥ 1000 copies/mL measured three months apart after 6 months on ART). 132 individuals with virological failure and three individuals who did not meet the criteria for virological failure had genotypic resistance tests.

Of the 135 individuals with genotype sequence available, 12 (8.9%) initiated ART within the trial whilst the remaining were on ART prior to joining the trial (Table 6.7). 89 (65.9%) were female. The median age was 32.6 years (IQR 26.4-39.5), similar between the two groups (p=0.783). The median CD4 count at initiation was 111 (IQR 60-193), similar in both groups (p=0.338). The group that was ART-experienced at entry had been on first-line ART significantly longer than those that initiated within the trial (median duration, 5.6 years vs. 1.3 yeas; p<0.0001) and have also been left on a failing ART regimen for longer (median 2.8 years vs.0.45 years, p=0.006). The majority of individuals were on tenofovir + lamivudine or emtricitabine backbone at the time of genotyping.

A switch to secondline ART occurred in 82/135 (60.7%) of individuals. 26 of the remaining 53 resuppressed (VL<1000) on the same first-line regimen. Of the 82 who switched to second-line ART, 67 (81.7%) achieved an HIV viral load < 400 copies/mL. Overall, 93/135 (68.9%) regained virological control. Of the 27 that did not switch to secondline ART, 9 were lost to follow up, 5 died and 5 transferred their care to a different clinic before they could be switched to secondline.

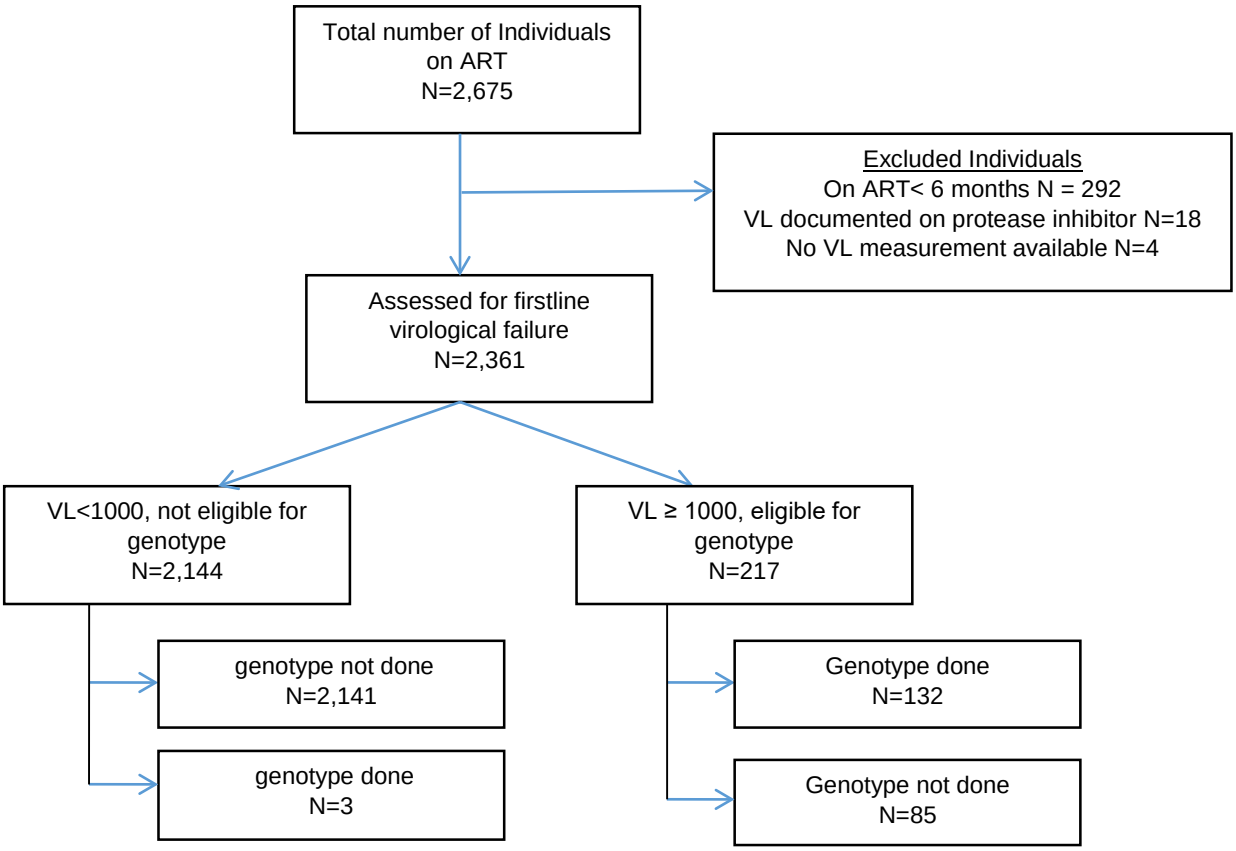


Figure 6.2 Profile of individuals with virological failure who underwent genotype testing

Table 6.7 Clinical and demographic characteristics of individuals who were genotyped

Characteristics	N	Initiated ART in DoH (ART experienced at entry) N = 123 (%)	N	Initiated ART within trial N=12 (%)	Total N=135
CD4 at Initiation (cells/mm^3)					
Median (IQR)	123	106 (60-191)	12	199 (80, 303)	111 (60-193)
≤350		88 (71.5)		10 (83.3)	98 (72.6)
>350		3 (2.4)		2 (16.7)	5 (3.7)
Missing		32 (26.0)		-	32 (23.7)
Viral load at time of genotype Log$_{10}$					
Median (IQR) years	123	4.3 (3.6-4.9)	12	4.2 (3.8-5.0)	4.3 (3.6-4.9)
ART duration on First-line					
Median (IQR) years	123	5.63 (3.71-7.10)	12	1.31 (0.92-1.80)	5.42 (2.94-7.01)
***Time on failing regimen**					
Median (IQR) years	100	2.84 (0.87-5.24)	9	0.45 (0.0-1.25)	2.42 (0.77-5.04)
Regimen at time of genotype	123		12		
ABC+3TC+EFV	123	1 (0.8)	12	-	1 (0.7)
AZT+3TC+EFV		13 (10.6)		3 (25.0)	16 (11.9)
D4T+3TC+EFV		29 (23.6)		2 (16.7)	31 (23.0)
D4T+3TC+NVP		11 (8.9)		-	11 (8.2)
TDF+3TC+EFV		27 (22.0)		-	27 (20.0)
TDF+3TC+NVP		3 (2.4)		-	3 (2.2)
TDF+FTC+EFV		39 (31.7)		7 (58.3)	46 (34.1)
Previous ART substitution					
NRTI substitution	123	46 (37.4)	12	1(8.3)	47 (34.8)
NNRTI substitution	123	17 (13.8)	12	-	17 (12.6)
Switched to second-line after genotype	123		12		

Characteristics	N	Initiated ART in DoH (ART experienced at entry) N = 123 (%)	N	Initiated ART within trial N=12 (%)	Total N=135
No		46 (37.4)		7 (58.3)	53 (39.3)
Yes		77 (62.6)		5 (41.7)	82 (60.7)
Age at initiation (Years)					
Median age (IQR)	123	32.6 (26.4-39.0)	12	33.1 (28.5-43.6)	32.6 (26.4-39.5)
Sex	123		12		
Female		84 (68.3)		5 (41.7)	89 (65.9)
Male		39 (31.7)		7 (58.3)	46 (34.1)

*Time on failing regimen is defined as the interval between the date of first viral load >1000 copies following 6 months on ART until switch to second-line ART

6.6.3.2 Description of acquired resistance mutations

One hundred and nineteen (88.2%) individuals had at least one drug resistance mutation at the time of genotype testing. The frequency of any NNRTI and NRTI mutations was 85.9% (116/135) and 80.0% (108/135) respectively (Figures 6.3 and 6.4). 18 (13.3%) individuals had three or more NNRTI mutations while 39 (28.9%) had three or more NRTI nutations

The most frequent mutation was the M184V/I (76.3%) associated with the use of lamivudine/emtricitabine followed by the K103N/S mutation (56.3%) which causes high level resistance to nevirapine and efavirenz. 105 (77.8%) individuals had mutations belonging to both NRTI and NNRTI drug classes; 58.3% in individuals who initiated ART within the trial, whilst 79.7% was in individuals ART-experienced at entry.

One individual had two of three thymidine analogue mutations (TAM) 1 pathway comprising M41L and T215Y. TAM 1 mutations cause high level resistance to zidovudine and stavudine and also results in reduced susceptibility to didanosine, abacavir and tenofovir. This individual also had two additional mutations; M184V associated with reduced lamivudine/emtricitabine susceptibility and V106M, which is associated with reduced susceptibility to efavirenz and nevirapine

Five individuals had all three thymidine analogue mutations 2 pathway, also arising from exposure to zidovudine and stavudine comprising D67N, K70R and K219Q/E and decrease their susceptibility but cause less cross resistance to tenofovir. All five individuals had the M184V mutation and NNRTI-associated mutations (three had K103N and two Y188 L) and one of them had the T215F, a thymidine analogue mutation.

The K65R mutation was present in the samples of 35 (25.9%) individuals and is known to cause high level resistance to tenofovir. This was present in 30/78 (38.5%) of individuals exposed to tenofovir compared to 5/57(8.8%) with no prior or current tenofovir exposure.

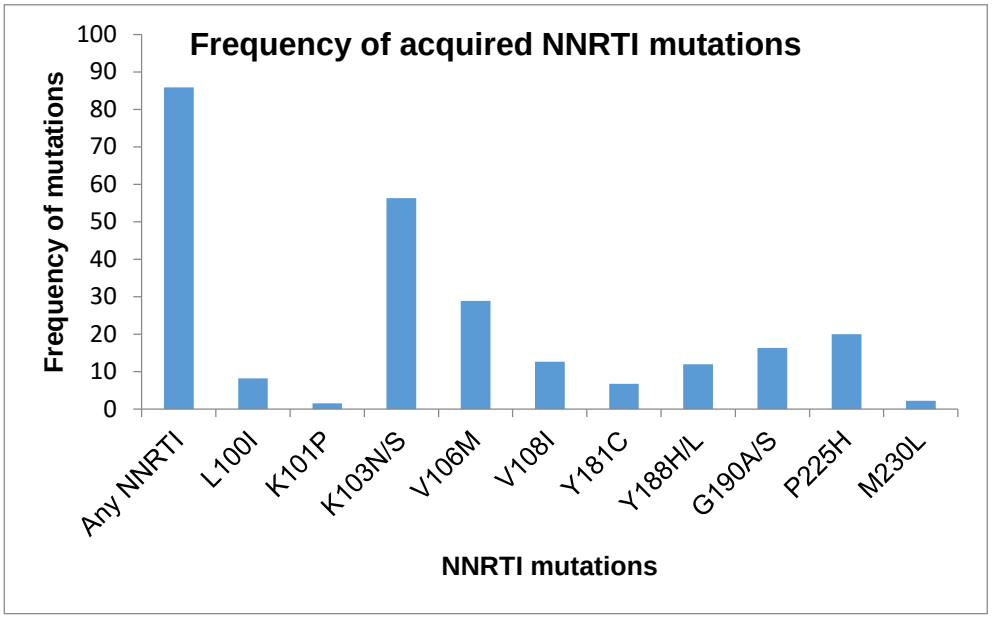


Figure 6.3 Frequency of acquired NNRTI mutations

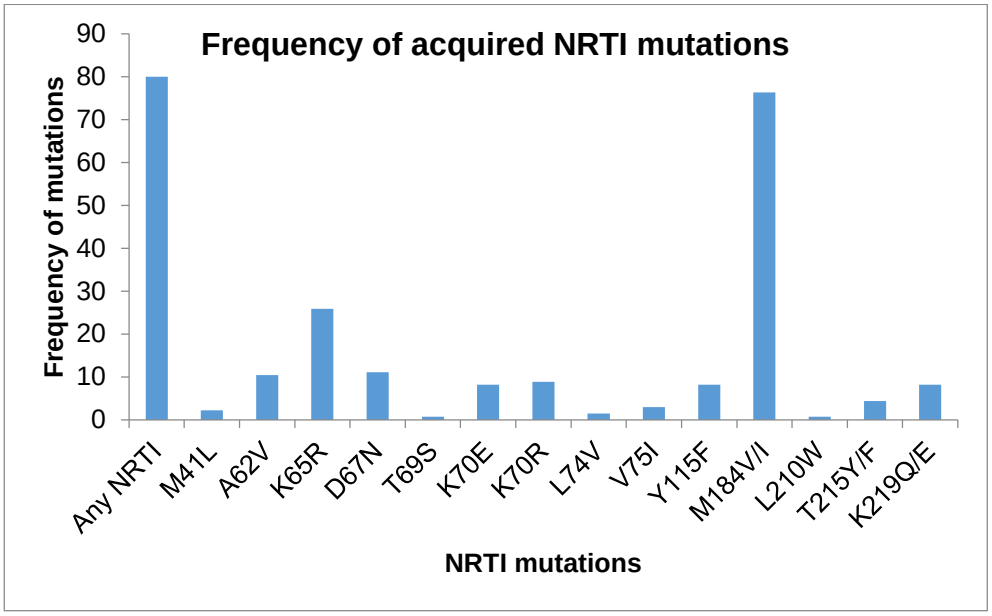


Figure 6.4 Frequency of acquired NRTI mutations

The median plasma viral load at virological failure was similar in individuals with and without the K65R mutation [35,504 copies/mL (IQR 4961-109,278) vs. 19,699 copies/mL (IQR 3,969-80,064); p=0.959].

Of the 26 individuals that resuppressed their HIV viral load on their first-line ART, 16 (61.5%) had at least one drug resistant mutation. 12 of the 16 had drug resistant mutations belonging to both the NRTI and NNRTI drug classes.

Eleven patients had a combination of TAMs with or without the K65R mutation (Table 6.8). Patients 1-5 all had the K65R mutation and only one TAM. The K65R reduces susceptibility to tenofovir while multiple TAMs are required to compromise zidovudine susceptibility. These patients would be expected to respond to a protease inhibitor based second-line ART with a zidovudine backbone. Patients 6-11 had multiple TAMs with no K65R mutation with Patient 9 having up to 4 TAMs (D67N, K70R, T215F, K219E). The Stanford algorithm interpretes these four TAMs as causing intermediate level resistance to tenofovir and high level resistance to zidovudine, hence the impact on second-line ART would be moderate to high as the protease inhibitor would be the only fully active drug in the combination for Patient 9. The impact of the TAMs on tenofovir for Patients 6,7,8,10 and 11 is described by the Stanford algorithm as low level resistance as there were three or fewer TAMs detected and except for patient 6 mostly belonged to TAM 2 pathway which cause less cross resistance to tenofovir.

Of the 12 participants who initiated ART within the trial, nine of them were assessed for pre-treatment drug resistance, three of whom had pre-treatment drug resistance mutations (Table 6.9). In patient 12, the K65R and Y181C mutations which were present pre-treatment in majority virus were absent after virological failure, despite a regimen containing tenofovir and efavirenz respectively. In patient 13, the L100I was present in minority virus and was not detected in majority virus after virological failure. Patient 13 had accrued an additional mutation (M184V) following virological failure. Patients 13 and 14, resuppressed on their first-line regimen without being switched to second-line ART.

Table 6.8 Thymidine analogue mutations with or without tenofovir resistance, including NNRTI mutations

Id	Mutations	Impact on first-line drugs				Expected impact on second-line ART
		ZDV	TDF	3TC/FTC	NVP/EFV	
1	M41L, K65R, K103N, V106M	S	HLR	S	HLR	None
2	M41L K65R, M184V, K103N, V106M	S	ILR	HLR	HLR	None
3	K65R, K70R, M184V, L100I, Y188L	S	ILR	HLR	HLR	None
4	K65R, D67N, V106M, Y181C	S	HLR	S	HLR	None
5	K65R, K219Q, M184V, L100I, M230L	S	ILR	HLR	HLR	None
6	M41L, T215Y, M184V, V106M	ILR	LLR	HLR	HLR	Minimal
7	D67N, K70R, K103N, M184V, K219Q, P225H	ILR	LLR	HLR	HLR	Minimal
8	D67N, K70R, K103N, V108I, M184V, K219E, P225H	IRL	LLR	HLR	HLR	Minimal
9	D67N, K70R, M184V, Y188L, T215F, K219E	HLR	ILR	HLR	HLR	Moderate to High
10	D67N, K70R, M184V, Y188L, K219Q	ILR	LLR	HLR	HLR	Minimal
11	D67N, K70R, K103N, M184V, K219E, P225H	ILR	LLR	HLR	HLR	Minimal

Id = Patient Identity, S = sensitive, HLR = high level resistance, ILR = intermediate level resistance, LLR = Low level resistance, ZDV = Zidovudine, TDF = Tenofovir, 3TC= Lamivudine, FTC = emtricitabine, NVP = Nevirapine, EFV = Efavirenz

Table 6.9 Drug resistance mutations present pre-treatment in participants who initiated ART within trial and developed acquired resistance

Id	Pre-treatment drug resistance	Acquired resistance	Sequence regimen	Resuppressed
12	K65R, M184V, Y181C, Y188L	M184V, Y188L	TDF, FTC, EFV	No
13	L100I (minority), V106M	V106M	ZDV, 3TC, EFV	Yes
14	K103N	M184V, K103N	TDF, FTC, EFV	Yes

6.7 Discussion

Firstly, I examined virological suppression at six and 12 months in individuals who initiated ART within the TasP trial and examined risk factors for virological suppression 6 months post-ART initiation. Secondly, I quantified virological suppression at the first clinic visit in individuals who were already ART-experienced at the time of enrolment and examined risk factors for virological suppression. Finally, I described acquired resistance at virological failure in the combined group, implications for transmission of the K65R mutation and potential impact of TAMs with or without K65R mutation on response to second-line ART.

I found high rates of virological suppression of 94% and 97% at six and 12 months respectively in individuals who initiated ART within the trial. Virological suppression rate at the first clinic visit was modest in individuals already established on ART at 79% after a median ART duration of 3.7 years. Amongst individuals who fulfilled the criteria for virological failure and who had genotype results available, 88% had at least one drug resistance mutation.

I restricted the risk factor analysis amongst individuals ART naïve at trial entry to the 6 month time-point as nearly all individuals achieved virological suppression at 12 months. The levels of virological suppression achieved of >90% exceeds the target for optimal programme performance suggested by WHO as part of the early warning indicators for HIV drug resistance (32).

In individuals who initiated ART within the trial, a higher CD4 count at initiation was associated with a significantly increased likelihood of virological suppression six months post-ART initiation. Good adherence was independently associated with increased likelihood of virological suppression even after adjusting for CD4 count at initiation. Adjusting for adherence in the association between CD4 count at initiation and virological suppression only slight attenuated the effect of CD4 count (3% difference in effect estimate). Results presented in Chapter 5 showed a lack of significant association between CD4 count at initiation and adherence measured by either VAS or PC. The question then arises as to why here an increased

virological suppression with increasing CD4 count at initiation was observed? This effect of CD4 count on virological suppression was similar even for individuals within the same adherence stratum, pointing to a biological effect due to level of CD4 counts rather than a behavioural effect. I could speculate that the viral quasi-species are less diverse in individuals with higher CD4 count as they are at an earlier stage of HIV infection, making the virus more susceptible to antiretroviral therapy. It is also possible that a relatively intact immune system could be synergistic with ART to achieve virological suppression.

I found no study in the African setting that examined the risk factors for virological suppression in individuals initiating ART at CD4 counts >350 cells/mm^3. The superior virological suppression in the short-term at higher CD4 at initiation is in agreement with one study conducted in a high-income setting that showed better virological suppression at higher CD4 counts (243). However, a review by Anglemyer et al (275) covering the period 1 Jan 1996 to 24 Aug 2012 found that the combined effect of early treatment on viral suppression amongst observational studies and one included randomised controlled trial was not statistically significant.

Being on a fixed-dose combination of tenofovir, emtricitabine and efavirenz was associated with increased virological suppression. This association is independent of the positive effect on adherence due to the convenient dosing of one tablet taken once day but likely due to the better tolerability of the combination compared to zidovudine based first-line ART (258). Having a high self-reported health status was also associated with increased likelihood of virological suppression corroborating other studies with similar findings (259). A dose-response relationship of decreased virological suppression with increasing viral load was also observed. An association between poor virological outcomes and high baseline viral load has also been reported in other studies (260-262).

The virological suppression of nearly 80% seen in those who were established on ART prior to the first clinic visit was similar in all quartiles of ART duration

examined. This falls short of the WHO target for optimal programme performance and further highlights the difference in level of care in a trial setting compared to a real life public health ART programme. In these individuals, higher CD4 count at initiation was associated with increased virological suppression as was seen in analysis of those who initiated ART within the trial. Older age was independently associated with increased virological suppression in line with findings elsewhere (176, 277-279). This was often attributed to better adherence in older individuals, however, I could not adjust for adherence in this analysis as there were no adherence data available at the time of transfer to the trial. Male sex was associated with a decreased likelihood of virological suppression, unlike in those who initiated ART within the trial. One notable difference to point out is that being on a fixed dose combination was not significantly associated with virological suppression in this group in contrast to what was seen in indiviudals who initiated ART within the trial. The majority of these individuals were initially on separate tablet regimens and were later switched to single tablet regimen by their primary care providers in line with changing South African treatment guidelines. Some of those switched could have been failing treatment at the time of switching even if this was not detected and not acted upon; very few people (<1%) had been switched to second-line therapy despite a virological suppression of 80%.

Reluctance to switch to second-line treatment may be linked to the fact that the South African public ART programme is predominantly nurse-led and nurses may not be sufficiently confident to switch patients to second-line ART due to inadequate training. All patients, especially those who were noted not to be virologically suppressed, were counselled on adherence at each repeat clinic visit. These patients would normally wait for a doctor to decide on management concerning the viral load results from a previous visit. Staff shortages meant doctors were not always available to service the primary care clinics (263). Patients who remain on failing regimen are likely to accumulate drug resistant mutations (264-266) which can be transmitted to sexual partners. A study in the same sub-district relating to the public HIV treatment programme showed that patients

remained on a failing regimen for a median duration of 27 months (14). Concern has been expressed that earlier initiation of ART could increase the numbers of individuals on ART with virological failure potentially increasing the levels of acquired resistance and the transmission of drug resistant virus.

High levels of drug resistance were observed in individuals with virological failure with nearly 90% of individuals having at least one drug resistant mutation. The M184V/I mutation which confers resistance to lamivudine and emtricitabine was the most common mutation followed by the K103N/S mutation which confers high level resistance to nevirapine and efavirenz. This reflects the current first-line ART regimen. This result is similar to another study on acquired resistance using data from the public ART programme between December 2010 and March 2012 in the communities adjacent to the trial communities (27). The main difference was in the frequency of tenofovir resistance which was 6% in that study but 25% in my study. This reflects an increase in the use of tenofovir following changing guidelines with tenofovir now the preferred NRTI for first-line ART unless clinically indicated otherwise. Second-line regimen is based on protease inhibitor with zidovudine being the preferred NRTI backbone, with either lamivudine or emtricitabine. There is some concern about increasing tenofovir resistance compromising the public ART programme if transmitted to sexual partners. Currently, the South African treatment guidelines do not allow for assessment of drug resistance prior to initiation of first-line ART (15). However there is still debate about the transmissibility of the K65R mutation with some studies reporting this mutation to be less transmissible (209, 214). I found no evidence of a difference in the median plasma viral load of viruses with or without the K65R mutation suggesting that viruses harbouring this mutation could be as fit as wild type virus and potentially transmissible, although numbers were small. This corroborates results from a study on acquired resistance which also reported on the transmissibility of the K65R mutation (215).

The WHO recommends surveillance of acquired resistance as this can inform policy on second-line and third-line regimens (216). In this study, only one patient

with four thymidine analogue mutations and NNRTI mutations was at risk of initiating a compromised standard second-line regimen, suggesting that the current public health approach may be adequate. However, individuals who initiated ART within the government programme had spent a median of nearly three years on a failing regimen without being switched to second-line ART at the time they arrived in a trial clinic. This could lead to accumulation of drug-resistant mutations (267) (265) which could compromise second-line ART. The situation was different in individuals that initiated ART within the trial with time spent on a failing regimen being an average of six months before switch to second-line ART. The former scenario could be due to a number of weaknesses in the public ART programme with limited resources compared to a trial setting. Health system factors such as staff shortages, inadequate knowledge and training of staff, lack of adherence to guidelines with respect to viral load monitoring and acting on results and patient-related factors such as frequent disengagement and reengagement with care could have contributed to delay in switching to second-line ART.

Nearly one in five individuals genotyped resuppressed their HIV viral load on their first-line ART, despite nearly half of those who resuppressed having drug resistant mutations belonging to both the NRTI and NNRTI drug class. Three-quarters of these individuals with dual class resistance were on fixed dose combination of tenofovir, emtricitabine and efavirenz and the remaining one-quarter on a combination of zidovudine, lamivudine and efavirenz. Re-suppression of viraemia in the presence of drug resistance mutations has also been reported in other studies (268-270). This reinforces the importance of adherence counselling in individuals suspected to have virological failure as a proportion of these individuals would regain virological control. Treatment guidelines should be followed with prompt switch to second-line in those with confirmed virological failure.

Of the 12 individuals who initiated ART within the trial and who were assessed for acquired resistance, nine had their pre-treatment samples assessed for pre-treatment drug resistance. The three who had evidence of pre-treatment resistance were compared with the mutations observed at the time of virological failure. There

were more mutations present pre-treatment for patient 12 compared to those present at the time of failure. The K65R and Y181C were not present at virological failure despite drug selective pressure from tenofovir and efavirenz. These mutations were not expected to be archived due to ongoing drug pressure. A possible explanation for their absence could be due to the difference in sequencing techniques employed for the assessment of the presence of these mutations. Next generation sequencing of the full HIV genome was used to assess for pre-treatment drug resistance while Sanger sequencing was used for assessment of the Pol gene at virological failure. It is possible that next generation sequencing could be more sensitive than Sanger sequencing even when the drug resistant mutants are present in majority virus. Laboratory error could be an alternative explanation. Patient 13 resuppressed on the same regimen with a genotypic sensitivity score of 2 (two active drugs) whilst patient 14 resuppressed on a regimen with a genotypic sensitivity score of 1 (one active drug). This could be due to emtricitabine maintaining some residual activity in the presence of the M184V mutation and the hypersensitivity to tenofovir that arises as a result of this mutation (271).

This study has some limitations. In those who initiated ART within the trial, I was only able to examine the impact of CD4 count at initiation on virological suppression 6 months post-ART initiation. The high virological suppression rate at 12 months post-ART initiation did not allow for this analysis and would suggest that high CD4 count at ART initiation may not be detrimental. The short-follow up duration meant I could not assess durability of virological suppression and long-term clinical outcomes. I have only included variables with few missing observations in the multivariable model to allow for comparisons between the univariable and multivariable models. This meant the independent effects of exposures with missing values could not be estimated.

For individuals ART-experienced at trial entry, no adherence data were measured prior to enrolment in the trial, and this factor could thus not be assessed. Furthermore, the baseline viral load for this population representing the first

measured viral load prior to ART initiation was missing for the majority of these participants, as many had their first viral load measurements after initiation of ART and the association between baseline viral load and virological response could not be assessed. Further, I excluded participants with missing CD4 count at initiation in the complete case analysis presented here. I explored potential biases that may have arisen as a result of excluding individuals with missing data. However, a strength of this analysis was the ability to assess long-term virological suppression in individuals who were ART-experienced at their first trial clinic visit as this reflected real life setting,

To summarise, increased CD4 count at initiation was associated with increased virological suppression both in the short-term for ART-naïve individuals initiating ART within the trial as well as in the long-term in ART experienced individuals arriving in the trial. The concerns about poorer adherence and risk of poor virological outcomes in individuals initiating ART at high CD4 count may be unfounded. The majority of individuals developed resistance mutations at virological failure, but the data showed that about 20% of them re-suppressed on their first-line regimen with reinforcement of adherence. Adherence support for people failing ART should be properly managed so that individuals are not left on failing ART regimen for prolonged periods of time. About 70% of individuals with virological failure achieved a viral load <1000 copies/mL following either only adherence support or a switch to second-line ART. Although the virological suppression rates observed in ART-experienced patients at entry in the trial falls well below the target of >90% at 12 months recommended by WHO (32), results from those who initiated ART within the trial suggest that achieving very high virological suppression rates is possible with appropriate support in the form of adherence counselling, tracking of individuals who failed to attend clinic appointments as well as improved healthcare provider-patient ratios.

Following the results of two large randomised trials (16, 17), the WHO now recommends ART initiation regardless of CD4 count for individual benefit (105). The results of this research are reassuring but long-term follow up will be required

before firm conclusions can be reached on virological and clinical outcomes of initiating ART at high CD4 counts. The parameters presented here would be valuable for modelling and predicting long-term outcomes on ART.

Chapter 7 Pre-treatment HIV drug resistance and response to first-line antiretroviral therapy

This chapter describes the estimation of the the prevalence of pre-treatment drug resistance and examines associated factors in individuals who were ART-naïve at enrolment in the trial clinics as well as in individuals who seroconverted during the trial period regardless of whether enrolled in trial clinics or not. I also examined the association between pre-treatment drug resistance, CD4 count at initiation and virological suppression.

7.1 Background

In 2015, 12 of the 17 million people who had been initiated on ART globally were resident in sub-Saharan Africa (6). The unprecedented ART scale-up, in public health terms, has immense individual clinical health benefits in terms of reduction in morbidity and mortality with the added public health effect of an anticipated reduction in population HIV incidence (14, 16, 17). However, an important challenge associated with this rapid and broad scale-up is the possible emergence of resistance in individuals failing ART which could then result in transmission of drug resistant HIV to sexual partners. Transmitted drug resistance (TDR) could potentially compromise public health ART programmes using standardised regimens because individuals infected with resistant virus may experience early virological failure (272, 273). The extent of transmitted drug resistance will depend on a number of factors such as ART coverage in the population and duration of ART roll-out, number of individuals failing ART with drug resistance, duration on failing regimen and viral load of individuals failing with drug resistance, influenced by the fitness of the viral mutants (274). ART roll out in South Africa started in 2004 with parsimonious indications for initiating ART (275) but since September 2016 all HIV-positive individuals are eligible for ART immediately after HIV diagnosis (15)

following the adoption of the most recent WHO ART guidelines (105). A mathematical model by Blower predicted that transmitted drug resistance would only reach 5%, the lower threshold set by WHO for surveillance of transmitted drug resistance, after 10 years of ART roll-out or once >30% of all HIV-infected population had been initiated on ART (180, 181, 276). Both these conditions have now been satisfied in South Africa with ART coverage estimated at 49% of all HIV-positive (6). Further, another model predicts that although the number of new HIV infections will decrease with the expansion of ART, the proportion of new infections with transmitted drug resistance will increase substantially (29).

WHO recommends the monitoring of pre-treatment HIV drug resistance in adults initiating ART in order to inform decisions about optimal first and second-line ART in public health programmes (277).

WHO classifies the prevalence of transmitted drug resistance for each drug class as low (<5%), moderate (5-15%) and high (>15%) and recommends a review of a country's ART regimen when the prevalence of drug resistant mutation to a specific drug class exceeds 15% (28). This is higher than the threshold of 10% recommended as the threshold to trigger baseline resistance testing in high-income countries (278) where baseline resistance testing and at virological failure is standard ART guidelines (279-281).

Although there have been many studies estimating the prevalence of transmitted/pre-treatment drug resistance in Africa, very few studies of the impact of the presence of this drug resistance mutations on virological outcomes have been published.

Therefore, I conducted a scoping review of the published literature on the prevalence of pre-treatment drug resistance, and its impact on virological outcomes in patients initiated on ART. Secondly, I quantified the prevalence of pre-treatment drug resistance in ART-naïve individuals and factors associated with the presence of pre-treatmentdrug resistance were identified. Further, I examined the

association between the presence of pre-treatment drug resistance, CD4 count at ART initiation and other factors with virological suppression.

7.2 Literature review

The aim of the literature review was to summarise published studies of the prevalence of pre-treatment drug resistance and their impact on virological outcomes in the African setting.

7.2.1 Prevalence of pre-treatment drug resistance and impact on virological outcomes

I searched Pubmed for studies that reported on prevalence of either transmitted drug resistance (TDR) or pre-treatment drug resistance (PDR), as well as those studies that additionally reported on their impact on virological ouctomes. The studies needed to have been published in Africa before 14 February 2017.

Search (((pretreatment) OR pre-treatment) OR transmitted) OR primary (1,561,680)

Search HIV (313,095)

Search Resistance (711,925)

Search Africa

Search ((ART) OR Antiretroviral) OR anti-retroviral (143,321)

Combining all search criteria above using the Boolean operator 'AND'
Search (((((((((pretreatment) OR pre-treatment) OR transmitted) OR primary)) AND HIV) AND Resistance) AND Africa)) AND (((ART) OR Antiretroviral) OR anti-retroviral)- 237

I assessed the titles of all 237 articles and excluded 154 articles not related to the topic of interest. I read the abstract of the remaining 83 articles and selected 74

articles for full review. Of these 74 articles, a further 10 were excluded after reading the full articles. Two of the excluded studies were either reviews or metanalysis (282, 283), one was a mathematical model (284), one was a commentary on a published study (285), one evaluated resistance in children (286), one was on WHO early warning indicators (287), one was on acquired resistance (250), one was a multicountry sub-study of the START trial with no data on the African cohorts (288), one pooled sequence data from other published studies (289) and in one, the numerators were not very clear (290). I summarised the remaining 64 published articles by studies that reported both on prevalence and impact of pre-treatment drug resistance on virological outcomes (6 studies) (272, 291-295) (Table 7.1) and studies that reported only on prevalence of pre-treatment drug resistance (58 studies) (206, 296-352) summarised in appendix N

7.3 Discussion of literature review

Different methods were used to estimate prevalence of transmitted drug/pre-treatment drug resistance in studies included in this literature review. In appendix L, I used the term transmitted resistance (TDR) to refer to studies that described steps taken to exclude individuals that might have had previous exposure to ART. Most of these studies applied the WHO threshold survey method of sequential sampling to estimate prevalence (28). In the threshold survey method, each survey required no more than 47 samples from individuals consecutively diagnosed with HIV (laboratory confirmation), <25 years old and if female, must not have had a previous pregnancy (because they could then have been exposed to ART for the prevention of mother-to-child transmission). Due to the small number of samples required, the threshold survey method does not allow for precise estimates of prevalence with 95% confidence intervals but allows for categorisation of prevalence of transmitted resistance above or below agreed thresholds using this sample size. With this threshold survey, if the first consecutive 34 samples showed no resistant mutation, then the survey is stopped and prevalence is classified as

<5% for all drug classes. If a resistant mutation is detected, then sampling continues to the maximum 47 samples required.

These thresholds are applied to categorise transmitted resistance to each drug class. Threshold surveillance has been superseded by the monitoring of pre-treatment drug resistance in ART naïve individuals (216) which approach recognises that individuals presenting to healthcare facilities as ART naïve may have had previous exposure to ART. In further discussion here, I will use the term pre-treatment drug resistance to refer to all forms of resistance in individuals who are presumed to be ART-naïve.

In all but two surveys, prevalence of pre-treatment drug resistance was below 15% overall, and to each of the three drug classes. In the two surveys in which prevalence exceeded 15%, one was a survey from Angola (299) with an overall prevalence of pre-treatment resistance of 16.3%, however the prevalence to each of the three drug classes remained below 15%. The other study was from Cameroon (308) but this study had a very small sample size of 21 individuals. In most surveys, the prevalence of pre-treatment resistance to the NNRTI drug class was highest, followed by the NRTI drug class resistance; resistance to PI drugs was lowest. The very low level of resistance to the PI drug class reflects the fact that second-line ART was introduced only relatively recently in these settings.
A recent survey of pre-treatment drug resistance conducted between March 2013 and October 2014 involving all nine provinces in South Africa showed a moderate level of pre-treatment resistance of 9% (95% CI 6.1-13.0) by Sanger sequencing of the HIV *Pol* genes (347). The majority of transmitted resistance mutation belonged to the NNRTI drug class (8.3%) with the K103N mutation being the most common (5.8%).

Table 7.1Published literature on the prevalence of pre-treatment drug resistance and the impact on virological outcomes

Author	Study setting	Study population/Sample size	Study design	Year of sampling	Outcome	Prevalence/effect estimate
Boender, TS (272)	13 clinical sites (PASER-M) in Kenya, Nigeria, South Africa, Uganda, Zambia & Zimbabwe	2579 ART naïve HIV positive individuals initiating ART	Prospective cohort	2007-2009	Proportion with PDR Impact of PDR on switching from 1st to 2nd line for presumed ART failure	5.5% had PDR PDR associated with switch to 2nd line adjusted hazard ratio (aHR) 3.80 (95% CI 1.49-9.68)
Chung, M (291)	Nairobi,Kenya	386 ART naïve individuals starting ART in a randomised trial of adherence. 356 initiated ART	Samples from a randomised trial Fixed dose combination of D4T/3TC/NVP	2006	Proportion with PDR using Oligonucleotide ligation assay for point mutations of NNRTI ((K103N, Y181C and G190A) and 3TC (M184V)	15/386 (3.9%) with PDR pre-ART. 13/356 (3.7%) with PDR in those who initiated ART. PDR associated with virological failure, Rate ratio 10.39 (95% CI, 3.23-32.41)
Hamers, R (292)	13 clinical sites in Kenya, Nigeria,, South Africa, Uganda, Zambia, and Zimbabwe (PASER-M)	2579 ART naïve HIV positive individuals initiating ART	Prospective cohort	March 2007 to September 2009	Proportion with PDR; Risk factors for virological failure (VL≥400 copies/mL)	175/2579 (7%) with PDR; 213/2115 (10%) had virological failure at 12 months on ART: PDR & fully active drug vs. no PDR; aOR 1.01 (95% CI 0.55-1.87);

Author	Study setting	Study population/Sample size	Study design	Year of sampling	Outcome	Prevalence/effect estimate
						PDR & partially active drug vs. no PDR aOR 2.13 (95% CI 1.44-3.14)
Lee, G (293)	Kampala and Mbarara, Uganda	81 HIV positive ART naïve from Kampala; 491 from Mbarara	Prospective cohort D4T/3TC/NVP	Kampala March 2002 to December 2004 Mbarara 2005 to 2010	Proportion with TDR	**Kampala**: 6/81 (7%) NRTI 3/81 (3.7%) NNRTI 1/81 (1.2%) PI 1/81 (1.2%) **Mbarara:** 15/491 (3%) NRTI 4/491 (0.8%) NNRTI 9/491 (1.8% PI: None Time to virological suppression (VL≤400 copies/mL) was similar in Kampala patients with and without PDR (97 days vs. 90 days; p=0.3) & for Mbarara patients PDR vs. No PDR (89 days vs. 85 days, p=0.05)
Mzingwane, M (294)	Pretoria, South Africa	65 HIV-positive individuals who initiated ART and had at least one follow up viral load	Cohort study	July 2013 and May 2014	Minority mutations on risk of virological failure (VL>1000 copies/mL after 6 months on ART)	8/65 (12.3%) with VF. Deep sequencing to 1% in 4 individuals with failure, 3 with VS and 1 with low level viraemia. All 8 individuals harboured mutations compromising EFV. No difference in mutation

Author	Study setting	Study population/Sample size	Study design	Year of sampling	Outcome	Prevalence/effect estimate
						frequency between the 4 with VF and 4 without VF. Active to both TDF/FTC
Rusine, J (295)	Kigali, Rwanda	158 HIV positive individuals who initiated ART with 12 months VL available. 109 with baseline resistance test	Nested within a prospective study	November 2007 and January 2010	Proportion with PDR. Risk factors for virological failure at 12 months	4/109 (3.6%) No impact of PDR on risk of failure but very few had PDR

The most recent published meta-analysis of studies of pre-treatment drug resistance was excluded from the literature review to avoid duplication of included studies (283) This meta-analysis comprised studies with at least a sample size of 25 HIV positive individuals published between 1 March 2000 and 31 December 2013. There was no regional restriction. The sub-Saharan African region comprised 95 studies from 32 countries with 11,536 individuals. The overall median prevalence pre-treatment resistance in sub-Saharan Africa was 2.8% (IQR CI 1.3%-5.6%); NRTI 0% (IQR 0%-2.4%), NNRTI 1.4% (IQR 0%-2.8%) and PI 0% (IQR 0%-1.4%). The meta-analysis showed that the odds of overall prevalence of pre-treatment resistance increases estimates by 1.09-fold (95% CI: 1.05–1.14) per year.

Six studies from the literature review summarised in Table 7.1 examined the impact of pre-treatment drug resistance on virological outcomes. These studies are heterogeneous and varied in size from 65 (294) to 2579 individuals (272, 292). The study by Hamers et al (292) and Boender et al (272) relate to the same cohort, used the same sample size and addressed outcomes which were only slightly different. In Hamers et al, the outcome was virological failure at 12 months whilst the Boender et al, the outcome was time to treatment switch to second-line ART for presumed failure. In these studies, genotypic drug resistance was defined using the International Antiviral Society USA mutation list of December, 2010.The study by Hamers and colleagues, reported pre-treatment drug resistance to at least one prescribed drug vs. no pre-treatment resistance was associated with virological failure (VL ≤400 copies/mL) after 12 months on ART (OR 2.13, 95% CI 1. 44–3. 14). Pre-treatment drug resistance on fully active drug compared to not having pre-resistance was not associated with virological failure, OR (1. 01, 95% CI: 0.55–1.87). A third of the participants in this study were on tenofovir-containing regimen combined with either lamivudine or emtricitabine and the third agent was either nevirapine or efavirenz. Nearly all the remaining individuals were either on a zidovudine or stavudine backbone.

The other study by Chung et al (291) in Kenya reported pre-treatment drug resistance in 15 (3.9%) of 386 assessed. Oligonucleotide assay was used to test the pre-treatment samples for point mutations that confer resistance to NNRTI (K103N, Y181C, G190A) and 3TC (M184V). 356 were initiated on a fixed dose combination stavudine, lamivudine and nevirapine within an adherence trial. They retrospectively examined the impact of transmitted NNRTI mutations on virological failure (VL ≥1,000) in individuals who completed 18 months of follow up. Among the 356 individuals who started ART, 51 (14.3%) developed virological failure, and 13 (3.65%) had pre-treatment drug resistance. In an multivariable cox regression model that adjusted for age, baseline viral load, adherence, employment and randomization assignment, pre-treatment drug resistance was independently associated with the risk of virological failure, HR 10.39 (95% CI, 3.23-32.41).

In the study by Rusine et al (295) in Kigali, Rwanda, 158 HIV-positive individuals who initiated ART as part of a larger prospective trial were evaluated for virological failure at 12 months. Genotypic resistance testing was done retrospectively on participants that had baseline and month 12 samples with a VL>1000 copies/mL. 89% of patients were on zidovudine + lamiviudine with either nevirapine or efavirenz. 7% were on stavudine and 4% on tenofovir containing regimen. 18 (11.4%) of 158 patients developed virological failure. Of 91 individuals who achieved virological suppression and had pre-treatment genotype available, only 1 (1.1%) had pre-treatment resistance. Of 16 individuals with virological failure who had pre-treatment genotype available, 3 (18.8%) had pre-treatment resistance ($p<0.001$). However in the univariable model, there was no association between pre-treatment drug resistance and virological failure. The number of events was very small in this study.

Lee et al (293), examined the impact of pre-treatment drug resistance on virological suppression (VL<400 copies/mL) at six months using retrospective samples from Kampala, an urban city and Mbarara, a rural setting, in Uganda.

Kaplan-Meier survival methods were used to estimate time to virological suppression in the groups with and without pre-treatment drug resistance. Of the 74 Kampala patients that were on ART (fixed dose combination of stavudine, lamivudine and nevirapine) and eligible, six had pre-treatment drug resistance and 67 (91%) achieved virological suppression overall. Virological suppression in 62/68 patients and 5/6 without and with pre-treatment drug resistance occurred at a median of 90 days (IQR 84–99) and 97 days (IQR 85–100), respectively (log-rank test, p = 0.3)

Of the 439 patients from Mbarara who were on ART and eligible, 13 had pre-treatment drug resistance, and 434 (99%) achieved virological suppression overall. Virological suppression in 422/426 patients and 12/13 patients without and with pre-treatment drug resistance occurred at a median of 85 days (IQR 83–97) and 89 days (IQR 83–172), respectively (log rank test p= 0.05). 86% were on nevirapine based ART, and 12% on efavirenz in combination with lamivudine and Zidovudine.The time to suppression estimates were not adjusted for baseline differences, but the authors commented that baseline characteristics did not differ significantly between the groups with and without pre-treatment drug resistance, except in the Kampala cohort in which the baseline CD4 count was significantly higher in those achieving virological suppression at 6 months than in those that did not (median 68 cells/mm^3 vs. 19 cells/mm^3, p=0.04).

The study by Mzingwane et al (294), with a sample size of 65 individuals on ART was equally too small to allow a firm conclusion to be drawn about the impact of pre-treatment drug resistance on virological outcomes. This is one of the very few studies in the African setting that has examined the impact of minority drug resistant variants on virological outcomes. Deep sequencing was done on the pre-treatment samples of four individuals with virological failure (VL ≥1000copies/mL after 6 months on ART), three with virological suppression (VL < 50 copies/mL) and one with low level viraemia (VL<1000 copies/mL) who were on a fixed dose combination of tenofovir/emtricitabine/efavirenz. The mutations identified at the 1% level of deep sequencing showed low to high level resistance to efavirenz.

Although there were TAMs identified in all eight individuals as well as other NRTI mutations, the viral isolates were susceptible to tenofovir and emtricitabine according to the Stanford algorithm. No conclusions can be drawn about the impact of minority variants on virological outcomes based on this study.

The study by Zoufaly et al (353) was not identified by the literature review above but through a separate review of the literature on impact of minority variants on treatment outcomes. I included it for two reasons; it was the only one from the African setting examining the impact of minority variants on virological outcomes and it had a moderately large sample size. This prospective study examined the impact of minority variants on virological failure (VL ≥ 1000 copies/mL) at 12 months in 300 individuals consecutively started on ART between January and October 2010 in rural Cameroon. Of the 238 individuals who had been on ART for 12 months, 38 (16%) experienced virological failure. Pre-treatment (baseline) resistance using next generation sequencing with a detection threshold of 1% was assessed in patients with virological failure and compared with an equal number of controls that had VL < 1000 copies/mL matched for age, sex, CD4 count and viral load at baseline. 17% of patients were on a tenofovir containing ART combined with either lamivudine or emtricitabine. The majority were on Zidovudine/lamivudine (83%) containing regimen. The third agent was nevirapine in 71% of cases, efavirenz (25%) and boosted lopinavir (5%). 17% of Pre-treatment drug resistance was identified in 6/30 (20%) patients with virological failure and 6/35 (17%) controls (p=0.77). In multivariable regression, the independent risk factors for virological failure were a lower baseline CD4 cell count (OR 1.47, 95% CI 1.02–2.08, per 100 cells/mm^3 lower) and a lower pill count adherence (OR 1.04, 95% CI 1.02–1.07 per 1% lower).

To summarise, two studies in the African setting (291, 292) showed an association between the presence of pre-treatment drug resistance and virological failure. Neither used a fixed dose combination of tenofovir, emtricitabine and efavirenz and it remains unclear whether a fixed dose combination of tenofovir, emtricitabine and efavirenz which has fewer side-effects compared to older regimen (258) and likely

increased adherence (354) can reduce the risk of virological failure from pre-treatment drug resistance.

7.4 Aims and objectives

The aim of this chapter was thus to quantify the prevalence of pre-treatment drug resistance in chronically and recently infected ART-naïve individuals, to examine risk factors for pre-treatment drug resistance as well as its impact on virological suppression in individuals receiving mainly fixed dose tenofovir, emtricitabine and efavirenz.

The primary objectives were:

- To estimate the prevalence of pre-treatment drug resistance in chronically and recently infected ART naïve individuals.
- To assess the risk factors for the presence of pre-treatment drug resistance
- To estimate the impact of pre-treatment drug resistance on virological suppression in individuals initiated on first-line ART

7.5 Methods

7.5.1 Study population

The study population has been described in Chapter 3.

Briefly, individuals eligible for inclusion of estimation of prevalence of pre-treatment drug resistance were:

- Recently infected adults enrolled in trial clinics: These were individuals who were ART naïve at their first trial clinic visit in whom a negative HIV ELISA test on dried blood spots collected during a home survey was followed by a positive ELISA test in one of the six-monthly home surveys. Hence these

individuals seroconverted during the trial and had plasma samples taken at the first clinic visit available for evaluation.

- Recently infected adults identified through serial dried blood spot ELISA tests as above, during the period of the trial, but who did not link to a trial clinic. As they did not attend the trial clinics, they did not have plasma samples available for evaluation.
- Chronically infected ART naïve adults enrolled in trial clinics: These were individuals who were not on ART at their first trial clinic visit nor had evidence of previous ART exposure and in whom there was no prior documentation of a negative ELISA test on dried blood spots at any point during the trial period from March 2012 to June 2016. Plasma samples were taken at the first clinic visit for evaluation of pre-treatment drug resistance.

The results of HIV ELISA tests done on DBS were not communicated to participants as the tests were not done in real time. The rapid HIV tests also offered to participants during the home surveys was used for clinical diagnosis of HIV status and for identifying participants who needed referral to trial clinics. Results were given to participants within 20 minutes.

7.5.2 Laboratory procedures

Total nucleic acid was extracted from two spots on the filter paper using NucliSENS *EasyMAG* technique in recently infected individuals who did not have plasma samples (Figure 7.1). Amplification of the *POL* gene using oneStep RT-PCR followed by nested PCR. Sanger sequencing of the *POL* gene spanning all the 99 protease and 300 reverse transcriptase amino acids was performed. Sanger sequencing only detected drug resistance mutation present in majority virus (i.e. >20%) on dried blood spots.

HIV whole genome sequences were generated (WGS) on Illumina MiSeq on plasma samples of chronically infected individuals and a subset of recently infected

individuals who had plasma samples available as described above. WGS were assembled using Geneious software and a 2% threshold was used to assess the presence of minority drug resistance variants, defined as representing less than 20% of the viral population with a minimum of 1000 reads. Hence three sensitivity thresholds for the detection of pre-treatment drug resistance were established; 2-5%, 5-20% (minority variants) and >20% (majority variants).

The sequencing was undertaken by research fellows at the Africa Health Research Institute laboratory in Durban.

Pre-treatment drug resistance mutations were assessed according to the WHO 2009 list for surveillance of drug resistance mutations as shown in Table 7.2 (355).

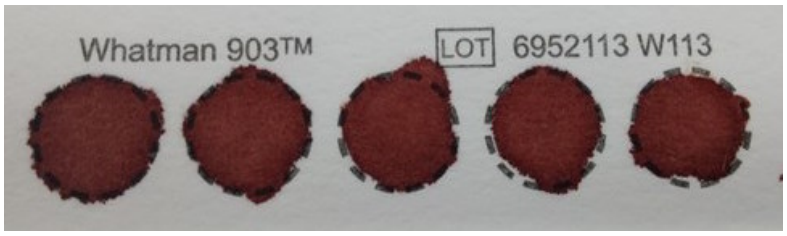


Figure 7.1 Dried blood spots collected on filter paper

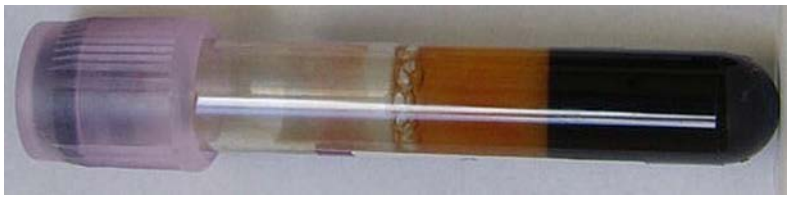


Figure 7.2 Blood sample collected in all ART naive individuals during the first clinic visit

Table 7.2 List of WHO 2009 surveillance drug resistance mutation

Nucleoside reverse transcriptase inhibitor mutations		Non-nucleoside reverse transcriptase inhibitor mutations		Protease inhibitor mutations	
M41	L	L100	I	L23	I
K65	R	K101	E, P	L24	I
D67	N, G, E	K103	N, S	D30	N
T69	D, Ins	V106	M, A	V32	I
K70	R, E	V179	F	M46	I, L
L74	V, I	Y181	C, I, V	I47	V, A
V75	M, T, A, S	Y188	L, H, C	G48	V, M
F77	L	G190	A, S, E	I50	V, L
Y115	F	P225	H	F53	L, Y
F116	Y	M230	L	I54	V, L, M, A, T, S
Q151	M			G73	S, T, C, A
M184	V, I			L76	V
L210	W			V82	A, T, F, S, C, M, L
T215	Y, F, I, S, C, D, V, E			N83	D
K219	Q, E, N, R			I84	V, A,C
				I85	V
				N88	D, S
				L90	M

7.5.3 Statistical analysis

The proportion with evidence of pre-treatment drug resistance and their 95% confidence interval was estimated initially for all groups (chronic and recent infection) at the >20% detection threshold including those with only dried blood spot samples.

Furthermore, the proportion with evidence of pre-treatment drug resistance including minority variants at >2% and >5% detection thresholds could only be estimated for those with plasma samples using next generation sequencing.

The proportion of individuals with pre-treatment drug resistance mutations was also described graphically according to drug classes as well as the mutations within each drug class for both chronically and recently infected participantcs.

For the examination of factors associated with the presence of pre-treatment drug resistance, only data from participants with plasma samples (in care in trial clinics) were used. Pre-treatment drug resistance was estimated using deep sequencing but only mutations present in majority virus were considered. Plasma samples were used to allow potential association between clinical exposure variables such as CD4 count and viral loads at the first clinic visit and presence of pre-treatment resistance to be examined . Other exposure variables considered for this analysis were age at presentation, sex, educational attainment, employment status and recency of infection (chronic/recent infection). I used logistic regression to estimate odds ratios and their 95% confidence intervals and the likelihood ratio tests to find the model which best fiited the data. I examined CD4 count at the first clinic visit and age as both categorical and continuous variables. The model with the linear forms of CD4 and age fitted better, hence this was selected. In the multivariable logistic regression model, I made no assumptions about the role of age and sex as in previous chapters. Only exposure variables with p values <0.15 in the univariable model were included in the multivariable model.

Individuals who initiated ART amongst those that were ART naïve at presentation to trial clinics were included in the analyses examining association between the

presence of pre-treatment drug resistance and virological suppression. This assessment was based on a time to event analysis using Kaplan-Meier survival methods with the origin set at the date of ART initiation. For inclusion in the analysis, individuals needed to have had at least one viral load measurement following ART initiation. The event was virological suppression (VL< 400 copies/mL). Follow up of individuals that did not achieve virological suppression was censored at the date of the last viral load measurement. Cox regression was used to estimate the hazard ratios and 95% confidence intervals for the association between pre-treatment drug resistance and other factors with virological suppression. Individuals with pre-treatment drug resistance were further subdivided into whether the resistance mutations were present in minority virus only (<20%) or in majority virus (>20%).

Other risk factors examined were CD4 count at ART initiation, viral load, age at ART initiation, sex and mean of adherence measured using VAS. Mean adherence was calculated by taking the average adherence in the visits prior to achieving virological suppression in those that suppressed or the average adherence in the visits prior to censoring in those that did not achieve virological suppression. I used likelihood ratio tests to examine whether CD4 count and age were best fitted in the model as categorical or linear exposures. There was no evidence of a departure from linearity, hence these variables were included in the model as linear variables.

In the multivariable Cox model, age and sex were forced into the model, regardless of significance in the univariable model because of reported association with virological suppression in the literature (255, 256). Other potential risk factors were only included in the model if p values <0.15 in the univariable model.

7.6 Results

7.6.1 Cohort profile

Figure 7.3 shows the breakdown of participants that contributed data to the analysis described in this chapter.

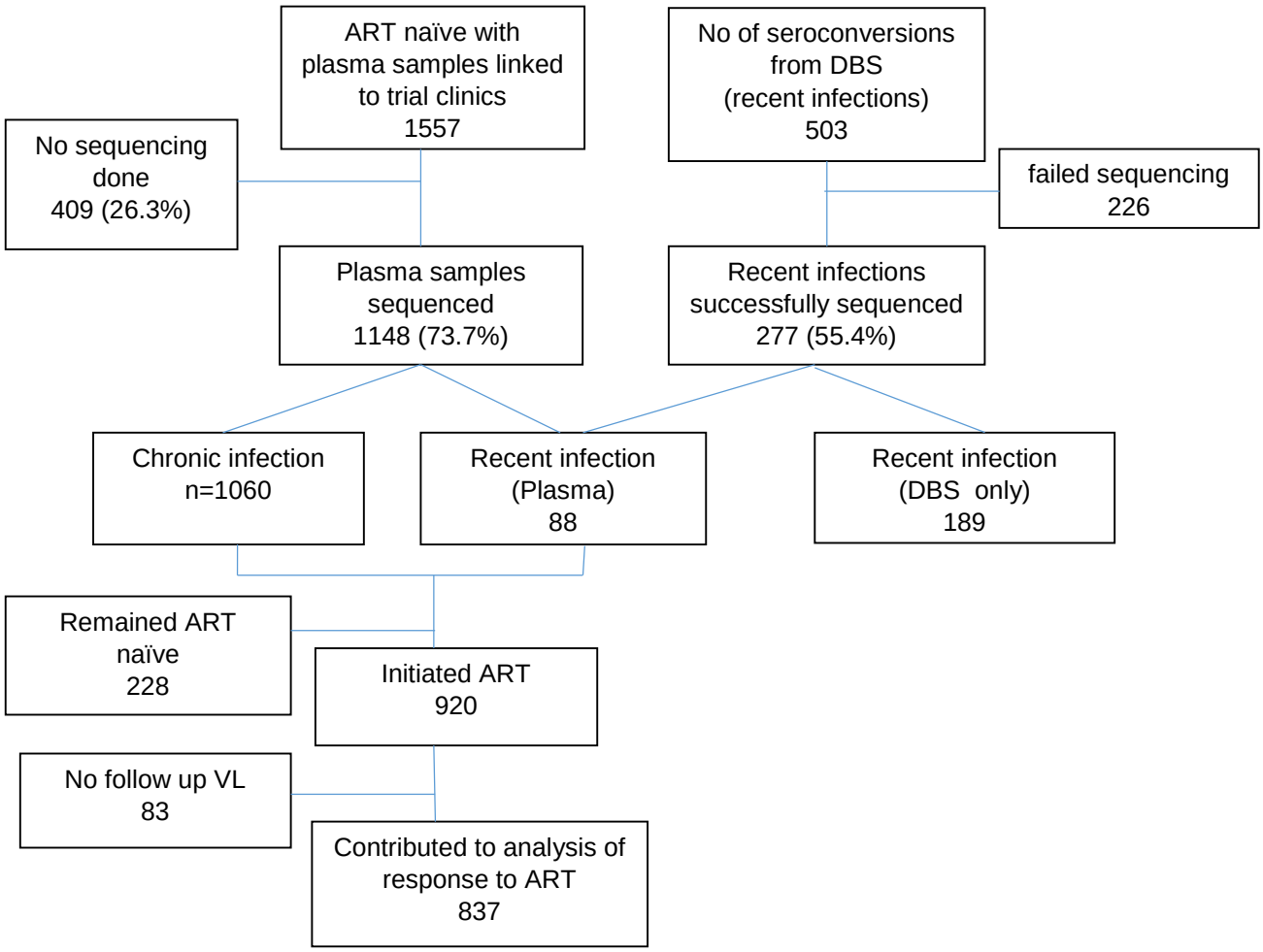


Figure 7.3 Cohort profile of participants with plasma sample and dried blood spots

Of the 503 individuals who seroconverted during the course of the trial (recent infections), 277 were successfully sequenced. Sequencing failed in the remaining 226 individuals for a variety of reasons mainly poor quality dried blood spots and failure to amplify the RNA. There was no significant difference in the sex distribution of those sequenced vs. not sequenced (88.2% of female sequenced vs 84.1% not sequenced; p=0.178). The median age of individuals with sequenced samples was similar to that of those whose samples were not sequenced [(22.3 years (IQR 19.5, 28.4) vs. 22.5 years (IQR 18.8, 27.3); p=0.751]. Of the 277 individuals with recent infection that were sequenced, 189 sequences were from dried blood spots and 88 sequences were from plasma.

Of the 1557 ART-naïve adults linked to trial clinics with plasma samples, samples from 1148 (73.7%) were successfully sequenced. There was a significant difference in the sex of those with sequenced plasma samples compared to those not sequenced (70.3% of female sequenced vs.77.2% not sequenced; p=0.008) with no difference in median age between the sequenced and not sequenced group (33.0 years vs. 33.5 years; p=0.685)

Of the 1148 plasma samples sequenced, 1060 were from individuals with chronic infection and 88 were from individuals with recent infections. 48/1060 (4.5%) of those with chronic infection had received ART for prevention of mother to child transmission. The total number of successful sequences was 1337, comprising 1148 plasma samples and 189 dried blood spot samples. This population was used to determine the overall prevalence of pre-treatment drug resistance at the 20% threshold of resistance detection.

7.6.2 Characteristics of participants

The majority of participants were under the age of 40 years, female, single and unemployed (Table 7.3). Amongst the participants who linked to care and provided plasma samples, the median CD4 count at presentation was 405 cells/mm^3 (IQR 261-559) and the median viral load was 4.5 Log10 copies/mL (IQR 4.0-5.2).

Table 7.3 Demographic and clinical characteristics of all participants surveyed for pre-treatment drug resistance

Characteristics	Complete cohort n=1337	Individuals without pre-treatment HIV drug resistance n=1221	Individuals with Pre-treatment HIV drug resistance n=116
Type of infection			
Chronic	1,060 (79.3)	969 (79.4)	91 78.5)
Recent	277 (20.7)	252 (20.6)	25 (21.6)
Age (Years)			
Median age	31.1 (24.3-43.4)	31.4 (24.3-44.4)	28.9 (23.0-35.4)
16-29	608 (45.5)	546 (44.7)	62 (53.5)
30-39	314 (23.5)	283 (23.2)	31 (26.7)
40-49	186 (13.9)	177 (14.5)	9 (7.8)
>50	213 (15.9)	201 (16.5)	12 (10.3)
Missing	16 (1.2)	14 (1.2)	2 (1.7)
Sex			
Female	973 (72.8)	888 (72.7)	85 (73.3)
Male	364 (27.2)	333 (27.3)	31 (26.7)
CD4 at presentation *			
Median (IQR) cells/mm^3	405 (261-559)	406 (261-560)	372 (263-527)
<350	448 (33.5)	405 (33.2)	43 (37.1)
350-500	300 (22.4)	272 (22.3)	28 (24.1)
>500	382 (28.6)	353 (28.9)	29 (25.0)
Missing	207 (15.5)	191 (15.6)	16 (13.8)
Viral load* copies/mL			
Median (Log10)	4.5 (4.0-5.2)	4.5 (3.9-5.2)	4.6 (4.1-5.1)
<10,000	311 (23.3)	289 (23.7)	22 (19.0)
10,000-100,000	479 (35.8)	431 (35.3)	48 (41.4)
>100,000	356 (26.6)	321 (26.3)	35 (30.2)
Missing	191 (14.3)	180 (14.7)	11 (9.5)

Characteristics	Complete cohort n=1337	Individuals without pre-treatment HIV drug resistance n=1221	Individuals with Pre-treatment HIV drug resistance n=116
Education			
Primary or less	512 (38.3)	463 (37.9	49 (42.2)
Some Secondary	519 (38.8)	474 (38.8)	45 (38.8)
Secondary or higher	302 (22.6)	280 (22.9)	22 (19.0)
Missing	4 (0.3)	4 (0.3)	0 (0.0)
Marital status			
Never married	1,184 (88.6)	1,071 (87.7)	113 (97.4)
Married	105 (7.9)	103 (8.4)	2 (1.7)
Divorced/Separated	44 (3.3)	43 (3.5)	1 (0.9)
Missing	4 (0.3)	4 (0.3)	0 (0.0)
Employment			
Employed	183 (13.7)	171 (14.0)	12 (10.3)
Student	117 (8.8)	106 (8.7)	11 (9.5)
Unemployed	1,032 (77.2)	939 (76.9)	93 (80.2)
Missing	5 (0.4)	5 (0.5)	0 (0.0)

*189 of the 277 individuals with recent infection provided DBS during the home survey but did not link to care, hence the high numbers of missing clinical variables. Information on all other variables obtained from household survey.

7.6.3 Prevalence of pre-treatment drug resistance

Of the 1337 individuals evaluated for the presence of any pre-treatment drug resistance, 116 had evidence of pre-treatment drug resistance in majority virus giving an overall estimated prevalence of 8.7% (95% CI 7.3-10.3) as shown in Figure 7.4. There was no evidence of differences in the proportion with pre-treatment drug resistance in the different subgroups; those with chronic infection previously exposed to ART for prevention of mother-to-child transmission (PMTCT); 8.6% (95% CI 7.0-10.4), chronic infection with no previous exposure to PMTCT 8.0 (95% CI 6.5-9.8) and recent infection 9.0 (95% CI 6.2-13.0). As exposure to PMTCT may not have been completely ascertained, prevalence of any pre-treatmentdrug resistance was also estimated in men only. Of the 364 samples from men evaluated, 31 had evidence of any pre-treatment drug resistance; prevalence 8.5% (95% CI 6.0-11.9) which is similar to the overall prevalence.

The prevalence of any pre-treatment drug resistance for the different sensitivity thresholds of detection of low frequency (minority) variants was also estimated, overall and for each drug class in those with chronic and recent infections.

7.6.3.1 Chronically infected individuals

The prevalence of any pre-treatment drug resistance in those with chronic infection for the three sensitivity thresholds examined >2%, >5% and >20 was 16.7%, 11.7% and 8.6% respectively as shown in Figure 7.5.

The majority of drug resistance mutations present for the respective thresholds belonged to the NNRTI drug class followed by the NRTI drug class and lastly the PI drug class.

At the >20% sensitivity threshold, the proportion of individuals with any NNRTI mutation was 88/1060 (8.3%); 74/88 (84.1%) of those with NNRTI mutations had only one NNRTI mutation, 12/88 (13.6%) had two NNRTI mutations and two people had three NNRTI mutations.

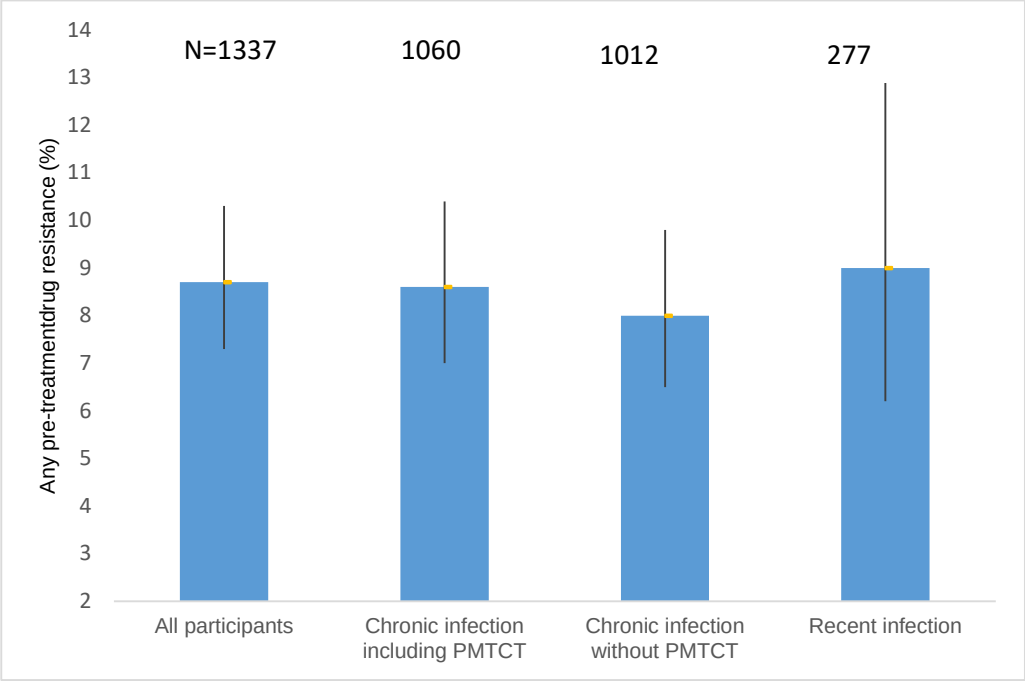


Figure 7.4 Prevalence of pre-treatment drug resistance in majority virus

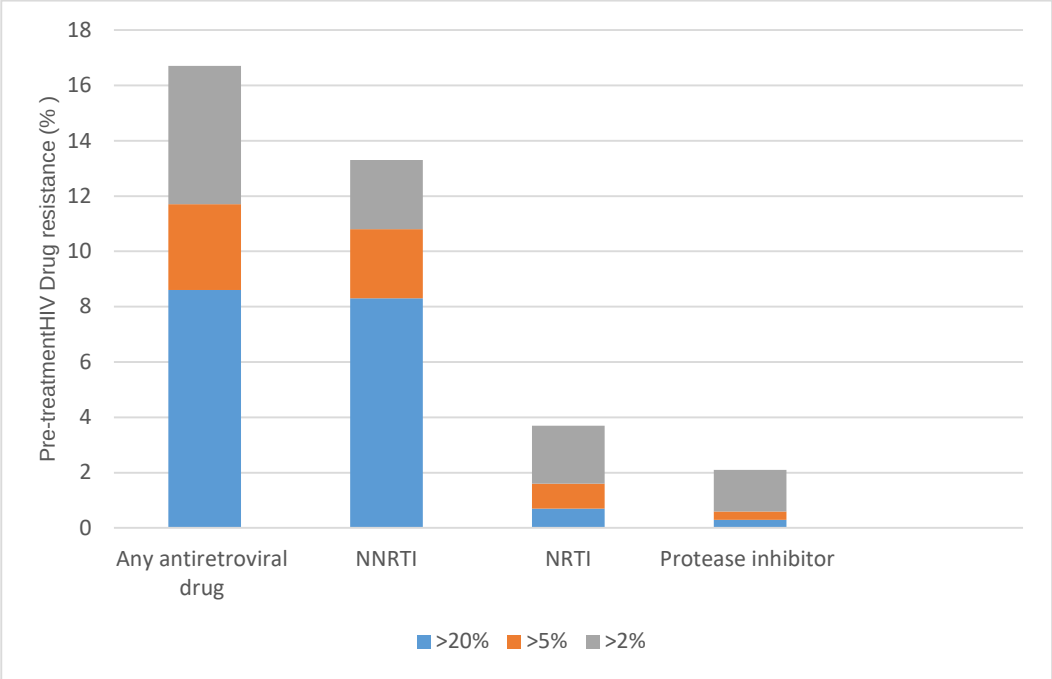


Figure 7.5 Prevalence of pre-treatment drug resistance in adults with chronic infection for different sensitivity thresholds

The proportion of individuals with any NNRTI mutation at the >2% and >5% thresholds were 141/1060 (13.3%) and 114/1060 (10.8%) respectively.

The proportion of individuals with any NRTI mutation at the 20% threshold was 7/1060 (0.7%); 3/7 (42.9%) of those with NRTI mutations had only one NRTI mutation and 4/7 (57.1%) had two NRTI mutations. The proportions at the >2% and >5% thresholds were 39/1060 (3.7%) and 17/1060 (1.6%) respectively.

The proportion of individuals with any PI mutation at the >20%, >5% and > 2% thresholds were 3/1060 (0.3%), 6/1060 (0.6%) and 23/1060 (2.2%) respectively.

No one had triple class resistance in majority virus (>20% threshold) but this was present in two individuals at the >2% threshold but not at the >5% threshold.
7/1060 (0.7%) had dual class resistance at >20% threshold; In six individuals, this was to both NNRTI and NRTI drug classes whilst in one individual it was to the NNRTI and PI drug classes.
21/1060 (2.0%) and 13/1060 (1.2%) had dual class resistance at >2% and >5% thresholds respectively.

The proportion of individuals with different drug resistance mutations at the different sensitivity thresholds of detection is shown in Figure 7.6.

For the NNRTI drug class the K103N/S mutation was the most frequent for the three thresholds being present in 102/1060 (9.6%), 90/1060 (8.5%) and 72/1060 (6.8%) of individuals at the >2%, >5% and >20% thresholds respectively

The second most prevalent NNRTI mutation was the V106M/A present in 19/1060 (1.8%), 14/1060 (1.3%) and 8/1060 (0.8%) of individuals at the >2%, >5% and >20% sensitivity thresholds. The G190A/E was the next most frequent mutation.
The most prevalent NRTI mutation at all sensitivity detection thresholds was the M184V/I. This was present in 12/1060 (1.1%), 6/1060 (0.6%) and 5/1060 (0.5%) of individuals at the >2%, >5% and >20% sensitivity thresholds respectively. The second most frequent NRTI mutation was the thymidine analogue mutation.

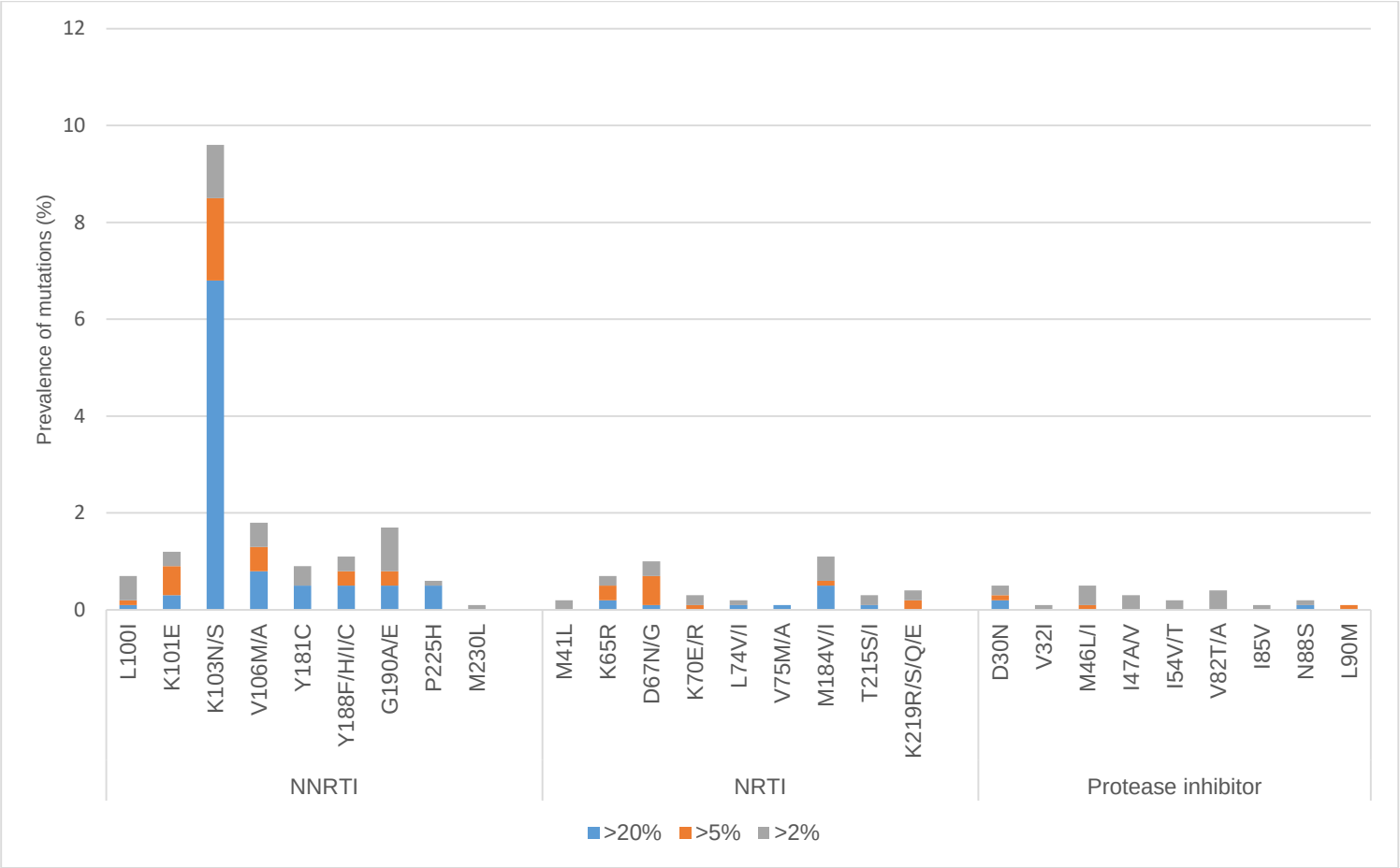


Figure 7.6 Prevalence of pre-treatment drug resistance mutation in adults with chronic infection for different sensitivity thresholds

D67N/G. This was present in 11/1060 (1.0%), 7/1060 (0.7%) and 1/1060 (0.1%) of individuals at the >2%, >5% and >20% sensitivity thresholds. The K65R was the next most frequent NRTI mutation; present in 7/1060 (0.7%), 5/1060 (0.5%) and 2/1060 (0.2%) of individuals at the >2%, >5% and >20% sensitivity thresholds respectively.

The most prevalent PI mutation was the D30N present in 5/1060 (0.5%), 3/1060 (0.3%) and 2/1060 (0.2%) of individuals at the >2%, >5% and >20% sensitivity thresholds respectively. The second most prevalent mutation was the M46L/I mutation. This was present at 5/1060 (0.5%) and 1/1060 (0.1%) at the >2% and >5% sensitivity thresholds respectively but absent in majority virus

7.6.3.2 Recently infected individuals

Of the 277 individuals with recent infection, 88 had plasma samples available for assessment of minority variants.

The prevalence of any pre-treatment drug resistance in those with recent infection for the three sensitivity thresholds examined >2%, >5% and >20 was 21.6%, 17.1% and 15.9% respectively as shown in Figure 7.7. The majority of the drug resistance mutations present for the respective thresholds belonged to the NNRTI drug class followed by the NRTI drug class and lastly the PI drug class. At the >20% sensitivity threshold, the prevalence of any NNRTI mutation was 12/88 (13.6%); 11/12 (91.7%) of those with NNRTI mutation had only one NNRTI mutation and 1/12 (8.3%) had two NNRTI mutations. The prevalence at the >5% and >2% thresholds were 12/88 (13.6%) and 15/88 (17.1%) respectively.

The prevalence of any NRTI mutation at the 20% threshold was 1/88 (1.1%) and this individual had only one NRTI mutation. The prevalence at the >5% and >2% thresholds were 2/88 (2.3%) and 5/88 (5.7%) respectively.

The prevalence of any PI mutation at the >20% and >5% thresholds was 1/88 (1.1%) in each case and 3/88 (3.4%) at >2% threshold.

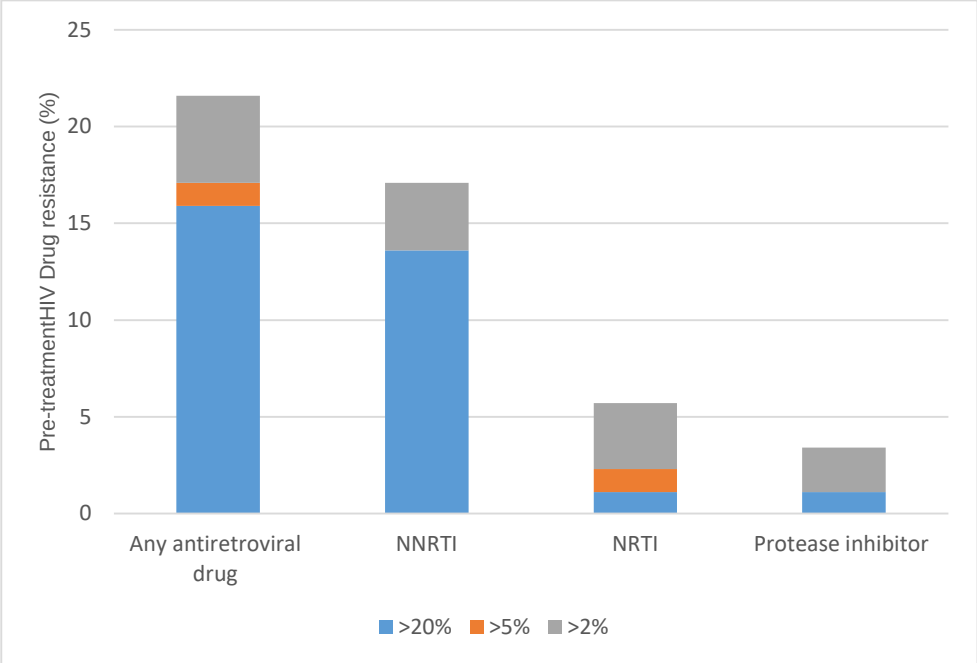


Figure 7.7 Prevalence of pre-treatment drug resistance in adults with recent infection for different sensitivity thresholds

No one had triple class resistance at >5% and >20% thresholds but this was present in low frequency at the >2% threshold in one individual. Two individuals had dual class resistance at >2% threshold but not at the >5% and >20% thresholds.

The K103N/S mutation was the most prevalent in the NNRTI drug class (Figure 7.8) for the three sensitivity thresholds being present in 11/88 (12.5%), 10/88 (11.4%) and 8/88 (9.1%) of individuals at the >2%, >5% and >20% thresholds respectively.

The second most prevalent NNRTI was the Y188C/F/H/I present in 3/88 (3.4%), 2/88 (2.3%) and 2/88 (2.3%) at the >2%, >5% and >20% sensitivity thresholds respectively. The next most prevalent mutations were the L100I, K101E and V106M/A present in similar proportions at all three sensitivity detection thresholds.

The D67N/G was the most prevalent NRTI mutation present at the >2% threshold in 2/88 (2.3%) individuals but absent at the >5% and >20% sensitivity thresholds. The next most prevalent mutation was the T215S/I present at 1/88 (1.1%), 1/88 (1.1%) and 1/88 (1.1%) at the >2%, >5% and >20% sensitivity thresholds respectively. The K219/S/Q/E was present at a similar proportion to the T215S/I at the >2% and >5% sensitivity thresholds but absent in the >20% threshold.

The PI mutations V82T/A was present in 1/88 (1.1%) of indiviuals at >2% detection threshold but not at >5% and 20% thresholds. The I85V was present in similar proportion of 1/88 (1.1%) at all three detection thresholds.

The prevalence of the different mutations was also estimated when all samples from recently infected individuals (88 plasma + 189 DBS) were taken into consideration.

As only Sanger sequencing was done on DBS to detect variants >20%, only the 20% detection threshold was taken into account for this estimation.

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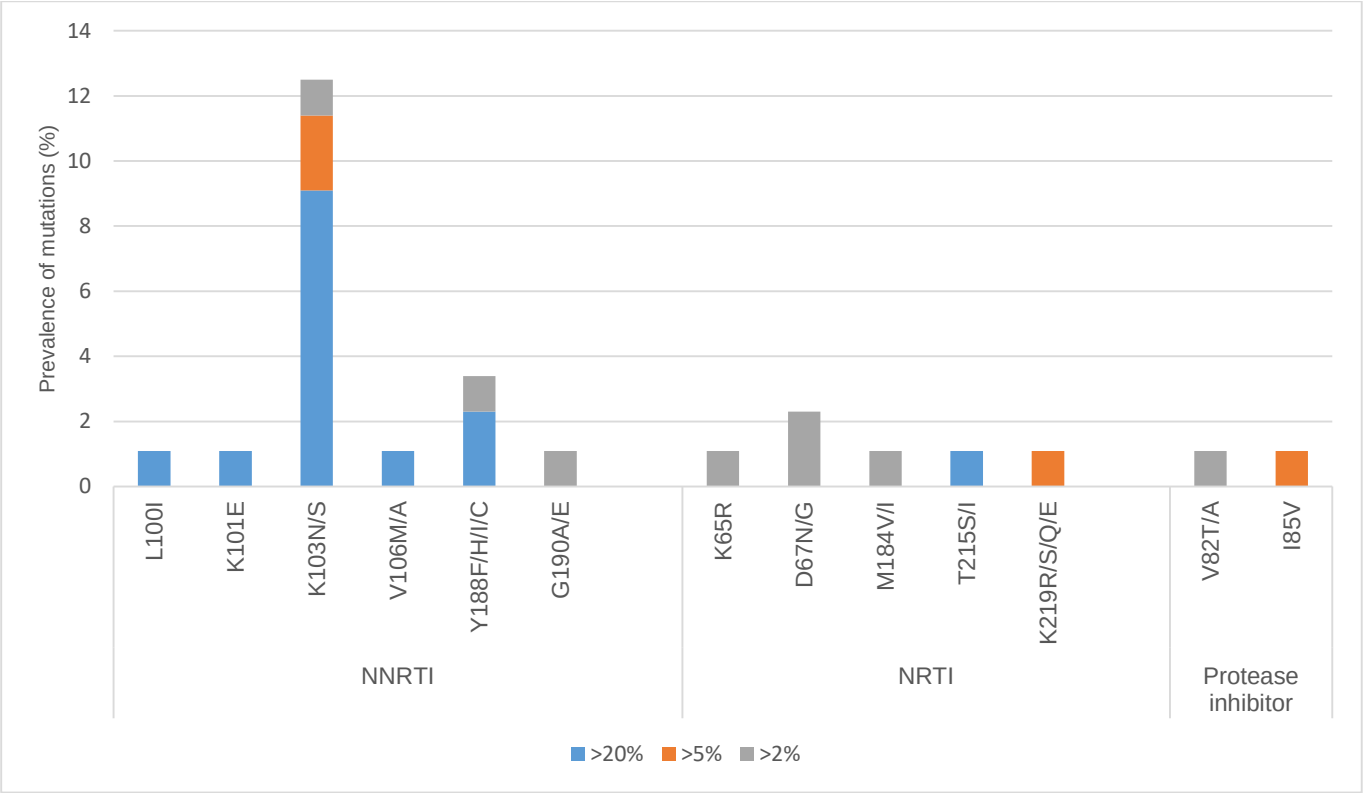


Figure 7.8 Frequency of pre-treatment drug resistance mutation in adults with recent oinfection

The prevalence of any NNRTI mutation was 7.9% (22/277). The K103N/S was the most prevalent at 16/277 (5.8%), followed by V106M/A, K101E and Y188F/H/I/C each at 2/277 (0.7%).

Two individuals had NRTI mutations in majority virus; one K65R and one T215S.

One individual had the PI mutation I85V

7.6.4 Factors associated with the presence of any pre-treatment drug resistance

Amongst individuals with plasma samples available (Table 7.4), Being recently infected was associated with double the odds of pre-treatment drug resistance compared to chronic infection in the univariable model; OR 2.01 (95% CI 1.09-3.71).

Older age was associated with a decreased probability of having any pre-treatment drug resistance; (OR 0.88, 95% CI 0.81-0.96 for every 5 years increase)

Being divorced or married was associated with lower likelihood of pre-treatment drug resistance; OR 0.21 (95% CI 0.03-1.55) and OR 0.20 (95% CI: 0.05-0.81), for divorced and married vs. single respectively.

On the multivariable regression model that adjusted for age, recency of infection and marital status, there was weak evidence that being recently infected compared to chronic infection was associated with nearly twice the odds of pre-treatment drug resistance; [aOR 1.87 (95% CI 1.00-3.51); p=0.06]. There was also weak evidence that being divorced or married compared to being single remained associated with decreased probability of pre-treatment drug resistance; [aOR 0.29 (95% CI 0.04-2.20) and aOR 0.26 (95% CI 0.06-1.13); P=0.05] for divorced and married vs. single respectively. After adjustment, age was no longer associated with pre-treatment drug resistance.

Table 7.4 Factors associated with the presence of any pre-treatment drug resistance*

Characteristics	Individuals with plasma samples n=1,148	Individuals without any pre-treatment HIV drug resistance in majority virus n= 1,043[#]	Individuals with any Pre-treatment HIV drug resistance in majority virus n= 105[#]	Univariable Odds ratio (95% CI)	P value	Multivariable odds ratio (95% CI)	P value
Type of infection					0.035		0.06
Chronic	1,060 (92.3)	969 (91.4)	91 (8.6)	1		1	
Recent	88 (7.7)	74 (84.1)	14 (15.9)	2.01 (1.09-3.71)		1.87 (1.00-3.51)	
Age (Years)							
Median age	33.0 (25.6-45.2)	33.4 (25.8-45.8)	30.0 (24.9-35.9)				
16-29	463 (40.2)	411 (88.8)	52 (11.2)				
30-39	298 (26.0)	267 (89.6)	31 (10.4)	0.88 (0.81-0.96)	0.003	0.94 (0.86-1.03)	0.178
40-49	178 (15.5)	169 (94.9)	9 (5.1)				
>50	202 (17.6)	190 (94.1)	12 (5.9)				
Missing[&]	7 (0.7)	6 (85.7)	1 (14.3)	-			
Sex					0.622		
Female	807 (70.3)	731(91.5)	76 (8.5)	1			
Male	341 (29.7)	312 (90.6)	29 (9.4)	0.89 (0.57-1.40)			

Characteristics	Individuals with plasma samples n=1,148	Individuals without any pre-treatment HIV drug resistance in majority virus n= 1,043[#]	Individuals with any Pre-treatment HIV drug resistance in majority virus n= 105[#]	Univariable Odds ratio (95% CI)	P value	Multivariable odds ratio (95% CI)	P value
CD4 at presentation							
Median (IQR) cells/mm^3	404 (261-559)	406 (261-560)	372 (263-527)				
<350	448 (39.0)	405 (90.4)	43 (9.6)				
350-500	299 (26.1)	271 (90.6)	28 (9.4)	0.99 (0.91-1.08)	0.857		
>500	379 (33.0)	350 (92.4)	29 (7.7)				
Missing[&]	22 (1.9)	17 (77.3)	5 (22.7)				
Viral load copies/mL					0.319		
Median (IQR)	33,660 (8,735-147,932)	32,786 (8,636-148,793)	36,979 (12230-133,500)				
<10,000	309 (26.9)	287 (92.9)	22 (7.1)	1			
10,000-100,000	478 (41.6)	430 (90.0)	48 (10.0)	1.46 (0.86-2.46)			
>100,000	356 (31.0)	321 (90.2)	35 (9.8)	1.42 (0.82-2.48)			
Missing[&]	5 (0.4)	5 (100.0)	0 (0.0)				
					0.330		

Characteristics	Individuals with plasma samples n=1,148	Individuals without any pre-treatment HIV drug resistance in majority virus n= 1,043[#]	Individuals with any Pre-treatment HIV drug resistance in majority virus n= 105[#]	Univariable Odds ratio (95% CI)	P value	Multivariable odds ratio (95% CI)	P value
Education							
Primary or less	483 (42.1)	434 (89.9)	49 (10.1)	1			
Some Secondary	426 (37.1)	386 (90.6)	40 (9.4)	0.92 (0.59-1.42)			
Secondary or higher	234 (20.4)	218 (93.2)	16 (6.8)	0.65 (0.36-1.17)			
Missing[&]	4 (0.4)	4 (100.0)	0 (0.0)				
Marital status					0.003		0.05
Never married	1,009 (87.9)	907 (89.9)	102 (10.1)	1		1	
Married	92 (8.0)	90 (97.8)	2 (2.2)	0.20 (0.05-0.81)		0.26 (0.06-1.13)	
Divorced/Separated	43 (3.8)	42 (97.7)	1 (2.3)	0.21 (0.03-1.55)		0.29 (0.04-2.20)	
Missing[&]	4 (0.4)	4 (100.0)	0 (0.0)				
					0.379		

Characteristics	Individuals with plasma samples n=1,148	Individuals without any pre-treatment HIV drug resistance in majority virus n= 1,043[#]	Individuals with any Pre-treatment HIV drug resistance in majority virus n= 105[#]	Univariable Odds ratio (95% CI)	P value	Multivariable odds ratio (95% CI)	P value
Employment							
Employed	166 (14.5)	155 (93.4)	11 (6.6)	1			
Student	60 (5.2)	53 (83.3)	7 (11.7)	1.86 (0.69-5.05)			
Unemployed	917 (79.8)	829 (90.5)	87 (9.5)	1.48 (0.77-2.83)			
Missing[&]	5 (0.6)	5 (100.0)	0 (0.0)				

*Analysis restricted to only individuals who linked to care and have more complete clinical information, hence only 88 of the 277 individuals with recent infection were included.
#Analysis was also restricted to mutations in majority virus only ascertained through deep sequencing & Missing category dropped from regression model (complete case analysis)

7.6.5 Impact of drug resistant mutations on virological suppression

Of the 1148 individuals linked to care, 920 initiated ART; of whom 837 had a viral load pre-ART and at least one follow up viral load after ART initiation (Figure 7.3). The 83 (9%) individuals with no follow up viral load data who were excluded were more likely to be younger than those included in analysis (median age 29.5 years; IQR (23.4, 41.6) vs 34.3 years; IQR (27.3, 46.6); p=0.027) and also more likely to be male (12.8% vs 7.4%; p =0.009). The prevalence of any pre-treatment drug resistance in those with missing viral load, who were excluded 16/83 (19.3%) was similar to those who had follow up viral load available 138/837 (16.5%); p=0.675. Reasons for missing viral loads included loss to follow up in 33 individuals (39.8%), death in seven (8.4%), transferred-out in 21 (25.3%) and no documented viral load in the remaining 22 (26.5%) still in care.

Table 7.5 summarises the characteristics of the 837 individuals that contributed to this analysis.

The median age of the cohort was 34.3 years, predominantly female, with 82.6% of the cohort having a mean adherence ≥ 95%. The median duration on ART was 1.36 years; IQR (0.91, 2.13). 96.3% were on fixed-dose combination of tenofovir, emtricitabine and efavirenz.

Figures 7.9 shows Kaplan-Meier curves for proportion of patients with virological suppression stratified by presence of any pre-treatment drug resistance. There was no evidence of a difference in virological suppression according to whether drug resistance mutations were present in minority or majority virus compared to no mutation (p=0.778).

The overall cumulative probability of virological suppression at 12 months was 97% (95% CI 96, 98).

Table 7.5 Baseline characteristics of individuals contributing to the analysis of virological suppression

Characteristics	Complete cohort n=837	No resistance n=699	Any resistance in minority virus only n=62	Any resistance in majority virus N=76
Age at initiation (Years)				
Median age	34.3 (27.3, 46.5)	35 (27.6,47.0)	30.8 (25.6, 39.8)	31.7 (27.0,40.5)
16-29	290 (34.6)	234 (33.5)	27 (43.6)	29 (38.2)
30-39	246 (29.4)	199 (28.5)	20 (32.3)	27 (35.5)
40-49	133 (15.9)	120 (17.2)	5 (8.1)	8 (10.5)
>50	166 (19.8)	144 (20.6)	10 (16.1)	12 (15.8)
Missing	2 (0.2)	2 (0.3)	0 (0.0)	0 (0.0)
Sex				
Female	599 (71.6)	489 (70.0)	54 (87.1)	56 (73.7)
Male	238 (28.4)	210 (30.0)	8 (12.9)	20 (26.3)
CD4 at initiation				
Median (IQR) cells/mm^3	348 (227, 480)	346 (221,477)	356 (276,559)	347 (245,481)
<=350	418 (49.9)	352 (50.4)	29 (46.7)	37 (48.7)
350-500	230 (27.5)	197 (28.2)	14 (22.6)	19 (25.0)
>500	182 (21.7)	147 (21.0)	17 (27.4)	18 (23.7)
Missing	87(0.8)	3 (0.4)	2 (3.2)	2 (2.6)
Viral load copies/mL				
Median (Log copies/mL)	4.6 (4.0, 5.2)	4.6 (4.0, 5.2)	4.3 (3.9, 5.1)	4.7 (4.2, 5.2)
<10,000	200 (23.9)	169 (24.2)	19 (30.7)	12 (15.8)
10,000-100,000	350 (41.8)	290 (41.5)	25 (40.3)	35 (46.1)
>100,000	285 (34.1)	238 (34.1)	18 (29.0)	29 (38.2)
Missing	2 (0.2)	2 (0.3)	0 (0.0)	0 (0.0)
Adherence (%)				
<95	126 (15.1)	109 (15.6)	8 (12.9)	9 (11.8)
≥95	691 (82.6)	573 (82.0)	53 (85.5)	65 (85.5)
Missing	20 (2.4)	17 (2.4)	1 (1.6)	2 (2.6)
ART regimen				
TDF+FTC+EFV	806 (96.3)	672 (96.1)	60 (96.8)	74 (97.4)
TDF+3TC+EFV	6 (0.7)	6 (0.9)	0 (0.0)	0 (0.0)
AZT+3TC+ EFV	18 (2.2)	14 (2.0)	2 (3.2)	2 (2.6)
D4T+3TC+EFV	1 (0.1)	1 (0.1)	0 (0.0)	0 (0.0)
AZT+3TC+PI	1 (0.1)	1 (0.1)	0 (0.0)	0 (0.0)
Missing	5 (0.6)	5 (0.7)	0 (0.0)	0 (0.0)

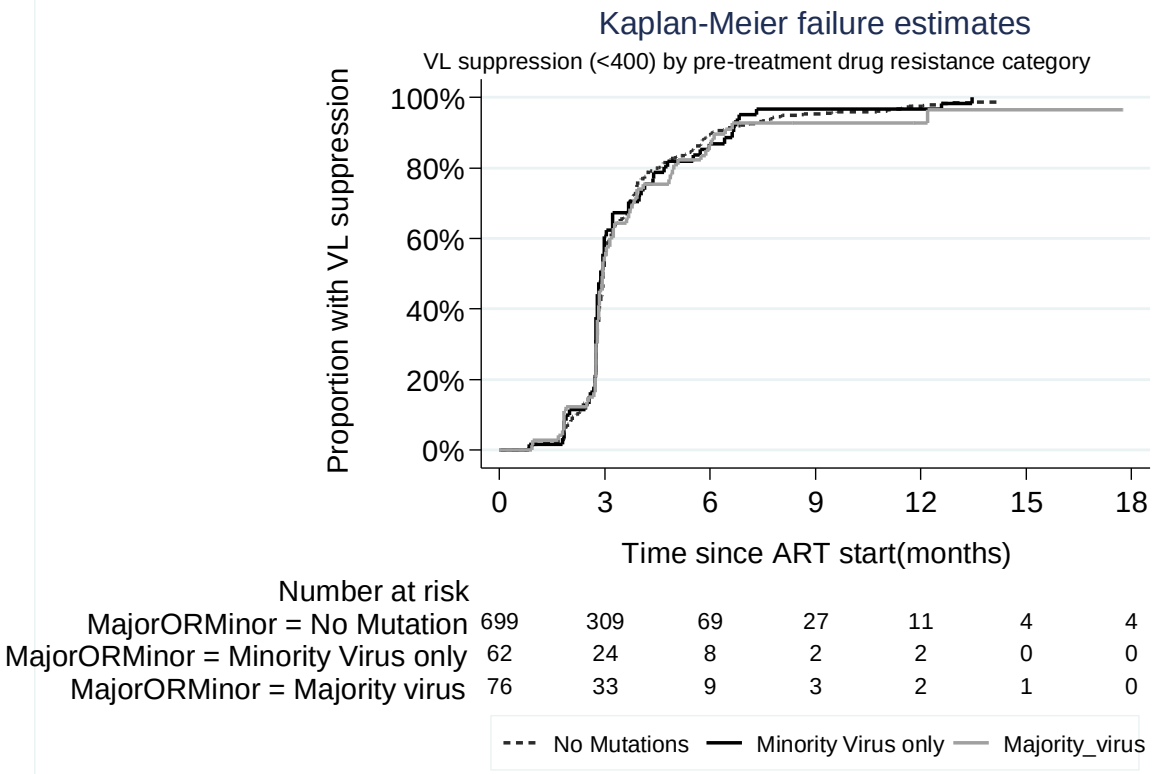


Figure 7.9 Kaplan Meier curves of the proportion of patients with virological suppression by presence of pre-treatment drug resistance

The median time to virological suppression, overall, was 2.96 months; IQR (2.76, 3.88), minority resistance mutation; 2.89 months (2.76, 4.14) and for those with resistance in majority virus, 2.96 months (2.76, 4.11).

Table 7.6 shows the association between the presence of pre-treatment drug resistance and other factors with virological suppression.

In the univariable Cox model, there was no evidence that pre-treatment drug resistance was associated with virological suppression. However being male and having a high viral load (VL>100,000) was associated with a decreased probability of virological suppression. Having a mean adherence of ≥95% was associated with an increased probability of virological suppression, HR 1.33 (95% CI 1.09-1.63)

In a multivariable model that adjusted for age, sex, CD4 count at initiation, viral load and adherence in Table 7.6, there was no evidence that pre-treatment drug resistance was significantly associated with virological suppression; adjusted HR 0.92 (95% CI 0.71-1.19) and aHR 1.03 (95% CI 1.00-1.06) for majority and minority variants respectively vs. no mutations. High baseline viral load (>100,000 vs. ≤10,000) was associated with a decreased probaility of virological suppression; aHR 0.76 (95% CI: 0.62-0.93) as was being male; aHR 0.84 (0.71-0.99). Higher adherence (≥95% vs. <95%) was associated with an increased probability of virological suppression; aHR 1.36 (95% CI: 1.11-1.66) and there was weak evidence that older age is associated with increased probability of virological suppression, [aHR 1.03 (95% CI 1.00-1.06); p=0.07].

I undertook a sensitivity analysis in which the presence of any pre-treatment drug resistance was substituted with the presence of any NNRT resistance mutation as the exposure variable of interest. Adjusting for the same factors as the original analysis, findings remained robust, also showing no evidence of an association between any NNRTI resistance mutation and virological suppression; adjusted HR 0.93, (95% CI: 0.71-1.21) and aHR 1.04 (95% CI: 0.74-1.46) for majority NNRTI and minority NNRTI variants respectively vs. no NNRTI mutations.

Table 7.6 Association of pre-treatment HIV drug resistance and other factors with virological suppression.

Characteristics	Univariable hazard ratio (95% CI)	P value	Multivariable hazard ratio (95% CI)	P value
Any pre-treatment drug resistance		0.783		0.815
No mutations present	1		1	
Minority mutations only	1.03 (0.80-1.34)		1.01 (0.77-1.32)	
Majority mutations	0.92 (0.72-1.19)		0.92 (0.71-1.19)	
Age at initiation/5 years	1.01 (0.99-1.04)	0.301	1.03 (1.00-1.06)	0.068
Sex		0.002		0.039
Female	1		1	
Male	0.79 (0.67-0.92)		0.84 (0.71-0.99)	
CD4 at initiation/100 cells/mm^3	1.02 (0.99-1.05)	0.18	1.00 (0.97-1.03)	0.928
Viral load copies/mL		0.0006		0.011
≤10,000	1		1	
10,000-100,000	0.92 (0.77-1.10)		0.90 (0.75-1.08)	
>100,000	0.72 (0.59-0.86)		0.75 (0.61-0.91)	

Characteristics	Univariable hazard ratio (95% CI)	P value	Multivariable hazard ratio (95% CI)	P value
Adherence (%)		0.003		0.041
<95	1		1	
≥95	1.33 (1.09-1.63)		1.36 (1.11-1.66)	

7.7 Discussion

This analysis examined the prevalence of pre-treatment drug resistance in a setting in rural South Africa where ART has been available since 2004/2005 (22) and where uptake/coverage has been in line with national figures (96).The overall prevalence of pre-treatment drug resistance in majority virus was 8.7% (95% CI 7.3-10.3), similar for chronic and recent infections. However, the prevalence doubled when minority variants were taken into account at the 2% detection threshold for those with chronic infection and increased 2.5 times for the small number of individuals with recent infection who had plasma samples available. The majority of the mutations identified in both majority and minority variants belonged to the NNRTI drug class and the K103N/S mutation was dominant. This mutation results in high level resistance to both nevirapine and efavirenz. Seven individuals had dual class resistance (six with NRTI & NNRTI drug classes and one with NNRTI and PI drug classes) in majority virus amongst those with chronic infection but no one had dual class resistance amongst those recently infected. Triple class resistance was absent for both groups in majority virus.

There was no evidence that the presence of any pre-treatment HIV drug resistance in minority or majority virus was significantly associated with virological suppression.

The moderate prevalence of pre-treatment drug resistance observed in this cohort is driven by the predominance of mutations to the NNRTI drug class which is classified as moderate level (5-15%) according to WHO threshold classification for the levels of transmitted drug resistance (28). The levels for the NRTI and PI drug classes remained <5% and is classified as low. A study with a much smaller sample size in communities adjacent to the TasP trial communities showed no pre-treatment drug resistance from 67 samples collected in 2010 using the WHO threshold survey method and a prevalence of 7.1% from 253 samples collected in 2012 from a population-based HIV surveillance (328). This is similar to the prevalence from my study that examined samples taken from March 2012 to June

2016. Another study that examined trend of transmitted drug resistance in KwaZulu-Natal was conducted amongst pregnant women between 2005 and 2009 (320) showing pre-treatment drug resistance to be <5% in all drug classes in 2007 with an increase in 2009 to 5-15% level in the NNRTI drug class but <5% in the PI and NRTI drug classes, consistent with the findings presented here. A more recent national survey, the first of its kind, reported a pre-treatment drug resistance prevalence of 9% from 277 sequences collected between March 2013 and October 2014 (347) which was also the prevalence from a representative sample from KwaZulu-Natal included in the national survey. A further similarity to my cohort was the predominance of the NNRTI drug class mutations, mainly the K103N while the main difference was that the K65R was the main NRTI mutation in the South African national survey whilst the lamivudine/emtricitabine associated mutation, M184V/I was the most frequent NRTI mutation in my study with only three individuals harbouring the K65R mutation. The paucity of the K65R, which is associated with tenofovir resistance, in my study is somewhat surprising, ≤1% with deep sequencing, considering that tenofovir has been in use in these communities since it was introduced in the South African public health ART programme in 2010. Furthermore, in Chapter 6, I showed that, amongst those with virological failure who were sequenced, 25% of them harboured the K65R mutation. A recent study showed that viruses harbouring the K65R mutation were equally as fit as those without this mutation (215) suggesting that the transmissibility of this mutation may not be diminished as some studies have suggested (209, 214). In my study, there was no difference in the median viral load at virological failure in viruses with and without the K65R mutation. This does not explain the low prevalence of K65R mutations in HIV-positive individuals with pre-treatment drug resistance.

Other studies have confirmed that population sequencing underestimates the true burden of resistant variants that may need to be taken into account when treatment decisions are made. I noticed a doubling in the prevalence of pre-treatment drug resistance when minority variants were taken into account similar to studies in high income countries (356) (357). The literature review I carried out did not reveal any

study conducted in South Africa which estimated the prevalence of low frequency minority variants in ART-naïve HIV-positive patients. One recent study in South Africa (294) of impact of pre-treatment minority variants on virological outcomes involved 65 patients on treatment but only eight pre-treatment samples were selected for deep sequencing and it was not possible to estimate prevalence or conclude on impact of minority variants. Other studies on minority variants have been in the context of PMTCT to examine the impact of short course Zidovudine and single dose nevirapine on the development of resistance mutations in minority virus (358, 359) which could compromise future ART in the mother.

In my study, there was no demonstrable impact on virological suppression as a result of the presence of resistant mutations in either majority or minority variants. This finding remained robust when analysis was restricted to presence of NNRTI mutations in minority or majority virus, despite nearly all individuals being on an NNRTI-based first-line regimen. These results contradict the findings from a multicountry study involving over 2000 patients, which concluded that the presence of pre-treatment drug resistance was associated with virological failure in one publication (292) or increased treatment switches for presumed virological failure in another publication (272). A few other smaller studies have not reached the same conclusions (293, 294).

The impact of minority variants on virological response remains a thorny subject with some studies, primarily from high income countries, showing an association with virological failure (197, 356, 360-366) and as many studies showing no evidence of an effect (294, 353, 367-374).

The majority of my cohort had single class resistance, primarily of the NNRTI drug class, even after accounting for minority variants. Hence, the genotypic sensitivity score (the number of active drugs) for the first-line regimen was two in about 99% of patients. It was possible that tenofovir and emtricitabine were potent enough to result in virological suppression. Furthermore, I only detected minority variants above the 2% threshold which is a potential limitation of the study as some studies

have shown that minority variants present at the 0.5-1% threshold could be clinically relevant (356, 361, 362, 364). It is possible that individuals classified as having no mutations in this study could actually harbour some clinically relevant minority variants thereby making it difficult to explore differences between groups, if any differences do indeed exist. Much lower cut-offs will result in increased artefacts from amplification errors or spontaneously generated mutants from error prone reverse transcriptase (375, 376). It is worth mentioning that these studies have used different technologies to identify low frequency variants, employed different sensitivity thresholds, different ART regimen and patient populations with varying duration of infection.

In addition, I found that a higher self-reported adherence was independently associated with virological suppression whilst individuals initiating ART at the highest viral load stratum were less likely to achieve virological suppression. A review of 10 studies on minority variants in high income countries, which concluded that the presence of minority variants was associated with increased virological failure, also examined the role of medication adherence in the same way as stratified in my study (364). They found an interaction between medication adherence and the risk of virological failure. In this study, having a good adherence partially compensated for the impact of minority variants, while the presence of minority variants coupled with an adherence <95% was associated with substantially increased risk of virological failure.

The association of high viral load with poorer virological outcomes as in my study, was also reported in other studies (377) (378) is independent of CD4 count at initiation and adherence. Nevertheless, individuals with high baseline viral load who are initiating ART will need to be counselled on adherence to maximise their chances of achieving virological suppression.

One of the strengths of this MD study lies in the large sample size in comparison to all but one study identified in Africa. As a result, I was able to estimate the prevalence of minority drug resistant variants, the first study to do this in the

African setting. It is also larger than the only other study in the African setting to examine the impact of minority variants on virological outcomes (353).

Secondly, the proportion of individuals with missing viral loads information was low (9%). There was no difference in the frequency of any pre-treatment drug resistance in those excluded as a result of missing viral load compared to those included, thus reducing the potential for selection bias as a result of missingness.

A limitation of the study was that only a third of patients with recent infection had plasma sample available for deep sequencing, the rest of the individuals had DBS, which only allowed Sanger sequencing. The prevalence of pre-treatment drug resistance estimated in majority virus using deep sequencing in these 88 individuals was significantly higher than that of the overall sample. This raises the question whether Sanger sequencing on DBS samples could have underestimated the prevalence of pre-treatment drug resistance in majority virus in recently infected patients. Furthermore, just over half of all recently infected patients were sequenced. There is no reason to suspect that individuals with pre-treatment drug resistance would have been more or less likely to be sequenced, making selection bias unlikely.

In conclusion, I found a moderate prevalence of pre-treatnent drug resistance in this cohort mainly driven by a high prevalence of resistance to the NNRTI drug class. This is not surprising as the preferred first-line ART in my cohort was efavirenz-based. Nevirapine, another NNRTI, was also used prior to the change to efavirenz. However, I found the low prevalence of transmitted K65R, associated with tenofovir use, surprising but encouraging.

I found no evidence of an association between the presence of resistant minority or majority variants with virological suppression. The health system needs to be strengthened to educate individuals initiating ART on adherence and prompt identification of individuals failing therapy with switching to second-line ART to prevent transmission of drug resistant HIV. The long-term clinical outcome and the

durability of virological suppression in individuals with resistant virus warrant further studies.

Chapter 8 Discussion

This thesis sets out to address the question whether earlier treatment of HIV will lead to drug resistance of the prevalence and form likely to make future elimination difficult in a HIV hyperendemic setting in rural KwaZulu-Natal, South Africa. To address this question, I used data from a large cluster randomised trial which I implemented, to investigate whether universal home-based HIV testing followed by immediate offer of ART regardless of CD4 count can result in population level reduction of HIV incidence. Within this trial, the overarching aim of my MD research was to establish whether treatment at high CD4 counts will lead to poor adherence to ART, as most would feel well at that stage and, therefore may not perceive ART to be immediately beneficial to their health. This could lead to virological failure and the development and transmission of drug resistant HIV. My main findings have been discussed in their respective chapters and are summarised below.

8.1 Main findings

8.1.1 Impact of CD4 count at ART initiation on adherence

Adherence measured by the visual analogue scale was optimal in 86% of visits during the first 12 months of ART and there was no evidence of a significant association between CD4 count at ART initiation and adherence during the first 12 months of ART. The median CD4 count at ART initiation for the cohort was 351 cells/mm^3 (IQR 236, 502) suggesting that most if not all cohort participants would have been in relatively good health, in contrast to findings from the only two studies identified in Africa (227, 228) in which CD4 count at ART initiation was examined as a factor associated with adherence. A recent systematic review showed that the mean CD4 count at ART initiation in sub-Saharan Africa has not improved in the last 10 years, at 152 cells/mm^3 in 2002 and 140 cells/mm^3 (222) in 2012, despite expanding ART treatment eligibility criteria and individuals presenting to care with a much higher CD4 count than was previously the case. In a sub-analysis in the

review, the situation in South Africa was similar. My study is the first in the African setting to examine factors associated with adherence in individuals who initiated ART regardless of CD4 cell count.

I also identified modifiable risk factors associated with non-optimal adherence such as food insecurity and not being on a single tablet regimen, like the fixed dose combination of tenofovir, emtricitabine and efavirenz. Male sex was associated with increased probability of non-optimal adherence, but this is not a risk factor that is modifiable. Studies to understand why adherence is poorer in men and how to intervene are required.

8.1.2 Virological suppression and acquired resistance

I examined the relationship between CD4 count at ART initiation, adherence and other factors with virological suppression in individuals ART-naïve at entry into the trial. I also examined the factors associated with virological suppression at entry into the trial in individuals who were ART-experienced at their first trial clinic visit. I quantified acquired resistance in individuals from both groups combined who developed virological failure.

Virological suppression was high, 94% and 97% at six and 12 months respectively, in individuals who initiated ART within the trial. On the other hand, virological suppression was modest at the first clinic visit in individuals already established on ART in the public ART programme at 79% after a median ART duration of 3.7 years. Amongst individuals who fulfilled the criteria for virological failure and had genotype results available, 88% had at least one drug resistance mutation.

Amongst individuals who initiated ART within the trial, a high CD4 count at initiation was associated with a significantly increased probability of virological suppression six months post-ART initiation. Optimal adherence (≥95%) was independently associated with increased probability of virological suppression after adjusting for CD4 count at initiation. The effect of CD4 count on virological suppression was similar even for individuals within the same adherence stratum, pointing to a

probable biological rather than a behavioural effect. This is the first study in the African setting examining factors associated with virological suppression in individuals initiating ART at CD4 counts >350 cells/mm^3. Other factors associated with increased probability of virological suppression were being on a single tablet regimen, older age and having a high self-reported health status. Being a student and having a high baseline viral load were associated with a decreased probability of virological suppression. The association of older age with increased virological suppression was often attributed to better adherence in older individuals. In my study, this association remained significant after adjusting for adherence.

The high virological suppression seen in individuals who initiated ART within the trial was not observed in those who were ART-experienced at trial entry suggesting a difference in the quality of care within the trial and the public ART programme. Virological suppression was 79% in individuals on ART at entry into the trial, although these individuals had been on ART for considerably longer than in those who initiated within the trial. These individuals initiated ART according to South African guidelines which had evolved from a CD4 of 200 cells/mm^3 to 500 cell/mm^3 as at the end of follow up in June 2016 (15, 22, 23, 379). The median CD4 count at ART initiation was much lower at 176 cells/mm^3 than in those initiating ART in the trial clinics. Nevertheless, we found some common factors associated with virological suppression between those who initiated ART within the trial and those already established on ART at trial entry. In those ART-experienced at trial entry, a high CD4 count at initiation was associated with increased probability of virological suppression which was also seen in those who initiated ART within the trial. Older age was also associated with increased probability of virological suppression. Male sex and non-disclosure of HIV-positive status to anyone was associated with a decreased probability of virological suppression. I could not examine the impact of adherence because there were no data available in this group prior to joining the trial. Despite 20% not being virologically suppressed and nearly all satisfying the definition for virological failure, no one was on second-line protease-based regimen at trial entry.

Not surprisingly, high levels of drug resistance were observed in individuals with virological failure with nearly 90% of individuals having at least one drug resistant mutation. Amongst those with virological failure, resistance mutations were more extensive in individuals ART-experienced at entry into the trial than in those who initiated ART within the trial. This could be because those ART-experienced at entry spent a comparatively longer time on a failing regimen without being switched to second-line ART, allowing for the development of drug resistant mutants.

The M184V/I mutation which confers resistance to lamivudine and emtricitabine was the most common mutation followed by the K103N/S mutation which confers high level resistance to nevirapine and efavirenz. This finding is consistent with the current first-line ART regimen in use within the trial and the public health ART programme (15). 25% of individuals who underwent genotype testing had the K65R mutation associated with tenofovir resistance. This reflects an increase in the use of tenofovir due to changing guidelines with tenofovir now being the preferred NRTI for first-line ART unless clinically indicated otherwise. Only one individual would have initiated a compromised second-line ART based on South African guidelines when I examined all individuals with extensive mutations comprising thymidine analogue mutations, with and without tenofovir resistance, and non-nucleoside reverse transcriptase inhibitor mutations. This is because switch to second-line ART is not informed by genotypic resistance test results.

20% of individuals genotyped resuppressed on the same regimen, even though nearly two-thirds of them had at least one drug resistant mutation present. The resistant mutations present at the time of virological failure was not very different from those present pre-treatment in the few individuals in which it was possible to assess resistance. Of the three individuals that had resistance both at baseline (pre-treatment) and at virological failure, two resuppressed on their first-line regimen.

8.1.3 Pre-treatment drug resistance and response to first line ART

I observed an overall moderate prevalence of pre-treatment drug resistance in majority virus of 8.7%, which was similar for both chronic and recent infections. However, the prevalence doubled when minority variants were taken into account at the 2% detection limit. The majority of the mutations identified in both majority and minority variants belonged to the NNRTI drug class and the K103N/S mutation was dominant. This mutation results in high level resistance to both nevirapine and efavirenz. The K65R mutation associated with tenofovir resistance was rare, despite being relatively common in individuals with virological failure raising further questions about the transmissibility of this mutation. Seven individuals had dual class resistance (six with NRTI & NNRTI drug classes and one with NNRTI and PI drug classes) in majority virus amongst those with chronic infection but no one had dual class resistance amongst those recently infected. Triple class resistance was absent for both groups in majority virus. This is the first study in the African setting to extensively report on the prevalence of pre-treatment minority drug resistant mutations and the biggest study to examine the impact of minority variants in the same setting.

There was weak evidence that those who were recently infected were more likely to have pre-treatment drug resistance than in those chronically infected. This analyses was done with the 88 recently infected individuals who had plasma samples with pre-treatment resistance identified using deep sequencing. The analyses including all 277 recently infected patients using Sanger sequencing (because of the 189 individuals with only dried blood samples), showed a similar prevalence between recently infected (9.0%) and chronically infected patients (8.0%) with confidence intervals that overlap. As the factor analysis for pre-treatment drug resistance included only a fraction of all those recently infected, It is difficult to conclude that there is a true difference between recently and chronically infected patients.

There was also weak evidence that being married or divorced was associated with decreased probability of pre-treatment drug resistance.

There was no evidence that the presence of any pre-treatment HIV drug resistance in minority or majority virus was significantly associated with virological suppression. Older age and optimal adherence (≥95%) were associated with increased probability of virological suppression while male sex and a high baseline viral load were associated with decreased probability of virological suppression. The findings were qualitatively similar when I examined the impact of having any non-nucleoside reverse transcriptase inhibitor mutations on virological suppression. The biggest study in Africa to date (292) found that pre-treatment drug resistance was associated with subsequent virological failure on treatment. Although, my thesis is focused on virological suppression, it is unlikely I would find an association of pre-treatment resistance with virological failure in my study as I was not able to demonstrate any difference in virological suppression in individuals with and without pre-treatment drug resistance.

8.2 Implications of results and contribution to knowledge

The latest WHO ART guidelines recommend ART for all HIV-positive individuals regardless of CD4 count (105) based primarily on the results of the START (16) and TEMPRANO (17) trials which both showed individual health benefits of initiating ART early and the potential for population level reduction of HIV incidence. ART has been demonstrated to reduce HIV acquisition within stable HIV serodiscordant couples (21); this has not been shown to be the case at the population level in a recent cluster randomised trial that investigated this (96). The ANRS 12249 trial showed no difference in HIV incidence between the intervention arm in which all HIV-positive individuals were offered ART regardless of CD4 count and the control arm in which ART was offered according to South African guidelines. Poor linkage of HIV-positive individuals to care was one of the reasons suggested for this lack of effect. However evidence from observational (14) and

ecological studies (380) (97) (99) suggest an impact of ART on population level reduction in HIV incidence.

South Africa has adopted the recent WHO guidelines since September 2016 (15). Despite this bold policy decision, there is a lack of good quality evidence in the African setting on the impact of high CD4 count at ART initiation on adherence. Concern has been expressed that individuals initiating ART at high CD4 count may not be motivated to adhere to treatment lifelong, given the absence of HIV-related symptoms and signs. The resulting poor adherence could lead to virological failure and the development and transmission of drug resistant HIV. I identified only two studies in the African setting in which individuals with high CD4 count at initiation were included in the assessment of factors for adherence (227, 228). Even though these studies included individuals with CD4 >350 cells/mm^3, the median CD4 counts at ART initiation in these studies were low; one study reported a high CD4 count at ART initiation to be associated with a poorer adherence (227) whilst the other study reported no association between CD4 count at ART initiation and adherence (228). In my study, I found no evidence of an association between CD4 count at ART initiation and adherence in individuals with a high median CD4 count at ART initiation. This finding is reassuring from a policy perspective, as universal HIV test and treat has since been implemented. My results corroborate findings of a systematic review that compared adherence in individuals initiating ART at high CD4 count in high income countries (226). I have only examined adherence during the first 12 months of ART. Over this relatively short period of time, optimal adherence was present in 86% of visits. This falls short of the >90% required as the target set by the WHO for the percentage of patients picking up their pills on time in the first 12 months post-ART initiation.This is one of the early warning indicators for the prevention of HIV drug resistance. On time pill pick up is used as a proxy to measure adherence, as many ART programmes found it easier to report this indicator rather than the previous WHO indicator that was based on actual adherence measurements (32). ART is required lifelong, hence optimal adherence to ART needs to be lifelong as well. Some studies have shown that adherence

wanes with time (133, 134), therefore continued vigilance and on-going adherence support will be required to maintain or improve adherence for optimal health outcomes to be sustained.

Virological suppression was very high (>90%) in individuals who initiated ART within the trial but only modest (79%) in individuals ART-experienced at entry.

I had assumed that given the lack of a statistically significant relationship between CD4 count at ART initiation and adherence amongst individuals who initiated ART within the trial, there would also be no significant relationship between CD4 count at ART initiation and virological suppression. I hypothesized that individuals with high CD4 count would have reduced adherence leading to reduced virological suppression in this group. Despite the lack of a statistically significant relationship between CD4 count and adherence, I found that having a high CD4 count at ART initiation was associated with an increased probability of virological suppression, even amongst individuals with the same level of adherence. To date, no study in the African setting has examined the relationship between individuals initiating ART at high CD4 count, and other risk factors with suppression. The TEMPRANO study conducted in Ivory Coast (17) reported virological suppression at 12 and 24 months in the immediate therapy arm in individuals initiating ART at CD4 <800 cells/mm^3 and the deferred therapy arm in those initiating according to standard of care. That study did not, however, explore risk factors for virological suppression at high CD4 count. My finding suggests that the association of high CD4 count with increased virological suppression is not just behavioural (that is due to high level of adherence in that group) but could also be biological. I found a 26% increase in the odds of virological suppression with every 100 units increase in CD4 count at initiation (p=0.009). The strength of this association makes it unlikely to be due to chance. If there is indeed a biological factor, then this is likely to be host-related. Perhaps, a relatively intact immune system is synergistic with the effect of ART. Some studies have shown lower levels of immune activation and better immune response in individuals who initiated ART earlier than those who initiated at low CD4 counts mediated through virological suppression (381-383). I found a similar

association when I examined those who were ART-experienced at trial entry. One explanation for the disparity in the virological suppression rates between those who initiated ART within the trial and those ART-experienced at trial entry could be the difference in quality of care provided. It could also be due to those initiating ART within the trial being prescribed a single tablet regimen (fixed dose combination of tenofovir, emtricitabine, efavirenz) which is easier to take and have a better side-effect profile whilst majority of those ART-experienced at trial entry were on separate tablet regimes, having started off with stavudine or zidovudine containing regimens which are known to be less tolerable due to side-effects (275).

Reluctance to switch to second-line treatment may be linked to the fact that the South African public ART programme is predominantly nurse-led and nurses may not be sufficiently confident to switch patients to second-line ART due to inadequate training. Patient care in the trial was mainly nurse-led, with me being available to see complicated patients including those with adherence issues. The public ART programme uses the same model of care, but scheduled visits to the primary care facilities by doctors from local district hospitals did not often happen due to poor staffing, hence nurses had to make nearly all the decisions. Whilst training the trial nurses, it emerged that in their previous employment, the nurses would interpret a viral load decrease from 100,000 copies/mL to 80,000 copies/mL in someone failing treatment as an improvement , and would reinforce adherence and advice continuation of the same regimen. Furthermore even with the availability of viral load tests in South Africa, patients from the public ART programme were not monitored as per guidelines, resulting in infrequent viral load tests. I estimated that just under a third of individuals who had been on ART for 12 months in the public ART programme had their 12 months viral load measured even after allowing a three month window. The public ART programme probably reflects reality but I have shown that it is possible to achieve high virological suppression rates with additional resources. Apart from the availability of doctors, additional resources provided in the trial were in the form of phone call and home

visits for people missing appointments and adherence support in the form of counselling and occasional assessment of the home situation.

I have shown that nearly 90% of people develop acquired resistance at virological failure. This has implications for the public ART programme because of the potential transmission of drug resistant HIV. With appropriate management, nearly 70% of people regained virological control either by a switch to second-line ART or resuppression on the same regimen. There was no indication that resistance test results would be required to guide a switch to second-line ART in individuals failing first-line therapy as I only found one case in which the second-line ART proposed by the South African guidelines was potentially compromised. The WHO recommends monitoring of acquired resistance to inform decisions about second and third-line ART in a public ART programme (216). Protease inhibitor-based second-line ART with zidovudine and lamivudine as recommended by the South African guideline appeared adequate as the majority of individuals were on tenofovir-based first line regimen. However challenges arise in individuals with renal problems because of drug stock outs affecting recommended alternatives (384). The potential drawback of protease inhibitor-based second-line regimen is the double boosting required in individuals taking rifampicin-based antituberculous chemotherapy (15, 385). The increased pill burden and increased toxicity of lopinavir/ritonavir makes this regimen less tolerable which could negatively impact adherence. There is a move within South Africa to switch to dolutegravir-based fixed dose combination for first-line ART, mainly dolutegravir and tenofovir alafenamide with either lamivudine or emtricitabine (386). This combination has never been used to date and clinical trials are planned to test the efficacy of this combination in South Africa. The main attraction is the high genetic barrier of dolutegravir to resistance (387, 388) with its more favourable toxicity profile compared to efavirenz-based first-line ART (389). However, there are limited data on the use of dolutegravir in patients with tuberculosis (390) and during pregnancy (388). On the other hand, protease-inhibitor based second-line ART is likely to

remain the standard of care in the near future in South Africa in particular and the African setting in general.

In a public health approach, baseline resistance testing is not used to guide ART initiation. However, the WHO recommends surveillance of pre-treatment drug resistance in individuals initiating ART as a way of assessing if the first-line ART in use in a country's ART programme is still appropriate (216). A review of the first-line ART used in a country is recommended if pre-treatment drug resistance to any of the drug class making up the first-line regimen exceeds 15% (28). This recommendation was based on the prevalence of the resistant mutant in majority virus. I found a moderate prevalence of pre-treatment drug resistance (according to the WHO classification of moderate prevalence (5-15%) of about 9% in my research cohort with a doubling of prevalence when minority resistant variants were taken into account. This was predominantly driven by resistance to the NNRTI drug class with a moderate prevalence (5-15%), specifically the K103N/S mutation. Pre-treatment drug resistance to the NRTI and PI drug classes was <5%, classified as low prevalence by the WHO. The high prevalence of transmitted K103N/S, associated with nevirapine and efavirenz resistance is not surprising as it reflects the regimen that has been in use in South Africa. Although, there was a higher prevalence of acquired M184V mutation, associated with lamivudine/emtricitabine resistance, one would expect this to be more commonly transmitted. However, viruses harbouring the M184V mutation are less fit, hence potentially less transmissible (271). This fitness cost to the virus also means it reverts to the more fit wild type virus even after it is transmitted (391). However, the K103N mutation is associated with a lower fitness cost to the virus, hence there is no actual advantage to the virus in reverting to wild-type, hence this mutation tends to persist (202, 203). The transmissibility of the K65R mutation remains a controversial subject with some studies suggesting that viruses harbouring this mutation are not easily transmissible (209) and other studies suggesting otherwise (215). In the small numbers of individuals sequenced at virological failure in my study, there was no evidence of a difference in the viral load of viruses with or

without the K65R mutation. It is unlikely that transmission of the K65R mutation or even NRTI drug class resistance, which is mainly tenofovir-based, will reach 15% in South Africa in the near future. Some individuals had three or more drug resistant mutations suggesting that they may have had previous exposure to ART.

My data did not show that the presence of pre-treatment drug resistance adversely affects virological outcomes in the short-term. This could be because tenofovir and emtricitabine were sufficient to bring about virological suppression, since the majority of participants had resistance to only efavirenz. All other studies that have shown that pre-treament drug resistance was associated with virological failure in the African setting did not evaluate patients taking a fixed dose combination of tenofovir, emtricitabine and efavirenz (292). It is possible that the contradictory findings could potentially be related to a difference in regimen and or the form of pre-treatment drug resistance present in those studies.

Clinical trials to compare current first-line regimen in South Africa (tenofovir, emtricitabine, efavirenz) with dolutegravir-based first-line ART combined with tenofovir alafenamide and lamivudine or emtricitabine are in advanced stage of planning (386). Increasing first-line options in South Africa will be a welcome development, as it is premature to comment on long-term clinical and virological outcomes in patient with pre-treatment drug resistance based on my findings.

In the short-term I found no evidence that treating HIV earlier will lead to drug resistance of the prevalence and form likely to make HIV elimination difficult. This is based on adherence to ART which is independent of CD4 count at initiation and superior virological suppression in individuals who initiated ART at higher CD4 counts. However, the majority of the few patients that failed developed resistance which can be transmitted to sexual partners. There was no evidence that the presence of pre-treatment drug resistance adversely impacted virological suppression in the short-term. The main challenge might be in increasing uptake of HIV test, so that individuals are aware of their HIV status. Improving public health facilities to maximise virological suppression and retention will also be key.

8.3 Limitations

I addressed specific limitations of my research in the respective chapters. An important general limitation of my research was that it was undertaken within a clinical trial, although there was no clinical exclusion criteria applied to the selection of participants. All HIV-positive individuals, 16 years and above and resident within the trial communities were eligible. In terms of clinical complexity, the patients were 'real life' patients, but benefitted from the close monitoring and improved care expected in a clinical trial. This was obvious based on the disparity in virological suppression rates between those initiated on ART within the trial and those arriving in the trial already on ART. All the patients have now been transitioned to the public ART programme with resources that may not be readily available to maintain the same level of care that the patients were used to receiving in the trial. It would be imperative to evaluate these patients now receiving care within the constraints of 'real life' resources.

8.4 Recommendations

Successful clinical outcomes on antiretroviral therapy include durable virological suppression and a reduction in morbidity and mortality (16, 17). There is strong evidence that HIV-positive individuals who are virologically suppressed on ART are less likely to transmit HIV to their sexual partners (21). Hence prevention of HIV transmission through use of ART is now an accepted public health HIV prevention strategy. The question is not so much whether HIV treatment is enough to bring about HIV elimination but if, despite optimisation of other prevention strategies, the anticipated increase in drug resistance resulting from treating HIV earlier will attenuate the impact of combination HIV prevention efforts.

I identified a difference in the quality of care received by ART-naïve individuals initiated on ART within the trial and those ART-experienced on trial entry. This inference is based on average 15% lower virological suppression in those ART-experienced at trial entry despite >3 years on ART on average. The subsequent

virological suppression following a switch to second-line protease inhibitor-based ART in those with virological failure amongst the ART-experienced suggests that they would do well given the right support and care. Optimal adherence is vital for virological suppression. Potential barriers to adherence could be due to either health system or patient-related factors (177). The South African ART guidelines now recommend ART initiation regardless of CD4 count which would result in an increase in the numbers of people requiring ART. With no planned increase in the work-force, this would result in increased out-patient waiting times and an overburdened and stressed workforce. It has been reported that the long-waiting times have meant some patients would leave the health facility without being seen and could be contributory factor to poor retention on ART (392, 393). A potential solution to this challenge would be through the introduction of a chronic disease model of care or differentiated care. This basically means identifying HIV-positive patients who are stable on ART whom it would be safe to reduce the frequency of clinic visits so they can receive their medications within the community (394, 395) The WHO defines stable HIV-positive individuals as those who have been on ART for ≥ 1 year, with no side-effects requiring regular monitoring, no active comorbidity or pregnancy, not currently breastfeeding and who understand their disease and are doing well on treatment (that is two consecutive viral load measurement <1000 copies/mL) (105). The rationale would be to create capacity within the clinics to allow time to be devoted to sicker patients while patients doing well on ART are incentivised with less clinic visits and dispensed ART for up to three months rather than monthly, which is the usual practice. This model has already been successfully implemented in a number of countries (395) including in some townships in South Africa (396, 397). However, in South Africa, this was the exception rather than the norm. In recognition of the pressure of increasing patient load on the health care facilities, the South African government introduced the Central Chronic Medicine Dispensing and Distribution (CCMDD) programme which allows medicine from repeat scripts to be dispensed and distributed to community pick up points (398). This model extends beyond just HIV to other chronic diseases

such as hypertension, diabetes and mental health. Stable patients are identified by healthcare staff in clinics and offered enrolment into the programme. Medication is issued for one month on the day of clinic visits, and if enrolled, subsequent medications are dispensed by a central point and distributed to a patient's preferred pick up point within the community set up for this purpose.There are systems in place to notify patients that their medications are ready for collections at their specified pick up point as well as systems for flagging up patients who failed to attend for their pick up. This system is currently being rolled out across South Africa. If it succeeds, it would provide immense benefits in curbing some of the barriers to medication adherence such as long waiting times and travel distance to clinic (392). However, in my study there was only a weak evidence of an association between travel distance to clinic and adherence amongst those who initiated ART within the trial. Having 22 trial clinics spread out across the communities must have made access easier for patients, but this might not be the case now the TasP trial communities are only served by three public ART clinics following the closure of trial clinics at the end of the trial.

I observed that, patients who had virological failure at their first trial clinic visit amongst those ART experienced were still taking their first line ART even though they had been failing for a number of years. There are a number of reasons why this situation could have arisen; lack of adherence to guidelines on patient monitoring, with only a third of ART-experienced patients observed to have documented 12 months viral load, failure to check results of blood tests after they have been requested, failure to transmit blood results from the laboratory in the local district hospital to the primary health care clinics and or nurses not feeling competent to act on abnormal results. The ART programme in South Africa is primarily nurse-led. These nurses were supposed to have received training on the "Nurse initiated management of antiretroviral therapy" (399, 400), however not all nurses have been able to go through this training programme. The few who have often seek employment with non-governmental organisations further depleting the public sector of much needed workforce. The nurses will often reinforce adherence

during each visit for patients failing treatment, with a referral for doctor review at the next clinic visit. In principle, primary health care clinics are visited weekly by doctors from the local district hospitals but these visits are not regular as a result of staff shortages in the hospital. Hence patients go through a cycle of adherence counselling at each visit with no decision taken about switching ART. This situation could partly be resolved by capacitating nurses to be able to switch patients to second-line therapy. Computers and fax machines for receiving results from the laboratory, are often not in good working order as repair or replacement sometimes take several months or in some cases does not happen. With the introduction of the ideal clinic initiative by the South African government (401) which basically means making primary health care clinics fit for purpose with infrastructure rehabilitation, adequate human resources and streamlined care through additional investments, it is anticipated that some of these challenges would be addressed in the coming years.

In the trial, there was a system in place for following up patients who missed clinic appointments, through phone calls from the counsellors or nurses in the trial clinics. If the patient failed to attend after a phone call or if they were not contacted, the patient would be visited in their homes by healthcare staff employed as trackers. All patients were asked for consent at the first clinic visit to allow home visits should they fail to attend their clinic appointments. This model already exists in the public ART programme, but its implementation is very clinic and area dependent. Currently nurses should be phoning patients who missed their appointments and offering them alternative appointments, but whether this happens or not depends on the motivation and time to do it. Community care givers who operate within clinic catchment areas have the responsibility of tracking patients who failed their clinic appointments and increasingly play important roles in community adherence clubs and as patient advocates (402). The community care givers will need to work in close collaboration with the clinics in their catchment areas so that there is bidirectional flow of information between them and

the facility-based health care workers so as to increase the effectiveness of the community care givers.

There should be ongoing surveillance of pre-treatment and acquired drug resistance in the population. Surveillance of pre-treament drug resistance will help inform choice of first-line regimen for the ART programme while surveillance of acquired resistance will help inform choice of second and third-line ART.

8.5 Future research

In the short-term, adherence in individuals initiating ART at CD4 > 350 cells/mm^3 has been as good as those initiating ART at lower CD4 counts. Individuals with higher CD4 counts were observed to have superior virological suppression. Pre-treatment drug resistance did not appear to have an impact on virological suppression over the short duration participants were followed up on ART. To address the question as to whether early treatment of HIV will lead to drug resistance of the form and prevalence likely to make HIV elimination difficult will require long-term studies in population initiating ART at higher CD4 counts. The future research question will focus on the long-term clinical outcomes of HIV-positive individuals initiating ART regardless of CD4 counts. HIV prevalence continues to rise because of a decrease in mortality due to ART and a failure of incidence to decrease at least in the community in which my research was conducted (96). This coupled with a fragile health system with poor patient monitoring will likely result in an increase in the numbers of individuals with virological failure. Furthermore as individuals on second-line therapy start to fail ART, decisions will need to be made as to what an appropriate third-line ART would be. Over the next 5 years, I would like to characterise the performance of the ART programme by evaluating long-term clinical outcomes and resistance patterns in the population. This would also identify gaps in the implementation of the recommendations I made above some of which are already being implemented. Data generated from this research will be used to parameterise models such as the

HIV synthesis model (403), which also takes into account the different resistance mutations and impact on drug activity, to make future projections about the impact of ART on HIV prevention.

8.6 Conclusion

Early initiation of antiretroviral therapy has proven individual and public health benefits. This research demonstrated that in the short-term, concerns about whether HIV-positive individuals treated at high CD4 counts, the majority of whom would be asymptomatic, would be motivated to adhere to ART may be unfounded. Virological suppression was superior in individuals who initiated ART at higher CD4 count, and this was not explained by adherence. Although drug resistance mutations continue to emerge in individuals failing ART with a consequent moderate prevalence in pre-treatment drug resistance, the levels do not seem to threaten the ART programme in the short-term. Long-term studies will be critical in assessing the durability of these favourable outcomes.

References

1. Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science (New York, NY). 1983;220(4599):868-71.

2. Gallo RC, Salahuddin SZ, Popovic M, Shearer GM, Kaplan M, Haynes BF, et al. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science (New York, NY). 1984;224(4648):500-3.

3. CDC. Kaposi's sarcoma and Pneumocystis pneumonia among homosexual men—New York City and California. 1981.

4. UNAIDS. FACT SHEET 2016 [cited 2016 10 February]. Available from: http://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf.

5. Sharp PM, Hahn BH. Origins of HIV and the AIDS pandemic. Cold Spring Harbor perspectives in medicine. 2011;1(1):a006841.

6. UNAIDS. Global AIDS Update 2016 2016 [cited 2016 7 Jan 2016]. Available from: http://www.unaids.org/sites/default/files/media_asset/global-AIDS-update-2016_en.pdf.

7. UNAIDS. 90-90-90 An ambitious treatment target to help end the AIDS epidemic 2014 [cited 2015 22/01/2015]. Available from: http://www.unaids.org/sites/default/files/media_asset/90-90-90_en_0.pdf.

8. Piot P, Abdool Karim SS, Hecht R, Legido-Quigley H, Buse K, Stover J, et al. Defeating AIDS--advancing global health. Lancet. 2015;386(9989):171-218.

9. National Department of Health SA, ,. The 2013 National Antenatal Sentinel HIV Prevalence Survey South Africa 2013 [cited 2017 10 Feb]. Available from: http://www.hst.org.za/sites/default/files/DoH%20Sentinel%20HIV%20Survey%20High%20Res%207102015.pdf.

10. South African National AIDS Council (SANAC). PROGRESS REPORT ON THE NATIONAL STRATEGIC PLAN FOR HIV, TB AND STIs (2012 – 2016) 2014

[cited 2017, 15 Feb]. Available from: http://sanac.org.za//wp-content/uploads/2015/12/SANAC-NSP-Progress-Report_6Nov2014_hires.pdf.

11. Shisana O, Rehle T, LC S, Zuma K, Jooste S, N Z, et al. South African National HIV Prevalence, Incidence and Behaviour Survey, 2012. . Cape Town: HSRC; 2014.

12. UNAIDS. South Africa HIV Epidemic Profile 2014 [cited 2017 12 Feb]. Available from: http://www.unaidsrstesa.org/wp-content/uploads/2015/05/UNAids-Profile-South-Africa.pdf-18-Feb.pdf.

13. Anderson SJ, Cherutich P, Kilonzo N, Cremin I, Fecht D, Kimanga D, et al. Maximising the effect of combination HIV prevention through prioritisation of the people and places in greatest need: a modelling study. Lancet. 2014;384(9939):249-56.

14. Tanser F, Barnighausen T, Grapsa E, Zaidi J, Newell ML. High coverage of ART associated with decline in risk of HIV acquisition in rural KwaZulu-Natal, South Africa. Science. 2013;339(6122):966-71.

15. National Department of Health SA, ,. Implementation of the universal test and treat strategy for HIV positive patients and differentiated care for stable patients 2016 [cited 2016, 17 Dec].

16. Group ISS, 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(9):795-807.

17. Group TAS, Danel C, Moh R, Gabillard D, Badje A, Le Carrou J, et al. A Trial of Early Antiretrovirals and Isoniazid Preventive Therapy in Africa. The New England journal of medicine. 2015;373(9):808-22.

18. Granich RM, Gilks CF, Dye C, De Cock KM, Williams BG. Universal voluntary HIV testing with immediate antiretroviral therapy as a strategy for elimination of HIV transmission: a mathematical model. Lancet. 2009;373(9657):48-57.

19. Merson MH, O'Malley J, Serwadda D, Apisuk C. The history and challenge of HIV prevention. Lancet. 2008;372(9637):475-88.

20. Quinn TC, Wawer MJ, Sewankambo N, Serwadda D, Li C, Wabwire-Mangen F, et al. Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group. The New England journal of medicine. 2000;342(13):921-9.

21. 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(6):493-505.

22. South Africa National Department of Health. National antiretroviral treatment guidelines 2004 [Available from: http://southafrica.usembassy.gov/media/2004-doh-art-guidelines.pdf.

23. South Africa National Department of Health. Clinical guidelines for the management of HIV & AIDS in adults and adolescents 2010 [cited 2014 25/05/2014]. Available from: http://www.sahivsoc.org/upload/documents/Clinical_Guidelines_for_the_Management_of_HIV_AIDS_in_Adults_Adolescents_2010.pdf.

24. South Africa National AIDS Council. South Africa National AIDS Council meeting in Blomfonteinon 12 August 2011 [Available from: http://www.setsoto.info/vbtube_show.php?s=c6f0b4875d711326c551c9cd92f70b50&tubeid=120&sti=South-African-National-Aids-Council-Meeting-in-Bloemfonteinon-12-August-2011.

25. Iwuji CC, Orne-Gliemann J, Tanser F, Boyer S, Lessells RJ, Lert F, et al. Evaluation of the impact of immediate versus WHO recommendations-guided antiretroviral therapy initiation on HIV incidence: the ANRS 12249 TasP (Treatment as Prevention) trial in Hlabisa sub-district, KwaZulu-Natal, South Africa: study protocol for a cluster randomised controlled trial. Trials. 2013;14:230.

26. 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(7):e21795.

27. Manasa J, Lessells RJ, Skingsley A, Naidu KK, Newell ML, McGrath N, 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. 2013;8(8):e72152.

28. Bennett DE, Myatt M, Bertagnolio S, Sutherland D, Gilks CF. Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. Antiviral therapy. 2008;13 Suppl 2:25-36.

29. Cambiano V, Bertagnolio S, Jordan MR, Pillay D, Perriens JH, Venter F, et al. Predicted levels of HIV drug resistance: potential impact of expanding diagnosis, retention, and eligibility criteria for antiretroviral therapy initiation. AIDS (London, England). 2014;28 Suppl 1:S15-23.

30. Van Damme L, Corneli A, Ahmed K, Agot K, Lombaard J, Kapiga S, et al. Preexposure prophylaxis for HIV infection among African women. The New England journal of medicine. 2012;367(5):411-22.

31. Marrazzo J, Ramjee, G, Nair, G. Pre-exposure prophylaxis for HIV in women: daily oral tenofovir, oral tenofovir/emtricitabine or vaginal tenofovir gel in the VOICE study (MTN 003). 20th Conference on Retroviruses and Opportunistic Infections, Atlanta, abstract 26LB, 2013. 2013.

32. World Health Organisation. GLOBAL REPORT ON EARLY WARNING INDICATORS OF HIV DRUG RESISTANCE 2016 [cited 2017, 15 Feb]. Available from: http://www.who.int/hiv/pub/drugresistance/ewi-hivdr-2016/en/.

33. UNAIDS. Combination HIV Prevention:Tailoring and Coordinating Biomedical, Behavioural and Structural Strategies to Reduce New HIV Infections. A UNAIDS Discussion Paper 2010. [Available from: http://www.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2011/20111110_JC2007_Combination_Prevention_paper_en.pdf.

34. Sexton J, Garnett G, Rottingen JA. Metaanalysis and metaregression in interpreting study variability in the impact of sexually transmitted diseases on susceptibility to HIV infection. Sexually transmitted diseases. 2005;32(6):351-7.

35. Glynn JR, Biraro S, Weiss HA. Herpes simplex virus type 2: a key role in HIV incidence. AIDS (London, England). 2009;23(12):1595-8.

36. Hayes R, Watson-Jones D, Celum C, van de Wijgert J, Wasserheit J. Treatment of sexually transmitted infections for HIV prevention: end of the road or new beginning? AIDS (London, England). 2010;24 Suppl 4:S15-26.

37. Deschamps MM, Pape JW, Hafner A, Johnson WD, Jr. Heterosexual transmission of HIV in Haiti. Annals of internal medicine. 1996;125(4):324-30.

38. Gray RH, Wawer MJ, Sewankambo NK, Serwadda D, Li C, Moulton LH, et al. Relative risks and population attributable fraction of incident HIV associated with symptoms of sexually transmitted diseases and treatable symptomatic sexually transmitted diseases in Rakai District, Uganda. Rakai Project Team. AIDS (London, England). 1999;13(15):2113-23.

39. Grosskurth H, Mosha F, Todd J, Mwijarubi E, Klokke A, Senkoro K, et al. Impact of improved treatment of sexually transmitted diseases on HIV infection in rural Tanzania: randomised controlled trial. Lancet. 1995;346(8974):530-6.

40. Ghys PD, Diallo MO, Ettiegne-Traore V, Satten GA, Anoma CK, Maurice C, et al. Effect of interventions to control sexually transmitted disease on the incidence of HIV infection in female sex workers. AIDS (London, England). 2001;15(11):1421-31.

41. Kaul R, Kimani J, Nagelkerke NJ, Fonck K, Ngugi EN, Keli F, et al. Monthly antibiotic chemoprophylaxis and incidence of sexually transmitted infections and HIV-1 infection in Kenyan sex workers: a randomized controlled trial. JAMA : the journal of the American Medical Association. 2004;291(21):2555-62.

42. White RG, Moodley P, McGrath N, Hosegood V, Zaba B, Herbst K, et al. Low effectiveness of syndromic treatment services for curable sexually transmitted infections in rural South Africa. Sexually transmitted infections. 2008;84(7):528-34.

43. Peters RP, Dubbink JH, van der Eem L, Verweij SP, Bos ML, Ouburg S, et al. Cross-sectional study of genital, rectal, and pharyngeal Chlamydia and gonorrhea in women in rural South Africa. Sexually transmitted diseases. 2014;41(9):564-9.

44. Watson-Jones D, Weiss HA, Rusizoka M, Changalucha J, Baisley K, Mugeye K, et al. Effect of herpes simplex suppression on incidence of HIV among women in Tanzania. The New England journal of medicine. 2008;358(15):1560-71.

45. Celum C, Wald A, Hughes J, Sanchez J, Reid S, Delany-Moretlwe S, et al. Effect of aciclovir on HIV-1 acquisition in herpes simplex virus 2 seropositive women and men who have sex with men: a randomised, double-blind, placebo-controlled trial. Lancet. 2008;371(9630):2109-19.

46. Fleming DT, Wasserheit JN. From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. Sexually transmitted infections. 1999;75(1):3-17.

47. Weiss HA, Quigley MA, Hayes RJ. Male circumcision and risk of HIV infection in sub-Saharan Africa: a systematic review and meta-analysis. AIDS (London, England). 2000;14(15):2361-70.

48. Auvert B, Taljaard D, Lagarde E, Sobngwi-Tambekou J, Sitta R, Puren A. Randomized, controlled intervention trial of male circumcision for reduction of HIV infection risk: the ANRS 1265 Trial. PLoS medicine. 2005;2(11):e298.

49. Bailey RC, Moses S, Parker CB, Agot K, Maclean I, Krieger JN, et al. Male circumcision for HIV prevention in young men in Kisumu, Kenya: a randomised controlled trial. Lancet. 2007;369(9562):643-56.

50. Gray RH, Kigozi G, Serwadda D, Makumbi F, Watya S, Nalugoda F, et al. Male circumcision for HIV prevention in men in Rakai, Uganda: a randomised trial. Lancet. 2007;369(9562):657-66.

51. Siegfried N, Muller M, Deeks JJ, Volmink J. Male circumcision for prevention of heterosexual acquisition of HIV in men. Cochrane database of systematic reviews (Online). 2009(2):CD003362.

52. Sanchez J, Sal YRVG, Hughes JP, Baeten JM, Fuchs J, Buchbinder SP, et al. Male circumcision and risk of HIV acquisition among MSM. AIDS (London, England). 2011;25(4):519-23.

53. Schiffner T, Sattentau QJ, Dorrell L. Development of prophylactic vaccines against HIV-1. Retrovirology. 2013;10:72.

54. Walker BD, Ahmed R, Plotkin S. Moving ahead an HIV vaccine: use both arms to beat HIV. Nature medicine. 2011;17(10):1194-5.

55. Flynn NM, Forthal DN, Harro CD, Judson FN, Mayer KH, Para MF, et al. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. The Journal of infectious diseases. 2005;191(5):654-65.

56. Pitisuttithum P, Gilbert P, Gurwith M, Heyward W, Martin M, van Griensven F, et al. Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. The Journal of infectious diseases. 2006;194(12):1661-71.

57. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet. 2008;372(9653):1881-93.

58. Gray GE, Allen M, Moodie Z, Churchyard G, Bekker LG, Nchabeleng M, et al. Safety and efficacy of the HVTN 503/Phambili study of a clade-B-based HIV-1 vaccine in South Africa: a double-blind, randomised, placebo-controlled test-of-concept phase 2b study. The Lancet infectious diseases. 2011;11(7):507-15.

59. NIH. NIH Discontinues Immunizations in HIV Vaccine Study 2013 [cited 2014; 10/09/2014]. Available from: http://www.niaid.nih.gov/news/newsreleases/2013/Pages/HVTN505April2013.aspx.

60. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. The New England journal of medicine. 2009;361(23):2209-20.

61. Hammer SM, Sobieszczyk ME, Janes H, Karuna ST, Mulligan MJ, Grove D, et al. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. The New England journal of medicine. 2013;369(22):2083-92.

62. Connor EM, Sperling RS, Gelber R, Kiselev P, Scott G, O'Sullivan MJ, et al. Reduction of maternal-infant transmission of human immunodeficiency virus

type 1 with zidovudine treatment. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. The New England journal of medicine. 1994;331(18):1173-80.

63. Townsend CL, Cortina-Borja M, Peckham CS, de Ruiter A, Lyall H, Tookey PA. Low rates of mother-to-child transmission of HIV following effective pregnancy interventions in the United Kingdom and Ireland, 2000-2006. AIDS (London, England). 2008;22(8):973-81.

64. Vernazza P, Hirshel B, Bernasconi E, Flepp M. Les personnes séropositives ne souffrant d'aucune autre MST et suivant un traitement antirétroviral efficace ne transmettent pas le VIH par voie sexuelle. Bulletins des médecins suisses. 2008;89:165-9.

65. Centers for Disease C, Prevention. Case-control study of HIV seroconversion in health-care workers after percutaneous exposure to HIV-infected blood--France, United Kingdom, and United States, January 1988-August 1994. MMWR Morbidity and mortality weekly report. 1995;44(50):929-33.

66. Schechter M, do Lago RF, Mendelsohn AB, Moreira RI, Moulton LH, Harrison LH, et al. Behavioral impact, acceptability, and HIV incidence among homosexual men with access to postexposure chemoprophylaxis for HIV. Journal of acquired immune deficiency syndromes (1999). 2004;35(5):519-25.

67. Drezzet J. Post exposure prophylaxis in raped women. IV International Conference in HIV Infection in women and children; Rio de Janeiro: Livro de Resumos. Universidade, Federal do Rio De Janeiro e Institute of Virology of Maryland 2002.

68. Harrison LH, Do Lago RF, Moreira RI, M S. Demand for post-sexual-exposure chemoprophylaxis for the prevention of HIV infection in Brazil [abstract 492]. . 7th Conference on Retroviruses and Opportunistic Infections; January 30--February 2, 2000; San Francisco, California 2000.

69. Hontelez JA, de Vlas SJ, Tanser F, Bakker R, Barnighausen T, Newell ML, et al. The impact of the new WHO antiretroviral treatment guidelines on HIV epidemic dynamics and cost in South Africa. PloS one. 2011;6(7):e21919.

70. Peterson L, Taylor D, Roddy R, Belai G, Phillips P, Nanda K, et al. Tenofovir disoproxil fumarate for prevention of HIV infection in women: a phase 2, double-blind, randomized, placebo-controlled trial. PLoS clinical trials. 2007;2(5):e27.

71. Abdool Karim Q, Abdool Karim SS, Frohlich JA, Grobler AC, Baxter C, Mansoor LE, et al. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. Science (New York, NY). 2010;329(5996):1168-74.

72. Marrazzo JM, Ramjee G, Richardson BA, Gomez K, Mgodi N, Nair G, et al. Tenofovir-based preexposure prophylaxis for HIV infection among African women. The New England journal of medicine. 2015;372(6):509-18.

73. 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(27):2587-99.

74. Thigpen MC, Kebaabetswe PM, Paxton LA, Smith DK, Rose CE, Segolodi TM, et al. Antiretroviral preexposure prophylaxis for heterosexual HIV transmission in Botswana. The New England journal of medicine. 2012;367(5):423-34.

75. Baeten JM, Donnell D, Ndase P, Mugo NR, Campbell JD, Wangisi J, et al. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. The New England journal of medicine. 2012;367(5):399-410.

76. Choopanya K, Martin M, Suntharasamai P, Sangkum U, Mock PA, Leethochawalit M, et al. Antiretroviral prophylaxis for HIV infection in injecting drug users in Bangkok, Thailand (the Bangkok Tenofovir Study): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet. 2013;381(9883):2083-90.

77. Rees H, Delany-Moretlwe S, Baron D, Lomba C, editors. FACTS 001 Phase III Trial of Pericoital Tenofovir 1% Gel for HIV Prevention in Women. CROI 2015; 2015 Feb 23-26, 2015; Seattle, Washington.

78. Molina JM, Capitant C, Spire B, Pialoux G, Cotte L, Charreau I, et al. On-Demand Preexposure Prophylaxis in Men at High Risk for HIV-1 Infection. The New England journal of medicine. 2015;373(23):2237-46.

79. McCormack S, Dunn DT, Desai M, Dolling DI, Gafos M, Gilson R, et al. Pre-exposure prophylaxis to prevent the acquisition of HIV-1 infection (PROUD): effectiveness results from the pilot phase of a pragmatic open-label randomised trial. Lancet. 2016;387(10013):53-60.

80. Baeten JM, Palanee-Phillips T, Brown ER, Schwartz K, Soto-Torres LE, Govender V, et al. Use of a Vaginal Ring Containing Dapivirine for HIV-1 Prevention in Women. The New England journal of medicine. 2016;375(22):2121-32.

81. Nel A, van Niekerk N, Kapiga S, Bekker LG, Gama C, Gill K, et al. Safety and Efficacy of a Dapivirine Vaginal Ring for HIV Prevention in Women. The New England journal of medicine. 2016;375(22):2133-43.

82. Musicco M, Lazzarin A, Nicolosi A, Gasparini M, Costigliola P, Arici C, et al. Antiretroviral treatment of men infected with human immunodeficiency virus type 1 reduces the incidence of heterosexual transmission. Italian Study Group on HIV Heterosexual Transmission. Archives of internal medicine. 1994;154(17):1971-6.

83. Melo LS, Lacerda HR, Campelo E, Moraes E, Ximenes RA. Survival of AIDS patients and characteristics of those who died over eight years of highly active antiretroviral therapy, at a referral center in northeast Brazil. The Brazilian journal of infectious diseases : an official publication of the Brazilian Society of Infectious Diseases. 2008;12(4):269-77.

84. Sullivan P, Kayitenkore K, Chomba E, Karita E, Mwananyanda L, Vwalika C, et al. Is the reduction of HIV transmission risk while prescribed antiretroviral therapy (ARVT) different for men and women? Results from discordant couples in Rwanda and Zambia. 5th IAS Conference on HIV Pathogenesis and Treatment. Abstract no. WEAC101. 2009.

85. Del Romero J, Castilla J, Hernando V, Rodriguez C, Garcia S. Combined antiretroviral treatment and heterosexual transmission of HIV-1: cross sectional and prospective cohort study. Bmj. 2010;340:c2205.

86. Donnell D, Baeten JM, Kiarie J, Thomas KK, Stevens W, Cohen CR, et al. Heterosexual HIV-1 transmission after initiation of antiretroviral therapy: a prospective cohort analysis. Lancet. 2010;375(9731):2092-8.

87. Lu W, Zeng G, Luo J, Duo S, Xing G, Guo-Wei D, et al. HIV transmission risk among serodiscordant couples: a retrospective study of former plasma donors in Henan, China. Journal of acquired immune deficiency syndromes (1999). 2010;55(2):232-8.

88. Reynolds SJ, Makumbi F, Nakigozi G, Kagaayi J, Gray RH, Wawer M, et al. HIV-1 transmission among HIV-1 discordant couples before and after the introduction of antiretroviral therapy. AIDS (London, England). 2011;25(4):473-7.

89. Birungi J, Wang H, Ngolobe M, Muldoon K, Khanakwa S, King R, et al., editors. Lack of effectiveness of antiretroviral therapy (ART) as an HIV prevention tool for serodiscordant couples in a rural ART program without viral load monitoring in Uganda. XIX International AIDS Conference; 2012, July 23-27; Washington, DC.

90. Jia Z, Mao Y, Zhang F, Ruan Y, Ma Y, Li J, et al. Antiretroviral therapy to prevent HIV transmission in serodiscordant couples in China (2003-11): a national observational cohort study. Lancet. 2013;382(9899):1195-203.

91. Oldenburg CE, Barnighausen T, Tanser F, Iwuji CC, De Gruttola V, Seage GR, 3rd, et al. Antiretroviral Therapy to Prevent HIV Acquisition in Serodiscordant Couples in a Hyperendemic Community in Rural South Africa. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2016;63(4):548-54.

92. Anglemyer A, Rutherford GW, Horvath T, Baggaley RC, Egger M, Siegfried N. Antiretroviral therapy for prevention of HIV transmission in HIV-discordant couples. Cochrane database of systematic reviews (Online). 2013;4:CD009153.

93. Attia S, Egger M, Muller M, Zwahlen M, Low N. Sexual transmission of HIV according to viral load and antiretroviral therapy: systematic review and meta-analysis. AIDS (London, England). 2009;23(11):1397-404.

94. Hayes R, Ayles H, Beyers N, Sabapathy K, Floyd S, Shanaube K, et al. HPTN 071 (PopART): rationale and design of a cluster-randomised trial of the

population impact of an HIV combination prevention intervention including universal testing and treatment - a study protocol for a cluster randomised trial. Trials. 2014;15:57.

95. AVAC, UNAIDS,. Antiretroviral Treatment for Prevention of HIV and Tuberculosis. 2013 update on current and planned research efforts 2014 [05/02/2015]. Available from: http://www.avac.org/sites/default/files/resource-files/ART%20for%20prevention%20study%20update%20report%20March%202014.pdf.

96. Iwuji C, Orne-Gliemann J, Balestre E, Larmarange J, Thiebaut R, Tanser F, et al. The impact of universal test and treat on HIV incidence in a rural South African population: ANRS 12249 TasP trial, 2012-2016. International AIDS Conference; July 18-22; Durban, South Africa 2016.

97. Fang CT, Hsu HM, Twu SJ, Chen MY, Chang YY, Hwang JS, et al. Decreased HIV transmission after a policy of providing free access to highly active antiretroviral therapy in Taiwan. The Journal of infectious diseases. 2004;190(5):879-85.

98. Gill VS, Lima VD, Zhang W, Wynhoven B, Yip B, Hogg RS, et al. Improved virological outcomes in British Columbia concomitant with decreasing incidence of HIV type 1 drug resistance detection. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2010;50(1):98-105.

99. Das M, Chu PL, Santos GM, Scheer S, Vittinghoff E, McFarland W, et al. Decreases in community viral load are accompanied by reductions in new HIV infections in San Francisco. PloS one. 2010;5(6):e11068.

100. Moore RD, Gebo KA, Lucas GM, Keruly JC. Rate of comorbidities not related to HIV infection or AIDS among HIV-infected patients, by CD4 cell count and HAART use status. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2008;47(8):1102-4.

101. Kowalska JD, Mocroft A, Ledergerber B, Florence E, Ristola M, Begovac J, et al. A standardized algorithm for determining the underlying cause of death in

HIV infection as AIDS or non-AIDS related: results from the EuroSIDA study. HIV clinical trials. 2011;12(2):109-17.

102. Baker JV, Peng G, Rapkin J, Abrams DI, Silverberg MJ, MacArthur RD, et al. CD4+ count and risk of non-AIDS diseases following initial treatment for HIV infection. AIDS (London, England). 2008;22(7):841-8.

103. Danel C, Gabillard D, Le Carrou J, Anglaret X, Moh R, Eholie S, et al. Early ART and IPT in HIV-Infected African Adults With High CD4 Count (Temprano Trial). CROI 2015; Feb 23-26, 2015; Seattle Washington2015.

104. National Institutes of Health. Starting antiretroviral treatment early improves outcomes for HIV-infected individuals 2015 [cited 2015 31 May 2015]. Available from: http://www.nih.gov/news/health/may2015/niaid-27.htm.

105. World Health Organisation. Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxix for HIV 2015 [cited 2016 9/11/2016]. Available from: http://apps.who.int/iris/bitstream/10665/186275/1/9789241509565_eng.pdf.

106. Greenberg AE, Hader SL, Masur H, Young AT, Skillicorn J, Dieffenbach CW. Fighting HIV/AIDS in Washington, D.C. Health affairs. 2009;28(6):1677-87.

107. Miller WC, Lesko CR, Powers KA. The HIV care cascade: simple concept, complex realization. Sexually transmitted diseases. 2014;41(1):41-2.

108. De Cock KM. Plus ca change ... antiretroviral therapy, HIV prevention, and the HIV treatment cascade. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014;58(7):1012-4.

109. Gill MJ, Krentz HB. Unappreciated epidemiology: the churn effect in a regional HIV care programme. International journal of STD & AIDS. 2009;20(8):540-4.

110. Rebeiro P, Althoff KN, Buchacz K, Gill J, Horberg M, Krentz H, et al. Retention among North American HIV-infected persons in clinical care, 2000-2008. Journal of acquired immune deficiency syndromes. 2013;62(3):356-62.

111. Hallett TB, Eaton JW. A side door into care cascade for HIV-infected patients? Journal of acquired immune deficiency syndromes. 2013;63 Suppl 2:S228-32.

112. Raymond A, Hill A, Pozniak A. Large disparities in HIV treatment cascades between eight European and high-income countries – analysis of break points 2014 [Available from: http://www.jiasociety.org/index.php/jias/article/view/19507.

113. Krentz HB, MacDonald J, Gill MJ. The impact of transfer patients on the local cascade of HIV care continuum. Journal of acquired immune deficiency syndromes. 2015;68(2):236-40.

114. Lourenco L, Colley G, Nosyk B, Shopin D, Montaner JS, Lima VD, et al. High levels of heterogeneity in the HIV cascade of care across different population subgroups in British Columbia, Canada. PloS one. 2014;9(12):e115277.

115. Takuva S, Puren A, Brown A, Delpech V, Macleod W, Pillay Y. Disparities in Engagement Within HIV Care in South Africa. CROI 2015; Feb 23-26, 2015; Seattle, Washington2015.

116. Kranzer K, van Schaik N, Karmue U, Middelkoop K, Sebastian E, Lawn SD, et al. High prevalence of self-reported undiagnosed HIV despite high coverage of HIV testing: a cross-sectional population based sero-survey in South Africa. PloS one. 2011;6(9):e25244.

117. UNAIDS. UNAIDS report on the global AIDS epidemic 2013 [22/08/2014]. Available from: http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf.

118. Campsmith ML, Rhodes PH, Hall HI, Green TA. Undiagnosed HIV prevalence among adults and adolescents in the United States at the end of 2006. Journal of acquired immune deficiency syndromes. 2010;53(5):619-24.

119. Marks G, Crepaz N, Senterfitt JW, Janssen RS. Meta-analysis of high-risk sexual behavior in persons aware and unaware they are infected with HIV in the United States: implications for HIV prevention programs. Journal of acquired immune deficiency syndromes. 2005;39(4):446-53.

120. McNairy ML, El-Sadr WM. Antiretroviral therapy for the prevention of HIV transmission: what will it take? Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014;58(7):1003-11.

121. Musheke M, Ntalasha H, Gari S, McKenzie O, Bond V, Martin-Hilber A, et al. A systematic review of qualitative findings on factors enabling and deterring uptake of HIV testing in Sub-Saharan Africa. BMC public health. 2013;13:220.

122. Deblonde J, De Koker P, Hamers FF, Fontaine J, Luchters S, Temmerman M. Barriers to HIV testing in Europe: a systematic review. European journal of public health. 2010;20(4):422-32.

123. Kranzer K, Govindasamy D, Ford N, Johnston V, Lawn SD. Quantifying and addressing losses along the continuum of care for people living with HIV infection in sub-Saharan Africa: a systematic review. Journal of the International AIDS Society. 2012;15(2):17383.

124. Plazy M, Farouki KE, Iwuji C, Okesola N, Orne-Gliemann J, Larmarange J, et al. Access to HIV care in the context of universal test and treat: challenges within the ANRS 12249 TasP cluster-randomized trial in rural South Africa. Journal of the International AIDS Society. 2016;19(1):20913.

125. Govindasamy D, Ford N, Kranzer K. Risk factors, barriers and facilitators for linkage to antiretroviral therapy care: a systematic review. AIDS (London, England). 2012;26(16):2059-67.

126. Rosen S, Fox MP. Retention in HIV care between testing and treatment in sub-Saharan Africa: a systematic review. PLoS Med. 2011;8(7):e1001056.

127. Fox MP, Rosen S. Patient retention in antiretroviral therapy programs up to three years on treatment in sub-Saharan Africa, 2007-2009: systematic review. Tropical medicine & international health : TM & IH. 2010;15 Suppl 1:1-15.

128. Marks G, Gardner LI, Craw J, Crepaz N. Entry and retention in medical care among HIV-diagnosed persons: a meta-analysis. AIDS (London, England). 2010;24(17):2665-78.

129. WHO. Adherence to long term therapies. Evidence for action 2003 [cited 2013 22/09/2013]. Available from: http://www.who.int/chp/knowledge/publications/adherence_full_report.pdf.

130. Paterson DL, Swindells S, Mohr J, Brester M, Vergis EN, Squier C, et al. Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. Annals of internal medicine. 2000;133(1):21-30.

131. Chi BH, Cantrell RA, Zulu I, Mulenga LB, Levy JW, Tambatamba BC, et al. Adherence to first-line antiretroviral therapy affects non-virologic outcomes among patients on treatment for more than 12 months in Lusaka, Zambia. International journal of epidemiology. 2009;38(3):746-56.

132. Maqutu D, Zewotir T, North D, Naidoo K, Grobler A. Factors affecting first-month adherence to antiretroviral therapy among HIV-positive adults in South Africa. African journal of AIDS research : AJAR. 2010;9(2):117-24.

133. Gardner EM, Burman WJ, Maravi ME, Davidson AJ. Durability of adherence to antiretroviral therapy on initial and subsequent regimens. AIDS patient care and STDs. 2006;20(9):628-36.

134. Mannheimer S, Friedland G, Matts J, Child C, Chesney M. The consistency of adherence to antiretroviral therapy predicts biologic outcomes for human immunodeficiency virus-infected persons in clinical trials. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2002;34(8):1115-21.

135. Mills EJ, Nachega JB, Bangsberg DR, Singh S, Rachlis B, Wu P, et al. Adherence to HAART: a systematic review of developed and developing nation patient-reported barriers and facilitators. PLoS Med. 2006;3(11):e438.

136. Patel A, Hirschhorn L, Fullem A, Ojikutu B, Oser R. Adult Aherence to Treatment and Retention in Care.: Arlington, VA: USAID | AIDSTAR-ONE PROJECT, Task Order 1.; 2010 [Available from: http://www.aidstar-one.com/focus_areas/treatment/resources/technical_briefs/adult_adherence_treatment_and_retention_care.

137. Clifford S, Barber N, Horne R. Understanding different beliefs held by adherers, unintentional nonadherers, and intentional nonadherers: application of the Necessity-Concerns Framework. Journal of psychosomatic research. 2008;64(1):41-6.

138. Oyugi JH, Byakika-Tusiime J, Ragland K, Laeyendecker O, Mugerwa R, Kityo C, et al. Treatment interruptions predict resistance in HIV-positive individuals purchasing fixed-dose combination antiretroviral therapy in Kampala, Uganda. AIDS (London, England). 2007;21(8):965-71.

139. Eholie SP, Tanon A, Polneau S, Ouiminga M, Djadji A, Kangah-Koffi C, et al. Field adherence to highly active antiretroviral therapy in HIV-infected adults in Abidjan, Cote d'Ivoire. J Acquir Immune Defic Syndr. 2007;45(3):355-8.

140. Horne R, Weinman J. Patients' beliefs about prescribed medicines and their role in adherence to treatment in chronic physical illness. Journal of psychosomatic research. 1999;47(6):555-67.

141. Horne R, Buick D, Fisher M, Leake H, Cooper V, Weinman J. Doubts about necessity and concerns about adverse effects: identifying the types of beliefs that are associated with non-adherence to HAART. International journal of STD & AIDS. 2004;15(1):38-44.

142. Horne R, Weinman J. Self-regulation and self-managementin asthma: exploring the role of illness perceptions and treatment beliefs in explaining non-adherence to preventer medication. Psychol Health. 2002;14:1-24.

143. Neame R, Hammond A. Beliefs about medications: a questionnaire survey of people with rheumatoid arthritis. Rheumatology. 2005;44(6):762-7.

144. Horne R, Chapman SC, Parham R, Freemantle N, Forbes A, Cooper V. Understanding Patients' Adherence-Related Beliefs about Medicines Prescribed for Long-Term Conditions: A Meta-Analytic Review of the Necessity-Concerns Framework. PloS one. 2013;8(12):e80633.

145. Berg KM, Arnsten JH. Practical and conceptual challenges in measuring antiretroviral adherence. Journal of acquired immune deficiency syndromes (1999). 2006;43 Suppl 1:S79-87.

146. Oyugi JH, Byakika-Tusiime J, Charlebois ED, Kityo C, Mugerwa R, Mugyenyi P, et al. Multiple validated measures of adherence indicate high levels of adherence to generic HIV antiretroviral therapy in a resource-limited setting. Journal of acquired immune deficiency syndromes (1999). 2004;36(5):1100-2.

147. Hubley AM, Zumbo B. A Dialectic on Validity: Where We Have Been and Where We Are Going. The Journal of General Psychology. 1996;1996;123(3):207-15.

148. Steiner JF, Koepsell TD, Fihn SD, TS. I. A general method of compliance assessment using centralized pharmacy records. Description and validation. Medical care. 1988;26(8):814-23.

149. Inciardi JF, Leeds AL. Assessing the utility of a community pharmacy refill record as a measure of adherence and viral load response in patients infected with human immunodeficiency virus. Pharmacotherapy. 2005;25(6):790-6.

150. Liu H, Golin CE, Miller LG, Hays RD, Beck CK, Sanandaji S, et al. A comparison study of multiple measures of adherence to HIV protease inhibitors. Annals of internal medicine. 2001;134(10):968-77.

151. Arnsten JH, Demas PA, Farzadegan H, Grant RW, Gourevitch MN, Chang CJ, et al. Antiretroviral therapy adherence and viral suppression in HIV-infected drug users: comparison of self-report and electronic monitoring. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2001;33(8):1417-23.

152. Hugen PW, Langebeek N, Burger DM, Zomer B, van Leusen R, Schuurman R, et al. Assessment of adherence to HIV protease inhibitors: comparison and combination of various methods, including MEMS (electronic monitoring), patient and nurse report, and therapeutic drug monitoring. Journal of acquired immune deficiency syndromes. 2002;30(3):324-34.

153. Murri R, Ammassari A, Gallicano K, De Luca A, Cingolani A, Jacobson D, et al. Patient-reported nonadherence to HAART is related to protease inhibitor levels. Journal of acquired immune deficiency syndromes. 2000;24(2):123-8.

154. Gandhi M, Ameli N, Bacchetti P, Anastos K, Gange SJ, Minkoff H, et al. Atazanavir concentration in hair is the strongest predictor of outcomes on antiretroviral therapy. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2011;52(10):1267-75.

155. DiMatteo MR, Giordani PJ, Lepper HS, Croghan TW. Patient adherence and medical treatment outcomes: a meta-analysis. Medical care. 2002;40(9):794-811.

156. DiMatteo MR. Variations in patients' adherence to medical recommendations: a quantitative review of 50 years of research. Medical care. 2004;42(3):200-9.

157. Paterson DL, Potoski B, Capitano B. Measurement of adherence to antiretroviral medications. Journal of acquired immune deficiency syndromes. 2002;31 Suppl 3:S103-6.

158. Hull MW, Lima VD, Hogg RS, Harrigan PR, Montaner JS. Epidemiology of treatment failure: a focus on recent trends. Current opinion in HIV and AIDS. 2009;4(6):467-73.

159. Lima VD, Harrigan R, Bangsberg DR, Hogg RS, Gross R, Yip B, et al. The combined effect of modern highly active antiretroviral therapy regimens and adherence on mortality over time. Journal of acquired immune deficiency syndromes. 2009;50(5):529-36.

160. Gatanaga H, Tsukada K, Honda H, Tanuma J, Yazaki H, Watanabe T, et al. Detection of HIV type 1 load by the Roche Cobas TaqMan assay in patients with viral loads previously undetectable by the Roche Cobas Amplicor Monitor. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2009;48(2):260-2.

161. Havlir DV, Bassett R, Levitan D, Gilbert P, Tebas P, Collier AC, et al. Prevalence and predictive value of intermittent viremia with combination hiv therapy. JAMA : the journal of the American Medical Association. 2001;286(2):171-9.

162. Nettles RE, Kieffer TL, Kwon P, Monie D, Han Y, Parsons T, et al. Intermittent HIV-1 viremia (Blips) and drug resistance in patients receiving HAART. JAMA : the journal of the American Medical Association. 2005;293(7):817-29.

163. Podsadecki TJ, Vrijens BC, Tousset EP, Rode RA, Hanna GJ. Decreased adherence to antiretroviral therapy observed prior to transient human immunodeficiency virus type 1 viremia. The Journal of infectious diseases. 2007;196(12):1773-8.

164. Grennan JT, Loutfy MR, Su D, Harrigan PR, Cooper C, Klein M, et al. Magnitude of virologic blips is associated with a higher risk for virologic rebound in HIV-infected individuals: a recurrent events analysis. The Journal of infectious diseases. 2012;205(8):1230-8.

165. World Health Organisation. Antiretroviral therapy for HIV infection in adults and adolescents. Recommendations for a public health approach, 2010 revision. 2010 [cited 2013 22/09/2013].

166. Rutherford GW, Anglemyer A, Easterbrook PJ, Horvath T, Vitoria M, Penazzato M, et al. Predicting treatment failure in adults and children on antiretroviral therapy: a systematic review of the performance characteristics of the 2010 WHO immunologic and clinical criteria for virologic failure. AIDS (London, England). 2014;28 Suppl 2:S161-9.

167. Moore DM, Awor A, Downing R, Kaplan J, Montaner JS, Hancock J, et al. CD4+ T-cell count monitoring does not accurately identify HIV-infected adults with virologic failure receiving antiretroviral therapy. Journal of acquired immune deficiency syndromes. 2008;49(5):477-84.

168. 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 (London, England). 2008;22(15):1971-7.

169. Badri M, Lawn SD, Wood R. Utility of CD4 cell counts for early prediction of virological failure during antiretroviral therapy in a resource-limited setting. BMC infectious diseases. 2008;8:89.

170. Department of Health RoSA, ,. National consolidated guidelines for the prevention of mother-to-child transmission of HIV (PMTCT) and the management of HIV in children, adolescents and adults 2014 [cited 2015 31 May 2015]. Available from: http://www.sahivsoc.org/upload/documents/HIV%20guidelines%20_Jan%202015.pdf.

171. Barth RE, van der Loeff MF, Schuurman R, Hoepelman AI, Wensing AM. Virological follow-up of adult patients in antiretroviral treatment programmes in sub-Saharan Africa: a systematic review. The Lancet infectious diseases. 2010;10(3):155-66.

172. 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(9):678-87.

173. Ferradini L, Jeannin A, Pinoges L, Izopet J, Odhiambo D, Mankhambo L, et al. Scaling up of highly active antiretroviral therapy in a rural district of Malawi: an effectiveness assessment. Lancet. 2006;367(9519):1335-42.

174. Kamya MR, Mayanja-Kizza H, Kambugu A, Bakeera-Kitaka S, Semitala F, Mwebaze-Songa P, et al. Predictors of long-term viral failure among ugandan children and adults treated with antiretroviral therapy. Journal of acquired immune deficiency syndromes. 2007;46(2):187-93.

175. Wester CW, Kim S, Bussmann H, Avalos A, Ndwapi N, Peter TF, et al. Initial response to highly active antiretroviral therapy in HIV-1C-infected adults in a public sector treatment program in Botswana. Journal of acquired immune deficiency syndromes. 2005;40(3):336-43.

176. Harries AD, Zachariah R, van Oosterhout JJ, Reid SD, Hosseinipour MC, Arendt V, et al. Diagnosis and management of antiretroviral-therapy failure in resource-limited settings in sub-Saharan Africa: challenges and perspectives. The Lancet infectious diseases. 2010;10(1):60-5.

177. Chesney MA. Factors affecting adherence to antiretroviral therapy. Clin Infect Dis. 2000;30 Suppl 2:S171-6.

178. Parienti JJ, Massari V, Descamps D, Vabret A, Bouvet E, Larouze B, et al. Predictors of virologic failure and resistance in HIV-infected patients treated with nevirapine- or efavirenz-based antiretroviral therapy. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2004;38(9):1311-6.

179. UNAIDS. World AIDS Day 2014 Report - Fact sheet 2014 [31 May 2015]. Available from: http://www.unaids.org/en/resources/campaigns/World-AIDS-Day-Report-2014/factsheet.

180. Vardavas R, Blower S. The emergence of HIV transmitted resistance in Botswana: "when will the WHO detection threshold be exceeded?". PloS one. 2007;2(1):e152.

181. Blower S, Bodine E, Kahn J, McFarland W. The antiretroviral rollout and drug-resistant HIV in Africa: insights from empirical data and theoretical models. AIDS (London, England). 2005;19(1):1-14.

182. Uy J, Armon C, Buchacz K, Wood K, Brooks JT, Investigators H. Initiation of HAART at higher CD4 cell counts is associated with a lower frequency of antiretroviral drug resistance mutations at virologic failure. Journal of acquired immune deficiency syndromes (1999). 2009;51(4):450-3.

183. Lima VD, Reuter A, Harrigan PR, Lourenco L, Chau W, Hull M, et al. Initiation of antiretroviral therapy at high CD4+ cell counts is associated with positive treatment outcomes. AIDS (London, England). 2015;29(14):1871-82.

184. Fogel JM, Hudelson SE, Ou SS, Hart S, Wallis C, Morgado MG, et al. Brief Report: HIV Drug Resistance in Adults Failing Early Antiretroviral Treatment: Results From the HIV Prevention Trials Network 052 Trial. Journal of acquired immune deficiency syndromes (1999). 2016;72(3):304-9.

185. 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(1):62-72.

186. Gupta R, Hill A, Sawyer AW, Pillay D. 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. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2008;47(5):712-22.

187. WHO. WHO HIV DRUG RESISTANCE REPORT 2012 2012 [Available from: http://apps.who.int/iris/bitstream/10665/75183/1/9789241503938_eng.pdf.

188. Klein CE, Chiu YL, Cai Y, Beck K, King KR, Causemaker SJ, et al. Effects of acid-reducing agents on the pharmacokinetics of lopinavir/ritonavir and ritonavir-boosted atazanavir. Journal of clinical pharmacology. 2008;48(5):553-62.

189. DeJesus E, Pozniak A, Gallant J, Arribas J, Zhou Y, Warren D, et al., editors. Improvement in Fasting Lipids but Minimal Recovery of Limb Fat Were Seen 96 Weeks After Switching from Lamivudine/Zidovudine Plus Efavirenz to Fixed-Dose Efavirenz/Emtricitabine/Tenofovir DF in HIV-Infected Patients. ICAAC 2009; 2009 September 12-15, 2009; San Francisco, CA, USA.

190. Hong SY, Fanelli TJ, Jonas A, Gweshe J, Tjituka F, Sheehan HM, et al. Household food insecurity associated with antiretroviral therapy adherence among HIV-infected patients in Windhoek, Namibia. Journal of acquired immune deficiency syndromes (1999). 2014;67(4):e115-22.

191. Tuller DM, Bangsberg DR, Senkungu J, Ware NC, Emenyonu N, Weiser SD. Transportation costs impede sustained adherence and access to HAART in a clinic population in southwestern Uganda: a qualitative study. AIDS Behav. 2010;14(4):778-84.

192. Buonaguro L, Tornesello ML, Buonaguro FM. Human immunodeficiency virus type 1 subtype distribution in the worldwide epidemic: pathogenetic and therapeutic implications. Journal of virology. 2007;81(19):10209-19.

193. Coutsinos D, Invernizzi CF, Xu H, Brenner BG, Wainberg MA. Factors affecting template usage in the development of K65R resistance in subtype C variants of HIV type-1. Antiviral chemistry & chemotherapy. 2010;20(3):117-31.

194. Coutsinos D, Invernizzi CF, Moisi D, Oliveira M, Martinez-Cajas JL, Brenner BG, et al. A template-dependent dislocation mechanism potentiates K65R

reverse transcriptase mutation development in subtype C variants of HIV-1. PloS one. 2011;6(5):e20208.

195. McColl DJ, Chappey C, Parkin NT, Miller MD. Prevalence, genotypic associations and phenotypic characterization of K65R, L74V and other HIV-1 RT resistance mutations in a commercial database. Antiviral therapy. 2008;13(2):189-97.

196. Fox J, Dustan S, McClure M, Weber J, Fidler S. Transmitted drug-resistant HIV-1 in primary HIV-1 infection; incidence, evolution and impact on response to antiretroviral therapy. HIV medicine. 2006;7(7):477-83.

197. Goodman DD, Zhou Y, Margot NA, McColl DJ, Zhong L, Borroto-Esoda K, et al. Low level of the K103N HIV-1 above a threshold is associated with virological failure in treatment-naive individuals undergoing efavirenz-containing therapy. AIDS (London, England). 2011;25(3):325-33.

198. Wittkop L, Gunthard HF, de Wolf F, Dunn D, Cozzi-Lepri A, de Luca A, et al. Effect of transmitted drug resistance on virological and immunological response to initial combination antiretroviral therapy for HIV (EuroCoord-CHAIN joint project): a European multicohort study. The Lancet infectious diseases. 2011;11(5):363-71.

199. Gupta RK, Hill A, Sawyer AW, Cozzi-Lepri A, von Wyl V, Yerly S, et al. Virological monitoring and resistance to first-line highly active antiretroviral therapy in adults infected with HIV-1 treated under WHO guidelines: a systematic review and meta-analysis. The Lancet infectious diseases. 2009;9(7):409-17.

200. 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(9):1453-61.

201. Nouhin J, Madec Y, Ngo-Giang-Huong N, Ferradini L, Nerrienet E. Increased risk of Q151M and K65R mutations in patients failing stavudine-containing first-line antiretroviral therapy in Cambodia. PloS one. 2013;8(8):e73744.

202. Jain V, Sucupira MC, Bacchetti P, Hartogensis W, Diaz RS, Kallas EG, et al. Differential persistence of transmitted HIV-1 drug resistance mutation classes. The Journal of infectious diseases. 2011;203(8):1174-81.

203. 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(9):1459-63.

204. Pingen M, Nijhuis M, de Bruijn JA, Boucher CA, Wensing AM. Evolutionary pathways of transmitted drug-resistant HIV-1. The Journal of antimicrobial chemotherapy. 2011;66(7):1467-80.

205. Gupta RK, Jordan MR, Sultan BJ, Hill A, Davis DH, Gregson J, et al. Global trends in antiretroviral resistance in treatment-naive individuals with HIV after rollout of antiretroviral treatment in resource-limited settings: a global collaborative study and meta-regression analysis. Lancet. 2012;380(9849):1250-8.

206. Hamers RL, Wallis CL, Kityo C, Siwale M, Mandaliya K, Conradie F, et al. HIV-1 drug resistance in antiretroviral-naive individuals in sub-Saharan Africa after rollout of antiretroviral therapy: a multicentre observational study. The Lancet infectious diseases. 2011;11(10):750-9.

207. Manasa J, Danaviah S, Tanser F, Pillay S, Mthiyane H, Wilkinson E, et al., editors. Increase in HIV Primary Drug Resistance in a Demographic Surveillance Area in Rural KwaZulu-Natal South Africa. CROI 2015; 2015 Feb 23-25, 2015; Seattle, Washington.

208. Little SJ, Frost SD, Wong JK, Smith DM, Pond SL, Ignacio CC, et al. Persistence of transmitted drug resistance among subjects with primary human immunodeficiency virus infection. Journal of virology. 2008;82(11):5510-8.

209. Cong ME, Heneine W, Garcia-Lerma JG. The fitness cost of mutations associated with human immunodeficiency virus type 1 drug resistance is modulated by mutational interactions. Journal of virology. 2007;81(6):3037-41.

210. Skhosana L, Steegen K, Bronze M, Lukhwareni A, Letsoalo E, Papathanasopoulos MA, 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. 2015;10(2):e0118145.

211. Sunpath H, Wu B, Gordon M, Hampton J, Johnson B, Moosa MY, et al. High rate of K65R for antiretroviral therapy-naive patients with subtype C HIV infection failing a tenofovir-containing first-line regimen. AIDS (London, England). 2012;26(13):1679-84.

212. Chan PA, Huang A, Kantor R. Low prevalence of transmitted K65R and other tenofovir resistance mutations across different HIV-1 subtypes: implications for pre-exposure prophylaxis. Journal of the International AIDS Society. 2012;15(2):17701.

213. Frankel FA, Invernizzi CF, Oliveira M, Wainberg MA. Diminished efficiency of HIV-1 reverse transcriptase containing the K65R and M184V drug resistance mutations. AIDS (London, England). 2007;21(6):665-75.

214. White KL, Margot NA, Wrin T, Petropoulos CJ, Miller MD, Naeger LK. Molecular mechanisms of resistance to human immunodeficiency virus type 1 with reverse transcriptase mutations K65R and K65R+M184V and their effects on enzyme function and viral replication capacity. Antimicrobial agents and chemotherapy. 2002;46(11):3437-46.

215. TenoRes Study G. 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;16(5):565-75.

216. World Health Organisation. HIV DRUG RESISTANCE SURVEILLANCE GUIDANCE: 2015 UPDATE 2015 [cited 2017 16 Feb]. Available from: http://apps.who.int/iris/bitstream/10665/204471/1/9789241510097_eng.pdf?ua=1.

217. Coates TJ, Richter L, Caceres C. Behavioural strategies to reduce HIV transmission: how to make them work better. Lancet. 2008;372(9639):669-84.

218. Zaidi J, Grapsa E, Tanser F, Newell ML, Barnighausen T. Dramatic increases in HIV prevalence after scale-up of antiretroviral treatment: a longitudinal population-based HIV surveillance study in rural kwazulu-natal. AIDS (London, England). 2013.

219. Department of Health. The South African antiretroviral treatment guidelines. 2013.

220. Manasa J, Lessells R, Rossouw T, Naidu K, Van Vuuren C, Goedhals D, et al. Southern African Treatment Resistance Network (SATuRN) RegaDB HIV drug resistance and clinical management database: supporting patient management, surveillance and research in southern Africa. Database : the journal of biological databases and curation. 2014;2014:bat082.

221. Kroenke K, Spitzer RL, Williams JB, Lowe B. An ultra-brief screening scale for anxiety and depression: the PHQ-4. Psychosomatics. 2009;50(6):613-21.

222. Siedner MJ, Ng CK, Bassett IV, Katz IT, Bangsberg DR, Tsai AC. Trends in CD4 count at presentation to care and treatment initiation in sub-Saharan Africa, 2002-2013: a meta-analysis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2015;60(7):1120-7.

223. Muya AN, Geldsetzer P, Hertzmark E, Ezeamama AE, Kawawa H, Hawkins C, et al. Predictors of Nonadherence to Antiretroviral Therapy among HIV-Infected Adults in Dar es Salaam, Tanzania. Journal of the International Association of Providers of AIDS Care. 2015;14(2):163-71.

224. Marconi VC, Wu B, Hampton J, Ordonez CE, Johnson BA, Singh D, et al. Early warning indicators for first-line virologic failure independent of adherence measures in a South African urban clinic. AIDS patient care and STDs. 2013;27(12):657-68.

225. Jain V, Byonanebye DM, Amanyire G, Kwarisiima D, Black D, Kabami J, et al. Successful antiretroviral therapy delivery and retention in care among asymptomatic individuals with high CD4+ T-cell counts above 350 cells/mul in rural Uganda. AIDS (London, England). 2014;28(15):2241-9.

226. Bock P, James A, Nikuze A, Peton N, Sabapathy K, Mills E, et al. Baseline CD4 Count and Adherence to Antiretroviral Therapy: A Systematic Review and Meta-Analysis. Journal of acquired immune deficiency syndromes (1999). 2016;73(5):514-21.

227. Charurat M, Oyegunle M, Benjamin R, Habib A, Eze E, Ele P, et al. Patient retention and adherence to antiretrovirals in a large antiretroviral therapy program in Nigeria: a longitudinal analysis for risk factors. PloS one. 2010;5(5):e10584.

228. Memiah P, Shumba C, Etienne-Mesubi M, Agbor S, Hossain MB, Komba P, et al. The effect of depressive symptoms and CD4 count on adherence to highly active antiretroviral therapy in sub-Saharan Africa. Journal of the International Association of Providers of AIDS Care. 2014;13(4):346-52.

229. Merlin JS, Westfall AO, Raper JL, Zinski A, Norton WE, Willig JH, et al. Pain, mood, and substance abuse in HIV: implications for clinic visit utilization, antiretroviral therapy adherence, and virologic failure. Journal of acquired immune deficiency syndromes (1999). 2012;61(2):164-70.

230. Kyser M, Buchacz K, Bush TJ, Conley LJ, Hammer J, Henry K, et al. Factors associated with non-adherence to antiretroviral therapy in the SUN study. AIDS care. 2011;23(5):601-11.

231. O'Connor JL, Gardner EM, Mannheimer SB, Lifson AR, Esser S, Telzak EE, et al. Factors associated with adherence amongst 5295 people receiving antiretroviral therapy as part of an international trial. The Journal of infectious diseases. 2013;208(1):40-9.

232. Haubrich RH, Little SJ, Currier JS, Forthal DN, Kemper CA, Beall GN, et al. The value of patient-reported adherence to antiretroviral therapy in predicting virologic and immunologic response. California Collaborative Treatment Group. AIDS (London, England). 1999;13(9):1099-107.

233. Langebeek N, Gisolf EH, Reiss P, Vervoort SC, Hafsteinsdottir TB, Richter C, et al. Predictors and correlates of adherence to combination antiretroviral therapy (ART) for chronic HIV infection: a meta-analysis. BMC medicine. 2014;12:142.

234. Weiser SD, Fernandes KA, Brandson EK, al. e. The association between food insecurity and mortality among HIV-infected individuals on HAART. J Acquir Immune Defic Syndr. 2009;52(3):342-9.

235. Weiser SD, Frongillo EA, Ragland K, al. e. Food insecurity is associated with incomplete HIV RNA suppression among homeless and marginally housed HIV-infected individuals in San Francisco. J Gen Intern Med. 2008;24:14-20.

236. Sanjobo N, Frich JC, Fretheim A. Barriers and facilitators to patients' adherence to antiretroviral treatment in Zambia: a qualitative study. SAHARA J : journal of Social Aspects of HIV/AIDS Research Alliance. 2008;5(3):136-43.

237. Unge C, Johansson A, Zachariah R, Some D, Van Engelgem I, Ekstrom AM. Reasons for unsatisfactory acceptance of antiretroviral treatment in the urban Kibera slum, Kenya. AIDS care. 2008;20(2):146-9.

238. Lankowski AJ, Siedner MJ, Bangsberg DR, Tsai AC. Impact of geographic and transportation-related barriers on HIV outcomes in sub-Saharan Africa: a systematic review. AIDS and behavior. 2014;18(7):1199-223.

239. Sanders R. Equity and Access to ART in Ethiopia: Activity Report. Washington DC; 2010.

240. Mahler HR, Kileo B, Curran K, Plotkin M, Adamu T, Hellar A, et al. Voluntary medical male circumcision: matching demand and supply with quality and efficiency in a high-volume campaign in Iringa Region, Tanzania. PLoS medicine. 2011;8(11):e1001131.

241. Gilbert L, Walker L. 'My biggest fear was that people would reject me once they knew my status…':stigma as experienced by patients in an HIV⁄AIDS clinic in Johannesburg, South Africa. Health and Social Care in the Community. 2010;18(2):139-46.

242. Wainberg MA, Friedland G. Public health implications of antiretroviral therapy and HIV drug resistance. JAMA : the journal of the American Medical Association. 1998;279(24):1977-83.

243. Zugna D, Geskus RB, De Stavola B, Rosinska M, Bartmeyer B, Boufassa F, et al. Time to virological failure, treatment change and interruption for individuals treated within 12 months of HIV seroconversion and in chronic infection. Antiviral therapy. 2012;17(6):1039-48.

244. Barnabas RV, van Rooyen H, Tumwesigye E, Murnane PM, Baeten JM, Humphries H, et al. Initiation of antiretroviral therapy and viral suppression after home HIV testing and counselling in KwaZulu-Natal, South Africa, and Mbarara district, Uganda: a prospective, observational intervention study. The lancet HIV. 2014;1(2):e68-e76.

245. Barnabas RV, van Rooyen H, Tumwesigye E, Brantley J, Baeten JM, van Heerden A, et al. Uptake of antiretroviral therapy and male circumcision after community-based HIV testing and strategies for linkage to care versus standard clinic referral: a multisite, open-label, randomised controlled trial in South Africa and Uganda. The lancet HIV. 2016;3(5):e212-20.

246. Vanobberghen FM, Kilama B, Wringe A, Ramadhani A, Zaba B, Mmbando D, et al. Immunological failure of first-line and switch to second-line antiretroviral therapy among HIV-infected persons in Tanzania: analysis of routinely collected national data. Tropical medicine & international health : TM & IH. 2015;20(7):880-92.

247. Farahani M, Vable A, Lebelonyane R, Seipone K, Anderson M, Avalos A, et al. Outcomes of the Botswana national HIV/AIDS treatment programme from 2002 to 2010: a longitudinal analysis. The Lancet Global health. 2014;2(1):e44-50.

248. Mfinanga SG, Kirenga BJ, Chanda DM, Mutayoba B, Mthiyane T, Yimer G, et al. Early versus delayed initiation of highly active antiretroviral therapy for HIV-positive adults with newly diagnosed pulmonary tuberculosis (TB-HAART): a prospective, international, randomised, placebo-controlled trial. The Lancet infectious diseases. 2014;14(7):563-71.

249. Datay MI, Boulle A, Mant D, Yudkin P. Associations with virologic treatment failure in adults on antiretroviral therapy in South Africa. Journal of acquired immune deficiency syndromes (1999). 2010;54(5):489-95.

250. Hanson DL, Adje-Toure C, Talla-Nzussouo N, Eby P, Borget MY, Kouadio LY, et al. HIV type 1 drug resistance in adults receiving highly active antiretroviral therapy in Abidjan, Cote d'Ivoire. AIDS research and human retroviruses. 2009;25(5):489-95.

251. Hong SY, Jonas A, DeKlerk M, Shiningavamwe A, Desta T, Badi A, et al. Population-based surveillance of HIV drug resistance emerging on treatment and associated factors at sentinel antiretroviral therapy sites in Namibia. Journal of acquired immune deficiency syndromes (1999). 2015;68(4):463-71.

252. 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. Journal of acquired immune deficiency syndromes (1999). 2015;68(5):527-35.

253. Ruperez M, Pou C, Maculuve S, Cedeno S, Luis L, Rodriguez J, et al. Determinants of virological failure and antiretroviral drug resistance in Mozambique. The Journal of antimicrobial chemotherapy. 2015;70(9):2639-47.

254. Jose S, Quinn K, Dunn D, Cox A, Sabin C, Fidler S, et al. Virological failure and development of new resistance mutations according to CD4 count at combination antiretroviral therapy initiation. HIV medicine. 2016;17(5):368-72.

255. Lodi S, Phillips A, Fidler S, Hawkins D, Gilson R, McLean K, et al. Role of HIV infection duration and CD4 cell level at initiation of combination anti-retroviral therapy on risk of failure. PloS one. 2013;8(9):e75608.

256. Fatti G, Mothibi E, Meintjes G, Grimwood A. Antiretroviral treatment outcomes amongst older adults in a large multicentre cohort in South Africa. PloS one. 2014;9(6):e100273.

257. Barth RE, Tempelman HA, Moraba R, Hoepelman AI. Long-Term Outcome of an HIV-Treatment Programme in Rural Africa: Viral Suppression despite Early Mortality. AIDS research and treatment. 2011;2011:434375.

258. Gallant JE, DeJesus E, Arribas JR, Pozniak AL, Gazzard B, Campo RE, et al. Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV. The New England journal of medicine. 2006;354(3):251-60.

259. Safren SA, Biello KB, Smeaton L, Mimiaga MJ, Walawander A, Lama JR, et al. Psychosocial predictors of non-adherence and treatment failure in a large scale multi-national trial of antiretroviral therapy for HIV: data from the ACTG A5175/PEARLS trial. PloS one. 2014;9(8):e104178.

260. Mujugira A, Celum C, Tappero JW, Ronald A, Mugo N, Baeten JM. Younger Age Predicts Failure to Achieve Viral Suppression and Virologic Rebound Among HIV-1-Infected Persons in Serodiscordant Partnerships. AIDS research and human retroviruses. 2016;32(2):148-54.

261. Tanner Z, Lachowsky N, Ding E, Samji H, Hull M, Cescon A, et al. Predictors of viral suppression and rebound among HIV-positive men who have sex with men in a large multi-site Canadian cohort. BMC infectious diseases. 2016;16(1):590.

262. Meloni ST, Chang CA, Eisen G, Jolayemi T, Banigbe B, Okonkwo PI, et al. Long-Term Outcomes on Antiretroviral Therapy in a Large Scale-Up Program in Nigeria. PloS one. 2016;11(10):e0164030.

263. Department of Labour South Africa. The Shortage of Medical Doctors in South Africa, Scarce and critical skills Research Project 2008. p. 1-97.

264. 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(2):377-86.

265. Levison JH, Orrell C, Losina E, Lu Z, Freedberg KA, Wood R. Early outcomes and the virological effect of delayed treatment switching to second-line therapy in an antiretroviral roll-out programme in South Africa. Antiviral therapy. 2011;16(6):853-61.

266. 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. Journal of acquired immune deficiency syndromes (1999). 2011;58(1):23-31.

267. Barth RE, Wensing AM, Tempelman HA, Moraba R, Schuurman R, Hoepelman AI. Rapid accumulation of nonnucleoside reverse transcriptase inhibitor-associated resistance: evidence of transmitted resistance in rural South Africa. AIDS (London, England). 2008;22(16):2210-2.

268. El-Khatib Z, Delong AK, Katzenstein D, Ekstrom AM, Ledwaba J, Mohapi L, 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. Journal of AIDS & clinical research. 2011;2(117).

269. Hoffmann CJ, Charalambous S, Sim J, Ledwaba J, Schwikkard G, Chaisson RE, et al. Viremia, resuppression, and time to resistance in human immunodeficiency virus (HIV) subtype C during first-line antiretroviral therapy in South Africa. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2009;49(12):1928-35.

270. Neogi U, Heylen E, Shet A, Chandy S, Shamsunder R, Sonnerborg A, et al. Long-term efficacy of first line antiretroviral therapy in Indian HIV-1 infected patients: a longitudinal cohort study. PloS one. 2013;8(1):e55421.

271. Diallo K, Gotte M, Wainberg MA. Molecular impact of the M184V mutation in human immunodeficiency virus type 1 reverse transcriptase. Antimicrobial agents and chemotherapy. 2003;47(11):3377-83.

272. Boender TS, Hoenderboom BM, Sigaloff KC, Hamers RL, Wellington M, Shamu T, et al. Pretreatment HIV drug resistance increases regimen switches in sub-Saharan Africa. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2015;61(11):1749-58.

273. Phanuphak P, Sirivichayakul S, Jiamsakul A, Sungkanuparph S, Kumarasamy N, Lee MP, et al. Transmitted drug resistance and antiretroviral treatment outcomes in non-subtype B HIV-1-infected patients in South East Asia. Journal of acquired immune deficiency syndromes (1999). 2014;66(1):74-9.

274. Bennett DE, Bertagnolio S, Sutherland D, Gilks CF. The World Health Organization's global strategy for prevention and assessment of HIV drug resistance. Antiviral therapy. 2008;13 Suppl 2:1-13.

275. Department of Health. National antiretroviral treatment guidelines. Pretoria, South Africa: Department of Health. 2004.

276. Blower S, Ma L, Farmer P, Koenig S. Predicting the impact of antiretrovirals in resource-poor settings: preventing HIV infections whilst controlling drug resistance. Current drug targets Infectious disorders. 2003;3(4):345-53.

277. World Health Organisation. Surveillance of HIV Drug Resistance in adults initiating antiretroviral therapy (pre-treatment HIV Drug resistance) 2014 [cited 2016 7 Jan 2016]. Available from: http://apps.who.int/iris/bitstream/10665/112802/1/9789241507196_eng.pdf?ua=1.

278. Weinstein MC, Goldie SJ, Losina E, Cohen CJ, Baxter JD, Zhang H, et al. Use of genotypic resistance testing to guide hiv therapy: clinical impact and cost-effectiveness. Annals of internal medicine. 2001;134(6):440-50.

279. British HIV Association. Guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy 2015 (2016 interim update) 2016 [cited 2016 7 Jan 2016]. Available from: http://www.bhiva.org/documents/Guidelines/Treatment/2016/treatment-guidelines-2016-interim-update.pdf.

280. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services [cited 2016 7 Jan 2016]. Available from: https://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf.

281. Little SJ, Holte S, Routy JP, Daar ES, Markowitz M, Collier AC, et al. Antiretroviral-drug resistance among patients recently infected with HIV. The New England journal of medicine. 2002;347(6):385-94.

282. Ssemwanga D, Lihana RW, Ugoji C, Abimiku A, Nkengasong J, Dakum P, et al. Update on HIV-1 acquired and transmitted drug resistance in Africa. AIDS reviews. 2015;17(1):3-20.

283. Rhee SY, Blanco JL, Jordan MR, Taylor J, Lemey P, Varghese V, et al. Geographic and temporal trends in the molecular epidemiology and genetic mechanisms of transmitted HIV-1 drug resistance: an individual-patient- and sequence-level meta-analysis. PLoS medicine. 2015;12(4):e1001810.

284. Wilson DP, Kahn J, Blower SM. Predicting the epidemiological impact of antiretroviral allocation strategies in KwaZulu-Natal: the effect of the urban-rural divide. Proceedings of the National Academy of Sciences of the United States of America. 2006;103(38):14228-33.

285. Parikh UM, Mellors JW. Pretreatment HIV-1 drug resistance is strongly associated with virologic failure in HIV-infected patients receiving partly active antiretroviral regimens. Future microbiology. 2012;7(8):929-32.

286. Ceccarelli L, Salpini R, Moudourou S, Cento V, Santoro MM, Fokam J, et al. Characterization of drug resistance mutations in naive and ART-treated patients infected with HIV-1 in Yaounde, Cameroon. Journal of medical virology. 2012;84(5):721-7.

287. Fokam J, Elat JB, Billong SC, Kembou E, Nkwescheu AS, Obam NM, et al. Monitoring HIV Drug Resistance Early Warning Indicators in Cameroon: A Study Following the Revised World Health Organization Recommendations. PloS one. 2015;10(6):e0129210.

288. Baxter JD, Dunn D, White E, Sharma S, Geretti AM, Kozal MJ, et al. Global HIV-1 transmitted drug resistance in the INSIGHT Strategic Timing of AntiRetroviral Treatment (START) trial. HIV medicine. 2015;16 Suppl 1:77-87.

289. Manasa J, Katzenstein D, Cassol S, Newell ML, de Oliveira T, Southern Africa T, et al. Primary drug resistance in South Africa: data from 10 years of surveys. AIDS research and human retroviruses. 2012;28(6):558-65.

290. Msimanga PW, Vardas E, Engelbrecht S. HIV-1 diversity in an antiretroviral treatment naive cohort from Bushbuckridge, Mpumalanga Province, South Africa. Virology journal. 2015;12:24.

291. Chung MH, Beck IA, Dross S, Tapia K, Kiarie JN, Richardson BA, et al. Oligonucleotide ligation assay detects HIV drug resistance associated with virologic failure among antiretroviral-naive adults in Kenya. Journal of acquired immune deficiency syndromes (1999). 2014;67(3):246-53.

292. Hamers RL, Schuurman R, Sigaloff KC, Wallis CL, Kityo C, Siwale M, et al. Effect of pretreatment HIV-1 drug resistance on immunological, virological, and

drug-resistance outcomes of first-line antiretroviral treatment in sub-Saharan Africa: a multicentre cohort study. The Lancet infectious diseases. 2012;12(4):307-17.

293. Lee GQ, Bangsberg DR, Muzoora C, Boum Y, Oyugi JH, Emenyonu N, et al. Prevalence and virologic consequences of transmitted HIV-1 drug resistance in Uganda. AIDS research and human retroviruses. 2014;30(9):896-906.

294. Mzingwane ML, Tiemessen CT, Richter KL, Mayaphi SH, Hunt G, Bowyer SM. Pre-treatment minority HIV-1 drug resistance mutations and long term virological outcomes: is prediction possible? Virology journal. 2016;13(1):170.

295. Rusine J, Asiimwe-Kateera B, van de Wijgert J, Boer KR, Mukantwali E, Karita E, 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. 2013;8(8):e64345.

296. Elmi Abar A, Jlizi A, Darar HY, Kacem MA, Slim A. HIV-1 drug resistance genotyping from antiretroviral therapy (ART) naive and first-line treatment failures in Djiboutian patients. Diagnostic pathology. 2012;7:138.

297. Abegaz WE, Grossman Z, Wolday D, Ram D, Kaplan J, Sibide K, et al. Threshold survey evaluating transmitted HIV drug resistance among public antenatal clinic clients in Addis Ababa, Ethiopia. Antiviral therapy. 2008;13 Suppl 2:89-94.

298. Bila DC, Young P, Merks H, Vubil AS, Mahomed M, Augusto A, et al. Evolution of primary HIV drug resistance in a subtype C dominated epidemic in Mozambique. PloS one. 2013;8(7):e68213.

299. Afonso JM, Bello G, Guimaraes ML, Sojka M, Morgado MG. HIV-1 genetic diversity and transmitted drug resistance mutations among patients from the North, Central and South regions of Angola. PloS one. 2012;7(8):e42996.

300. Aghokeng AF, Kouanfack C, Laurent C, Ebong E, Atem-Tambe A, Butel C, et al. Scale-up of antiretroviral treatment in sub-Saharan Africa is accompanied by increasing HIV-1 drug resistance mutations in drug-naive patients. AIDS (London, England). 2011;25(17):2183-8.

301. Aghokeng AF, Vergne L, Mpoudi-Ngole E, Mbangue M, Deoudje N, Mokondji E, et al. Evaluation of transmitted HIV drug resistance among recently-infected antenatal clinic attendees in four Central African countries. Antiviral therapy. 2009;14(3):401-11.

302. Ajoge HO, Gordon ML, Ibrahim S, Shittu OS, Ndung'u T, Olonitola SO. Drug resistance pattern of HIV type 1 isolates sampled in 2007 from therapy-naive pregnant women in North-Central Nigeria. AIDS research and human retroviruses. 2012;28(1):115-8.

303. Ayouba A, Lien TT, Nouhin J, Vergne L, Aghokeng AF, Ngo-Giang-Huong N, et al. Low prevalence of HIV type 1 drug resistance mutations in untreated, recently infected patients from Burkina Faso, Cote d'Ivoire, Senegal, Thailand, and Vietnam: the ANRS 12134 study. AIDS research and human retroviruses. 2009;25(11):1193-6.

304. Bartolo I, Zakovic S, Martin F, Palladino C, Carvalho P, Camacho R, et al. HIV-1 diversity, transmission dynamics and primary drug resistance in Angola. PloS one. 2014;9(12):e113626.

305. Bartolo I, Casanovas J, Bastos R, Rocha C, Abecasis AB, Folgosa E, et al. HIV-1 genetic diversity and transmitted drug resistance in health care settings in Maputo, Mozambique. Journal of acquired immune deficiency syndromes (1999). 2009;51(3):323-31.

306. Bonney EY, Addo NA, Ntim NA, Addo-Yobo F, Bondzie P, Aryee KE, et al. Low level of transmitted HIV Drug resistance at two HIV care centres in Ghana: a threshold survey. Ghana medical journal. 2013;47(2):82-6.

307. Bruzzone B, Saladini F, Sticchi L, Mayinda Mboungou FA, Barresi R, Caligiuri P, et al. Prevalence of HIV-1 Subtypes and Drug Resistance-Associated Mutations in HIV-1-Positive Treatment-Naive Pregnant Women in Pointe Noire, Republic of the Congo (Kento-Mwana Project). AIDS research and human retroviruses. 2015;31(8):837-40.

308. Burda ST, Viswanath R, Zhao J, Kinge T, Anyangwe C, Tinyami ET, et al. HIV-1 reverse transcriptase drug-resistance mutations in chronically infected

individuals receiving or naive to HAART in Cameroon. Journal of medical virology. 2010;82(2):187-96.

309. Bussmann H, de la Hoz Gomez F, Roels TH, Wester CW, Bodika SM, Moyo S, et al. Prevalence of transmitted HIV drug resistance in Botswana: lessons learned from the HIVDR-Threshold Survey conducted among women presenting for routine antenatal care as part of the 2007 national sentinel survey. AIDS research and human retroviruses. 2011;27(4):365-72.

310. Castelbranco EP, da Silva Souza E, Cavalcanti AM, Martins AN, de Alencar LC, Tanuri A. Frequency of primary resistance to antiretroviral drugs and genetic variability of HIV-1 among infected pregnant women recently diagnosed in Luanda-Angola. AIDS research and human retroviruses. 2010;26(12):1313-6.

311. Charpentier C, Bellecave P, Cisse M, Mamadou S, Diakite M, Peytavin G, et al. High prevalence of antiretroviral drug resistance among HIV-1-untreated patients in Guinea-Conakry and in Niger. Antiviral therapy. 2011;16(3):429-33.

312. Derache A, Maiga AI, Traore O, Akonde A, Cisse M, Jarrousse B, et al. Evolution of genetic diversity and drug resistance mutations in HIV-1 among untreated patients from Mali between 2005 and 2006. The Journal of antimicrobial chemotherapy. 2008;62(3):456-63.

313. Derache A, Traore O, Koita V, Sylla A, Tubiana R, Simon A, et al. Genetic diversity and drug resistance mutations in HIV type 1 from untreated patients in Bamako, Mali. Antiviral therapy. 2007;12(1):123-9.

314. Eshleman SH, Laeyendecker O, Parkin N, Huang W, Chappey C, Paquet AC, et al. Antiretroviral drug susceptibility among drug-naive adults with recent HIV infection in Rakai, Uganda. AIDS (London, England). 2009;23(7):845-52.

315. Fokam J, Takou D, Santoro MM, Akonie HZ, Kouanfack C, Ceccherini-Silberstein F, et al. Short Communication: Population-Based Surveillance of HIV-1 Drug Resistance in Cameroonian Adults Initiating Antiretroviral Therapy According to the World Health Organization Guidelines. AIDS research and human retroviruses. 2016;32(4):329-33.

316. Haidara A, Chamberland A, Sylla M, Aboubacrine SA, Cisse M, Traore HA, et al. High level of primary drug resistance in Mali. HIV medicine. 2010;11(6):404-11.

317. Handema R, Terunuma H, Kasolo F, Kasai H, Sichone M, Yamashita A, et al. Prevalence of drug-resistance-associated mutations in antiretroviral drug-naive Zambians infected with subtype C HIV-1. AIDS research and human retroviruses. 2003;19(2):151-60.

318. Hassan AS, Mwaringa SM, Obonyo CA, Nabwera HM, Sanders EJ, Rinke de Wit TF, et al. Low prevalence of transmitted HIV type 1 drug resistance among antiretroviral-naive adults in a rural HIV clinic in Kenya. AIDS research and human retroviruses. 2013;29(1):129-35.

319. Huang KH, Goedhals D, Fryer H, van Vuuren C, Katzourakis A, De Oliveira T, et al. Prevalence of HIV type-1 drug-associated mutations in pre-therapy patients in the Free State, South Africa. Antiviral therapy. 2009;14(7):975-84.

320. Hunt GM, Ledwaba J, Basson AE, Moyes J, Cohen C, Singh B, et al. Surveillance of transmitted HIV-1 drug resistance in Gauteng and KwaZulu-Natal Provinces, South Africa, 2005-2009. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2012;54 Suppl 4:S334-8.

321. Huruy K, Maier M, Mulu A, Liebert UG. Limited increase in primary HIV-1C drug resistance mutations in treatment naive individuals in Ethiopia. Journal of medical virology. 2015;87(6):978-84.

322. Kamoto K, Aberle-Grasse J. Surveillance of transmitted HIV drug resistance with the World Health Organization threshold survey method in Lilongwe, Malawi. Antiviral therapy. 2008;13 Suppl 2:83-7.

323. Kantor R, DeLong A, Balamane M, Schreier L, Lloyd RM, Jr., Injera W, et al. HIV diversity and drug resistance from plasma and non-plasma analytes in a large treatment programme in western Kenya. Journal of the International AIDS Society. 2014;17:19262.

324. Kantor R, Smeaton L, Vardhanabhuti S, Hudelson SE, Wallis CL, Tripathy S, et al. Pretreatment HIV Drug Resistance and HIV-1 Subtype C Are

Independently Associated With Virologic Failure: Results From the Multinational PEARLS (ACTG A5175) Clinical Trial. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2015;60(10):1541-9.

325. Kasang C, Kalluvya S, Majinge C, Stich A, Bodem J, Kongola G, et al. HIV drug resistance (HIVDR) in antiretroviral therapy-naive patients in Tanzania not eligible for WHO threshold HIVDR survey is dramatically high. PloS one. 2011;6(8):e23091.

326. Koizumi Y, Ndembi N, Miyashita M, Lwembe R, Kageyama S, Mbanya D, et al. Emergence of antiretroviral therapy resistance-associated primary mutations among drug-naive HIV-1-infected individuals in rural western Cameroon. Journal of acquired immune deficiency syndromes (1999). 2006;43(1):15-22.

327. Maiga AI, Fofana DB, Maiga AC, Diallo F, Ait-Arkoub Z, Daou F, et al. Transmitted antiretroviral drug resistance in newly HIV-infected and untreated patients in Segou and Bamako, Mali. AIDS research and human retroviruses. 2013;29(1):182-6.

328. Manasa J, Danaviah S, Lessells R, Elshareef M, Tanser F, Wilkinson E, et al. Increasing HIV-1 Drug Resistance Between 2010 and 2012 in Adults Participating in Population-Based HIV Surveillance in Rural KwaZulu-Natal, South Africa. AIDS research and human retroviruses. 2016;32(8):763-9.

329. Maphalala G, Okello V, Mndzebele S, Gwebu P, Mulima N, Dlamini S, et al. Surveillance of transmitted HIV drug resistance in the Manzini-Mbabane corridor, Swaziland, in 2006. Antiviral therapy. 2008;13 Suppl 2:95-100.

330. Masimba P, Kituma E, Klimkait T, Horvath E, Stoeckle M, Hatz C, et al. Prevalence of drug resistance mutations and HIV type 1 subtypes in an HIV type 1-infected cohort in rural Tanzania. AIDS research and human retroviruses. 2013;29(9):1229-36.

331. Mensch BS, Gorbach PM, Kelly C, Kiepiela P, Gomez K, Ramjee G, et al. Characteristics Associated with HIV Drug Resistance Among Women Screening for an HIV Prevention Trial in KwaZulu-Natal, South Africa. AIDS and behavior. 2015;19(11):2076-86.

332. Mungati M, Mhangara M, Gonese E, Mugurungi O, Dzangare J, Ngwende S, et al. Pre-treatment drug resistance among patients initiating antiretroviral therapy (ART) in Zimbabwe: 2008-2010. BMC research notes. 2016;9:302.

333. Nazziwa J, Njai HF, Ndembi N, Birungi J, Lyagoba F, Gershim A, et al. Short communication: HIV type 1 transmitted drug resistance and evidence of transmission clusters among recently infected antiretroviral-naive individuals from Ugandan fishing communities of Lake Victoria. AIDS research and human retroviruses. 2013;29(5):788-95.

334. Ndembi N, Hamers RL, Sigaloff KC, Lyagoba F, Magambo B, Nanteza B, et al. Transmitted antiretroviral drug resistance among newly HIV-1 diagnosed young individuals in Kampala. AIDS (London, England). 2011;25(7):905-10.

335. Ndembi N, Lyagoba F, Nanteza B, Kushemererwa G, Serwanga J, Katongole-Mbidde E, et al. Transmitted antiretroviral drug resistance surveillance among newly HIV type 1-diagnosed women attending an antenatal clinic in Entebbe, Uganda. AIDS research and human retroviruses. 2008;24(6):889-95.

336. Nwobegahay J, Selabe G, Ndjeka NO, Manhaeve C, Bessong PO. Low prevalence of transmitted genetic drug resistance in a cohort of HIV infected naive patients entering antiretroviral treatment programs at two sites in northern South Africa. Journal of medical virology. 2012;84(12):1839-43.

337. Nyombi BM, Holm-Hansen C, Kristiansen KI, Bjune G, Muller F. Prevalence of reverse transcriptase and protease mutations associated with antiretroviral drug resistance among drug-naive HIV-1 infected pregnant women in Kagera and Kilimanjaro regions, Tanzania. AIDS research and therapy. 2008;5:13.

338. Onsongo S, Abidi SH, Khamadi S, Shah R, Kageha S, Ojwang P, et al. Prevalence of Transmitted Drug Resistance Mutations in HIV-1-Infected Drug-Naive Patients from Urban and Suburban Regions of Kenya. AIDS research and human retroviruses. 2016;32(3):220-5.

339. Onywera H, Maman D, Inzaule S, Auma E, Were K, Fredrick H, et al. Surveillance of HIV-1 pol transmitted drug resistance in acutely and recently

infected antiretroviral drug-naive persons in rural western Kenya. PloS one. 2017;12(2):e0171124.

340. Parboosing R, Naidoo A, Gordon M, Taylor M, Vella V. Resistance to antiretroviral drugs in newly diagnosed, young treatment-naive HIV-positive pregnant women in the province of KwaZulu-Natal, South Africa. Journal of medical virology. 2011;83(9):1508-13.

341. Pillay V, Ledwaba J, Hunt G, Rakgotho M, Singh B, Makubalo L, et al. Antiretroviral drug resistance surveillance among drug-naive HIV-1-infected individuals in Gauteng Province, South Africa in 2002 and 2004. Antiviral therapy. 2008;13 Suppl 2:101-7.

342. Rowley CF, MacLeod IJ, Maruapula D, Lekoko B, Gaseitsiwe S, Mine M, et al. Sharp increase in rates of HIV transmitted drug resistance at antenatal clinics in Botswana demonstrates the need for routine surveillance. The Journal of antimicrobial chemotherapy. 2016;71(5):1361-6.

343. Sigaloff KC, Mandaliya K, Hamers RL, Otieno F, Jao IM, Lyagoba F, et al. Short communication: High prevalence of transmitted antiretroviral drug resistance among newly HIV type 1 diagnosed adults in Mombasa, Kenya. AIDS research and human retroviruses. 2012;28(9):1033-7.

344. Somda A, Sangare L, Soro M, Yameogo S, Bazie B, Bigirimana F, et al. Surveillance of transmitted drug-resistant HIV among young pregnant women in Ouagadougou, Burkina Faso. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2012;54 Suppl 4:S317-9.

345. Somi GR, Kibuka T, Diallo K, Tuhuma T, Bennett DE, Yang C, et al. Surveillance of transmitted HIV drug resistance among women attending antenatal clinics in Dar es Salaam, Tanzania. Antiviral therapy. 2008;13 Suppl 2:77-82.

346. Ssemwanga D, Kapaata A, Lyagoba F, Magambo B, Nanyonjo M, Mayanja BN, et al. Low drug resistance levels among drug-naive individuals with recent HIV type 1 infection in a rural clinical cohort in southwestern Uganda. AIDS research and human retroviruses. 2012;28(12):1784-7.

347. Steegen K, Carmona S, Bronze M, Papathanasopoulos MA, van Zyl G, Goedhals D, et al. Moderate Levels of Pre-Treatment HIV-1 Antiretroviral Drug Resistance Detected in the First South African National Survey. PloS one. 2016;11(12):e0166305.

348. Tebit DM, Sangare L, Tiba F, Saydou Y, Makamtse A, Somlare H, et al. Analysis of the diversity of the HIV-1 pol gene and drug resistance associated changes among drug-naive patients in Burkina Faso. Journal of medical virology. 2009;81(10):1691-701.

349. Tshabalala M, Manasa J, Zijenah LS, Rusakaniko S, Kadzirange G, Mucheche M, et al. Surveillance of transmitted antiretroviral drug resistance among HIV-1 infected women attending antenatal clinics in Chitungwiza, Zimbabwe. PloS one. 2011;6(6):e21241.

350. Wadonda-Kabondo N, Banda R, Moyo K, M'Bang'ombe M, Chiwaula M, Porter C, et al. Prevalence of transmitted HIV drug resistance among newly diagnosed antiretroviral therapy-naive pregnant women in Lilongwe and Blantyre, Malawi. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2012;54 Suppl 4:S324-7.

351. Weidle PJ, Kityo CM, Mugyenyi P, Downing R, Kebba A, Pieniazek D, et al. Resistance to antiretroviral therapy among patients in Uganda. Journal of acquired immune deficiency syndromes (1999). 2001;26(5):495-500.

352. Bussmann H, Novitsky V, Wester W, Peter T, Masupu K, Gabaitiri L, et al. HIV-1 subtype C drug-resistance background among ARV-naive adults in Botswana. Antiviral chemistry & chemotherapy. 2005;16(2):103-15.

353. Zoufaly A, Jochum J, Hammerl R, Nassimi N, Raymond Y, Burchard GD, et al. Virological failure after 1 year of first-line ART is not associated with HIV minority drug resistance in rural Cameroon. The Journal of antimicrobial chemotherapy. 2015;70(3):922-5.

354. Blanco JL, Montaner JS, Marconi VC, Santoro MM, Campos-Loza AE, Shafer RW, et al. Lower prevalence of drug resistance mutations at first-line virological failure to first-line therapy with atripla vs. tenofovir +

emtricitabine/lamivudine + efavirenz administered on a multiple tablet therapy. AIDS (London, England). 2014;28(17):2531-9.

355. Bennett DE, Camacho RJ, Otelea D, Kuritzkes DR, Fleury H, Kiuchi M, et al. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. PloS one. 2009;4(3):e4724.

356. Simen BB, Simons JF, Hullsiek KH, Novak RM, Macarthur RD, Baxter JD, et al. Low-abundance drug-resistant viral variants in chronically HIV-infected, antiretroviral treatment-naive patients significantly impact treatment outcomes. The Journal of infectious diseases. 2009;199(5):693-701.

357. Metzner KJ, Rauch P, Walter H, Boesecke C, Zollner B, Jessen H, et al. Detection of minor populations of drug-resistant HIV-1 in acute seroconverters. AIDS (London, England). 2005;19(16):1819-25.

358. Samuel R, Julian MN, Paredes R, Parboosing R, Moodley P, Singh L, et al. HIV-1 Drug Resistance by Ultra-Deep Sequencing Following Short Course Zidovudine, Single-Dose Nevirapine, and Single-Dose Tenofovir with Emtricitabine for Prevention of Mother-to-Child Transmission. Journal of acquired immune deficiency syndromes (1999). 2016;73(4):384-9.

359. Samuel R, Paredes R, Parboosing R, Moodley P, Gordon M. Minority HIV-1 drug-resistant mutations and prevention of mother-to-child transmission: perspectives for resource-limited countries. AIDS reviews. 2014;16(4):187-98.

360. Paredes R, Lalama CM, Ribaudo HJ, Schackman BR, Shikuma C, Giguel F, et al. Pre-existing minority drug-resistant HIV-1 variants, adherence, and risk of antiretroviral treatment failure. The Journal of infectious diseases. 2010;201(5):662-71.

361. Johnson JA, Li JF, Wei X, Lipscomb J, Irlbeck D, Craig C, et al. Minority HIV-1 drug resistance mutations are present in antiretroviral treatment-naive populations and associate with reduced treatment efficacy. PLoS medicine. 2008;5(7):e158.

362. Geretti AM, Fox ZV, Booth CL, Smith CJ, Phillips AN, Johnson M, et al. Low-frequency K103N strengthens the impact of transmitted drug resistance on

virologic responses to first-line efavirenz or nevirapine-based highly active antiretroviral therapy. Journal of acquired immune deficiency syndromes (1999). 2009;52(5):569-73.

363. Metzner KJ, Giulieri SG, Knoepfel SA, Rauch P, Burgisser P, Yerly S, et al. Minority quasispecies of drug-resistant HIV-1 that lead to early therapy failure in treatment-naive and -adherent patients. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2009;48(2):239-47.

364. Li JZ, Paredes R, Ribaudo HJ, Svarovskaia ES, Metzner KJ, Kozal MJ, et al. Low-frequency HIV-1 drug resistance mutations and risk of NNRTI-based antiretroviral treatment failure: a systematic review and pooled analysis. JAMA : the journal of the American Medical Association. 2011;305(13):1327-35.

365. Avila-Rios S, Garcia-Morales C, Matias-Florentino M, Romero-Mora KA, Tapia-Trejo D, Quiroz-Morales VS, et al. Pretreatment HIV-drug resistance in Mexico and its impact on the effectiveness of first-line antiretroviral therapy: a nationally representative 2015 WHO survey. The lancet HIV. 2016;3(12):e579-e91.

366. Cozzi-Lepri A, Noguera-Julian M, Di Giallonardo F, Schuurman R, Daumer M, Aitken S, et al. Low-frequency drug-resistant HIV-1 and risk of virological failure to first-line NNRTI-based ART: a multicohort European case-control study using centralized ultrasensitive 454 pyrosequencing. The Journal of antimicrobial chemotherapy. 2015;70(3):930-40.

367. Peuchant O, Thiebaut R, Capdepont S, Lavignolle-Aurillac V, Neau D, Morlat P, et al. Transmission of HIV-1 minority-resistant variants and response to first-line antiretroviral therapy. AIDS (London, England). 2008;22(12):1417-23.

368. Balduin M, Oette M, Daumer MP, Hoffmann D, Pfister HJ, Kaiser R. Prevalence of minor variants of HIV strains at reverse transcriptase position 103 in therapy-naive patients and their impact on the virological failure. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 2009;45(1):34-8.

369. Jakobsen MR, Tolstrup M, Sogaard OS, Jorgensen LB, Gorry PR, Laursen A, et al. Transmission of HIV-1 drug-resistant variants: prevalence and effect on

treatment outcome. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2010;50(4):566-73.

370. Metzner KJ, Rauch P, Braun P, Knechten H, Ehret R, Korn K, et al. Prevalence of key resistance mutations K65R, K103N, and M184V as minority HIV-1 variants in chronically HIV-1 infected, treatment-naive patients. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 2011;50(2):156-61.

371. Messiaen P, Verhofstede C, Vandenbroucke I, Dinakis S, Van Eygen V, Thys K, et al. Ultra-deep sequencing of HIV-1 reverse transcriptase before start of an NNRTI-based regimen in treatment-naive patients. Virology. 2012;426(1):7-11.

372. Lataillade M, Chiarella J, Yang R, Schnittman S, Wirtz V, Uy J, et al. Prevalence and clinical significance of HIV drug resistance mutations by ultra-deep sequencing in antiretroviral-naive subjects in the CASTLE study. PloS one. 2010;5(6):e10952.

373. Nicot F, Saune K, Raymond S, Jeanne N, Carcenac R, Lefebvre C, et al. Minority resistant HIV-1 variants and the response to first-line NNRTI therapy. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 2015;62:20-4.

374. Callegaro A, Di Filippo E, Astuti N, Ortega PA, Rizzi M, Farina C, et al. Early clinical response and presence of viral resistant minority variants: a proof of concept study. Journal of the International AIDS Society. 2014;17(4 Suppl 3):19759.

375. Johnson JA, Li JF, Wei X, Lipscomb J, Bennett D, Brant A, et al. Simple PCR assays improve the sensitivity of HIV-1 subtype B drug resistance testing and allow linking of resistance mutations. PloS one. 2007;2(7):e638.

376. Johnson JA, Geretti AM. Low-frequency HIV-1 drug resistance mutations can be clinically significant but must be interpreted with caution. The Journal of antimicrobial chemotherapy. 2010;65(7):1322-6.

377. Phillips AN, Staszewski S, Weber R, Kirk O, Francioli P, Miller V, et al. HIV viral load response to antiretroviral therapy according to the baseline CD4 cell

count and viral load. JAMA : the journal of the American Medical Association. 2001;286(20):2560-7.

378. Deeks SG. Determinants of virological response to antiretroviral therapy: implications for long-term strategies. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2000;30 Suppl 2:S177-84.

379. South Africa National Department of Health. The South African Antiretroviral Treatment Guidelines 2013 [cited 2013. Available from: http://www.sahivsoc.org/upload/documents/2013%20ART%20Treatment%20Guidelines%20Final%2025%20March%202013%20corrected.pdf.

380. Montaner JS, Lima VD, Barrios R, Yip B, Wood E, Kerr T, et al. Association of highly active antiretroviral therapy coverage, population viral load, and yearly new HIV diagnoses in British Columbia, Canada: a population-based study. Lancet. 2010;376(9740):532-9.

381. Jain V, Hartogensis W, Bacchetti P, Hunt PW, Hatano H, Sinclair E, et al. Antiretroviral therapy initiated within 6 months of HIV infection is associated with lower T-cell activation and smaller HIV reservoir size. The Journal of infectious diseases. 2013;208(8):1202-11.

382. Burdo TH, Lentz MR, Autissier P, Krishnan A, Halpern E, Letendre S, et al. Soluble CD163 made by monocyte/macrophages is a novel marker of HIV activity in early and chronic infection prior to and after anti-retroviral therapy. The Journal of infectious diseases. 2011;204(1):154-63.

383. Okulicz JF, Le TD, Agan BK, Camargo JF, Landrum ML, Wright E, et al. Influence of the timing of antiretroviral therapy on the potential for normalization of immune status in human immunodeficiency virus 1-infected individuals. JAMA internal medicine. 2015;175(1):88-99.

384. Bateman C. Drug stock-outs: inept supply-chain management and corruption. South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde. 2013;103(9):600-2.

385. la Porte CJ, Colbers EP, Bertz R, Voncken DS, Wikstrom K, Boeree MJ, et al. Pharmacokinetics of adjusted-dose lopinavir-ritonavir combined with rifampin in healthy volunteers. Antimicrobial agents and chemotherapy. 2004;48(5):1553-60.

386. Venter W, Kaiser B, Pillay Y, Conradie F, Gomez GB, Clayden P, et al. Cutting the cost of South African antiretroviral therapy using newer, safer drugs. South African Medical Journal. 2017;107(1):28-30.

387. Rathbun RC, Lockhart SM, Miller MM, Liedtke MD. Dolutegravir, a second-generation integrase inhibitor for the treatment of HIV-1 infection. The Annals of pharmacotherapy. 2014;48(3):395-403.

388. Kandel CE, Walmsley SL. Dolutegravir - a review of the pharmacology, efficacy, and safety in the treatment of HIV. Drug design, development and therapy. 2015;9:3547-55.

389. Walmsley SL, Antela A, Clumeck N, Duiculescu D, Eberhard A, Gutierrez F, et al. Dolutegravir plus abacavir-lamivudine for the treatment of HIV-1 infection. The New England journal of medicine. 2013;369(19):1807-18.

390. Cevik M, Vincent R, McGann H. Use of dolutegravir in combination with rifampicin-based TB therapy in HIV/TB co-infected patients: real-world experience from Leeds, UK. HIV Drug Therapy, Glasgow 2016; 23-26 Oct; Glasgow2016.

391. Paquet AC, Baxter J, Weidler J, Lie Y, Lawrence J, Kim R, et al. Differences in reversion of resistance mutations to wild-type under structured treatment interruption and related increase in replication capacity. PloS one. 2011;6(1):e14638.

392. Naidoo L, Mahomed OH. Impact of Lean on patient cycle and waiting times at a rural district hospital in KwaZulu-Natal. Afr J Prm Health Care Fam Med. 2016;8(1):2071-928.

393. Masango-Makgobela AT, Govender I, Ndimande JV. Reasons patients leave their nearest healthcare service to attend Karen Park Clinic, Pretoria North. Prm Health Care Fam Med. 2013;5(1).

394. Differentiated Care. DIFFERENTIATED CARE FOR HIV: A DECISION FRAMEWORK FOR ANTIRETROVIRAL THERAPY DELIVERY 2016 [cited 2017,

20 Feb]. Available from: http://www.differentiatedcare.org/Portals/0/adam/Content/yS6M-GKB5EWs_uTBHk1C1Q/File/Decision%20Framework%20REPRINT%20web.pdf.

395. Duncombe C, Rosenblum S, Hellmann N, Holmes C, Wilkinson L, Biot M, et al. Reframing HIV care: putting people at the centre of antiretroviral delivery. Tropical medicine & international health : TM & IH. 2015;20(4):430-47.

396. Grimsrud A, Sharp J, Kalombo C, Bekker LG, Myer L. Implementation of community-based adherence clubs for stable antiretroviral therapy patients in Cape Town, South Africa. Journal of the International AIDS Society. 2015;18:19984.

397. Luque-Fernandez MA, Van Cutsem G, Goemaere E, Hilderbrand K, Schomaker M, Mantangana N, et al. Effectiveness of patient adherence groups as a model of care for stable patients on antiretroviral therapy in Khayelitsha, Cape Town, South Africa. PloS one. 2013;8(2):e56088.

398. Health Systems Trust & National Department of Health. Central Chronic Medicine Dispensing and Distribution Programme (CCMDD) 2015 [cited 2017, 15 Feb]. Available from: http://www.hst.org.za/projects/central-chronic-medicine-dispensing-and-distribution-programme-ccmdd.

399. Sanne I, Orrell C, Fox MP, Conradie F, Ive P, Zeinecker J, et al. Nurse versus doctor management of HIV-infected patients receiving antiretroviral therapy (CIPRA-SA): a randomised non-inferiority trial. Lancet. 2010;376(9734):33-40.

400. Miles K, Clutterbuck DJ, Seitio O, Sebego M, Riley A. Antiretroviral treatment roll-out in a resource-constrained setting: capitalizing on nursing resources in Botswana. Bulletin of the World Health Organization. 2007;85(7):555-60.

401. Health Systems Trust. The Ideal Clinic Programme 2015-2016 [cited 2017, 20 Feb]. Available from: http://www.hst.org.za/sites/default/files/Ideal_Clinic_Programme_WEB20Oct2015.pdf.

402. Mwai GW, Mburu G, Torpey K, Frost P, Ford N, Seeley J. Role and outcomes of community health workers in HIV care in sub-Saharan Africa: a systematic review. Journal of the International AIDS Society. 2013;16:18586.

403. Phillips AN, Cambiano V, Nakagawa F, Brown AE, Lampe F, Rodger A, et al. Increased HIV incidence in men who have sex with men despite high levels of ART-induced viral suppression: analysis of an extensively documented epidemic. PloS one. 2013;8(2):e55312.

Appendices

Appendix A TasP Home-based individual questionnaire

Appendix B Consent form for home-based questionnaire

Appendix C Consent form for HIV testing

Appendix D Clinical history and examination form

Appendix E Clinic baseline form

Appendix F Clinic follow up form

Appendix G Ethics approval to commence main trial in control arm, Biomedical Research Ethics Committee, UKZN (BREC)

Appendix H Full ethics approval main trial, BREC

Appendix I Ethics recertification for main trial 2015 to 2016

Appendix J Approval, Medicines Control Council of South Africa

Appendix K Ethics approval, Brighton and Sussex Medical School

Appendix L Ethics approval, UCL

Appendix M Association between CD4 count at initiation, pill count adherence and other factors with virological suppression at 6 months

Appendix N Studies of prevalence of pre-treatment drug resistance in Africa

Appendix 0 Publication in AIDS & Clinical Research: The Art of HIV Elimination: Past and Present Science

Appendix A: TasP Home-based Individual Questionnaire

Ukuphila kwami, ukuphila kwethu

Africa Centre TasP Trial

IQ
v22 May 2014

Individual Questionnaire

BSID
TasP ID
Visit Date
Fieldworker

1. Individual Identification

Surname
Maiden name
First Name(s) 1
2.
Sex O Male O Female
Date of Birth
South African ID Y Y M M D D N/A
Cell 1.
2.

2. Operational Details

A. Fieldworker attempts

	Staff member	Attempt Date	Comment
1.			
2.			
3.			
4.			

B. Tracker / Special Task Team Attempts

1.		Y Y Y Y M M D	
2.		Y Y Y Y M M D	
3.		Y Y Y Y M M D	

3. Education and Marital Status

3.1 What is the highest level of education you have completed?

- O None
- O Some Primary
- O Completed Primary
- O Some secondary
- O Completed Secondary
- O Higher
- O Don't know
- O Refused

3.2 What is your current marital status?

- O Never been married
- O Engaged
- O Married (monogamous)
- O Married (polygamous)
- O Divorced/Separated
- O Widowed
- O Refused

3.3 Are you currently living with a husband/wife/partner?

- O Yes
- O No
- O Refused

4. Employment and Income

I would now like to ask you about your employment status, any social grants that you receive, and any regular income you receive each month.

4.1 Are you currently in employment?

- O Yes, full-time
- O Yes, Part-time
- O No → 4.3
- O Don't know
- O Refused

4.2 Are you self-employed or an employee?

- O Self-employed → 4.4
- O Employee → 4.4
- O Don't know
- O Refused

4.3 If you are not currently doing anything to earn money, then are you:

- O Studying
- O Looking for work
- O Nothing (not looking)
- O Retired / Old age
- O Don't know
- O Pregnant
- O Other
  - → *Please specify* _____
- O Sick or injured
- O Refused

4.4 Do you receive any Government Grant for yourself or on behalf of someone else?

- O Yes
  - → How many of each type of grant do you receive? *If none put zero*
    - Child support ___ grants
    - Foster care ___ grants
    - Disability (Care Dependancy) ___ grants
    - Old age pension ___ grants
    - Other ___ grants
      - → *Specify* _____
- O No, None
- O Don't know
- O Refused

4.5 Do you receive a regular income (money) other than a government grant. For example money you receive from an employer?

- O Yes
  - → 4.6 How much? R _____
    - O Don't know
    - O Refused
- O No
- O Don't know
- O Refused

5. Attitudes towards HIV testing

5.1 There are many places to get an HIV test. Which is the best place to get tested?

- O Home
- O Clinic
- O Other
- O Refused
- O Hospital
- O Private Doctor
- O Don't know
- O Counselling Centre
- O Mobile testing unit

5.2 Do you know your HIV status?

- O Yes
- O No
- O Not sure
- O Refused

5.3 When was the last time you had an HIV test? *(Explain we are NOT asking about the test result)*

- O In the last 6 months
- O More than a year ago
- O Refused
- O 6 months to 1 year ago
- O Never HIV-tested → Q5.7

5.4 Where did you test the last time you had an HIV test?

- O Home
- O Ante-natal clinic
- O Mobile testing unit
- O Refused
- O Hospital
- O Other Clinic
- O Other
- O Counselling Centre
- O Private Doctor
- O Don't know

5.5 Would you be willing to share your HIV test results with this study?

- O Yes
- O No
- O Not sure
- O Refused

→ Q5.6

5.6 What is your HIV status?

- O Positive
- O Negative
- O Not sure
- O Refused

5.7 According to you, when do you think people should test for HIV?

Tick all that apply

- ☐ When they feel sick
- ☐ When suggested by a counsellor or healthcare professional
- ☐ When they have had unprotected sex
- ☐ Regularly, as part of looking after their health → 5.6 How often?
  - O Once a year
  - O Every six months
  - O More often than once every 6 months
  - O Don't know
  - O Refused
- ☐ Don't know
- ☐ Refused

Please tell me whether you agree or disagree with the following statements.	Agree	Disagree	Don't know	Refused
5.8 People in your community do not blame people for having HIV.	O	O	O	O
5.9 People in your community avoid people with HIV.	O	O	O	O
5.10 I believe antiretroviral drugs make people with HIV less infectious.	O	O	O	O
5.11 I am less worried about HIV now treatments have improved.	O	O	O	O
5.12 If I were HIV-positive, I would want to start taking ARVs as soon as possible.	O	O	O	O
5.13 People in my community are more willing to talk openly about HIV than they were a year or so ago.	O	O	O	O
5.14 People in this community are less worried about HIV than they were a year or so ago.	O	O	O	O

5.15 Do you know someone with HIV? (Explain that we're NOT asking for their names)

- O Yes
- O No → Section 6
- O Not sure
- O Refused

→ 5.13 Who do you know? *Tick all that apply*

- ☐ I am HIV-positive myself
- ☐ Someone in my family
- ☐ One of my friends
- ☐ Someone in my community

6. Alcohol

6.1 Do you ever drink alcohol?

- O Yes
- O No
- O Refused

→ 6.2 How many times in the past six months, have you had more than three big bottles of beer and/or more than 6 glasses of other alcoholich drinks to drink on one occasion?

- O Never or only once
- O Less than once a month
- O Once monthly
- O Once weekly
- O Every or nearly every week
- O Every or nearly every day
- O Refused

7. Safety and security.

I shall now ask about safery and security in your community.

Please tell me whether you agree or disagree with the following statements.		Agree	Disagree	Don't know	Refused
7.1	Safety and security are major issues in this community.	O	O	O	O
7.2	I always feel safe in my community.	O	O	O	O
7.3	I have been a victim of crime in the last 12 months.	O	O	O	O
7.4	I have been a victim of a sexual crime in the last 12 months	O	O	O	O
7.5	In the last 12 months I have been forced to have sex that I didn't want, either by my regular partner or by someone else.	O	O	O	O
7.6	I would feel able to report a crime of a sexual nature to the police	O	O	O	O

8. Health care Expenditure

I shall now ask about how you provide for your own health care and how much you had to pay for that. In the LAST FOUR WEEKS, have you used any of the following healthy services?

8.1 Ask about each one, record how many visits (or inpatient days) and the cost. (Don't know=96/9996, 98/9998=Refused)

Type of facility or service	Visits/inpatient days (if none, put zero)	Cost (If none, put zero)
Primary care clinic	(times) |__|__|	R |__|__|__|__|
Chemist/pharmacy	(times) |__|__|	R |__|__|__|__|
A hospital emergency/outpatient department	(times) |__|__|	R |__|__|__|__|
Inpatient stay in hospital	(days) |__|__|	R |__|__|__|__|
A private doctor	(times) |__|__|	R |__|__|__|__|
A traditional healer	(times) |__|__|	R |__|__|__|__|

9. Sexual Relationships

9.1 Have you ever had sex? *(Explain what is meant by 'Having sex)'*

O Yes O No → Section 10 O Refused

→ 9.2 How old were you when you first had sex?

____ years If under 12, comment: _____

O Don't know

O Refused

9.3 How many sexual partners have you had in your lifetime? ____ partners

9.4 How many sexual partners, in total, have you had in the last 12 months? ____ partners

Sometimes people have more than one relationship at the same time

9.5 How many relationships are you in at the moment? ____ relationships

9.6 In the last 12 months have you had sex with someone who you know for certain was HIV Positive?

O Yes O No O Refused

The following questions are about sexual partners in the last year. (If no partners in the last 12 months reported then ask only about the most recent partner only to be sure to indicate carefully the time since that partner in item 45). No names will be used during analysis and only group data will be presented

.	Most Recent Partner	Previous Partner 1	Previous Partner 2
9.7 Remembering the most recent/previous time you had sex, what was your relationship to that partner at the time?	O Current spouse (at the time) O Current regular partner (at the time) O Former spouse / regular partner O Casual partner O Other	O Current spouse (at the time) O Current regular partner (at the time) O Former spouse / regular partner O Casual partner O Other	O Current spouse (at the time) O Current regular partner (at the time) O Former spouse / regular partner O Casual partner O Other

9.8 Did you know this partner's HIV status?	○ Yes → 9.9 ○ No → 9.11 ○ Don't know ○ Refused	○ Yes → 9.9 ○ No → 9.11 ○ Don't know ○ Refused	○ Yes → 9.9 ○ No → 9.11 ○ Don't know ○ Refused
9.9 Are you willing to share this partner's HIV status	○ Yes ○ No ○ Don't know ○ Refused	○ Yes ○ No ○ Don't know ○ Refused	○ Yes ○ No ○ Don't know ○ Refused
9.10 What is this partner's HIV status?	○ Positive ○ Negative ○ Don't know ○ Refused	○ Positive ○ Negative ○ Don't know ○ Refused	○ Positive ○ Negative ○ Don't know ○ Refused
9.11 Are you still in a sexual relationship with this partner?	○ Yes ○ No ○ Don't know ○ Refused	○ Yes ○ No ○ Don't know ○ Refused	○ Yes ○ No ○ Don't know ○ Refused
9.12 When was the last time you had sex with this partner?	__ Number ○ Days ○ Months ○ Weeks ○ Years ○ DK ○ Rfs	__ Number ○ Days ○ Months ○ Weeks ○ Years ○ DK ○ Rfs	__ Number ○ Days ○ Months ○ Weeks ○ Years ○ DK ○ Rfs
9.13 Is this partner older, younger or about the same age?	○ Older ○ Younger ○ Same age ○ Don't know	○ Older ○ Younger ○ Same age ○ Don't know	○ Older ○ Younger ○ Same age ○ Don't know
9.14 About how many years [older / younger]? *(Record actual number or 98=Don't know)*	__ years younger/older	__ years younger/older	__ years younger/older
9.15 Where does this partner normally reside?	○ With Member ○ Elsewhere in this Isigodi ○ Outside this Isigodi ○ Don't know ○ Refused	○ At this homestead ○ Elsewhere in this Isigodi ○ Outside this Isigodi ○ Don't know ○ Refused	○ At this homestead ○ Elsewhere in this Isigodi ○ Outside this Isigodi ○ Don't know ○ Refused
Always enter local area	His/Her Isigodi ____ Local area ____	His/Her Isigodi ____ Local area ____	His/Her Isigodi ____ Local area ____
9.16 Did you use a condom the last time you had sex with this partner?	○ Yes ○ No ○ Don't know ○ Refused	○ Yes ○ No ○ Don't know ○ Refused	○ Yes ○ No ○ Don't know ○ Refused
9.17 How long were you / have you been sexually involved with this partner?	__ Number ○ Days ○ Months ○ Weeks ○ Years ○ DK ○ Rfs	__ Number ○ Days ○ Months ○ Weeks ○ Years ○ DK ○ Rfs	__ Number ○ Days ○ Months ○ Weeks ○ Years ○ DK ○ Rfs
9.18 How many times have you had sexual intercourse with this partner in the last three months?	No. of times __ Don't know ○ Refused ○	No. of times __ Don't know ○ Refused ○	No. of times __ Don't know ○ Refused ○

9.19	On how many of these occasions did you and your partner use condoms	No. of times ⌊__⌋⌊__⌋ Don't know ○ Refused ○		No. of times ⌊__⌋⌊__⌋ Don't know ○ Refused ○		No. of times ⌊__⌋⌊__⌋ Don't know ○ Refused ○	

10. Parenthood and Pregnancy *(Females only)*

10.1 Have you ever been pregnant?

○ Yes ○ No → 10.4 ○ Refused

→ 10.2 How many children have you had? *(include those that have died)*

⌊__⌋⌊__⌋ children ○ Don't know ○ Refused

10.3 Some women have children with more than one man. How many fathers do your children have?

⌊__⌋⌊__⌋ fathers ○ Don't know ○ Refused

10.4 Do you plan to have a(nother) child?

○ Yes, I would like another child one day ○ No ○ Don't know

○ Yes, I am trying to have another child now ○ No, I can no longer have children ○ Refused

○ Yes, I am already pregnant

Skip to Section 12

11. Parenthood and circumcision *(Males only)*

11.1 Have you fathered any children?

○ Yes ○ No → 11.4 ○ Don't know → 11.4 ○ Refused → 11.4

→ 11.2 How many children have you fathered *(include those that have died)*

⌊__⌋⌊__⌋ children ○ Don't know ○ Refused

11.3 Some men have children with more than one woman. How many mothers do your children have?

⌊__⌋⌊__⌋ mothers ○ Don't know ○ Refused

11.4 Do you plan to have a(nother) child?

○ Yes, I would like another child one day ○ No ○ Don't know ○ Refused

○ Yes, I am trying to have another child now

○ Yes, my wife/partner is already pregnant

11.5 Are you circumcised?

○ Yes ○ No → Section 12 ○ Refused

→ 11. Where was the circumcision carried out?

○ Government hospital ○ Other ○ Don't know

○ Private clinic / hospital → *Specify* ⌊_____⌋ ○ Refused

○ Dept. of Health camp (MCC)

11.7 When was the circumcision carried out?

○ As an infant ○ Don't know ○ Refused

○ As a child or teenager

○ As an adult

11.8 Was this for cultural and/or health reasons?

○ Cultural reasons ○ Don't know ○ Refused

○ Health reasons

○ Both

○ Neither

12. Quality of Life

12.1 Which of the following best describes your mobility today?

- O I have no problems in walking about.
- O I have some problems walking about.
- O I am confined to bed.
- O Refused

12.2 Which of the following best describes your ability to care for yourself today?

- O I have no problems with self-care
- O I have some problems with washing or dressing myself
- O I am unable to wash or dress myself
- O Refused

12.3 Which of the following best describes your ability to do your usual activities today?
(e.g. work, study, housework, family or leisure activities)

- O I have no problems performing my usual activities.
- O I have some problems performing my usual activities.
- O I am unable to perform my usual activities.
- O Refused

12.4 Which of the following best describes your level of pain or discomfort today?

- O I have no pain or discomfort.
- O I have moderate pain or discomfort.
- O I have extreme pain or discomfort.
- O Refused

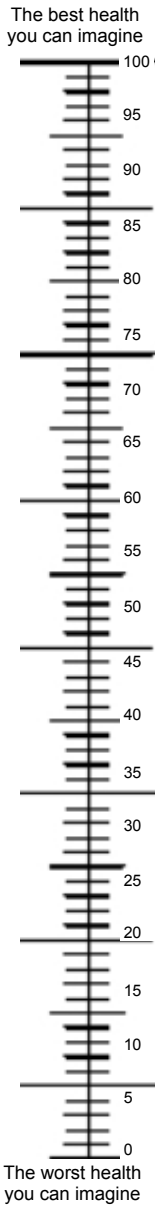
12.5 Which of the following best describes your level of anxiety or depression today?

- O I am not anxious or depressed.
- O I am moderately anxious or depressed.
- O I am extremely anxious or depressed.
- O Refused

12.6 I would like to know how good or bad you rate your health TODAY

- This scale is numbered from 0 to 100
- 100 means the BEST health you can imagine.
- 0 means the WORST health you can imagine.
- Mark an X on the scale to indicate how your health is today.
- Now please write the number you marked on the scale in the box below

Your health today = ☐☐☐



13. HIV Testing

13.1 Do you wish to consider HIV testing with one of the Ukuphila kwami, ukuphila kwethu staff today?

- O Yes
- O No

→ 13.2 Why not? *Tick all that apply*

- ☐ I know my status is positive
- ☐ I know my status is negative
- ☐ I don't want to disclose my status to anyone
- ☐ I am afraid to know my status
- ☐ I can only test with my partner
- ☐ I would be afraid if my partner knew my status
- ☐ Other
- ☐ Refused

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Appendix B: Consent form for home-based questionnaire (English and IsiZulu)

This English version is NOT for use in the field

Ukuphila kwami, ukuphila kwethu

Africa Centre TasP Trial

CE1
v31 Mar 2014

BSID
TasP ID
Visit Date
Fieldworker

PARTICIPANT SIGNATURE SHEET
INDIVIDUAL QUESTIONS AND DBS COLLECTION

Title of the research study:

Ukuphila kwami, ukuphila kwethu - Antiretroviral Treatment as Prevention (TasP) -A cluster-randomized trial in Hlabisa sub-district, KwaZulu-Natal
ANRS 12249
Protocol V2.0- 9 January2014
Sponsor: ANRS - National AIDS Research Agency, Paris, France
Coordinating Centre: Africa Centre for Health and Population Studies, University of KwaZulu-Natal, Somkhele, South Africa
This research study was approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal on 2 February 2012 and 6 July 2012.

Age of participant: ____ years If below 18 years, parent or guardian must sign to indicate their consent to the child's participation in the study.

Parent / guardian's name (print)	Parent / guardian's signature	Date
_____	_____	_____

Participation consent:

I have been told about the above research study by a trained counsellor. I understand my participation in this study is voluntary. No one can force me to participate.

I, _____ agree to participate in this research study being done by the Africa Centre. I have received and understood the study information sheet. I understand the benefits, difficulties and the implications for my family and myself of participating in this research study. I understand that the test for HIV is voluntary. I have been told where and when I can see a counsellor and obtain an HIV test if I do not want to have one today.

I consent to the following:

1) Answer the counsellor's questions about myself, my general health, my attitudes and beliefs about HIV, my personal relationships and sexual behaviour. This takes about 15 minutes.

2) Provide a very small blood specimen - 5 dots dried into a piece of paper. To do this requires a tiny prick of one of my fingers. Once the paper with the bloodspots is dry, the counsellors will place it in an envelope. All the papers collected will be stored in a laboratory and only used for other research studies relating to HIV. I understand that confidentiality is kept about these samples because they are coded and the laboratory does not know my identity.

3) Discuss with the counsellor about taking the important step of learning my HIV status through a process of HIV counseling and testing (HCT). I will be counseled separately about this, and asked to sign a separate consent form like the ones used in the Department of Health clinics indicating my agreement to have an HIV test. Having an HIV test today is not obligatory.

I know that I can leave the research study at any time without prejudice and that my treatment by the Health Services and by Africa Centre staff will be exactly the same whether or not I choose to take part. I also understand that I am not giving up any of my legal rights by signing this informed consent document.

Participant's name (print)	Participant's signature (Persons who cannot write may mark with X)	Date
_____	_____	_____
Name of staff member who administered consent (print)	Staff Member's signature	Date
_____	_____	_____
Witness' name (print) *	Witness' signature	Date
_____	_____	_____

Stick DBS Specimen Id barcode here

* Witness required only if the participant cannot write or if the participant asks for one.

This trial is conducted in accordance with international Guidelines for Good Clinical Practice in Clinical Trials and with the approval of both the University of KwaZulu-Natal Biomedical Research Ethics Committee (6 July 2012) and the SA Medicines Control Council (28 June 2012).

Contact details:
Biomedical Research Ethics Administration, University of KwaZulu-Natal, Research Office, Westville Campus, Govan Mbeki Building
Private Bag X 54001, Durban, 4000, KwaZulu-Natal. Tel: 27 31 2604769 - Fax: 27 31 2604609 - Email: BREC@ukzn.ac.za
SA Medicines Control Council, Department of Health, Private Bag X828, PRETORIA 0001. Fax: 27 12 395 8775; Email: nkambp@health.gov.za

Printed 28 May 2014 11:56

Ukuphila kwami, ukuphila kwethu

Africa Centre TasP Trial

PARTICIPANT SIGNATURE SHEET

INDIVIDUAL QUESTIONS AND DBS COLLECTION

Round 1

CZ1

v31 Mar 2014

BSID

TasP ID

Visit Date Y Y Y Y M M D D

Fieldworker

Isihloko Socwaningo:
Ukuphila kwami, ukuphila kwethu - Ukuvikela ngokusetshenziswa kwemishanguzo (TasP) -Ucwaningo olwenziwa ezigodini ezingaphansi kwesifunda sakwaHlabisa lungekho uhlelo oluqondile lokukhetha abantu, KwaZuluNatali
ANRS 12249
Uhlelo lwemithetho V2.0 - 9 January 2014
Umxhasi: ANRS - National AIDS Research Agency, Paris, France
Isikhungo esixhumanisayo: Africa Centre for Health and Population Studies, University of KwaZulu-Natal, Somkhele, South Africa
Lolucwaningo luphasiswe ikomidi elibhekelela amalungelo kwezocwaningo lwezempilo lase Nyuvesi yakwaZulu-Natali mhlaka - 2 Feb 2012 nangomhlaka 6 July 2012.

Ubudala ____ iminy

Uma ingaphansi kuka-18, umzali noma umbheki kumele asayine ukuvumela ingane ukuthi ibambe

Igama lomzali /mbheki (Loba)	Ukusayina komzali / mbheki	Usuku
_____	_____	_____

Iphepha lemvume lokubamba iqhaza:

Sengichazeliwe ngocwaningo olungenhla ngumaluleki oqeqeshiwe. Ngiyaqondisisa ukuthi ukubamba iqhaza kwami kulolucwaningo kungokuzikhethela kwami. Akekho namunye ongangiphoqelela ukubamba iqhaza.

Mina, _____ ngiyavuma ukuba yingxenye yocwaningo olwenziwa yi-Africa Centre. Ngilitholile iphepha lolwazi futhi ngaliqondisisa. Ngiyakuqonda ukuhlomula, ubunzima kanye nemthelela okungaba nawo emndenini wami nakimi ukuba yingxenye yaloluchwaningo. Ngiyaqonda ukuthi ukuhlolela igciwane le-HIV kungokuzikhethela kwami. Ngichazeliwe ukuthi ngingamubona kuphi nanini umaluleki ngihlolele i-HIV uma ngingathandi ukuhlolwa namuhla.

Ngiyavuma kulokhu okulandelayo:

1) Ukuphendula umaluleki imibuzo emayelana nami, isimo sempilo yami, indlela engicabanga ngayo, nezinkolelo zami nge-HIV, ubudlelwano enginabo nokuziphatha kwami ngezocansi. Lokhu kuzothatha imizuzu engevile kweyishumi nanhlanu.

2) Ukunikezela ngeconsi legazi - amaconsi amahlanu omisiwe esiqeshini esincane sephepha. Ukwenza lokhu kuzodinga ukuthi ngichofozwe kancane emunweni owodwa. Uma iphepha elinamaconsi egazi selomile, umaluleki uyobe eselifaka emvilophini. Wonke amaphepha aqoqiwe azobekwa e-laboratory ayosetshenziselwa kuphela olunye ucwaningo oluphathelene ne-HIV. Ngiyaqonda ukuthi ukuthathwa kwalama sampula egazi kugcineka kuyimfihlo ngoba kusetshenziswa amakhodi ne-laboratory angeke yazi ukuthi yimina.

3) Ukuxoxisana nomaluleki ngokuthatha igxathu elibalulekile lokwazi ngesimo sami se-HIV ngohlelo lokwalulekwa nokuhlolela i-HIV (HCT). Ngizokwalulekwa ngokwahlukile ngalokhu, ngisayine iphepha lemvume elifana nelisetshenziswa emitholampilo yoMnyango Wezempilo elizoveza ukuvuma kwami ukuhlolela i-HIV. Ukuhlolela i-HIV namuhla akusiyona impoqo.

Igama lobamba iqhaza (loba)	Obamba iqhaza uyasayina (Kongakwazi ukubhalo loba u X)	Usuku
Igama lomsebenzi onikezele ngephepha lemvume (loba)	Ukusayina komsebenzi	Usuku
Igama likafakazi (loba) *	Ufakazi uyasayina	Usuku

* Ufakazi udingeka kuphela uma obambe iqhaza engakwazi ukubhala noma ecela ukuba nofakazi.

Stick DBS Specimen Id barcode here

Lolucwaningo lwenziwa ngokulandela imigomo yomhlaba ye-Good Clinical Practice in Clinical Trials futhi luphasiswe yikomidi elibhekele amalungelo kwezocwaningo lwezempilo laseNyuvesi yaKwaZulu-Natali (Mhlaka 6 July 2012) nesigungu esilawula ngezemithi eNingizimu Africa (mhlaka 28 June 2012).

Imininingwane yekomidi:
Biomedical Research Ethics Administration, University of KwaZulu-Natal, Research Office, Westville Campus, Govan Mbeki Building
Private Bag X 54001, Durban, 4000, KwaZulu-Natal. Tel: 27 31 2604769 - Fax: 27 31 2604609 - Email: BREC@ukzn.ac.za
SA Medicines Control Council, Department of Health, Private Bag X828, PRETORIA 0001. Fax: 27 12 395 8775; Email: nkambp@health.gov.za

Appendix C: Consent for HIV testing (English and IsiZulu)

PARTICIPANT SIGNATURE SHEET
HOME-BASED HIV TESTING

BSID
TasP ID
Visit Date
Fieldworker

Title of the research study:

Ukuphila kwami, ukuphila kwethu - Antiretroviral Treatment as Prevention (TasP) -A cluster-randomized trial in Hlabisa sub-district, KwaZulu-Natal
ANRS 12249
Protocol V2.0 - 9 January 2014
Sponsor: ANRS - National AIDS Research Agency, Paris, France
Coordinating Centre: Africa Centre for Health and Population Studies,
University of KwaZulu-Natal, Somkhele, South Africa
This research study was approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal on 2 February 2012 and 6 July 2012.

HIV testing consent:

I, …… hereby give my fully informed consent to be tested for HIV antibodies. I have been counselled by a trained HIV counsellor with knowledge of HIV issues. We have discussed the advantages and disadvantages of doing the test and I fully understand the implications of having a test and the impact it may have on my life. I understand that the test for HIV is voluntary and that I will receive my result today if I wish. I consent to being followed up should there be a need to refer me to another facility or facilitate attendance to the trial clinics to ensure that I have received all the necessary support and services available. This follow-up can take the form of a visit or a phone call from a member of the study team.

I give permission to the study team to work at and capture data from my clinic records for the purpose of this research.

I know that I can leave the research study at any time and refuse to receive my HIV test result without prejudice and that my treatment by the Health Services and by Africa Centre staff will be exactly the same whether or not I choose to take part. I also understand that I am not giving up any of my legal rights by signing this informed consent document.

_____	_____	_____
Participant's name (print)	Participant's signature (Persons who cannot write may mark with X)	Date
_____	_____	_____
Name of staff member who administered consent (print)	Staff Member's signature	Date
_____	_____	_____
Witness' name (print) *	Witness' signature	Date

* Witness required only if the participant cannot write or if the participant asks for one.

Ukuphila kwami, ukuphila kwethu

Africa Centre TasP Trial

BSID
TasP ID
Visit Date Y Y Y Y M M D D
Fieldworker

PARTICIPANT SIGNATURE SHEET

HOME-BASED HIV TESTING

Isihloko Socwaningo:

Ukuphila kwami, ukuphila kwethu - Ukuvikela ngokusetshenziswa kwemishanguzo (TasP) -Ucwaningo olwenziwa ezigodini ezingaphansi kwesifunda sakwaHlabisa lungekho uhlelo oluqondile lokukhetha abantu, KwaZulu-Natali
ANRS 12249
Uhlelo lwemithetho V2.0 - 9 January 2014
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Lolucwaningo luphasiswe ikomidi elibhekelela amalungelo kwezocwaningo lwezempilo lase Nyuvesi yakwaZulu-Natali mhlaka - 2 Feb 2012 nangomhlaka 6 July 2012.

Iphepha lemvume yokuhlolela i-HIV:

Mina, …… ngiyavuma ngokuphelele ukuthi ngihlolelwe isandulela ngculazi. Sengikhulumile nomaluleki oqeqeshiwe futhi onolwazi nge-HIV. Sixoxisene ngemiphumela yokuhlolwa kwegazi engaba mihle noma ibe mibi ngaqonda ngokuphelele imithelela engabakhona ngokuhlola empilweni yami. Ngiyaqonda ukuthi ukuhlolelwa i-HIV kungokokuzikhethela kwami futhi ngizothola imiphumela yami namuhlanje uma ngifuna. Ngiyavuma ukulandelelwa uma kunesidingo sokuba ngidluliselwe kwesinye isikhungo noma ngikhuthazwe ukuhambela imitholampilo yocwaningo ukuqinisekisa ukuthi ngikuthola konke ukwesekwa nezinsiza ezikhona. Lokhu kulandelelwa kungaba ngokuvakashelwa noma ukushayelwa ucingo ilungu lethimba locwaningo.

Ngiyalinika ithimba locwaningo imvume yokuthi lingasebenzisa futhi luqophe imininingwane yami yasemtholampilo ngenhloso yalolucwaningo.

Ngiyaqonda ukuthi ngingalushiya ucwaningo noma ingasiphi isikhathi futhi nginganqaba ukuthatha imiphumela yami yesandulela ngculaza ngaphandle kokucwaswa ngokwenzenjalo kanti futhi nokuphathwa kwami ngabasebenzi bezempilo nabakwa Africa Centre kuzofana uma ngivuma noma nginqaba ukubamba iqhaza. Ngiyaqonda futhi ukuthi alikho ilungelo engililahlayo ngokusayina lelifomu lemvume.

_____	_____	_____
Igama lobamba iqhaza (loba)	Obamba iqhaza uyasayina (Kongakwazi ukubhalo loba u X)	Usuku
_____	_____	_____
Igama lomsebenzi onikezele ngephepha lemvume (loba)	Ukusayina komsebenzi	Usuku
_____	_____	_____
Igama likafakazi (loba) *	Ufakazi uyasayina	Usuku

* Ufakazi udingeka kuphela uma obambe iqhaza engakwazi ukubhala noma ecela ukuba nofakazi.

Lolucwaningo lwenziwa ngokulandela imigomo yomhlaba ye-Good Clinical Practice in Clinical Trials futhi luphasiswe yikomidi elibhekele amalungelo kwezocwaningo lwezempilo laseNyuvesi yaKwaZulu-Natali (mhlaka 6 July 2012) nesigungu esilawula ngezemithi eNingizimu Africa (mhlaka 28 June 2012).

Imininingwane yekomidi:

Biomedical Research Ethics Administration, University of KwaZulu-Natal, Research Office, Westville Campus, Govan Mbeki Building
Private Bag X 54001, Durban, 4000, KwaZulu-Natal. Tel: 27 31 2604769 - Fax: 27 31 2604609 - Email: BREC@ukzn.ac.za

SA Medicines Control Council, Department of Health, Private Bag X828, PRETORIA 0001. Fax: 27 12 395 8775; Email: nkambp@health.gov.za

Counselling checklist for HIV testing

Pretest HIV counselling

- [ ] Explore the participant's understanding of HIV and AIDS

Explain to them about

- [ ] HIV Transmission
- [ ] Prevention of HIV
- [ ] The benefits of treatment and why adherence is important.
- [ ] Explain the window period with respect to current test

Assess the participant's risk of HIV.
Find out about:

- [ ] Their previous HIV tests
- [ ] Their partner history
- [ ] Assess their use of condoms

Assess the implications of results for the participant:

- [ ] What are the implications of negative, positive, and indeterminate test results for participant, partner and family
- [ ] Explore if would disclose, and coping mechanisms and support
- [ ] Explore their fears and concerns

Post-test HIV counselling

HIV positive results

- [ ] Check participant understands results
- [ ] Ask how participant feels
- [ ] Check their plans for the day
- [ ] Check for mental health risk (some of this is simple observation of body language etc)
- [ ] Referral for psychosocial support (e.g support groups)
- [ ] Check their plans for disclosure
- [ ] Discuss condom use to prevent transmission
- [ ] Refer to TasP clinic for CD4 check, further care and support
- [ ] Offer revisit for further support

HIV negative results

- [ ] Check participant understands results
- [ ] Revisit Window period and the need for repeat test 3 months after exposure
- [ ] Safer sex, ABC
- [ ] Circumcision for males

Appendix D: Clinical history and examination form

Clinical History and Examination

To be completed by Trial Nurse

CHE
v10 June 2014

TasP ID
Clinic
Visit Date Y Y Y Y M M D D
Visit Type O Protocol → Week
O Non-protocol
Nurse

1. Participant Identification

Surname | First Name(s) 1 | 2.

2. CD4 testing/result

Should a CD4 test be carried out this visit? O Yes → *Take blood specimen and start PIMA test machine*
CD4 should normally be taken at Baseline, 3m, 6m, 12m, 18m and 24m, 30m, 36m, 42m, 48m visits
Result CD4 Count
or: Why no result?
O No

3. Blood glucose test

Was a blood glucose test carried out? O Yes → Result . mmol/L
O No

4. Past TB Treatment *Ask ONLY at Baseline Visit*

Have you previously been treated for TB? O Yes O No
→ *Complete details below*

	Date treatment started	TB Site		Regimen				Treatment completed?	
		PTB	EPTB	1	2	MDR	Unk	Yes	No
1.	Y Y Y Y M M D D	O	O	O	O	O	O	O	O
2.	Y Y Y Y M M D D	O	O	O	O	O	O	O	O

5. Current TB Treatment

Are you currently on TB treatment? O Yes → *Give details. If possible use information from the participant's TB card.*

Date current treatment started	TB Site			Regimen			
	Smear+ PTB	Smear- PTB	EPTB	1	2	MDR	XDR
Y Y Y Y M M D D	O	O	O	O	O	O	O

→ *Section 7*

O No

6. TB Screening

		Yes	No
a.	Have you had a cough for 24 hours or longer?	O	O
b.	Have you recently coughed up blood in the sputum?	O	O
c.	Have you experienced loss of appetite?	O	O
d.	Have you lost weight?	O	O
e.	Have you been sweating unusually at night?	O	O
f.	Have you had recurrent fever or chills lasting more than 3 days?	O	O
g.	Have you experienced chest pain?	O	O
h.	Have you had difficulty in breathing or fast breathing?	O	O
i.	Have you experienced swellings in the neck, armpits or elsewhere?	O	O
j.	Are you currently taking Isoniazid Preventive Therapy (IPT)?	O	O
k.	Have you been on IPT in the past?	O	O

If ALL questions were answered "No" then prescribe IPT as per protocol.

If any TB symptoms are present (i.e. one or more questions a. to i. answered "Yes"), then investigate as per TB guidelines and refer as appropriate.

7. STI Screening

Males	Yes	No
a. Have you noticed any swelling in your groin?	O	O
b. Do you have any ulcers or sores in your genital area?	O	O
c. Do you have pain when you pass urine?	O	O
d. Do you have any lower abdominal pain?	O	O
e. Do you have any discharge from your penis?	O	O
f. Have you noticed any swelling in your scrotum?	O	O

Females	Yes	No
a. Have you noticed any swelling in your groin?	O	O
b. Do you have any ulcers or sores in your genital area?	O	O
c. Do you have pain when you pass urine?	O	O
d. Do you have any lower abdominal pain?	O	O
e. Do you have a vaginal discharge which is increased in amount or changed in smell or colour?	O	O
f. Have you experienced vulval itching or burning?	O	O

8. Other Symptoms

	Yes	No	
a. Do you have diarrhoea today?	O	O	*If "Yes"* → *Obtain stool sample for microscopy.*
b. Have you had generalised itching of the skin for one month or more?	O	O	
c. Do you have a generalised skin rash?	O	O	
d. Have you suffered from recurrent blisters or sores on and around the lips?	O	O	
e. Have you experienced any problems with your vision?	O	O	
f. Do you have difficulty or pain when swallowing?	O	O	
g. Do you have a headache that has been getting worse or is persistent?	O	O	*If "Yes"* → *Consider referral to Hlabisa hospital for lumbar puncture.*

9. Hospitalisation etc.

9.1 In the last six months, or since last clinic visit, have you been admitted to hospital (slept there)?
O Yes → Which hospital? _____ Details: _____
O No

9.2 In the last six months, or since last clinic visit, have you seen any other healthcare provider?
O Yes → Details: _____ *State who and where etc.*
O No

10. Other Chronic Condition

	Yes	No	
a. Do you suffer from diabetes mellitus? *(High blood sugar)*	O	O	
b. Do you suffer from hypertension? *(High blood pressure)*	O	O	
c. Do you suffer from epilepsy?	O	O	
d. Do you suffer from any mental health condition?	O	O	*If "Yes"* → Specify: _____
e. Do you suffer from asthma/COPD?	O	O	
f. Do you suffer from arthritis?	O	O	
g. Have you ever suffered a stroke?	O	O	
h. Any other chronic conditions?	O	O	*If "Yes"* → Specify: _____

11. Concomitant medication not recorded in ART or TB regimen

Medication	Dose and frequency	Date started *If started since last visit* (Y Y Y Y M M D D)	Date stopped *If stopped since last visit* (Y Y Y Y M M D D)	or Ongoing?
a. Cotrimoxazole				☐
b. Isoniazid				☐
c. Pyridoxine				☐
Others: (specify)				
d.				☐
e.				☐
f.				☐

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12. Adverse Events *(only ask people on ART)*

Since the last visit, or in last 6 months, have you experienced any symptoms which you feel are caused by your ART drugs? O Yes O No → *Section 12*

	Symptom	Date started *If started since last visit*	Date stopped *If stopped since last visit*	or Ongoing?	Grading *1=mildest, 4=severest see ANRS grading scale*
a.	Nausea / vomiting	Y Y Y Y M M D D	Y Y Y Y M M D D	☐	
b.	Diarrhoea	Y Y Y Y M M D D	Y Y Y Y M M D D	☐	
c.	Loss of appetite	Y Y Y Y M M D D	Y Y Y Y M M D D	☐	
d.	Abdominal pain	Y Y Y Y M M D D	Y Y Y Y M M D D	☐	
e.	Jaundice	Y Y Y Y M M D D	Y Y Y Y M M D D	☐	
f.	Skin rash / itching	Y Y Y Y M M D D	Y Y Y Y M M D D	☐	
g.	Headache	Y Y Y Y M M D D	Y Y Y Y M M D D	☐	
h.	Dizziness	Y Y Y Y M M D D	Y Y Y Y M M D D	☐	
i.	Fatigue	Y Y Y Y M M D D	Y Y Y Y M M D D	☐	
j.	Insomnia	Y Y Y Y M M D D	Y Y Y Y M M D D	☐	
k.	Abnormal dreams	Y Y Y Y M M D D	Y Y Y Y M M D D	☐	
l.	Difficulty concentrating	Y Y Y Y M M D D	Y Y Y Y M M D D	☐	
m	Painful feet	Y Y Y Y M M D D	Y Y Y Y M M D D	☐	
n.	Change in body shape	Y Y Y Y M M D D	Y Y Y Y M M D D	☐	
o.	Other:	Y Y Y Y M M D D	Y Y Y Y M M D D	☐	

13. Pregnancy and contraception *(Females only)*

13.1 Are you pregnant? O Yes → LMP date Y Y Y Y M M D D

Confirmed by urine test? O Yes O No

O No → Are you trying to get pregnant now? O Yes O No

13.2 Do you use any form of contraception? O Yes → Which method(s)? *Tick all that apply*

- ☐ Injectables
- ☐ Oral contraceptive pills
- ☐ Condoms
- ☐ Others → Specify:

O No

13.3 Gravida ___ 13.4 Para ___

14. General examination

		Yes	No
a.	Wasting?	O	O
b.	Jaundice?	O	O
c.	Anaemia?	O	O
d.	Clubbing?	O	O
e.	Cyanosis?	O	O
f.	Pedal Oedema?	O	O
g	Lymphadenopathy > 1cm?	O	O

15. Mouth and Skin

Mouth lesions		Yes	No
a.	Herpes simplex?	O	O
b.	Angular stomatitis?	O	O
c.	Oral thrush?	O	O
d.	Kaposi's sarcoma?	O	O
e.	Oral hairy leukoplakia?	O	O
f.	Other	O	O

Any skin lesions? O Yes → a. Description ___

b. Distribution ___

O No

If 'Yes' → Specify: ___

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16. Respiratory system

a. Breathless at rest?	O Yes	O No	Respiratory rate ___ Breaths / min	
b. Percussion notes	O Normal	O Dull		
c. Air entry	O Normal both sides	O Reduced left side	O Reduced right side	O Reduced both sides
d. Breath sounds	O Vesicular	O Bronchial		
e. Added sounds	O None	O Crackles	O Ronchi	

17. Abdomen

Are there any abdominal abnormalities which warrant referral to the Trial doctor?

O Yes → Details _____
Refer to doctor in Section 26l

O No

18. Genitourinary system - Males

	Yes	No	If Yes, describe
a. Urethral discharge?	O	O	
b. Inguinal swelling?	O	O	
c. Ulcers?	O	O	
d. Lumps?	O	O	
e. Other	O	O	

19. Genitourinary system - Females

	Yes	No	If Yes, describe or give reasons
a. Vulval ulcers?	O	O	
b. Inguinal swelling?	O	O	
c. Vaginal discharge	O	O	
d. Lumps?	O	O	
e. Any pelvic abnormalities which warrant referral to the Trial doctor?	O	O	*Refer to doctor in Section 26l*
f. Pap smear taken?	O	O	*(Take at baseline, then yearly)*

20. General Clinical impression and diagnoses

	Diagnosis	ICD10 code
1.		.
2.		.
3.		.

21. WHO staging

WHO Stage O 1 O 2 O 3 O 4

Reasons for staging _____

22. ART Eligibility *For participants who ARE NOT already on ART*

Is this participant eligible for ART? O Yes →
Will Atripla be appropriate if relevant laboratory investigations are normal?
O Yes → *Section 23*
O No → Why not? _____
Refer to doctor in Section 26l

O No, because participant is in Control Cluster, and their CD4 count is over 350 and clinical criteria are not met.

23. ART Review *For participants who ARE already on ART*

Is a possible change in ART regimen indicated?

O Yes or unsure → Details _____
Refer to doctor in Section 24l

O No

24. Drug Prescriptions

		Date prescribed	Dose and frequency	Period covered
A) Prophylaxis				
1	Cotrimoxazole	Y Y Y Y M M D D		Days
2	Isoniazid	Y Y Y Y M M D D		Days
3	Pyridoxine	Y Y Y Y M M D D		Days
B) STI Treatment				
1		Y Y Y Y M M D D		Days
2		Y Y Y Y M M D D		Days
3		Y Y Y Y M M D D		Days
4		Y Y Y Y M M D D		Days
C) Other				
1		Y Y Y Y M M D D		Days
2		Y Y Y Y M M D D		Days
3		Y Y Y Y M M D D		Days
4		Y Y Y Y M M D D		Days

25. ART (Use codes AZT, d4T, etc)

	Date started (Enter only if new)	Date stopped (Only if stopping)	Ongoing?	Dose/Freq	Reason for stopping	Period covered (days)
1	Y Y M M D D	Y Y M M D D	☐			
2	Y Y M M D D	Y Y M M D D	☐			
3	Y Y M M D D	Y Y M M D D	☐			
4	Y Y M M D D	Y Y M M D D	☐			

26. Action Plan

	Yes	No	Not Applic.
a. Referred for ART counselling?	O	O	O
b. Blood taken for Hlabisa Lab? *(U&Es, LFTs, glucose, lipids, FBC & HepBsAg if indicated)*	O	O	O
c. Blood taken for Africa Centre Durban Lab? *20mls for plasma storage and HIV RNA Viral Load*	O	O	O
d. Blood taken for genotypic resistance testing (Durban)?	O	O	O
e. Urinalysis?	O	O	O
f. Urine Beta-HGC pregnancy test?	O	O	O
g. Sputum M/C?	O	O	O
h. Pap smear taken? *(Take at baseline, then at 12 and 24 months)*	O	O	O
i. Blood taken for Hlabisa Lab 10% QC CD4 test?	O	O	O

Take Viral Load specimen at baseline then, if on ART, at 3, 6, 12, 18 and 24, 30, 36, 42, 48 m

Stick VL Specimen Id barcode here

If Yes → Result: Protein ____ *(0, 1 , 2 or 3)*
Result: Blood ____ *(0, 1 , 2, 3 or 4)*

	Yes	No	If Yes, give description and reason
j. Referred to Primary Healthcare Clinic?	O	O	
k. Referred to Hlabisa Hospital?	O	O	
l. Referred to TasP Trial doctor?	O	O	→ *CDR referral form*
m Any other actions taken?	O	O	

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27. Comments

Form must be signed by Trial Nurse

Nurse name: _____ Signature: _____ Date Y Y Y Y M M D D

Print

Appendix E: Clinic baseline form

Clinic Baseline Visit (Counsellor)

00127160

TasP ID
Clinic
Visit Date
Counsellor

1. Participant Identification

Identity confirmed and fingerprint registered? ○ Yes ○ No

Surname First Name(s) 1 2.

2. Anthropometry and vitals

Weight . kg Pulse per min.

Height cm Blood pressure / (Sys. / Dia.)

3. HIV Clinical Information

3.1 Date of HIV diagnosis

3.2 Are you currently taking ART? ○ Yes →
- Date initiated
- Drug regimen: 1. 2. 3. 4.
- ART provider: ○ Hlabisa programme → Clinic ○ Other → Specify
- Do you want to transfer your HIV care to this trial clinic? ○ Yes → *Transfer-in form* ○ No
- *Form complete*

○ No

3.3 Have you been on ART at any time in the past? ○ Yes →
- What was this for? ○ HIV treatment ○ Pregnancy ○ PEP → *Refer to Trial doctor*
- Why are you not on ART currently? ○ Patient choice ○ Stopped by doctor ○ Other → *Specify*

○ No

Form must be reviewed and signed by Trial Nurse

Nurse name: (Print) Signature: Date reviewed

Appendix F: Clinic follow up form

Clinic Follow-up Visit

To be completed by Trial Counsellor

CFU
v7 May 2012

TasP ID
Clinic
Visit Date
Visit Type O Protocol → Week
O Non-protocol
Counsellor

1. Participant Identification

Identity confirmed by fingerprint? O Yes O No

Surname First Name(s) 1 2.

2. Anthropometry and vitals

Weight . kg Pulse per min.

Blood pressure / Sys. Dia.

If participant is NOT on ART → Form complete

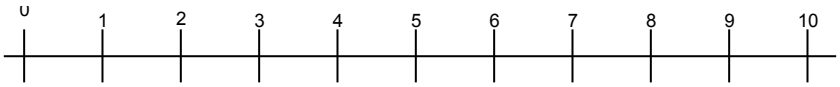
3. Adherence Checks

Carry out adherence checks monthly i.e. protocol visits

	Yes	No
3.1 When you feel better, do you sometimes stop taking your pills?	O	O
3.2 Thinking back over the last 4 days, have you missed any of your pills?	O	O
3.3 Sometimes, if you feel worse when you take your pills, do you stop taking them?	O	O

3.4 Adherence Visual Analogue Scale

Ask the patient to reflect on the last 4 days and point to their estimate of level of adherence



Score ____ %

3.5 Adherence Pill Identification Test

Ask the participant to identify their pills

Drug (use codes AZT, D4T etc)	Knows name? Yes	Knows name? No	Knows no. of pills Yes	Knows no. of pills No	When are pills taken? Morning time	When are pills taken? Evening time	Acceptable? Yes	Acceptable? No
1.	O	O	O	O	:	:	O	O
2.	O	O	O	O	:	:	O	O
3.	O	O	O	O	:	:	O	O
4.	O	O	O	O	:	:	O	O

3.6 Adherence pill count.

Did the participant return the pill containers? O Yes → % Adherence = (Dispensed − Returned) / Expected to be taken x 100 = (−) / x 100 = %

O No

Form must be reviewed by Trial Nurse

Nurse name: Print Signature: Date reviewed

Appendix G: Ethics approval for Control arm

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

RESEARCH OFFICE
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 260-4609
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/ResearchEthics/BiomedicalResearchEthics.aspx

02 February 2012

Prof. M-L Newell
Africa Centre for Health & Population Studies
Mtubatuba
3935

Dear Prof Newell

PROTOCOL: A cluster randomised trial to evaluate the effectiveness of antiretroviral treatment immediately on HIV diagnosis on reducing HIV incidence: the Treatment as Prevention trial in Hlabisa sub-district, rural KwaZulu-Natal. REF: BFC104/11

The Biomedical Research Ethics Committee (BREC) has considered the abovementioned application.

The study was provisionally approved by a sub-committee of the Biomedical Research Ethics Committee on 17 January 2012 pending appropriate responses to queries raised. Your responses dated 10 November 2011 to queries raised on 02 August 2011 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The activities described in your letter to BREC dated January 26th 2012 may commence with immediate effect. Full and final approval of the study will only be given pending response from the MCC.

This provisional approval is valid for one year from **02 February 2012.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/ResearchEthics11415.aspx.
BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The following Committee members were present at the meeting that took place on 13 December 2011:

Professor Doug Wassenaar. Chair
Professor Viren Rambiritch, Pharmacology
Professor Steven Collings, Psychiatry
Dr R Govender, Family Medicine
Dr Tim Hardcastle, Surgery - Trauma
Dr Z Khumalo - KZN Health (External)
Professor Dennis Pudifin, Medicine
Professor Chris Rout, Department of Anaesthesia
Dr Divendra Singh, Department of Anaesthesia
Professor Joyce Tsoka-Gwegweni, School of Medicine
Mrs T Makhanya, External
Professor Rajendra Bhimma - Paediatrics & Child Health
Professor Anne Coutsoudis - Paediatrics & Child Health
Professor Jerome Singh - Legal Representative

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

PROFESSOR D R WASSENAAR
Chair: Biomedical Research Ethics Committee

Appendix H: Full ethics approval

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

RESEARCH OFFICE
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
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Tel: 27 31 2604769 - Fax: 27 31 260-4609
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/ResearchEthics/BiomedicalResearchEthics.aspx

06 July 2012

Prof. M-L Newell
Africa Centre for Health & Population Studies
Mtubatuba
3935

Dear Prof Newell

PROTOCOL: A cluster randomised trial to evaluate the effectiveness of antiretroviral treatment immediately on HIV diagnosis on reducing HIV incidence: the Treatment as Prevention trial in Hlabisa sub-district, rural KwaZulu-Natal. REF: BFC104/11

The Biomedical Research Ethics Committee (BREC) has considered the abovementioned application.

The study was provisionally approved by a sub-committee of the Biomedical Research Ethics Committee on 02 February 2012 pending a response from the Medicine Controls Council. Your responses dated 29 June 2012 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval and may begin as from 06 July 2012.

This approval is valid for one year from **06 July 2012.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The following Committee members were present at the meeting that took place on 13 December 2011:

Professor Doug Wassenaar. Chair
Professor Viren Rambiritch, Pharmacology
Professor Steven Collings, Psychiatry
Dr R Govender, Family Medicine
Dr Tim Hardcastle, Surgery - Trauma
Dr Z Khumalo - KZN Health (External)
Professor Dennis Pudifin, Medicine
Professor Chris Rout, Department of Anaesthesia
Dr Divendra Singh, Department of Anaesthesia
Professor Joyce Tsoka-Gwegweni, School of Medicine
Mrs T Makhanya, External
Professor Rajendra Bhimma - Paediatrics & Child Health
Professor Anne Coutsoudis - Paediatrics & Child Health
Professor Jerome Singh - Legal Representative

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

[redacted]

PROFESSOR D R WASSENAAR
Chair: Biomedical Research Ethics Committee

Appendix I: Ethics recertification

UNIVERSITY OF
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RESEARCH OFFICE
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Tel: 27 31 2604769 - Fax: 27 31 260-4609
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

22 September 2015

Prof. M-L Newell
Africa Centre for Health & Population Studies
Mtubatuba
3935

Dear Prof Newell

PROTOCOL: A cluster randomised trial to evaluate the effectiveness of antiretroviral treatment immediately on HIV diagnosis on reducing HIV incidence: the Treatment as Prevention trial in Hlabisa sub-district, rural KwaZulu-Natal. REF: BFC104/11.

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved: 06 July 2015
Expiration of Ethical Approval: 05 July 2016

I wish to advise you that your application for Recertification received 31 July 2015 for the above protocol has been noted and approved by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

This approval will be **ratified** by a full Committee at its next meeting taking place on **13 October 2015.**

Yours sincerely

[redacted]

Ms A Marimuthu
Senior Administrator: Biomedical Research Ethics

Appendix J: Approval, Medicines Control Council of South Africa

0123958775

health

Department:
Health
REPUBLIC OF SOUTH AFRICA

MEDICINES CONTROL COUNCIL
The Registrar of Medicines, Private Bag X828, PRETORIA, 0001
Tel 012 395 8000 Fax 012 395 9201
Tel: *Inquiries:*
Fax: *Reference:*

FAX AND MAIL TO:

PROF M NEWELL

Africa Centre
University of Kwazulu-Natal
P.O Box 192
Mtubatuba
3935

MS N NGOBENI
N2/19/8/2
Datum * Date:28 June 2012

Tel: 012 395 8127

Fax: 012 395 8775

Fax: 0355507565

Dear Prof M Newell

TITLE: **A cluster randomised trial comparing the impact of immediate versus WHO recommendations guided ART initiation on HIV incidence. The ANRS 12249 TasP (Treatment as Prevention) trial in Hlabisa sub-district, KwaZulu-Natal, South Africa.**

RE : APPLICATION TO CONDUCT STUDY

We acknowledge receipt of your letter dated 19 January 2012 with the following documents:

a. Study Protocol version 1.2 dated 30 November 2011
b. Package inserts for trial drugs
c. Trial Insurance certificate
d. Signed declarations, CVs, GCP certificates, Proof of malpractice insurance of the following trialists:
   - Prof M Newell - Principal Investigator - Africa Centre for Health and Population Studies
   - Dr K Naidu - Sub investigator - Africa Centre for Health and Population Studies
   - Dr R Bland - Sub investigator - Africa Centre for Health and Population Studies
   - Dr R Lessells - Sub investigator- Africa Centre for Health and Population Studies
   - Dr J Viljoen - Sub investigator- Africa Centre for Health and Population Studies
   - Dr C Iwuji - Sub-investigator- Africa Centre for Health and Population Studies

Please note that the study may proceed once Ethics Committee has granted approval.

Yours faithfully,

[redacted]

MS N NGOBENI

FOR AND ON BEHALF OF REGISTRAR OF MEDICINES

N20120335

Appendix K: Ethics approval, Brighton and Sussex Medical School

BSMS Research Governance & Ethics Committee (RGEC)

Chair: Professor Kevin Davies
Deputy Chair: Professor Bobbie Farsides

Secretary: Miss Caroline Brooks
Tel: 01273 696955 ext. 3905 (Monday – Wednesday) Tel: 01273 872855 (Thursday and Friday) Caroline.Brooks@bsuh.nhs.uk

Applications and general enquiries: rgec@bsms.ac.uk

Brighton and Sussex Medical School
Medical Teaching Building
University of Sussex
Falmer
Brighton
BN1 9PX

09/09/2013

Professor Melanie Newport
Brighton and Sussex Medical School
Medical Research Building
University of Sussex
Falmer
Brighton
BN1 9PS

Dear Professor Newport

Full Study Title:	**Virological failure and HIV resistance in a test and treat approach to prevent HIV transmission**
R&D Ref No. :	**13/033/NEW**

I am writing to inform you that the Brighton and Sussex Medical School Research Governance and Ethics Committee (RGEC) has now assessed your application and granted **Research Governance Approval** to proceed with the above named project.

This letter acknowledges that you have all the necessary internal regulatory approvals.

Conditions of Approval

The approval covers the period stated in the Research Governance & Ethics Committee (RGEC) application and will be extended in line with any amendments agreed by the RGEC. Research must commence within 12 months of the issue date of this letter. Any delay beyond this may require a new review of the project resources.

Amendments

Project amendment details dated after the issue of this approval letter should be emailed to RGEC for formal approval.

Monitoring

The Medical School has a duty to ensure that all research is conducted in accordance with the Research Governance Framework and if appropriate. In order to ensure compliance the department undertakes random audits. If your project is selected for audit you will be given 4 weeks notice to prepare all documentation for inspection.

It is your responsibility to inform me in the event of early termination of the project or if you fail to complete the work.

I wish you luck with your project.

Yours sincerely

Professor Kevin Davies
Chair of the BSMS Research Governance and Ethics Committee

Appendix L: Ethics approval, UCL

UCL RESEARCH ETHICS COMMITTEE
ACADEMIC SERVICES

18 June 2015

Professor Kholoud Porter
MRC Clinical Trials Unit
UCL

Dear Professor Porter

Notification of Ethical Approval
Project ID: 6604/001: Will earlier treatment lead to drug resistance of the form and prevalence likely to compromise future elimination of HIV?

Further to your satisfactory responses to the committee's comments, I am pleased to confirm in my capacity as Chair of the UCL Research Ethics Committee (REC) that your study has been approved by the REC for the duration of the project i.e. until June 2016.

Approval is subject to the following conditions:

1. You must seek Chair's approval for proposed amendments to the research for which this approval has been given. Ethical approval is specific to this project and must not be treated as applicable to research of a similar nature. Each research project is reviewed separately and if there are significant changes to the research protocol you should seek confirmation of continued ethical approval by completing the 'Amendment Approval Request Form':

2. It is your responsibility to report to the Committee any unanticipated problems or adverse events involving risks to participants or others. Both non-serious and serious adverse events must be reported.

   Reporting Non-Serious Adverse Events
   For non-serious adverse events you will need to inform Helen Dougal, Ethics Committee Administrator (ethics@ucl.ac.uk), within ten days of an adverse incident occurring and provide a full written report that should include any amendments to the participant information sheet and study protocol. The Chair or Vice-Chair of the Ethics Committee will confirm that the incident is non-serious and report to the Committee at the next meeting. The final view of the Committee will be communicated to you.

   Reporting Serious Adverse Events
   The Ethics Committee should be notified of all serious adverse events via the Ethics Committee Administrator immediately the incident occurs. Where the adverse incident is unexpected and serious, the Chair or Vice-Chair will decide whether the study should be terminated pending the opinion of an independent expert. The adverse event will be considered at the next Committee meeting and a decision will be made on the need to change the information leaflet and/or study protocol.

On completion of the research you must submit a brief report (a maximum of two sides of A4) of your findings/concluding comments to the Committee, which includes in particular issues relating to the ethical implications of the research.

With best wishes for the research.

Yours sincerely

Professor John Foreman
Chair of the UCL Research Ethics Committee

Cc:
Collins Iwuji, Applicant
Dr Richard Gilson

Academic Service, 2 Taviton Street,
University College London Gower Street London WC1E 6BT
Tel: +44 (0)20 3108 4312
Email: ethics@ucl.ac.uk
http://ethics.grad.ucl.ac.uk/

Appendix M: The association between CD4 count at initiation, adherence measured by PC and other factors with virological suppression at 6 months

Socio-demographic characteristics	Virological suppression n (%)	Crude odds ratio (95% CI)	P value	*Adjusted odds ratio (95% CI)	P value
Clinical characteristics					
CD4 at Initiation (cells/mm^3) n=906					
CD4 at initiation/100 units					
≤350	396/440 (90.0)				
350-500	236/242 (97.5)	1.49 (1.25-1.77)	<0.0001	1.27 (1.05-1.53)	0.009
>500	217/224 (96.9)				
Adherence (Pill count) % N=898			0.065		0.373
<95	159/175 (90.9)	1		1	
≥95	685/723 (94.7)	1.81 (0.99-3.34)		1.37 (0.69-2.70)	
Viral load at presentation (copies/mL) **n=908**			<0.0001		0.001
<10,000	315/320 (98.4)	1		1	
10,000-100,000	297/313 (94.9)	0.29 (0.11-0.81)		0.33 (0.11-0.97)	
>100,000	239/275 (86.9)	0.10 (0.04-0.27)		0.17 (0.06-0.47)	
First line Regimen n=903			<0.0001		<0.0001
Non Fixed dose combination	24/34 (70.6)	1		1	
Fixed dose combination (Atripla)	822/869 (94.5)	7.29 (3.29-16.12)		10.67 (4.02-28.34)	
Age at initiation (Years) **n=907**					
16-29	283/301 (94.0)				
30-39	249/266 (93.6)	1.00 (0.90-1.11)	0.996	1.17 (1.02-1.34)	0.02
40-49	139/153 (90.9)				
>50	179/187 (95.7)				
			0.023		0.188
Sex n=908					
Female	630/664 (94.9)	1		1	
Male	221/244 (90.6)	0.52 (0.30-0.90)		0.63 (0.32-1.24)	

Socio-demographic characteristics	Virological suppression n (%)	Crude odds ratio (95% CI)	P value	*Adjusted odds ratio (95% CI)	P value
Educational attainment n=905			0.873		
Primary or less	371/396 (93.7)	1			
Some Secondary	435/465 (93.6)	0.98 (0.56-1.69)			
At least completed secondary	42/44 (95.5)	1.42 (0.32-6.19)			
Marital status n= 905			0.361		
Never been married/Engaged	739/792 (93.3)	1			
Married	73/76 (96.1)	1.74 (0.53-5.72)			
Divorced/Separated	36/37 (97.3)	2.58 (0.35-19.20)			
Employment status n=905			0.022		0.017
Employed	131/140 (93.6)	1		1	
Student	29/36 (80.6)	0.28 (0.10-0.83)		0.15 (0.04-0.56)	
Unemployed	688/729 (94.4)	1.15 (0.55-2.43)		0.80 (0.34-1.90)	
Distance from home to trial clinic (Km) n= 908					
Distance to trial clinic/unit increase			0.878		
≤1.3	424/453 (93.6)	1			
>1.3	427/455 (93.9)	1.04 (0.61-1.78)			
Other characteristics					
Self-reported health status n=907			0.003		0.009
≤80	490/534 (91.8)	1		1	
>80	360/373 (96.5)	2.49 (1.32-4.69)		2.48 (1.22-5.06)	
HIV status disclosure to anyone n=894			0.946		
Yes	721/769 (93.8)	1			
No	117/125 (93.6)	0.97 (0.45-2.11)			
			0.912		
HIV status disclosure to current partner n=875					
Yes	454/482 (94.2)	1			
No	245/260 (94.2)	1.01 (0.53-1.92)			
Not applicable (No partner)	124/133 (93.2)	0.85 (0.39-1.85)			
Food insecurity n= 896			0.511		

Socio-demographic characteristics	Virological suppression n (%)	Crude odds ratio (95% CI)	P value	*Adjusted odds ratio (95% CI)	P value
Yes	532/571 (93.2)	1			
No	301/318 (94.7)	1.30 (0.72-2.33)			
Don't know	6/7 (85.7)	0.44 (0.05-3.74)			
ART will improve health n= 893			0.298		
Yes	810/863 (93.9)	1			
No	11/12 (91.7)	0.72 (0.09-5.68)			
Don't know	15/18 (83.3)	0.33 (0.09-1.17)			
Worried about side effects of ART n=887			0.926		
Yes	713/762 (93.6)	1			
No	37/39 (94.8)	1.27 (0.30-5.43)			
Don't know	81/86 (94.2)	1.11 (0.43-2.87)			
Agree that ART will reduce transmission n= 880			0.858		
Yes	635/680 (93.4)	1			
No	66/71 (93.0)	0.94 (0.36-2.44)			
Don't know	122/129 (94.6)	1.24 (0.54-2.80)			
Psychological distress (PHQ4) n=879			0.272		
None	670/713 (94.0)	1			
Mild	135/145 (93.1)	0.87 (0.42-1.77)			
Moderate	12/12 (100.0)	-			
Severe	7/9 (77.8)	0.22 (0.05-1.11)			

*Adjusted for CD4 count at initiation, PC adherence, baseline viral load, whether on fixed dose combination ART, age, sex, employment status, self-reported health status

Appendix N: Published studies of the prevalence of pre-treatment drug resistance in the African setting

Author	Study setting	Study population/Sample size	Study design	Year of sampling	Outcome	Prevalence/effect estimate
Bussmann, H (296)	Francistown & Gaborone in Botswana	39 HIV positive pregnant women from Francistown and 33 samples from Gaborone	Threshold survey	July to Sept 2007 antenatal HIV surveillance samples	Proportion with TDR	<5% to all drug classes in Francistown. Could not be determined in Gaborone as fewer than 34 samples were obtained
Abar, A (297)	Djibouti	19 ART-naive Health centre attendees	Cross sectional	Oct to Dec 2009	Proportion with PDR	2/19 (10.5%), Only NNRTI
Abegaz, W (298)	Ethiopia	Specimens from 39 HIV positive women attending 7 ANC sites	*Threshold survey	6 April to 8 Aug 2005	Proportion with TDR	None
Bila, D (299)	Maputo and Beira in Mozambique	Pregant women attending ANC-based surveillance sites. 75 protease & 64 reverse transcriptase (RT) sequences in 2007; 114 protease and 123 RT in 2009	*Threshold survey	March to June 2007 & 2009	Proportion with TDR	<5% for all three 3 drug classes in Maputo for 2007 & 2009. No NNRTI mutations in 2007 for Beira, 5-15% threshold for NNRTI in 2009
Afonso, J (300)	Angola	86 ART naïve HIV positive individuals	Repeat cross sectional	Pooled samples from August 2008, July 2009, November 2010	Proportion with TDR	14/86 (16.3%); NRTI:10.5%, NNRTI:14%. PI 0%

Author	Study setting	Study population/Sample size	Study design	Year of sampling	Outcome	Prevalence/effect estimate
Aghokeng, A (300)	Cameroon	369 ART naïve individuals	Cross sectional	1996 to 2007	Proportion with PDR at different time periods using WHO 2009 SDRM list	**Urban** 1996-1999: 0/61(0.0%) 2001: 1/53 (1.9%) 2002: 2/49 (4.1%) 2007: 10/81 (12.3%) **Rural** 2006-2007: 6/125 (4.8%)
Aghokeng, A (301)	Congo, Central African Republic, Chad, Cameroon	Women<25 years, sentinel survey. Congo & Central African Republic-insufficient samples, Chad: 55 samples Yaounde: 58 Douala:57	Threshold survey	End of 2006 and mid-2007	Proportion with TDR	Chad: no resistance mutations after sequencing first 34 samples (<5%) all drug classes. Yaounde: No PI mutations for the first 34 sequences (<5%), but 1 NNRTI and NNRTI. Additional 10 sequences yielded no further mutations for NRTI (<5%) but 1 for NNRTI. Maximum 47 sequences done; NNRTI (5-15%). Douala: PI (<5%), NRTI (5-15%), NNRTI (<5%)

Author	Study setting	Study population/Sample size	Study design	Year of sampling	Outcome	Prevalence/effect estimate
Ajoge,H (302)	North Central Nigeria	28 Pregnant women attending ANC clinics	Cross sectional	August to November 2007	Proporton with TDR	PI: no major mutations NRTI: No major mutations NNRTI: 2/28 (7.1%)
Ayouba, A (303)	Bobo Dioulasso, Burkina Faso Abidjan,Cote d'Ivoire Dakar, Senegal	Mainly pregnant women except in Senegal which included VCT attendees of both sex. Burkina Faso: 51 Cote d'Ivoire: 48 Senegal: 48	Threshold survey, modified to include nonpregnant women with CD4>500	Not specified, paper published 2009	Proportion with TDR	<5% for all three drug classes in all sites
Bartolo, I (304)	Luanda, Angola	138 Drug naïve HIV-positive individuals attending hospital	Cross sectional	Samples from 2009	Proportion with PDR	No Major PI mutations. NNRTI: 1/138 NRTI: No mutations
Bartolo, I (305)	Maputo, Mozambique	104 Drug naïve attending public and private hospitals	Cross sectional	2002-2004	Proportion with PDR	No major PI mutations. NNRTI: 4/104 NRTI: 1/104
Bonney, M (306)	Ghana	47 Pregnant women attending ANC	Threshold survey	Oct 2007 to Feb 2009	Proportion with TDR	<5% for all drug classes
Bruzzone, B (307)	Pointe Noire, Congo	68 ART naïve pregnant women	Cross sectional	Sept 2005 to Dec 2008	Proportion with PDR	PDR 6/68(8.8%); NRTI: 5.9%, NNRTI 4.4%, PI 2.9

Author	Study setting	Study population/Sample size	Study design	Year of sampling	Outcome	Prevalence/effect estimate
Burda, S (308)	SouthWest and North-West Cameroon	21 ART naïve individuals selected at random from ART clinic attendees	Cross sectional	2006 and 2007	Proportion of PDR	5/21 (24%) with PDR NRTI (14.3%) NNRTI (19.0%) PI: Not specified
Bussmann, H (309)	Northern and Southern Botswana	70 HIV-positive women attending ANC and men with STIs randomly selected from 22 health districts	Cross sectional	July to September 2001	Proportion with PDR	No mutations to all three drug classes identified
Castelbranco, E (310)	Luanda, Angola	35 HIV-positive women attending ANC	Cross sectional	Nov 2008 to Jan 2009	Proportion with TDR	2/35 (5.7%) with TDR No PI mutations. NRTI (2.9%) NNRTI (5.7%)
Charpentier, C (311)	Conakry, Guinea and Niamey, Niger	93 ART newly diagnosed HIV positive patients from VCT centre in Guinea; 92 samples from Niger	Cross sectional	September 2009	Proportion with PDR	**Guinea** 8/93 (8.6%) with PDR. NRTI 2/93 (2.1%) NNRTI 7/93 (7.5%) PI 1/93 (1%) **Niger** 6/92 (6.5%) with PDR NRTI 2/92 (2.2%) NNRTI 5/92 (5.4%) PI 1/92 (1.1%)

Author	Study setting	Study population/Sample size	Study design	Year of sampling	Outcome	Prevalence/effect estimate
Derache, A (312)	Bamako and Segou Mali	193 HIV-positive ART naïve individuals recruited consecutively from 2 clinics (186 amplified for reverse transcriptase)	Cross-sectional	May 2006	Proportion with PDR	11.5% with PDR PI 2/193 (1%) NRTI 3/186 (1.6%) NNRTI 18/186 (10.0%)
Derache, A (313)	Bamako, Mali	98 HIV-positive ART naïve recruited from one clinic	Cross sectional	May 2005	Proportion with PDR	2/98 (2%) with PDR PI no mutations NRTI 1/98 (1.0%) NNRTI 1/98 (1.0%)
Eshleman, S (314)	Rakai, Uganda	104 seroconverters	Longitudinal cohort with annual testing	1998-2003	Proportion with TDR	6/104 (5.8%) with PDR PI: 3/104 (2.9%) NRTI: 3/104 (2.9%) NNRTI: None
Fokam, J (315)	Yaounde, Cameroon	49 ART naïve + 4 with ≥ 3 months of ART interruption (Total 53)	Prospective enrollment	January to March 2014	Proportion with PDR	2/53 (3.8%) with PDR. NRTI 1/53 NNRTI 1/53 PI mutation: None
Haidara, A (316)	Mali	101 ART naïve individuals	Prospectively enrollment	July 2007 to October 2008	Proportion with PDR	10/101 (9.9%) with PDR NRTI 5% NNRTI 6% PI 0%

Author	Study setting	Study population/Sample size	Study design	Year of sampling	Outcome	Prevalence/effect estimate
Hamers, R (206)	Kenya, Nigeria, South Africa, Uganda, Zambia and Zimbabwe	Kenya 404, Nigeria 186, South Africa 561, Uganda 570, Zambia 525, Zimbabwe 190	Cross sectional study	March 2007 and September 2009	Proportion with TDR	Kenya 4.7%, Nigeria 1.6%, South Africa 3.6%, Uganda 11.6%, Zambia 5.0%, Zimbabwe 2.6%
Handema, R (317)	Zambia	28 newly diagnosed HIV positive pregnant women attending ANC	Cross-sectional	August 2000	Proportion with TDR	No mutations observed to all drug classes
Hassan, A (318)	Kenya	182 HIV positive individuals entering care	2 cross sectional studies	Between July 2008 and June 2010	Proportion with PDR	2/182 (1.1%) overall; NRTI 1/181 (0.5%) PI 1/181 (0.5%)
Huang, K (319)	Free State, South Africa	390 HIV-positive individuals recruited at the first clinic visit	Cross sectional	February to September 2006	Proportion with TDR	16/390 (4.1%) with TDR
Hunt, G (320)	Gauteng & KwaZulu-Nata, South Africa	196 HIV positive women in Gauteng & 158 in KwaZulu-Natal	Threshold survey	9 surveys conducted between 2005 and 2009	Proportion with TDR	**Gauteng;** 2005: <5% for NRTI & NNRTI, 2006-2009: <5% for all drug classes each year. **KwaZulu-Natal** 2005 & 2008:Insufficient numbers 2007: <5% to all drug classes. 2009:<5% for PI & NRTI,

Author	Study setting	Study population/Sample size	Study design	Year of sampling	Outcome	Prevalence/effect estimate
						5-15% for NNRTI
Huruy, K (321)	Northern and NorthWest Ethiopia	83 HIV positive individuals recruited from health facilities	Cross sectional	2010	Proportion with TDR	6/83 (7.2%); NNRTI 3/83 (3.6%) NRTI 1/83 (1.2%) PI 2/83 (2.4%)
Kamoto, K (322)	Lilongwe, Malawi	54 HIV positive pregnant women attending antenatal clinics	Threshold survey	Full article could not be retrieved, abstract reviewed	Proportion with TDR	No resistant mutations identified (<5% to all drug classes)
Kantor, R (323)	Western Kenya	58 HIV positive ART naïve individuals	Cross sectional	May 2006 to November 2007	Proportion with TDR	4/58 (7%) NRTI 4/58 (7%) NNRTI 2/58 (3.4%) PI: no mutations
Kantor R (324)	Brazil, Hailti, India, Malawi, Peru, USA Malawi, South Africa & Zimbabwe	Malawi: 29 South Africa 29 Zimbabwe: 28 comprising 33% of all participants contributing to estimate of baseline resistance in the subcohort	Randomised trial of time to failure comparing three different regimens	Enrolment from 2005 to 2007 and completion of follow up in May 2010	Proportion with PDR	Malawi: 6.9% (95% CI 0%–16.7%) South Africa: 3.5% (95% 0%–10.5%) Zimbabwe:0%.
					Time to virologic failure	In analysis involving all 9 countries: PDR associated with virologic failure: (aHR, 2.26 [95% CI,1.03–4.95])

Author	Study setting	Study population/Sample size	Study design	Year of sampling	Outcome	Prevalence/effect estimate
Kasang, C (325)	Mwanza, Tanzania	88 HIV positive patients enrolled as part of a clinical trial	Nested cohort	June 2007 to February 2009,	Proportion with PDR	13/88 (14.8%) NRTI 7/88 NNRTI 6/88 PI 5/88
Koizumi, Y (326)	Rural Western Cameroon	54 HIV positive ART naïve attending ANC/STD clinics (30 women, 24 men)	Cross sectional	February 2004	Proportion with PDR	PI 4/54 (7.4%) NRTI 2/51 (3.9%) NNRTI 3/51 (5.9%)
Maiga, A (327)	Bamako and Segou, Mali	51 consecutive patients testing HIV positive at VCT sites	Cross sectional	March 2010	Proportion with TDR	4/51 (7.8%) NRTI 2/51 (3.9%) NNRTI 2/51 (3.9%) PI 1/51 (2.0%)
Manasa, J (328)	Rural KwaZulu-Natal, South Africa	Seroconverters from annual HIV surveillance; 67 in 2010, 381 in 2011, 253 in 2012	Cross sectional	2010 to 2012	Trend in TDR	36/701 (5.1%) Overall TDR NNRTI 32/701 (4.6%) NRTI 10/701 (1.4%) PI: None 2010: No TDR 2011: 18/381 (4.7%) 2012: 18/253 (7.1%)
Maphalala, G (329)	Manzini-Mbabane, Swaziland	61 HIV positive women attending ANC	Threshold survey	July to August 2006	Proportion with TDR	No mutations after first 34 sequences <5% to all drug classes.

Author	Study setting	Study population/Sample size	Study design	Year of sampling	Outcome	Prevalence/effect estimate
Masimba, P (330)	Ifakara Tanzania	HIV positive ART naibe patients enrolled from a clinic 120 in 2005-2007; 119 in 2009	Cross sectional	2005-2007 2009		2005-2007: 8.4% overall NNRT 3.4% NNRTI 7.6% PI: None 2009 (3.3% overall) NRTI 0.8% NNRTI 3.3% PI: None
Mensch, B (331)	Durban, South Africa	352 samples in women who presented for screening in the VOICE trial	Nested within a trial	Aug 2010 and June 2011	Proportion with PDR	26/352 (7.4%)
Mungati, M (332)	Zimbabwe	1483 HIV positive individuals sequentially recruited from 12 ART initiation sites	Cross sectional	2008, 2009 & 2010	Proportion with PDR	93/1483 (6.3%) NNRTI 4.9% NRTI 13.5% PI 0.7%
Nazziwa, J (333)	Masaka, Wakiso,Mukono, Uganda	47 Seroconverters identified from prospective sampling of HIV negative individuals	Cohort/Threshold survey		Prevalence of TDR	5-15% for NNRTI <5% for both PI and NRTI
Ndembi, N (334)	Kampala, Uganda	70 individuals testing HIV positive at VCT sites	Cross sectional	Feb 2009 to Feb 2010 May 2009 to May 2010	Proportion with TDR	6/70 (8.6%) with TDR NNRTI 4.3% NRTI 2.9% PI 1.4%

Author	Study setting	Study population/Sample size	Study design	Year of sampling	Outcome	Prevalence/effect estimate
Ndembi, N (335)	Entebbe, Uganda	46 newly diagnosed pregnant women attending ANC; 13-22 years	Threshold survey	2006 to 2007	Proportion with TDR	<5% for all drug classes
Nwobegahay, J (336)	Limpopo, South Africa	80 HIV positive individuals preparing for ART initiation	Cross sectional	Feb 2008 to December 2008	Proportion with PDR	2/80 (2.5%) with PDR NNRTI 1/80 (1.3%) NRTI 1/80 (1.3%) PI: None
Nyombi, B (337)	Kagera & Kilimanjaro, Tanzania	100 HIV-positive ART naïve pregnant women & 61 post-natal women who received single dose nevirapine	Cross sectional	September and December 2005	Proportion with TDR in naïve	NRTI 3% NNRTI 4% PI: None
					Proporion with resistance in nevirapine exposed women	NRTI 1.6% NNRTI 11.5% PI: None
Onsongo, S (338)	Thika and Nairobi, Kenya	63 HIV positive ART naïve individuals recruited frm health facilities	Cross sectional	Not specified	Proportion with protease inhibitor PDR	No major PI mutations
Onywera, H (339)	Rural Western Kenya	87 HIV positive individuals confirmed to be recently infected using avidity assays	Nested within a cross sectional population survey of HIV incidence	September to Nov 2012	Proportion with TDR	8/87(9.2%) NNRTI 6.9% NRTI 4.6% PI 1.2%

Author	Study setting	Study population/Sample size	Study design	Year of sampling	Outcome	Prevalence/effect estimate
Parboosing, R (340)	Rural KwaZulu-Natal, South Africa	65 HIV positive pregnant women attending ANC who took VCT, stopped after sequencing 47 samples	Threshold survey	February to April 2009	Proportion with TDR;	<5% for all drug classes (1NNRTI mutation detected)
Pillay, V (341)	Gauteng, South Africa	Women attending antenatal clinics; 65 in 2002, 48 in 2004	Threshold survey	Samples from 2002 and 2004 annual ANC HIV seroprevalence survey	Proportion with TDR	<5% for all drug classes for both periods. (No mutations in 2002, & 2 NRTI mutations in 2004)
Rowley, C (342)	Gaborone, Molepolole, Mochudi in Botswana	234 HIV positive pregnant women from ANC and 188 HIV positive individuals from infectious diseases clinic (IDCC)	Cross sectional	April 2012 to December 2015	Proportion with PDR	**ANC** 12/234 (5.1%) ***Gaborone*** 2012: 3/105 (2.9%) 2014-15: 6/62 (9.7%) ***Molepole*** (n=34) no resistance mutations ***Mochudi*** (n=33) 3/33 (9.1%) **IDCC** 4/188 (2.1%) ***Gaborone***: 4/115 (3.5%) ***Molepole*** (n=49) no resistance ***Mochudi*** (n=24) no resistance
Sigaloff, K (343)	Mombasa, Kenya	68 newly diagnosed HIV positive attendees of VCT sites in Kenya	Cross sectional study	May 2009 to March 2010	Proportion with TDR	9/68 (13.2%) NRTI 1.5% NNRTI 7.4%

Author	Study setting	Study population/Sample size	Study design	Year of sampling	Outcome	Prevalence/effect estimate
						PI 4.4%
Somda, A (344)	Ouagadougou, Burkina Faso	52 HIV positive women attending antenatal clinics; first 47 samples sequenced	Threshold survey	June 2008 to July 2009	Proportion with TDR	5-15% for both NRTI and NNRTI. No protease mutations
Somi, G (345)	Dar es Salaam, Tanzania	50 HIV positive pregnant women, attending antenatal clinics, first 39 samples sequenced	Threshold survey	November 2005 to February 2006	Proportion with TDR	No mutations identified after the first 39 sequences <5% for all drug classes
Ssemwanga, D (346)	SouthWestern Uganda	72 seroconverters in a rural clinical cohort	Longitudinal cohort	Feb 2004 to Jan 2010	Proportion with TDR	1/72 (1.4%) NNRTI:1/72 (1.4%) PI: None NRTI: None
Steegen, K (347)	National survey, South Africa	277 HIV positive-individuals attending a health facility for ART initiation	Cross sectional survey	March 2013 and October 2014	Proportion with PDR	25/277 (9%) NNRTI: 23/277 (8.3%) NRTI 7/277 (2.5%) PI 2/277 (0.7%)
Tebit, D (348)	Ouagadougou, Burkina Faso	104 HIV positive ART naïve individuals	Cross sectional	November 2004 to November 2006	Proportion wih PDR	12.5% with PDR NRTI 10.6% NNRTI 6.1% PI: None

Author	Study setting	Study population/Sample size	Study design	Year of sampling	Outcome	Prevalence/effect estimate
Tshabalala, M (349)	Chitungwiza, Zimbabwe	236 HIV positive pregnant women (236 sequences for RT and 175 for PR)	Cross sectional	2006 to 2007	Proportion wih TDR	2/236 (0.8%) NNRTI: 1/236 NRTI: None PI 1/236
Wadonda-Kabondo, N (350)	Lilongwe, Blantyre in Malawi	HIV positive pregnant women attending ANC. Blantyre: 54 Lilongwe: 55	Threshold survey	2006 & Jan 2009 to April 2009	Proportion wih TDR	**Blantyre** 3 individuals with RT (2 with NRTI & 2 with NNRTI mutations in first 47 samples (<5% to all drug classes) **Lilongwe** 3 with NNRTI in first 47 samples (5-15%) <5% for PI and NRTI
Weidle, P (351)	Uganda	11 HIV positive patients who initiated ART	Retrospective analysis of stored samples	Stored samples from 1996 to 1998	Proportion wih TDR	No mutations in RT and PR

Appendix o: Publication in AIDS and Clinical Research, The Art of HIV Elimination, Past and Present Science

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Review Article Open Access

The Art of HIV Elimination: Past and Present Science

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Abstract

Introduction: Remarkable strides have been made in controlling the HIV epidemic, although not enough to achieve epidemic control. More recently, interest in biomedical HIV control approaches has increased, but substantial challenges with the HIV cascade of care hinder successful implementation. We summarise all available HIV prevention methods and make recommendations on how to address current challenges.

Discussion: In the early days of the epidemic, behavioural approaches to control the HIV dominated, and the few available evidence-based interventions demonstrated to reduce HIV transmission were applied independently from one another. More recently, it has become clear that combination prevention strategies targeted to high transmission geographies and people at most risk of infections are required to achieve epidemic control. Biomedical strategies such as male medical circumcision and antiretroviral therapy for treatment in HIV-positive individuals and as pre-exposure prophylaxis in HIV-negative individuals provide immense promise for the future of HIV control. In resource-rich settings, the threat of HIV treatment optimism resulting in increased sexual risk taking has been observed and there are concerns that as ART roll-out matures in resource-poor settings and the benefits of ART become clearly visible, behavioural disinhibition may also become a challenge in those settings. Unfortunately, an efficacious vaccine, a strategy which could potentially halt the HIV epidemic, remains elusive.

Conclusion: Combination HIV prevention offers a logical approach to HIV control, although what and how the available options should be combined is contextual. Therefore, knowledge of the local or national drivers of HIV infection is paramount. Problems with the HIV care continuum remain of concern, hindering progress towards the UNAIDS target of 90-90-90 by 2020. Research is needed on combination interventions that address all the steps of the cascade as the steps are not independent of each other. Until these issues are addressed, HIV elimination may remain an unattainable goal.

Keywords: HIV; Combination HIV prevention; Antiretroviral therapy; Post-exposure prophylaxis; Pre-exposure prophylaxis; HIV vaccines; HIV cascade

Introduction

As the HIV epidemic approaches its fourth decade, effective prevention remains elusive in the communities most affected by the virus. An estimated 36.9 million people were living with HIV globally by end 2014 [1] of whom 70% in sub-Saharan Africa. In 2014, an estimated 1.4 million people acquired HIV infection; 66% of these new infections and 66% of all HIV-related deaths occurred in sub-Saharan Africa, a region disproportionately affected by the epidemic. Remarkable strides have been made recently towards combating the epidemic and increasing antiretroviral therapy (ART) coverage with considerable reduction in mortality and morbidity [2], such that in 2014, 40% of all people living with HIV were receiving ART. Following the results of the START [3] and TEMPRANO [4] trials, the World Health Organisation (WHO) now recommends ART regardless of CD4 count [5], a policy that would maximise both the individual and population health benefit of ART. This aligns with the recent UNAIDS target of 90:90:90 (90% of people living with HIV aware of their HIV status, 90% of people diagnosed HIV-positive on ART, 90% of people on ART virologically suppressed) in 2020 [6], but will require huge financial investments and commitments from governments to bear fruit.

It is now well-recognised that prevention approaches need to be combined to accelerate the effective prevention of HIV acquisition and transmissions [7]; HIV programme planning have now moved from the implementation of single preventive methods to combination context-specific prevention approaches, for which evidence of effectiveness exists.

This paper reviews currently available HIV prevention methods, highlighting the strengths and weaknesses of past prevention approaches, draws attention to the present array of prevention armamentarium available and conceptualises how these could be combined towards the goal of HIV elimination.

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HIV Prevention

Behavioural prevention

Behavioural prevention approaches include: delaying onset of first intercourse, decreasing the number of sexual partners, increasing the number of sexual acts protected, counselling and testing for HIV including repeat HIV testing, encouraging adherence to biomedical HIV prevention strategies, decreasing sharing of needles and syringes and reducing substance use [8].

A 2010 review of behavioural intervention trials, with HIV incidence as main outcome, showed no significant reduction in HIV incidence in any of the nine randomised-controlled trials studied [9]. Project Accept (HPTN 043), is a cluster-randomised trial evaluating whether a multicomponent social and behavioural prevention approach could reduce HIV incidence. In this trial community-based versus facility-based HIV counselling and testing showed no significant reduction in HIV incidence overall (relative risk [RR] 0·86, 95% CI 0·73–1·02) although there was a significant reduction in HIV incidence in he sub-group of women > 24 years of age (RR=0·70, 0·54–0·90) [10].

Structural interventions

HIV-associated structural factors are defined as the physical, social, cultural, organizational, community, economic, legal or policy aspects of the environment that impede or facilitate persons' efforts to avoid HIV infection [11] For example, laws that discriminate against certain HIV risk groups such as men who have sex with men (MSM) or injecting drug users may stigmatise and hinder access to HIV prevention services. Similarly, cultural norms which perpetuate gender inequity may leave women economically dependent on men and unable to negotiate condom use for fear of abandonment [12]. Interventions addressing these factors tend to be complex and context-specific; they do not seek to address risky behaviours directly, but address the prevailing circumstances which give rise to risky behaviours, acting on factors distal to the HIV outcome of interest. Distal factors may impact the outcome through multiple causal pathways making them difficult to evaluate; replication in other environments is challenging. One of the structural interventions receiving attention recently is the use of social cash transfers to encourage safer sex and a reduction in HIV acquisition. A randomised control trial in Lesotho using a lottery scheme as an incentive to reduce risky sexual behaviour showed a 25% (OR 0.75, 95% CI 0.58 – 0.97) reduction in HIV incidence over 2 years [13]. A cluster randomised trial (CRT) in Malawi showed that cash incentives to young women and their households reduced HIV prevalence by 64% at 18 months; but making the payments conditional on school attendance made no difference to the reduction in HIV infection observed [14]. However a recently concluded CRT in South Africa showed that a conditional cash transfer to young women and men tied to HIV testing, participation in life skills training and academic attainment reduced the incidence of HSV-2 by 30% but did not have an impact on HIV incidence after 24 months [15]. Similarly, another recently concluded randomised trial in South Africa found that cash transfer which is conditional on 80% school attendance by young women showed no reduction in HIV incidence after 3 years [16]. These results suggest that the effectiveness of social cash transfer could be context specific.

Treatment of sexually transmitted infections (STIs)

Substantial evidence exists from observational studies suggesting an increased risk of HIV acquisition with both curable STIs and genital herpes [17,18]. STIs have also been associated with increased HIV infectiousness, although this has not been quantified directly in observational studies [19]. HIV-STI co-infection appears more likely to result in HIV transmission than infection with HIV alone [20,21]

However, nine randomised trials to date (four cluster randomised trials, two individual randomised trials on treating curable STIs and three individual randomised trials on Herpes suppressive therapy) have together failed to confirm the hypothesis that STI treatment would reduce HIV transmission and acquisition [19]. Of the four cluster-randomised trials examining the impact of STI treatment on HIV incidence, only the Mwanza trial in Tanzania showed syndromic treatment of STIs to be associated with a 40% significant reduction in HIV incidence [22]. Various factors may explain the differences in effect between trials, including differences in the HIV epidemic phase, enhanced interventions in the control group, and higher prevalence of STIs in the Mwanza trial compared to the other sites [19].

Syndromic treatment of STIs focusses on patients presenting with symptoms, but provision of inadequate treatment and poor adherence could result in low effectiveness of syndromic treatment, which was estimated to be only 13% for curable STIs in rural KwaZulu-Natal [23]. Further, a significant proportion of STIs are asymptomatic [24] and the large pool of untreated individuals with asymptomatic STIs will continue to transmit HIV. This situation coupled with poor uptake of partner notification could result in significant rates of STI reinfections and will likely impact HIV transmission and acquisition.

The effect of herpes simplex virus (HSV) suppressive therapy on HIV incidence has been evaluated in two randomised trials; the first one in high-risk HSV-2 positive, HIV negative women in Tanzania [25] and the second involving women from three sites in Africa (Harare, Lusaka, Johannesburg) and MSM from Peru and the USA [26]. In these trials, treating HIV negative, HSV-2 positive individuals with aciclovir did not result in decreased HIV acquisition. A third randomised trial investigated the impact of HSV-2 suppressive therapy in HIV positive individuals on the risk of HIV transmission. Although suppressive therapy with acyclovir reduced HIV plasma viral load by about 0.25 $\log^{10}$ and genital ulcers due to HSV-2 by 73%, there was no significant effect on HIV transmission (RR 0.92, 95% CI 0.60-1.41).

Although, these results are disappointing there remains compelling biological and epidemiological evidence that STIs are co-factors for HIV acquisition and transmission [27] and treatment of STIs should be part of the HIV care and prevention programme.

Male circumcision

A meta-analysis of 27 published observational studies on male circumcision in sub-Saharan Africa [28] provided evidence that male circumcision protects against HIV acquisition.

Male circumcision was shown to be protective against HIV acquisition in three randomised controlled trials in South Africa, Uganda and Kenya [29-31]; in pooled analysis the combined incidence risk ratio (IRR) at 12 months was 0.50 (95% CI 0.34-0.72) and 0.46 (95% CI 0.34-0.62) at 21 or 24 months [32].

These observations in heterosexual HIV acquisition raised the question of whether this protection would also be observed in MSM. However, an observational analysis of data from a randomised controlled trial of HSV-2 suppressive therapy to prevent HIV acquisition found no evidence that circumcision was associated with reduced HIV incidence in MSM who practised predominantly insertive sex (RR 0.31, 95% CI: 0.06-1.51) [33]

Non-ART vaginal microbicides

Initial research involving microbicides focused on non-ART related compounds, with a recent shift to ART-related compounds following multiple failures of the former to demonstrate effectiveness in the prevention of HIV acquisition in women.

These earlier compounds were surfactants (nonoxynol-9) which disrupt the cell membranes of bacteria and viruses, polyanions (Carraguard, cellulose sulphate and PRO 2000) which interfere with the attachment of the virus to target cells in the mucosa and vaginal milieu, and protectors (BufferGel) which render the vagina acidic. In an acidic environment, sperms and viruses are inactivated or killed [34]. A recent meta-analysis of 13 randomised controlled trials involving 35,905 HIV negative women from Africa, India, Thailand and the United States of America between 1996 -2011 showed no protective effects on HIV acquisition (RR 0.97, 95% CI: 0.87-1.08) [35]. This meta-analysis included mostly non-ART related microbicides; five trials of nonoxynol-9, two trials of SAVVY, two of cellulose sulphate, one of Carraguard, one of PRO 2000 and one of BufferGel and one ART-related microbicide (CAPRISA 004 with 1% vaginal tenofovir gel). More adverse events due to genital lesions were reported in the nonoxynol-9 trials while these events were similar in both the microbicide and placebo arms of the other trials.

Antiretroviral treatment

The efficacy of antiretroviral therapy at preventing HIV transmission has been demonstrated in a variety of clinical scenarios such as in the prevention of mother-to-child [36,37], and heterosexual transmission [38], which led to the declaration that an HIV infected individual who is on ART and has undetectable viral loads for at least 6 months with no STIs is sexually non-infectious [39]. Other uses include post-exposure prophylaxis in HIV-negative individuals after occupational or sexual exposure to body fluids from known or suspected HIV-positive individuals [40-43].

Oral and topical ART-based pre-exposure prophylaxis: More recently, studies have shown that ART could also be used by HIV-negative individuals prior to exposure to HIV to prevent HIV acquisition, known as pre-exposure prophylaxis (PrEP).

Table 1 summarises the 11 trials on pre-exposure prophylaxis using

Author	Study setting	Sample size contributing data	Study Population	Intervention/Control	Follow-up time/Person years	HIV seroconversions	Impact on HIV incidence (95% CI)
Peterson, L 2007[44]	Ghana, Cameroun, Nigeria	936	18-35 year old high risk HIV negative women	Intervention: Oral daily tenofovir (TDF) Control: Placebo	476	Intervention: 2 Control: 6	Rate ratio (RR) 0.35 (0.03-1.93)
Abdool Karim, Q 2010[45] (CAPRISA 004)	South Africa	889	18-40 year old HIV-negative women	Intervention: coitally administered 1% vaginal gel formulation of TDF Control: Placebo	1341	Intervention:38 Control:60	RR 0.61(0.40-0.94)
Grant RM, 2010[51] (iPrEX study)	Peru, Ecuador, South Africa, Brazil, Thailand, USA	2499	>18 years, HIV negative MSM or transgender	Intervention: Oral daily tenofovir/emtricitabine (TDF-FTC) Control: Placebo	3324	Intervention: 36 Control: 64	44% reduction (15-63)
Thigpen MC, 2012[52] (TDF2 Study)	Botswana	1219	18-39 years, HIV negative men and women	Intervention: Oral daily TDF-FTC Control: Placebo	1563	Intervention:9 Control:24	62.2% reduction (21.5-83.4)
Baeten J.M, 2012[53] Partners PrEP Study	Kenya, Uganda	4747	Heterosexual HIV serodiscordant couples	Interventions: i) Once daily oral TDF ii) Once daily TDF-FTC Control: Placebo	7830	Interventions: TDF 17 TDF/FTC: 13 Control: 52	67% reduction due to TDF (44-81) 75% reduction due to TDF/FTC (55-87)
Van Damme L, 2012[46] FEM-PrEP Study	Kenya, South Africa, Tanzania	2056	18-35 years, HIV negative women	Intervention: Oral daily TDF-FTC Control: Placebo	1407	Interventions: 33 Control: 35	Hazard ratio (HR) 0.94 (0.59-1.52)
Marrazzo J, 2013[54] VOICE Study	South Africa, Zimbabwe, Uganda	5029	Mean age 25.3 years, HIV negative women	Intervention: i) Oral daily TDF ii)Oral daily TDF/FTC iii) 1% TDF vaginal gel Control: i) Oral placebo ii) Placebo vaginal gel	5509	Interventions: i) oral TDF 52 ii) oral TDF-FTC 61 iii) Vaginal TDF gel: 61 Control i) Placebo for oral TDF: 35 ii) Placebo for oral TDF/FTC: 60 iii) Placebo for vaginal gel: 70	HR for Oral TDF 1.49 (0.97-2.3) HR for oral TDF/FTC 1.04 (0.7-1.5) HR for vaginal TDF gel 0.85 (0.6-1.2)
Choopanya K, 2013 [55] Bangkok Tenofovir study	Bangkok, Thailand	2413	20-60 years, HIV negative and reported injecting drug use within the past year	Intervention: Oral tenofovir Control: Placebo	9665	Intervention: 17 Placebo: 33	Efficacy of tenofovir 48.9% (9.6-72.2)
Rees H, 2015 FACTS 001	South Africa	2029	HIV negative women, 18-30 years	Intervention: Pericoital 1% vaginal gel formulation of Tenofovir Control: Placebo	3036	Intervention: 61 Control: 62	IRR 1.0 (0.7-1.4)
McCormack S, 2015 PROUD	England	544	HIV negative MSM, ≥18 years	Immediate: oral daily TDF/FTC Deferred: Oral daily TDF/FTC after 12 months	465	Immediate: 3 Deferred: 20	86% reduction (64-96)
Molina J-M, 2015 IPERGAY	France, Canada	400	HIV negative adult MSM	Intervention: On demand TDF/FTC Control: Placebo		Intervention: 2 Control: 14	86% reduction (39.4-98.5)

Table 1: Oral and ART-based topical pre-exposure prophylaxis.

ART completed to date. The first trial evaluating the effectiveness of once daily oral tenofovir for pre-exposure prophylaxis was conducted in three sites in Ghana, Cameroun and Nigeria among high risk HIV-negative women aged 18-35 years [44]. The Nigeria and Cameroun sites were closed prematurely for unspecified reasons and as a result this trial lacked statistical power because of the small number of HIV seroconversions observed. In the CAPRISA 004, a proof-of-concept phase II trial including 889 HIV negative women, 1% tenofovir gel compared to placebo was shown to significantly decrease HIV acquisition, (RR 0.63, 95% CI 0.43-0.93) [45]. However, the results of three other PrEP trials, FEM-PrEP [46] , VOICE [47] and FACTS 001 [48] conducted in women have been very disappointing with none of them demonstrating any efficacy. Substudies of adherence within these large trials showed that there was poor adherence to the study drug which could explain the lack of efficacy observed.

The placebo arm of two other PreP trials – IPERGAY [49] and PROUD [50] - were terminated early because of marked reduction in HIV acquisition in the intervention arm compared to the placebo arm

ART in HIV-discordant partnerships: Table 2 summarises the nine observational studies and one randomised-controlled trial evaluating the effectiveness of ART in preventing HIV transmission from the index to the HIV-uninfected partner. A Cochrane review and meta-analysis [56] of these observational studies identified 2112 HIV transmissions: 1,016 among ART-treated couple and 1096 in those not taking ART. The combined rate ratio for the nine observational studies was 0.58 (95% CI: 0.17-0.75).

The one trial was a multicentre randomised-controlled trial (HPTN 052) [38] involving 1763 stable serodiscordant couples from 9 countries (Table 2) which reported findings in 2011. HIV infected individuals with CD4 counts between 350-550 cells/μL and in a stable relationship with an uninfected partner were randomly allocated to receive ART immediately (early therapy) or delayed until CD4 count decreased below 250 cells/mm^3 or development of clinical symptoms (deferred therapy). This study was stopped early because of clear efficacy of ART in preventing transmission in the early therapy arm. There were 39 HIV transmissions in total of which 28 were virologically linked to the infected partner; of the linked transmissions, 27 occurred in the deferred and one in the early therapy group (HR 0.04, 95% CI: 0.01-0.27). Besides the clear public health significance of this finding, there was also a clinical benefit to the individual if randomised to the early therapy arm.

An earlier meta-analysis [57] reviewed observational studies of HIV transmission involving individuals on and not on ART from 11 cohorts comprising 5021 heterosexual couples and 461 HIV

Author	Study setting	No of couples	Study population	Study design/ intervention	Follow-up duration in person years	ART status of index case &sero-conversions (n)	Effect estimate (95% CI)
Musicco, M 1994[62]	Italy	436	Female sexual partners of HIV-infected males majority of whom were injecting drug users	Observational/ Zidovudine (ZDV) monotherapy	740	Partners of men not on ZDV:21 Partners of men on ZDV: 6	Risk lower in partners of treated Men RR 0.50 (0.1-0.9)
Melo MG, 2008[63]	Brazil	93	Female index case: 67 Male index case: 26	Observational/ 41 on triple ART 52 not on ART	Not stated	Partner on ART: 0 Partner not on ART : 6	Risk lower if partner on ART RR 0.10 (0.01-1.67)
Sullivan P, 2009[64]	Rwanda, Zambia	2993	HIV discordant couples	Observational	5609	Partner on ART: 4 Partner not on ART: 171	Risk lower if partner on ART RR 0.21 (0.08-0.59)
Del Romero, J 2010[65]	Spain	424	Stable sexual couples	Observational *144 couples with index partner on triple ART *47 couples on mono/dual ART *341 couples with index partner not on ART	1355	Partner on ART: 0 Partner not on ART: 5	Risk lower if partner on ART RR 0.21 (0.01-3.75)
Donnell D, 2010[66]	Botswana, Kenya, Rwanda, South Africa, Tanzania, Uganda, Zambia	3381	HIV serodiscordant partners	Prospective cohort 349 initiated ART 3082 did not initiate ART	4831	Partner on ART: 1 Partner not on ART: 102	Risk lower if partner on ART 0.08 (0.00-0.57
Lu W, 2010[67]	China	1927	HIV serodiscordant couples Male index 1092 Female index 835	Prospective cohort 1369 on ART 558 not on ART	4918	Partner on ART: 66 Partner not on ART: 18	RR 1.44 (0.85-2.44)
Reynolds SJ, 2011[68]	Uganda	250	HIV discordant couples Male index: 145 Female index: 155	Prospective cohort 32 initiated ART 218 not on ART	513	Partner on ART: 0 Partner not on ART: 42	RR 0.10 (0.01-1.64)
Cohen MS, 2011[38]	Botswana, Kenya, Malawi, South Africa, Zimbabwe, Brazil, India, Thailand, USA	1763	Stable HIV-discordant 97% heterosexual	Randomised controlled Immediate versus deferred ART	3152	Early therapy: 1 Deferred therapy: 27	RR 0.04 (0.01-0.27)
Birungi J, 2012 [69]	Uganda	586	Serodiscordant couples	348 ART-eligible couples initiated 238 not eligible for ART	Median follow up of 1.3 years	ART group : 9 Non-ART group: 8	RR 0.91 (0.38-2.20)
Jia Z, 2012 [70]	China	38, 862	Serodiscordant couples	24057 ART-treated 14,805 non-ART group	101,295	ART-group: 935 ART-naïve: 696	RR 0.74 (0.65-0.84)

Table 2: ART for preventing HIV transmission in HIV discordant partnerships.

transmission events. The HIV transmission risk in the five studies of individuals on ART, irrespective of viral load, was 0.46 (95% CI: 0.19-1.09) based on five transmissions and 1098 person years of follow up. When this meta-analysis was restricted to the two studies in which individuals had undetectable viral load, no transmission was recorded in 291 person years of follow up with an upper confidence limit of 1.27 per 100 person years.It is now established that ART is effective at preventing transmission in stable heterosexual couples, it remains unknown whether ART will be similarly effective at preventing HIV transmission at the population level. An observational study from rural KwaZulu-Natal suggests this to be the case [58]; and this question is currently being addressed by four randomised trials [59-61].

HIV vaccines

Recent HIV vaccine research has focused on antibody-based strategies following isolation of potent highly broadly neutralising monoclonal antibodies from infected individuals [71]. However, both arms of the adaptive immune system have important roles to play against HIV infection and or disease [71,72]. Neutralising antibody response aim to prevent acquisition of HIV infection, while cytotoxic T lymphocytes (CTL) response, which only recognises infected host cells, could play a role in controlling viral replication and disease progression. It is unclear if robust CTL response can eradicate HIV infection in humans [71].

Only one of the six HIV vaccine trials completed to date showed a protective efficacy (Table 3).

The VAX004 (North America and the Netherlands) and VAX003 (Thailand) were protein subunit trials using rgp120 monomers as immunogens aiming to elicit neutralising antibodies. Both failed to show significant protection against HIV acquisition [73,74].

Another vaccine approach is based on recombinant viral vectors engineered to express the gene of interest. The recombinant adenovirus serotype 5 was used as the vector for the Step (North and South America, the Caribbean and Australia) and Phambili (South Africa) trials [75,76]. These trials assessed the ability of these vaccines to stimulate the cellular immune responses. The Step trial was terminated early on the grounds of futility and lack of control of early viraemia in those who became infected. Enrolment in the Phambili trial was stopped because of the results observed in the Step trial.

The HVTN 505 (USA), was a phase 11b DNA vaccine trial that evaluated a DNA prime expressing Gag, Pol, Nef and Env with a recombinant adenovirus serotype 5 boost expressing Gag, Pol and Env. This trial was also halted prematurely for futility [77].

The RV144 vaccine trial in Thailand employed a combination of vaccine approaches [78], comprising a canary pox viral vector prime expressing Env, Gag and Pol followed by a protein subunit vaccine boost (AIDSVAX B/E). The vaccine efficacy was 31% (95% CI, 1.1 to 52.1) after 3.5 years. To date, this remains the only vaccine trial to demonstrate some protection against HIV acquisition.

Mathematical modelling

Mathematical modelling has played a pivotal role in the understanding of HIV pathogenesis by elucidating virus kinetics in terms of virus production and clearance from blood and CD4 T-lymphocytes depletion [80,81]. This showed that HIV replicated at a very rapid rate and demonstrated the superiority of combination therapy over single drug therapy on virus kinetics. This early models also examined the role of long-lived and latently infected cell populations in the blood and the question as to whether combination therapy would be adequate to eradicate or cure HIV in an individual arose as a hypothesis. Later models have identified third and fourth phase decays in HIV kinetics through the use of single copy assays [82]. This discovery as opposed to the initial two-phase decay proposed in earlier models implies that combination therapy may not be sufficient to eliminate HIV within an individual.

Models have also played significant roles in generating important hypothesis about the impact of immediate ART on HIV elimination from the general population. The model by Granich et al. [83] generated a lot of interest in this regard. This model predicted that yearly HIV testing followed by immediate ART coupled with male circumcision, behaviour change programmes, condoms and treatment of STIs could reduce HIV incidence to less than one case per 1000 per year within 10 years and reduce the prevalence of HIV to less than 1% within 50 years. However the assumptions used to parameterise the model may be overly optimistic as the impact of such approaches have been shown by more recent models to be sensitive to factors such as uptake of HIV testing, linkage to care and ART coverage and the nature of the sexual networks [84-86]. There are challenges in achieving the sort of coverage required as illustrated by the leaks in the HIV care cascade described below.

Mathematical models, in combination with empirical research would play pivotal role in understanding interventions and their expected impact on HIV prevention and elimination.

Barriers to HIV Elimination

HIV care cascade

For ART to succeed as an effective HIV prevention method, there

Author	Vaccine trial (randomised-placebo controlled	Vaccine type	Sample size	Population	Phase	Intended immune response	Results
Flynn et al; 2005 [73]	VAX004	Protein: rgp120	5400	Mostly high-risk MSM	III	Antibodies, CD4+ T cells	6% (-17 to 24)
Pitisuttithum et al; 2006 [74]	VAX003	Protein: rgp120	2500	Injection drug users	III	Antibodies, CD4+ T cells	0.1% (-30.8 to 23.8)
Rerks-Ngarm et al; 2009 [78]	RV144	Pox/protein: ALVAC/ grp120	16,403	Low risk heterosexuals	III	Antibodies, CD4+ & CD8 T cells	31% (1.1-52.1)
Buchbinder, SP et al; 2008 [75]	HVTN 502/Merck 023 (STEP)	Adenovirus type 5 (Ad5) gag/pol/nef	3000	High risk MSM, heterosexual men and women	IIb	CD8+ & CD4+ T cells	HR 1.2 (0.6-2.2)
Gray et al; 2011b [76]	HVTN 503 (Phambili)	Ad5 gag/pol/nef	801; original target of 3000	Heterosexual men and women	IIb	CD8+ & CD4+ T cells	HR 1·25 (0·76-2·05)
Hammer, S 2013 [79]	HVTN 505	DNA-Ad5 gag/pol/ nef/env	2504	High risk MSM	IIb	Antibodies, CD4+ & CD8+ T cells	-25% (-121.2 to 29.3)

Table 3: Summary of HIV vaccine trials and outcomes.

needs to be good coverage in all the steps of the HIV care pathway. The entry point into this pathway is HIV testing. Those testing HIV-positive need to be willing to initiate ART even when not clinically indicated for their own health, retained in care and be adherent lifelong. Equally important are those who tested negative. They should be aware of methods to protect themselves from HIV acquisition and be willing to test for HIV repeatedly for those who become HIV-positive to be linked to the care pathway.

HIV testing and linkage to care

HIV testing is necessary for linkage to HIV care and treatment. For HIV elimination, large numbers of individuals have to be willing to test for HIV regularly and those testing positive need to be linked to care and started on ART. However, despite the availability of effective treatment for HIV, 36% of individuals in SSA have never been tested for HIV [2], with low perception of risk, concerns about confidentiality and fear of disclosure, stigma and discrimination suggested as explanations. Gender inequity that leaves women economically dependent on men may undermine the ability of women to seek HIV testing [87-89].

Further, studies have shown a huge drop between the numbers of people taking an HIV test and linked to care. A systematic review and meta-analysis of eleven studies in SSA estimated that only 57% (95% CI, 48-66) of those diagnosed HIV positive are linked to care [90]. Another meta-analysis of studies in the USA estimated that 69% (95% CI, 66-71) of individuals diagnosed with HIV entered into care averaged over the time intervals from 1995 to 2009 [91]. Substantially higher numbers of individuals need to be linked to care for treatment assessment to realise the goal of HIV elimination. In a review of studies examining the barriers to linkage, the most commonly identified factors include transport costs and distance to clinics. Others include concerns about disclosure and stigma, staff shortages, long clinic waiting times, male sex and younger age [92].

Adherence/Retention in Care

The WHO defines adherence as "the extent to which a person's behaviour-taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider" [93]. Adherence to ART is vital for viral suppression [94], which is important for optimal treatment outcomes and for prevention of HIV transmission [95]. Studies reporting on routine treatment programmes with differing ART initiation CD4 thresholds have shown that individuals starting treatment at higher CD4 counts are less likely to adhere consistently than individuals starting at lower CD4 counts [96,97]. However in the HPTN 052 on stable sero-discordant couples, adherence measured by pill count of at least 95% was seen in 79% of participants in early therapy group (CD4 350-550 cells/mm^3) compared to 74% in the delayed therapy group (CD4<250 cells/mm^3) [38]. This may not be reflective of real life situations. It remains to be seen how evidence from the START [3] and TEMPRANO [4] trials which suggest individual benefit to early ART would influence adherence.

In the pre-exposure prophylaxis studies in which participants were aware that they were using the prescribed medications to prevent HIV transmission, adherence measured by drug levels was poor [46,54]. A meta-analysis involving 37 qualitative and 47 quantitative studies on barriers and facilitators to adherence identified fear of disclosure, concomitant substance abuse, forgetfulness, suspicions of treatment, complex regimens, high pill burden, decreased quality of life, work and family responsibilities, falling asleep and access to medications as the main adherence barriers [98]. Retention in HIV care takes two forms: pre-ART retention refers to retention in care of individuals not yet eligible for ART while retention on ART refers to individuals who remain in care after initiating ART. A review of four studies in South Africa and one in Malawi estimated that the median proportion of patients retained in pre-ART care was 45% when CD4 eligibility threshold was 200 cells/mm^3 [90]. A review of 14 studies reporting proportions of patients retained in care from ART eligibility to ART initiation estimates a median of 68% (range 14-84%) [99]. For retention in care after initiating ART, a systematic review of 33 studies reporting on 39 patient cohorts estimated that 65% of patients are retained in ART care (range 58%-72%) after 36 months [100]. Factors that impact retention in care include challenges that relate to housing, transportation to clinics, mental health and drug abuse which would need to be addressed in affected individuals. Provider-patient relationship and clinic opening hours are other issues that need to be addressed to improve retention in care [91].

Virological failure and drug resistance

Initiation of treatment early in the course of infection before symptoms develop results in large proportions of HIV-infected individuals on ART; if adherence is indeed sub-optimal, this could lead to significant rates of virological failure and the likely development of drug resistance. Studies in sub-Saharan Africa of people who started treatment on the basis of conservative guidelines showed that 15-25% of patients had HIV-RNA >400 copies/mL 6-36 months after starting ART [101], consistent with findings from the Hlabisa HIV Treatment and Care programme with an estimated 15% of patients having HIV RNA >400 copies/mL 12 months after starting ART [102]. A further study from this latter cohort showed that 86% of individuals failing first-line ART with detectable VL had at least one drug-resistant mutation [103] with high levels of NNRTI- (83%) and NRTI-(81%) associated mutations; the median time spent on a failing regimen was 27 months (IQR 17-41). The long duration spent on failing ART with accumulation of resistant mutations could be a possible explanation for the 15% of patients with virological failurewhose second-line regimen was compromised.

With increasing exposure of larger numbers of people to longer durations of ART, those developing ART resistance could potentially transmit resistant virus to their sexual partners, which would result in increasing numbers of new infections due to resistant virus [104,105]. A recent evaluation of transmitted resistance in 11 regions in six sub-Saharan African countries including South Africa estimated prevalence of transmitted drug resistance in South Africa of 1.1%, but 12.3% in Kampala, Uganda [106]. Increasing prevalence of transmitted resistance would necessitate more complex and more expensive first-line regimen which could impact on adherence and result in lack of virological suppression and increased transmissions making HIV elimination difficult.

Risk compensation

HIV has become a chronic condition, and some individuals may be less concerned about HIV than thirty years ago [107], which, coupled with the knowledge that ART may prevent HIV transmission, could lead to increased high risk sexual behaviour, known as risk compensation. However, studies in resource-limited settings with high HIV prevalence have not shown an increase in risky sexual behaviour amongst individuals initiating ART. In a Ugandan study , an increase in sexual activity following ART was accompanied by a 70% reduction in the number of unprotected sexual acts with a partner known to be HIV negative or of unknown serostatus [108]. In a longitudinal study in

South Africa on HIV-infected individuals with pre-ART and post-ART follow-up over seven years, high risk sexual behaviour following ART initiation was reduced [109]. A recent ecological study from a rural South African surveillance site found no evidence of an increase in high risk sexual behaviour at the population level following the expansion of ART availability, instead there was an increase in reported condom use at last sex with regular partners [110].

However, many studies in MSM in the developed world have shown an increase in high risk sexual behaviour following the introduction of ART coinciding with an increase in HIV incidence [111,112]. Whilst the frequency of HIV testing increased during this period, this was not sufficient to account for the observed increase in the number of new diagnoses [112].

As ART roll-out in sub-Saharan Africa is relatively recent, it is important to maintain on-going surveillance in risk behaviour in this region as this may change as more people become aware of the benefit of ART to prevent transmission.

Conclusion

Remarkable strides have been made in the past decade in potentially curbing the HIV epidemic, although numbers of new infections remain unacceptably high. No HIV prevention approach is 100% efficacious; all require behaviour change as individuals need to have the agency to decide which of the prevention methods best meet their needs at any particular point in time. The optimal way to tackle the epidemic is likely to be through combination HIV prevention [113,114], which combines behavioural change, treatment of STIs, ART for HIV positives and for pre-exposure prophylaxis for HIV negatives, male medical circumcision and structural approaches (Figure 1). It is now recognised that even within generalised epidemics, there are many microepidemics, hence interventions need to be focussed in nature by targeting areas of high transmission geographies and people at most risk of infections including key populations [115]

Which and how these interventions are combined may vary by setting using the "know your epidemic, know your response" concept [116]. A modelling study calibrated using the Kenyan HIV epidemic showed that combination of interventions which are deployed in a focused manner as opposed to a uniform manner with a fixed budget applied to both scenarios resulted in more substantial decrease in the incidence of HIV infections [117]. This focused intervention approach requires that the HIV epidemic in a particular setting is characterised to subnational level.The partner demonstration project, which included high risk serodiscordant couples in Kenya and Uganda combined ART given to the HIV positive partner with PrEP given to the HIV negative partner resulted in a 96% reduction in HIV transmission from the HIV positive to the HIV-negative partner.

Research is needed into how the cascade of care can be strengthened from the point of HIV testing to linkage of individuals to care and virological suppression. This would be necessary steps to maximise the impact the new WHO guidelines which recommend ART regardless of CD4 count [5].

Factors which act as barriers and facilitators for each step of the cascade need to be understood both at the individual and health care system level so that appropriate interventions can be put in place.

Novel drug formulations that require infrequent administration would be a welcome addition to the HIV prevention armamentarium, as this strategy has the potential to increase adherence [118].

Although the HIV vaccine field has been disappointing with no sufficiently efficacious vaccine currently available, lessons have been learnt from the research with improved insight as to how HIV evades the immune system. This is not the time to relax, rather to intensify efforts in this area because an efficacious preventive vaccine would be required in addition to other biomedical intervention in order to make HIV elimination an attainable goal. The vaccine efficacy required to achieve this would need to be modelled in combination with other prevention approaches [119]. Substantial investments with smart health-financing, integrating of health services and political commitment would be required to achieve the goal of HIV elimination [119].

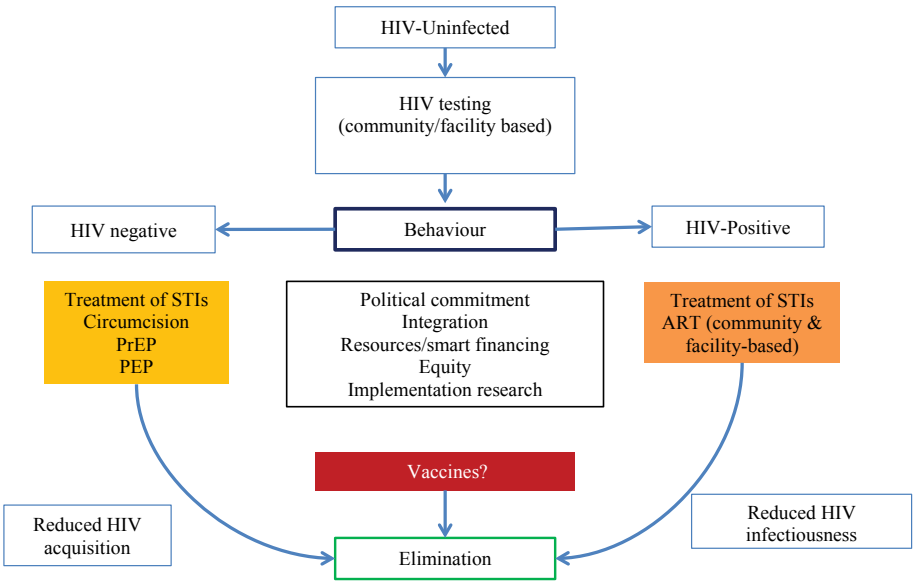

Figure 1: Combination interventions required to eliminate HIV.

Competing interests

CI has received honoraria for services rendered to Gilead Sciences. All other authors declare no competing interest.

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Authors contributions

CI did the literature search and wrote the first draft of the review. NM, TDO, KP, DP,MF, MN and MLN extensively reviewed the article and made substantial contributions that improved the overall quality of the work. All the authors read and approved the final version of the manuscript.

References

1. UNAIDS (2015) How AIDS changed everything. MDG 6: 15 years, 15 lessons of hope from the AIDS response.
2. UNAIDS (2013) UNAIDS report on the global AIDS epidemic 2013.
3. INSIGHT START Study Group, Lundgren JD, Babiker AG, Gordin F, Emery S, et al. (2015) Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection. N Engl J Med 373: 795-807.
4. TEMPRANO ANRS 12136 Study Group, Danel C, Moh R, Gabillard D, Badje A, et al. (2015) A Trial of Early Antiretrovirals and Isoniazid Preventive Therapy in Africa. N Engl J Med 373: 808-822.
5. WHO (2015) Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis to ART.
6. UNAIDS (2014) 90-90-90 An ambitious treatment target to help end the AIDS epidemic.
7. Merson MH, O'Malley J, Serwadda D, Apisuk C (2008) The history and challenge of HIV prevention. Lancet 372: 475-488.
8. Coates TJ, Richter L, Caceres C (2008) Behavioural strategies to reduce HIV transmission: how to make them work better. Lancet 372: 669-684.
9. Ross DA (2010) Behavioural interventions to reduce HIV risk: what works? AIDS 24 Suppl 4: S4-14.
10. Coates TJ, Kulich M, Celentano DD, Zelaya CE, Chariyalertsak S, et al. (2014) Effect of community-based voluntary counselling and testing on HIV incidence and social and behavioural outcomes (NIMH Project Accept; HPTN 043): a cluster-randomised trial. Lancet Glob Health 2: e267-277.
11. Sumartojo E (2000) Structural factors in HIV prevention: concepts, examples, and implications for research. AIDS 14 Suppl 1: S3-10.
12. Gupta GR, Parkhurst JO, Ogden JA, Aggleton P, Mahal A (2008) Structural approaches to HIV prevention. Lancet 372: 764-775.
13. Björkman-Nyqvist M, Corna L, D de Walque, J Svensson (2013) Evaluating the Impact of Short Term Financial Incentives on HIV and STI Incidence Among Youth in Lesotho: A Randomised Trial. Sex Transm Infect 89: A325.
14. Baird SJ, Garfein RS, McIntosh CT, Ozler B (2012) Effect of a cash transfer programme for schooling on prevalence of HIV and herpes simplex type 2 in Malawi: a cluster randomised trial. Lancet 379: 1320-1329.
15. Abdool Karim Q, Leask K, Kharsany A, Humphries H, Ntombela F, et al. (2015) Impact of conditional cash incentives on HSV-2 and HIV prevention in rural South African high school students: results of the CAPRISA 007 cluster randomized controlled trial; abstract TUAC0101LB. in IAS. 2015. Vancouver.
16. Pettifor A (2015) HPTN 068 conditional cash transfer to prevent HIV infection among young women in South Africa: results of a randomized controlled trial. in IAS 2015. Vancouver.
17. Sexton J, Garnett G, Røttingen JA (2005) Metaanalysis and metaregression in interpreting study variability in the impact of sexually transmitted diseases on susceptibility to HIV infection. Sex Transm Dis 32: 351-357.
18. Glynn JR, Biraro S, Weiss HA (2009) Herpes simplex virus type 2: a key role in HIV incidence. AIDS 23: 1595-1598.
19. Hayes R, Watson-Jones D, Celum C, van de Wijgert J, Wasserheit J (2010) Treatment of sexually transmitted infections for HIV prevention: end of the road or new beginning? AIDS 24 Suppl 4: S15-26.
20. Deschamps MM, Pape JW, Hafner A, Johnson WD Jr (1996) Heterosexual transmission of HIV in Haiti. Ann Intern Med 125: 324-330.
21. Gray RH, Wawer MJ, Sewankambo NK, Serwadda D, Li C, et al. (1999) Relative risks and population attributable fraction of incident HIV associated with symptoms of sexually transmitted diseases and treatable symptomatic sexually transmitted diseases in Rakai District, Uganda. Rakai Project Team. AIDS 13: 2113-2123.
22. Grosskurth H, Mosha F, Todd J, Mwijarubi E, Klokke A, et al. (1995) Impact of improved treatment of sexually transmitted diseases on HIV infection in rural Tanzania: randomised controlled trial. Lancet 346: 530-536.
23. White RG, Moodley P, McGrath N, Hosegood V, Zaba B, et al. (2008) Low effectiveness of syndromic treatment services for curable sexually transmitted infections in rural South Africa. Sex Transm Infect 84: 528-534.
24. Peters RP, Dubbink JH, van der Eem L, Verweij SP, Bos ML, et al. (2014) Cross-sectional study of genital, rectal, and pharyngeal Chlamydia and gonorrhea in women in rural South Africa. Sex Transm Dis 41: 564-569.
25. Watson-Jones D, Weiss HA, Rusizoka M, Changalucha J, Baisley K, et al. (2008) Effect of herpes simplex suppression on incidence of HIV among women in Tanzania. N Engl J Med 358: 1560-1571.
26. Celum C, Wald A, Hughes J, Sanchez J, Reid S, et al. (2008) Effect of aciclovir on HIV-1 acquisition in herpes simplex virus 2 seropositive women and men who have sex with men: a randomised, double-blind, placebo-controlled trial. Lancet 371: 2109-2119.
27. Fleming DT, Wasserheit JN (1999) From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. Sex Transm Infect 75: 3-17.
28. Weiss HA, Quigley MA, Hayes RJ (2000) Male circumcision and risk of HIV infection in sub-Saharan Africa: a systematic review and meta-analysis. AIDS 14: 2361-2370.
29. Auvert B, Taljaard D, Lagarde E, Sobngwi-Tambekou J, Sitta R, et al. (2005) Randomized, controlled intervention trial of male circumcision for reduction of HIV infection risk: the ANRS 1265 Trial. PLoS Med 2: e298.
30. Bailey RC, Moses S, Parker CB, Agot K, Maclean I, et al. (2007) Male circumcision for HIV prevention in young men in Kisumu, Kenya: a randomised controlled trial. Lancet 369: 643-656.
31. Gray RH, Kigozi G, Serwadda D, Makumbi F, Watya S, et al. (2007) Male circumcision for HIV prevention in men in Rakai, Uganda: a randomised trial. Lancet 369: 657-666.
32. Siegfried N, Muller M, Deeks JJ, Volmink J (2009) Male circumcision for prevention of heterosexual acquisition of HIV in men. Cochrane Database Syst Rev: CD003362.
33. Sánchez J, Sal Y Rosas VG, Hughes JP, Baeten JM, Fuchs J, et al. (2011) Male circumcision and risk of HIV acquisition among MSM. AIDS 25: 519-523.
34. Ramjee G, Kamali A, McCormack S (2010) The last decade of microbicide clinical trials in Africa: from hypothesis to facts. AIDS 24 Suppl 4: S40-49.
35. Obiero J, Mwethera PG, Hussey GD, Wiysonge CS (2012) Vaginal microbicides for reducing the risk of sexual acquisition of HIV infection in women: systematic review and meta-analysis. BMC Infect Dis 12: 289.
36. Connor EM, Sperling RS, Gelber R, Kiselev P, Scott G, et al. (1994) Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. N Engl J Med 331: 1173-1180.
37. Townsend CL, Cortina-Borja M, Peckham CS, de Ruiter A, Lyall H, et al. (2008) Low rates of mother-to-child transmission of HIV following effective pregnancy interventions in the United Kingdom and Ireland, 2000-2006. AIDS 22: 973-981.
38. Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, et al. (2011) Prevention of HIV-1 infection with early antiretroviral therapy. N Engl J Med 365: 493-505.
39. Vernazza P, Hirschel B, Bernasconi E, Flepp M (2008) Les personnes séropositives ne souffrant d'aucune autre MST et suivant un traitement antirétroviral efficace ne transmettent pas le VIH par voie sexuelle. Bulletins des médecins suisses 89: 165-169.

40. CDC (1995) Case-control study of HIV seroconversion in health-care workers after percutaneous exposure to HIV-infected blood--France, United Kingdom, and United States, January 1988-August 1994. MMWR Morb Mortal Wkly Rep 44: 929-933.

41. Schechter M, do Lago RF, Mendelsohn AB, Moreira RI, Moulton LH, et al. (2004) Behavioral impact, acceptability, and HIV incidence among homosexual men with access to postexposure chemoprophylaxis for HIV. J Acquir Immune Defic Syndr 35: 519-525.

42. Drezzet J (2002) Post exposure prophylaxis in raped women, in IV International Conference in HIV Infection in women and children. Rio de Janeiro: Livro de Resumos. Universidade, Federal do Rio De Janeiro e Institute of Virology of Maryland.

43. Harrison LH (2000) Demand for post-sexual-exposure chemoprophylaxis for the prevention of HIV infection in Brazil [abstract 492]. In 7th Conference on Retroviruses and Opportunistic Infections. 2000: San Francisco, California.

44. Peterson L, Taylor D, Roddy R, Belai G, Phillips P, et al. (2007) Tenofovir disoproxil fumarate for prevention of HIV infection in women: a phase 2, double-blind, randomized, placebo-controlled trial. PLoS Clin Trials 2: e27.

45. Abdool Karim Q, Abdool Karim SS, Frohlich JA, Grobler AC, Baxter C, et al. (2010) Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. Science 329: 1168-1174.

46. Van Damme L, Corneli A, Ahmed K, Agot K, Lombaard J, et al. (2012) Preexposure prophylaxis for HIV infection among African women. N Engl J Med 367: 411-422.

47. Marrazzo JM, Ramjee G, Richardson BA, Gomez K, Mgodi N, et al. (2015) Tenofovir-based preexposure prophylaxis for HIV infection among African women. N Engl J Med 372: 509-518.

48. Helen Rees, Sinead A. Delany-Moretlwe, Carl Lombard, Deborah Baron, Ravindre Panchia, et al. (2015) FACTS 001 Phase III Trial of Pericoital Tenofovir 1% Gel for HIV Prevention in Women.

49. Jean-Michel Molina, Catherine Capitant, Bruno Spire, Gilles Pialoux, Christian Chidiac, et al. (2015) On Demand PrEP With Oral TDF-FTC in MSM: Results of the ANRS Ipergay Trial. Seattle, Washington.

50. McCormack S, Dunn DT, Desai M, Dolling DI, Gafos M, et al. (2015) Pre-exposure prophylaxis to prevent the acquisition of HIV-1 infection (PROUD): effectiveness results from the pilot phase of a pragmatic open-label randomised trial. Lancet.

51. Grant RM, Lama JR, Anderson PL, McMahan V, Liu AY, et al. (2010) Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. N Engl J Med 363: 2587-2599.

52. Thigpen MC, Kebaabetswe PM, Paxton LA, Smith DK, Rose CE, et al. (2012) Antiretroviral preexposure prophylaxis for heterosexual HIV transmission in Botswana. N Engl J Med 367: 423-434.

53. Baeten JM, Donnell D, Ndase P, Mugo NR, Campbell JD, et al. (2012) Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. N Engl J Med 367: 399-410.

54. Marrazzo JM, Ramjee G, Nair G, Palanee T, Mkhize B, et al. (2013) Pre-exposure prophylaxis for HIV in women: daily oral tenofovir, oral tenofovir/emtricitabine or vaginal tenofovir gel in the VOICE study (MTN 003). 20th Conference on Retroviruses and Opportunistic Infections, Atlanta.

55. Choopanya K, Martin M, Suntharasamai P, Sangkum U, Mock PA, et al. (2013) Antiretroviral prophylaxis for HIV infection in injecting drug users in Bangkok, Thailand (the Bangkok Tenofovir Study): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet 381: 2083-2090.

56. Anglemyer A, Rutherford GW, Egger M, Siegfried N (2013) Antiretroviral therapy for prevention of HIV transmission in HIV-discordant couples. Cochrane Database Syst Rev 4: CD009153.

57. Attia S, Egger M, Müller M, Zwahlen M, Low N (2009) Sexual transmission of HIV according to viral load and antiretroviral therapy: systematic review and meta-analysis. AIDS 23: 1397-1404.

58. Tanser F, Bärnighausen T, Grapsa E, Zaidi J, Newell ML (2013) High coverage of ART associated with decline in risk of HIV acquisition in rural KwaZulu-Natal, South Africa. Science 339: 966-971.

59. Iwuji CC, Orne-Gliemann J, Tanser F, Boyer S, Lessells RJ, et al. (2013) Evaluation of the impact of immediate versus WHO recommendations-guided antiretroviral therapy initiation on HIV incidence: the ANRS 12249 TasP (Treatment as Prevention) trial in Hlabisa sub-district, KwaZulu-Natal, South Africa: study protocol for a cluster randomised controlled trial. Trials 14: 230.

60. Hayes R, Ayles H, Beyers N, Sabapathy K1, Floyd S, et al. (2014) HPTN 071 (PopART): rationale and design of a cluster-randomised trial of the population impact of an HIV combination prevention intervention including universal testing and treatment - a study protocol for a cluster randomised trial. Trials 15: 57.

61. AVAC, UNAIDS (2014) Antiretroviral Treatment for Prevention of HIV and Tuberculosis. 2013 update on current and planned research efforts.

62. Musicco M, Lazzarin A, Nicolosi A, Gasparini M, Costigliola P, et al. (1994) Antiretroviral treatment of men infected with human immunodeficiency virus type 1 reduces the incidence of heterosexual transmission. Italian Study Group on HIV Heterosexual Transmission. Arch Intern Med 154: 1971-1976.

63. Melo MG, Santos BR, De Cassia Lira R, Varella IS, Turella ML, et al. (2008) Sexual transmission of HIV-1 among serodiscordant couples in Porto Alegre, southern Brazil. Sex Transm Dis 35: 912-915.

64. Sullivan P, Kayitenkore K, Chomba E, Karita E, Mwananyanda L, et al. (2009) Is the reduction of HIV transmission risk while prescribed antiretroviral therapy (ARVT) different for men and women? Results from discordant couples in Rwanda and Zambia. 5th IAS Conference on HIV Pathogenesis and Treatment. Abstract no. WEAC101.

65. Del Romero J, Castilla J, Hernando V, Rodríguez C, García S (2010) Combined antiretroviral treatment and heterosexual transmission of HIV-1: cross sectional and prospective cohort study. BMJ 340: c2205.

66. Donnell D, Baeten JM, Kiarie J, Thomas KK, Stevens W, et al. (2010) Heterosexual HIV-1 transmission after initiation of antiretroviral therapy: a prospective cohort analysis. Lancet 375: 2092-2098.

67. Lu W, Zeng G, Luo J, Duo S, Xing G, et al. (2010) HIV transmission risk among serodiscordant couples: a retrospective study of former plasma donors in Henan, China. J Acquir Immune Defic Syndr 55: 232-238.

68. Reynolds SJ, Makumbi F, Nakigozi G, Kagaayi J, Gray RH, et al. (2011) HIV-1 transmission among HIV-1 discordant couples before and after the introduction of antiretroviral therapy. AIDS 25: 473-477.

69. Josephine Birungi, Jeong Eun Min, Katherine A. Muldoon, Pontiano Kaleebu, Rachel King, et al. (2012) Lack of effectiveness of antiretroviral therapy (ART) as an HIV prevention tool for serodiscordant couples in a rural ART program without viral load monitoring in Uganda. In XIX International AIDS Conference, Washington, DC.

70. Jia Z, Mao Y, Zhang F, Ruan Y, Ma Y, et al. (2013) Antiretroviral therapy to prevent HIV transmission in serodiscordant couples in China (2003-11): a national observational cohort study. Lancet 382: 1195-1203.

71. Schiffner T, Sattentau QJ, Dorrell L (2013) Development of prophylactic vaccines against HIV-1. Retrovirology 10: 72.

72. Walker BD, Ahmed R, Plotkin S (2011) Moving ahead an HIV vaccine: use both arms to beat HIV. Nat Med 17: 1194-1195.

73. Flynn NM, Forthal DN, Harro CD, Judson FN, Mayer KH, et al. (2005) Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. J Infect Dis 191: 654-665.

74. Pitisuttithum P, Gilbert P, Gurwith M, Heyward W, Martin M, et al. (2006) Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. J Infect Dis 194: 1661-1671.

75. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, et al. (2008) Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet 372: 1881-1893.

76. Gray GE, Allen M, Moodie Z, Churchyard G, Bekker LG, et al. (2011) Safety and efficacy of the HVTN 503/Phambili study of a clade-B-based HIV-1 vaccine in South Africa: a double-blind, randomised, placebo-controlled test-of-concept phase 2b study. Lancet Infect Dis 11: 507-515.

77. NIH (2013) NIH Discontinues Immunizations in HIV Vaccine Study.

78. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, et al. (2009) Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med 361: 2209-2220.

79. Hammer SM, Sobieszczyk ME, Janes H, Karuna ST, Mulligan MJ, et al. (2013)

Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. N Engl J Med 369: 2083-2092.

80. Perelson AS, Nelson PW (1999) Mathematical analysis of HIV-1 dynamics in vivo. Siam Review 41: 3-44.

81. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, et al. (1995) Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature 373: 123-126.

82. Perelson AS, Ribeiro RM (2013) Modeling the within-host dynamics of HIV infection. BMC Biol 11: 96.

83. Granich RM, Gilks CF, Dye C, De Cock KM, Williams BG (2009) Universal voluntary HIV testing with immediate antiretroviral therapy as a strategy for elimination of HIV transmission: a mathematical model. Lancet 373: 48-57.

84. Eaton JW, Johnson LF, Salomon JA, Bärnighausen T, Bendavid E, et al. (2012) HIV treatment as prevention: systematic comparison of mathematical models of the potential impact of antiretroviral therapy on HIV incidence in South Africa. PLoS Med 9: e1001245.

85. Dodd PJ, Garnett GP, Hallett TB (2010) Examining the promise of HIV elimination by 'test and treat' in hyperendemic settings. AIDS 24: 729-735.

86. Hontelez JA, Lurie MN, Bärnighausen T, Bakker R, Baltussen R, et al. (2013) Elimination of HIV in South Africa through expanded access to antiretroviral therapy: a model comparison study. PLoS Med 10: e1001534.

87. McNairy ML, El-Sadr WM (2014) Antiretroviral therapy for the prevention of HIV transmission: what will it take? Clin Infect Dis 58: 1003-1011.

88. Musheke M, Ntalasha H, Gari S, McKenzie O, Bond V, et al. (2013) A systematic review of qualitative findings on factors enabling and deterring uptake of HIV testing in Sub-Saharan Africa. BMC Public Health 13: 220.

89. Deblonde J, De Koker P, Hamers FF, Fontaine J, Luchters S, et al. (2010) Barriers to HIV testing in Europe: a systematic review. Eur J Public Health 20: 422-432.

90. Kranzer K, Govindasamy D, Ford N, Johnston V, Lawn SD (2012) Quantifying and addressing losses along the continuum of care for people living with HIV infection in sub-Saharan Africa: a systematic review. J Int AIDS Soc 15: 17383.

91. Marks G, Gardner LI, Craw J, Crepaz N (2010) Entry and retention in medical care among HIV-diagnosed persons: a meta-analysis. AIDS 24: 2665-2678.

92. Govindasamy D, Ford N, Kranzer K (2012) Risk factors, barriers and facilitators for linkage to antiretroviral therapy care: a systematic review. AIDS 26: 2059-2067.

93. WHO (2003) Adherence to long term therapies. Evidence for action.

94. Paterson DL1, Swindells S, Mohr J, Brester M, Vergis EN, et al. (2000) Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. Ann Intern Med 133: 21-30.

95. Quinn TC, Wawer MJ, Sewankambo N, Serwadda D, Li C, et al. (2000) Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group. N Engl J Med 342: 921-929.

96. Chi BH, Cantrell RA, Zulu I, Mulenga LB, Levy JW, et al. (2009) Adherence to first-line antiretroviral therapy affects non-virologic outcomes among patients on treatment for more than 12 months in Lusaka, Zambia. Int J Epidemiol 38: 746-756.

97. Maqutu D, Zewotir T, North D, Naidoo K, Grobler A (2010) Factors affecting first-month adherence to antiretroviral therapy among HIV-positive adults in South Africa. Afr J AIDS Res 9: 117-124.

98. Mills EJ, Nachega JB, Bangsberg DR, Singh S, Rachlis B, et al. (2006) Adherence to HAART: a systematic review of developed and developing nation patient-reported barriers and facilitators. PLoS Med 3: e438.

99. Rosen S, Fox MP (2011) Retention in HIV care between testing and treatment in sub-Saharan Africa: a systematic review. PLoS Med 8: e1001056.

100. Fox MP, Rosen S (2010) Patient retention in antiretroviral therapy programs up to three years on treatment in sub-Saharan Africa, 2007-2009: systematic review. Trop Med Int Health 15 Suppl 1: 1-15.

101. Harries AD, Zachariah R, van Oosterhout JJ, Reid SD, Hosseinipour MC, et al. (2010) Diagnosis and management of antiretroviral-therapy failure in resource-limited settings in sub-Saharan Africa: challenges and perspectives. Lancet Infect Dis 10: 60-65.

102. Mutevedzi PC, Lessells RJ, Rodger AJ, Newell ML (2011) Association of age with mortality and virological and immunological response to antiretroviral therapy in rural South African adults. PLoS One 6: e21795.

103. Manasa J, Lessells RJ, Skingsley A, Naidu KK, Newell ML, et al. (2013) 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 8: e72152.

104. Bennett DE, Myatt M, Bertagnolio S, Sutherland D, Gilks CF (2008) Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. Antivir Ther 13 Suppl 2: 25-36.

105. Cambiano V, Bertagnolio S, Jordan MR, Pillay D, Perriëns JH, et al., (2014) Predicted levels of HIV drug resistance: potential impact of expanding diagnosis, retention, and eligibility criteria for antiretroviral therapy initiation. AIDS 28: S15-23.

106. Hamers RL, Wallis CL, Kityo C, Siwale M, Mandaliya K, et al. (2011) HIV-1 drug resistance in antiretroviral-naive individuals in sub-Saharan Africa after rollout of antiretroviral therapy: a multicentre observational study. Lancet Infect Dis 11: 750-759.

107. Scandlyn J (2000) When AIDS became a chronic disease. West J Med 172: 130-133.

108. Bunnell R, Ekwaru JP, Solberg P, Wamai N, Bikaako-Kajura W, et al. (2006) Changes in sexual behavior and risk of HIV transmission after antiretroviral therapy and prevention interventions in rural Uganda. AIDS 20: 85-92.

109. Venkatesh KK, de Bruyn G, Lurie MN, Mohapi L, Pronyk P, et al. (2010) Decreased sexual risk behavior in the era of HAART among HIV-infected urban and rural South Africans attending primary care clinics. AIDS 24: 2687-2696.

110. McGrath N, Eaton JW, Bärnighausen TW, Tanser F, Newell ML (2013) Sexual behaviour in a rural high HIV prevalence South African community: time trends in the antiretroviral treatment era. AIDS 27: 2461-2470.

111. Katz MH, Schwarcz SK, Kellogg TA, Klausner JD, Dilley JW, et al. (2002) Impact of highly active antiretroviral treatment on HIV seroincidence among men who have sex with men: San Francisco. Am J Public Health 92: 388-394.

112. Bezemer D, de Wolf F, Boerlijst MC, van Sighem A, Hollingsworth TD, et al. (2008) A resurgent HIV-1 epidemic among men who have sex with men in the era of potent antiretroviral therapy. AIDS 22: 1071-1077.

113. Holmes KK (2007) King Kennard Holmes--chair of the Department of Global Health of The University of Washington. Interviewed by Marc Vandenbruaene. Lancet Infect Dis 7: 516-20.

114. UNAIDS (2010) Combination HIV Prevention:Tailoring and Coordinating Biomedical, Behavioural and Structural Strategies to Reduce New HIV Infections. A UNAIDS Discussion Paper.

115. Jones A, Cremin I, Abdullah F, Idoko J, Cherutich P, et al. (2014) Transformation of HIV from pandemic to low-endemic levels: a public health approach to combination prevention. Lancet 384: 272-279.

116. UNAIDS (2007) Practical guidelines for intensifying HIV prevention. Towards universal access.

117. Anderson SJ, Cherutich P, Kilonzo N, Cremin I, Fecht D, et al. (2014) Maximising the effect of combination HIV prevention through prioritisation of the people and places in greatest need: a modelling study. Lancet 384: 249-256.

118. Boffito M, Jackson A, Owen A, Becker S (2014) New approaches to antiretroviral drug delivery: challenges and opportunities associated with the use of long-acting injectable agents. Drugs 74: 7-13.

119. Piot P, Abdool Karim SS, Hecht R, Legido-Quigley H, Buse K, et al. (2015) Defeating AIDS--advancing global health. Lancet 386: 171-218.

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